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Marcelo Gonzalez-Ortiz *Editor*

Advances in Maternal-Fetal Biomedicine

Cellular and Molecular Mechanisms of Pregnancy Pathologies

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"The organism is the consequence of a historical process that goes on from the moment of conception until the moment of death; at every moment gene, environment, chance, and the organism as a whole are all participating". (Lewontin & Lewis, The Dialectical Biologist, Harvard University Press, 1985)

About This Book

This textbook aims to describe physiological and pathophysiological mechanisms that underlie human maternal-fetal interactions. The book emphasizes the structure and development of the fetoplacental unit, the endocrine and nutritional regulation of fetal development, nitric oxide signaling, solute carriers function, and ion channels regulation in healthy pregnancies and diseases like preeclampsia, gestational diabetes, and maternal obesity, among others. Also, we highlight some novelty mechanisms associated with language impairment in children, the use of serotonin inhibitors or cannabis during pregnancy, and maternal conditions' potential impact on cerebrovascular development in newborns and infants. The cellular and molecular understanding of maternal-fetal physiology and pathophysiology will allow the readers to understand the impact of diseases or conditions that are highly prevalent in pregnant women.

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About the Editor

Marcelo González-Ortiz earned a PhD in Physiological Sciences from the Pontificia Universidad Católica de Chile in 2009. From 2009 to 2018, he led the Vascular Physiology Lab at the Department of Physiology, and since 2018, he has been the head of the Maternal-Fetal Research Lab at the Department of Obstetrics and Gynecology at the Faculty of Medicine at Universidad de Concepción in Chile. His research is focused on studying the regulation mechanisms of nitric oxide synthesis and calcium-activated potassium channels in the human placenta under both physiological and pathophysiological conditions. Currently, Dr. González-Ortiz's research projects concentrate on gestational diabetes and the effects of COVID-19 on the placenta and children.

Feto-placental Unit: From Development to Function

1

Ambart Covarrubias, Macarena Aguilera-Olguín, Ivo Carrasco-Wong, Fabián Pardo, Pamela Díaz-Astudillo, and Sebastián San Martín

Abstract

The placenta is an intriguing organ that allows us to survive intrauterine life. This essential organ connects both mother and fetus and plays a crucial role in maternal and fetal well-being. This chapter presents an overview of the morphological and functional aspects of human placental development. First, we describe early human placental development and the characterization of the cell types found in the human placenta. Second, the human placenta from the second trimester to the term of gestation is reviewed, focusing on the morphology and specific pathologies that affect the placenta. Finally, we focus on the placenta's primary functions, such as oxygen and nutrient transport, and their importance for placental development.

Keywords

Placenta morphology · Development · Oxygen · Nutrient transport

1.1 Development of the Placenta

1.1.1 The Origin of Trophoblast

The first cell lineage to differentiate during development is the trophoblast, and the first organ to be formed in mammals is the placenta. The placental formation is required to establish a maternal-fetal vascular interface that can supply the needs of the developing embryo (Maltepe and Fisher 2015). On day three post-fertilization (dpf), the 8-cell embryo undergoes compaction to become a morula, a compact, smooth spherical structure. Consequently, an ionic gradient is generated, drawing water into the center of the mass and expanding it to form a blastocyst (~day 4.5 dpf) (Selwood and Johnson 2006). The blastocyst consists of two different cell populations: the inner cell mass (ICM) and the trophectoderm (TE). The placenta arises from the TE, the outer layer of the blastocyst. The trophectoderm layer is formed around 5 days post-fertilization (dpf), and the part that is contiguous with the underlying ICM (called polar TE) attaches to the colum-

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nar epithelium of the uterine mucosa (Fig. 1.1a). The earliest stages of implantation – initial interaction between the TE and uterine epithelium – have not yet been observed in human embryos; it appears that the blastocyst is orientated so that the embryonic pole is opposed to the endometrium first, as in the macaque (Boyd and Hamilton 1970). Also, morphological observation of early human pregnant hysterectomy specimens and studies in higher primates suggest that the TE fuses after attachment to the endometrium to form the primary syncytium (3) at day 6 ~ 7 dpf. This first stage, lasting from day 7 to day 8 dpf, is defined as the prelacunar period (Benirschke et al. 2012a) (Fig. 1.1a). The endometrium surrounds the blastocyst throughout the days, differentiated into a transient tissue termed decidua (Schlafke and Enders 1975). The uterine epithelium is reconstituted over the implantation site (Benirschke et al. 2012a). The syncytiotrophoblast (STB) quickly increases and forms a mantle over the surface of the blastocysts, supported by the proliferation and fusion of the underlying cytotrophoblast cells (Benirschke et al. 2012a) (CTB) (Fig. 1.1b).

1.1.2 Lacunar Stage

Around day 8 dpf, fluid-filled spaces appear within the syncytiotrophoblastic mass (Fig. 1.1c). The spaces rapidly enlarge and become confluent, forming a system of lacunae; this is the lacunar stage, which lasts from day 8 to 13 dpf. The separating lamellae and pillar of STB are called the trabeculae. First, STB surrounds and erodes the decidual glands; then the glandular secretions contribute to the fluid in the lacunae (Burton et al. 2007). Simultaneously, the CTB cells, beneath the STB mantle, proliferate and penetrate the trabeculae; they extend to the tip of the trabeculae and spread laterally to form the cytotrophoblast shell between the villi and decidua (Benirschke et al. 2012a). The formation of the lacunae subdivides the trophoblastic covering of the blastocyst into three layers: (A) the primary chorionic plate, facing the original blastocyst cavity; (B) the lacunar system together with the trabeculae; and (C) the cytotrophoblastic shell, facing the endometrium, which is the forerunner of the basal plate (Turco and Moffett 2019). The cytotrophoblastic shell develops around day 14 dpf (Fig. 1.1d). The cells of the shell are rounded and contain large amounts of glycogen. Individual cytotrophoblastic cells leave the shell to invade into decidua (maternal surface). These cells are referred to as extravillous trophoblasts and start the process of trophoblast invasion. The invading cells reach the inner third of the myometrium, where they undergo fusion to form multinucleated giant cells (Al-Lamki et al. 1999).

1.1.3 Villous Stage

By day 13 dpf, the CTB cells beneath the STB rapidly proliferate to protrude into the cavities and form branches, representing the primary villi, the villous stage of placentation. The primary villi are formed by a cytotrophoblastic core covering syncytiotrophoblasts (Fig. 1.1d). The villous trees are constituted by proliferation and branching; gaps turn into interspersed space.

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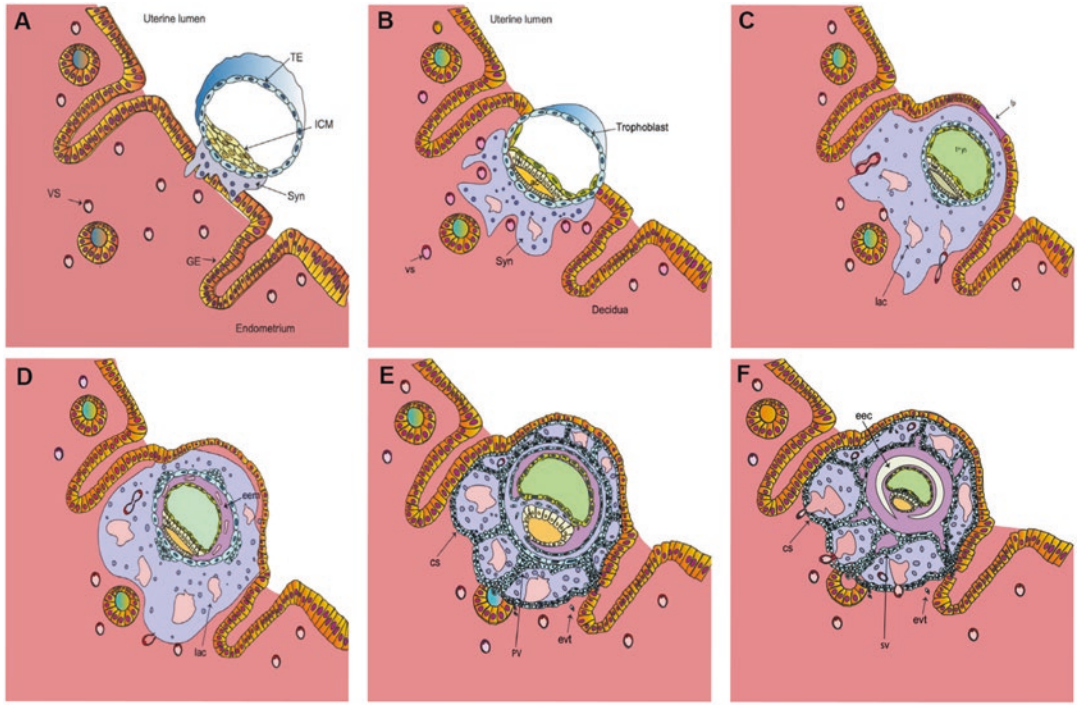

Fig. 1.1 Diagram showing the early stages of the placental development after blastocyst implantation. (**a**, **b**) Prelacunar stages, (**c**) lacunar stage, (**d**) transition from lacunar to primary villous stages, (**e**) primary villous stages, and (**f**) secondary villous stage. 1° *ys* primary yolk sac, *ac* amniotic cavity, *cs* cytotrophoblastic shell, *eec* extra-embryonic coelom, *eem* extra-embryonic mesoderm, *evt* extravillous trophoblast, *fp* fibrin plug, *GE* glandular epithelium, *ICM* inner cell mass, *lac* lacunae, *pv* primary villous, *sv* secondary villous, *syn* syncytium, *TE* trophectoderm, *vs* blood vessel

Soon, mesenchymal cells invade the primary villi to form the secondary villi (Fig. 1.1f). By day 18 dpf, fetal capillaries appear within the villi's mesenchyme, marking the development of tertiary villi (Castellucci et al. 1990). The capillaries are derived from hemangioblastic progenitor cells, locally differentiated from the mesenchyme (Robin et al. 2009). The villous tree proliferates and forms a villous system enlarged by progressive branching from the chorionic plate. The placenta's main structures are developed by the end of the first trimester. During the second and third trimesters, there is an increase in functional capacity due to the enlarged surface area of the chorionic villi and a decrease in the maternal-fetal distance (Benirschke et al. 2012a; Burton et al. 2007; Schlafke and Enders 1975; Turco and Moffett 2019).

1.1.4 Cell Types of Human Placenta

A variety of cells are involved in forming the placenta. Using single-cell RNA sequencing with no marker selection, it is possible to differentiate at least five clusters of cells: trophoblast, stromal fibroblast cells, Hofbauer cells, antigen-presenting cells, and endothelial cells (Sun et al. 2019). However, the primary cell type in the placenta is the trophoblast.

1.1.4.1 Trophoblastic Cell Linage

The trophectoderm gives rise to the epithelial trophoblast lineages of the placenta. A Dutch embryologist used "trophoblast" to describe the cells transporting nutrients from or to the growing fetus and generating a barrier between fetus and mother (Pijnenborg and Vercruysse 2013).

The word trophoblast is derived from the Greek "tropho," meaning feed. A variety of human trophoblast subtypes have been identified until now.

The mature human placenta contains two main types of epithelial trophoblasts: (1) syncytiotrophoblast (STB) and (2) extravillous trophoblast (EVT). Also, using scRNA-seq in first-trimester placentas, two distinct trophoblast differentiation pathways have been solved, along with several subtypes (Vento-Tormo et al. 2018). Finally, Sun et al. reported at least seven unique trophoblast subclusters from placentas in ongoing human pregnancies at 11–13 weeks gestation (Sun et al. 2019).

(a) *Cytotrophoblast.* The mononuclear villous trophoblast, also known as cytotrophoblast (CTB), forms a single layer of cells that lines the stromal cell core. Traditionally, CTB is considered germinative, replenishing STB and EVT because they are mitotic and express proliferative markers (Liu et al. 2018). The CTBs have a cuboidal shape when forming a continuous layer in early pregnancy. As it expands, the CTB layers become thinner and discontinuous, and in a term placenta, a thin syncytial layer mostly separates the villus core from the maternal blood (Handwerger 2010). Single-cell seq may help identify different cells and subtypes that have not yet been recognized. Liu et al. used single-cell seq to identify new subtypes of CTB cells: a proliferative subtype, which may serve as the group that replenishes the CTB pool; a non-proliferative, syncytin-2-positive cell subtype, which proved to be the progenitor cell of the STB; and a non-proliferative, syncytin-2-negative subtype. Identifying the progenitor cells for CTBs is crucial for understanding human placental lineage commitment (Liu et al. 2018).

(b) *Syncytiotrophoblast.* The STB, the outer layer of all villous trees, is a continuous syncytial layer with a brush border in direct contact with maternal glandular secretions at the beginning of the gestation and, later, with maternal blood flowing into the intervillous space. The brush border (microvilli) increases its surface area by several folds (Teasdale and Jean-Jacques 1985). The STB is a highly polarized epithelial layer. A multinucleated layer with no cell borders may facilitate diffusion across this epithelial layer and protect the fetus from pathogens (Turco and Moffett 2019). Also, it is the primary site of the synthesis and secretion of hormones; at least 102 polypeptide hormone genes were detected in STB by Liu et al. (2018) (Liu et al. 2018). Furthermore, STB functions as an immunological barrier because it does not express any human leukocyte antigen (HLA) molecules, resulting in the allogeneic fetus not being recognized by the circulating immune cells as foreign tissue (Yelavarthi et al. 1991). The STB also expresses the neonatal Fc receptor, which permits the transport of maternal IgG antibodies into the fetal circulation (Roopenian and Akilesh 2007).

(c) *Extravillous trophoblasts (EVT).* Classically, it has been thought that the EVTs migrate from the shell and the anchoring villi through the decidual following two pathways of differentiation: (1) interstitial trophoblasts (iEVT), which invade the maternal decidualized endometrium attaching the placenta to the uterus, and (2) endovascular trophoblasts (eEVT), which invade maternal spiral arteries establishing the uteroplacental blood flow. However, recently, a new pathway has been described: (3) endoglandular trophoblasts (gEVT), which invade the uterine glands, allowing histiotrophic nutrition (Moser et al. 2015). All have invasive capacity and differentiate from typical proliferative CTB cells in the cell column's basal layer (Kaufmann and Castellucci 1997).

  (i) *iEVT* cells are pleomorphic; some have a small fusiform shape, and others are large polygonal. They have tetraploid nuclei and are non-cycling, showing changes in senescence (Velicky et al. 2018). In addition, they specifically express placenta-specific protein 8 (PLAC8), which might play an essential role during the invasion process (Chang

et al. 2018). In healthy pregnancies, the iEVT cells invade the inner third of the myometrium and fuse to form placental bed giant cells (Turco and Moffett 2019).

(ii) *eEVT cells* are morphologically different from iEVT cells and express CD56 (NCAM) (Burrows et al. 1994). It was recently reported that eEVT cells express CD59 to evade complement-dependent cytotoxicity, which may confer some protection against maternal complement (Sato 2020; Ueda et al. 2019). eEVT cells invade the uterine spiral arteries, and some invade the vessel media and replace the endothelium, while others accumulate in the lumen of the spiral arteries, forming plugs. The process remodels the arteries into the dilated vessels without maternal vasomotor control (Pijnenborg 1996). Moreover, at the end of the first trimester (12–13 weeks of pregnancy), the plugs are disintegrated, creating the onset of the uteroplacental blood flow (hemotrophic nutrition). Zhou et al. described the molecular mimicry of the invading trophoblast-expressing endothelial adhesion molecules. They observed a reduced expression of E-cadherin; an upregulation of VE-cadherin, PECAM1, and VCAM1; and the acquisition of α5β3 and α1β1 (Zhou et al. 1997a, b); however, the mimicry model is questioned by other researchers (Floridon et al. 2000; Lyall et al. 2001). It is thought that the most critical role of the eEVT is to ensure placental blood perfusion. Increased arterial blood flow into the intervillous space contributes to a successful pregnancy. Besides, impaired spiral artery remodeling is considered one of the leading causes of preeclampsia (Pijnenborg et al. 2006).

(iii) *gEVT cells* are trophoblasts that penetrate uterine glands to form the interstitial side and replace the glandular epithelium. These cells open the lumen of the glands toward the intervillous space, releasing glandular secretion into the intervillous space and helping with the embryo's histiotrophic nutrition before establishing the uteroplacental blood flow (Moser et al. 2015, 2018). gEVT cells express the pro-invasive proteins MMP1, MMP9, and integrin B1 (ITGB1) (Weiss et al. 2016). Finally, it is thought that the defining feature of EVT differentiation is the upregulation of the non-classical MHC class I HLA-G and HLA-C molecules (King et al. 2000; Kovats et al. 1990). Interestingly, Sun et al. (2019) have reported after using scRNA-seq that HLA-G, the classical cell type-specific marker for EVT cells, is only detected in a portion of the EVT population (Sun et al. 2019).

1.1.4.2 Non-trophoblastic Cell Lineage

The placenta contains various cell types in the villi's stroma, including fibroblasts and immune and vascular cells. These cells are thought to arise from the extraembryonic mesenchyme. However, the origin of these cells in humans is uncertain. First, it was proposed that these cells derived from the mesenchymal core and originated from the primitive cytotrophoblast layer (Boyd and Hamilton 1970), which may be possible via an epithelial-mesenchymal transition (Mobley et al. 2017). Later, it was proposed that the extraembryonic mesenchyme originated from the hypoblast, a derivative of ICM (Boss et al. 2018).

(a) *Fibroblasts (FBs).* Fibroblasts are the most abundant cell type reported by single-cell RNA sequencing (scRNA-seq) of first-trimester villi (Sun et al. 2019; Suryawanshi et al. 2018). This population contains cells at different stages of differentiation, including undifferentiated mesenchymal stem cells, differentiated fibroblasts, and myofibroblasts (Sun et al. 2019). FBs share the expression of extracellular matrix genes such as collagens (COL1A1, COL1A2, and COL3A) and non-collagenous glycoproteins (LAMA2 and LAMC3), proteoglycans (DCN), and matrix metalloproteinase (TIMP1 and TIMP3) (Sun

et al. 2019; Suryawanshi et al. 2018). In addition, two populations of FBs can be distinguished by the presence or absence of the DLK1, an imprinted gene encoding an endocrine signaling molecule at high concentration in maternal circulation during late pregnancy (Cleaton et al. 2016; Liu et al. 2018; Turco and Moffett 2019; Vento-Tormo et al. 2018). Lastly, DLK1 cells have similarities to pericytes (Suryawanshi et al. 2018).

(b) *Fetal Endothelial Cells.* These cells develop from hemangioblast populations in the mesenchyme (Dempsey 1972; Robin et al. 2009). The immature endothelial cells express a specific pattern of genes, such as EGFL7, which is known to regulate vascular morphogenesis (Parker et al. 2004). In addition, they also express ACKR1, PCDH17, FAM167B, AQP1, HP, and IFI27 (Suryawanshi et al. 2018). Lastly, endothelial cells are usually the least abundant cell population shown by scRNA-seq, specifically expressing PECAM1 and CD93 (Sun et al. 2019).

(c) *Decidual Natural Killer (dNK) Cells.* In humans, CD56 bright CD16-NK cells are mainly present in the endometrium, accounting for 20% of leucocytes in the proliferative phase, increasing up to 40–50% in the secretory phase, and reaching a maximum of 70–80% in the early pregnancy decidua (Liu et al. 2017). The dNK cells diminish midway through gestation, and only a few are present at term (Sato 2020). However, during the first trimester of pregnancy, dNK cells are found near migrating interstitial trophoblasts and in the area of spiral arteries during remodeling (Helige et al. 2014). This evidence suggests that dNK cells are involved in spiral artery remodeling (Smith et al. 2009).

A combination of killer immunoglobulin-like receptors (KIRs) on the maternal dNK cells and HLA-C molecules on the fetal trophoblast could influence pregnancy success. Women with two KIR A haplotypes and fetuses with paternally derived HLA-C2 carry the highest risk of obstetric complications associated with defective placentation (Moffett et al. 2017).

In vitro studies have shown that activated dNK cells can enhance trophoblast invasion by producing soluble factors such as interleukin-8, interferon-inducible protein-10, and GM-CFS (Sato 2020). Furthermore, interactions between maternal KIRs and fetal HLA-C genetic variants are associated with pregnancy disorders such as preeclampsia, in which trophoblast invasion is deficient (Moffett and Colucci 2015). However, a detailed understanding of the cellular interaction in the decidual that supports early pregnancy is lacking.

1.2 Placenta During the Final Embryonic Stage and the Fetal Period

The placenta is responsible for many of the functions that take place during gestation (Turco and Moffett 2019). The size of the placenta is related to the efficacy of the organ and its function, whereby specific phenotypic characteristics are associated with fetal programming and postnatal diseases (Moffett and Colucci 2015).

Throughout pregnancy, the chorionic villus matures into the tertiary villus in proportion to the development and size of the embryo (Hayward et al. 2016; Pollheimer et al. 2018). Around weeks 6–8, the placenta's average volume is approximately 27.5 cm^3 (Hayward et al. 2016), and its length is approximately 32.5 mm (Walter et al. 2020), while the embryo reaches a cephalocaudal length of 7 mm (Elhelaly et al. 2020). It has been previously described that intervillous blood flow generally starts in the first trimester at 12 weeks of pregnancy (Fadl et al. 2017). In establishing the intervillous circulation, the placental volume can fluctuate between 40 and 45 mL (Farina 2016). At this stage, the placenta has reached a thickness of 15.2 mm (Azagidi et al. 2020) and an approximate weight of 36 gr. Similarly, at this point, the embryo has reached

an average weight of 23 gr and a cephalocaudal length of 8 cm (Azagidi et al. 2020; Sadler 2015).

Around week 14, the placental volume doubles to an average of 60–80 mL (Farina 2016; Hasegawa et al. 2015), while the placental thickness and weight increase to approximately 17.9 mm (Hasegawa et al. 2015) and 51 gr, respectively. At the same time, the fetus reaches a weight of 44 gr and a cephalocaudal length of 10 cm (Rosete Nogueira and Cardoso 2019; Sadler 2015). Although the placental growth has been described as a continuous process throughout pregnancy (Agwuna et al. 2016), the ratio between fetal and placental weight increases in the third trimester. This change occurs due to a deceleration in placental growth and a doubling of fetal weight between weeks 20 and 40 (Hayward et al. 2016). At this point, the placenta weighs approximately 316 gr (Nascente et al. 2020) and has a thickness of roughly 31.94 mm (Azagidi et al. 2020). In comparison, the fetus weighs about 908.7 gr (Nascente et al. 2020) and has a cephalocaudal length of 27 cm (Sadler 2015), establishing a 1:3 ratio between placental and fetal weight (Hayward et al. 2016).

At birth, the placenta has a diameter between 15 and 20 cm, a thickness of 2–4 cm, and weighs around 500 gr (Prieto-Gómez et al. 2018; Zaidi et al. 2016), reaching a ratio of 1:6 to 1:8 between placental and fetal weight. However, the ratio may vary since male newborns' placentas are often bigger than female ones (Pollheimer et al. 2018; Prieto-Gómez et al. 2018). In addition, the placental vasculature is over 550 km long and has a total surface area of 15 m^2 (McDonald et al. 2016).

The size of the placenta carries increased significance at various gestational ages, and its size has allowed us to identify or predict the occurrence of maternal and fetal disorders. For example, a small placenta for gestational age can be related to fetal growth restriction or congenital anomalies. In contrast, increased placental weight is associated with maternal anemia, diabetes, and abnormal umbilical cord insertion, among other abnormalities (Khong et al. 2019).

1.2.1 Fetal Annexes

1.2.1.1 Umbilical Cord

The umbilical cord forms between weeks 4 and 6 of gestation from the connecting stalk and connects the developing embryo to the placenta (Bosselmann and Mielke 2015). In the early embryonic stage, the umbilical cord is short. It develops from the center of the implantation site, where it is connected to the fetal gut in the proximal region until week 10. It then moves to the abdominal region, facilitating its elongation and placement at the embryo's umbilicus (Fahmy 2018). The umbilical vein allows for nutrients and oxygen, while the two umbilical arteries transport deoxygenated blood away from the fetus. These structures are inserted in the placenta and are surrounded by a substance called Wharton's Jelly (Wilke et al. 2018).

The location of cord insertion can be described as central, eccentric, marginal (battledore), or membranous (velamentous) insertions, depending on the site of insertion in the placenta (Chang and Aw 2019; Ismail et al. 2017). Central and eccentric cord insertions correspond to 90% of the insertions, while marginal and velamentous cord insertions are considered abnormal or pathological and represent 7% and 1% of cord insertions, respectively (Ismail et al. 2017).

Central cord insertions occur in the placental disc's central region of the fetal surface (Fig. 1.2a). While cord insertions occur outside the central area, no more than 1 cm from the placental border, they are called eccentric or paracentric insertions (Fig. 1.2b) (Ismail et al. 2017). Marginal insertions are characterized by cord insertion within less than 3 cm of the placental margins. The distance between the outer cord and placental margins is less than 2 cm (Fig. 1.2c) (Nkwabong et al. 2021). In the case of membranous or velamentous insertions, the umbilical cord blood vessels are not surrounded by Wharton's Jelly (Suzuki and Kato 2015). Here the vessels are inserted into the fetal membranes but not into the placenta (Fig. 1.2d) (Sinkin et al. 2018).

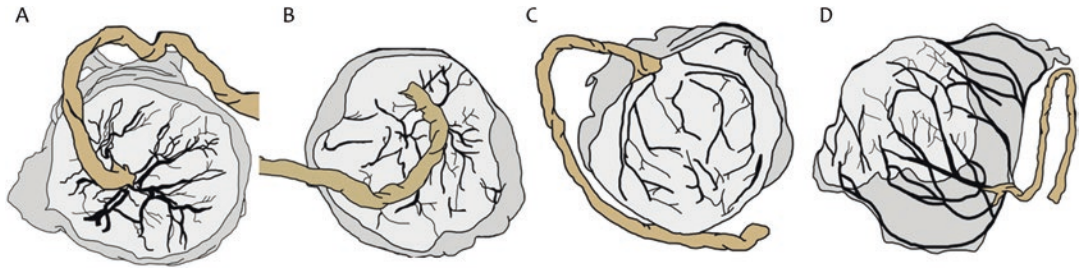

Fig. 1.2 Umbilical cord insertion types. (**a**) central, (**b**) eccentric, (**c**) marginal and (**d**) velamentous

Existing evidence suggests that abnormal umbilical cord insertion is related to an alteration in fetal growth (Padula et al. 2016). The pathogenesis is still unknown. However, three different theories have been postulated: abnormal implantation or polarity theory (orientation of the fetal pole relative to the endometrial surface), the theory of trophotropism (where the growth of the placenta depends on blood supply and atrophies in the areas where it is not sufficient), and, lastly, the theory of abnormal placental growth due to diminished chorionic villus (Ismail et al. 2017). In addition, abnormal cord insertion has been associated with premature labor, small for gestational age fetuses, intrauterine growth restriction, abnormal fetal heartbeat frequency, neonatal death, abruption, and other complications (Suzuki and Kato 2015).

An abnormal umbilical cord insertion may accompany an abnormal placenta (Khong et al. 2019). Placentas of varying shapes can therefore be classified as a bipartite placenta or bilobed placenta, meaning that they are formed by two lobes of similar sizes where the umbilical cord usually attaches between the two lobes (Dabkowska et al. 2020) or as a multilobed placenta, containing similar characteristics to the bipartite placenta but possessing more than two similar-sized lobes (Khong et al. 2019). In addition, a succenturiate placenta can also develop and is characterized by one or more accessory lobes of smaller size (Stelzl et al. 2017), which are located on the membranes apart from the placental disc (Kumari et al. 2015).

Finally, a circumvallate placenta is another anomalously shaped placenta whereby the chorionic plate is smaller than the basal plate, causing the membranes to fold towards the chorionic surface (AboEllail et al. 2015). The umbilical cord generally has a marginal insertion (Sharma et al. 2017). The membranous placenta, also called the diffuse placenta, is characterized by a gestational sac covered totally or partially by chorionic villi (Sharma et al. 2017). In contrast, the fenestrated placenta is characterized by the absence of the central portion of the placenta (Zaidi et al. 2016).

1.2.1.2 Extraembryonic Membranes

The extraembryonic membranes, the yolk sac, the allantois, and the amnion are formed during the three-layered embryo stage (Kruepunga et al. 2018). The yolk sac provides nutrients and gas exchange while also providing protein synthesis, hematopoiesis, and the formation of the gastrointestinal tract before the placental circulation is established (Odland Karlsen et al. 2019). It forms after the 7th or 8th day of development (Juul and Christensen 2018), and the size increases progressively until it begins to involute at the end of the embryonic period (Odland Karlsen et al. 2019). The yolk sac floats within the exocoelomic cavity and is connected to the embryo's midgut by the yolk duct (Cindrova-Davies et al. 2017). The yolk sac develops in two stages. First, the visceral and parietal endoderm differentiate into the primary yolk sac in the primary stage. Then, in the secondary stage, the secondary yolk sac forms from the remains of the primary yolk sac and has no contact with the trophoblast. Later, as a result of the expansion of the amnion, a reduction of the exocoelomic cavity and regression of the yolk sac takes place (Dong and Yang 2018).

The allantois corresponds to an endodermic excrescence of the posterior intestine. It develops

blood vessels and is associated with the development of the bladder (Al-Lamki et al. 1999). This extraembryonic membrane derives from the endoderm and splanchnic mesoderm (Hafez 2017). It forms from a hindgut (endoderm) evagination and a diverticulum covered by mesoderm (Bazer and Johnson 2018). The allantois blood vessels will become the umbilical vein and arteries, and as the development progresses, they will be called the urachus (McDonald et al. 2016).

The amniotic membrane is one of the innermost extraembryonic membranes derived from the epiblast and forms the amniotic cavity. It contains the amniotic fluid and is part of the fetal membrane. It is covered by the chorion, which serves as a protective barrier and provides limited space to allow fetal movement (Ramuta and Kreft 2018; Verbruggen et al. 2017). In the beginning, the chorion and the amnion are separated, but around weeks 17–20 of gestation, they form a mature chorioamniotic membrane (Muench et al. 2017). It comprises a monolayer of epithelial cells, the basal lamina, and the avascular stroma (Ramuta and Kreft 2018; Vo et al. 2017). In addition, it has been described as a metabolically active membrane since it maintains the homeostasis of the amniotic fluid (Mohan et al. 2017).

1.2.2 Amniotic Fluid

Amniotic fluid is generated at the beginning of pregnancy from the mother's plasma, consisting of 98% water, nutrients (protein, lactate, carbohydrates, lipids, phospholipids, urea, and electrolytes), hormones (prolactin, growth hormone), placental hormone, human chorionic gonadotropin, adrenocorticotropic hormone, chorionic thyrotropin and luteinizing hormone-releasing factor and antibodies (Lim et al. 2017; Rani et al. 2018). The amniotic fluid is transferred to the fetal gut through pinocytosis, diffusion, and absorption (Lim et al. 2017). The amniotic fluid volume changes radically throughout gestation, beginning at week 10 with 20 ml, increasing to 630 mL at 22 weeks, 770 mL at 28 weeks, and decreasing to 515 mL at 41 weeks (Beall et al. 2012). One of the amniotic fluid's primary functions is to protect against mechanical events or shocks and promote the development of the skeletal, muscular, pulmonary, and gastrointestinal tracts (Rani et al. 2018).

The production of amniotic fluid occurs during two different gestational periods: early and late gestation. In early pregnancy, during the first trimester and before the amniotic cavity formation, the fluid in the coelomic cavity maintains direct contact with the chorionic villi mesenchyme, providing nutrients to the embryo until the 12th week of gestation. During this period, the amniotic fluid has a composition similar to maternal plasma, suggesting that it is the product of maternal plasma ultrafiltration (Murphy and Koos 2018). Later, it disappears, and the amniotic cavity arises, where the amniotic fluid increases the space for fetal growth. In late pregnancy, after 12 weeks, the volume of amniotic fluid is composed mainly of maternal serum, and during the second and third trimesters, it will also contain large amounts of fetal urine (Fig. 1.3). Finally, pulmonary and gastrointestinal secretions and excretions from the umbilical cord and placental surface will become part of the amniotic fluid composition (Boopathy and Sathiya 2018; Fitzsimmons and Bajaj 2022).

1.2.2.1 Amniotic Fluid Pathologies

One of the alterations in amniotic fluid is the volume produced, which is evaluated in patients of at least 24 weeks gestation. Two alterations are identified: oligohydramnios is a decrease in amniotic fluid volume. It occurs due to decreased fetal urine, which generates chronic hypoperfusion of the fetus or leakage of the amniotic membrane, allowing amniotic fluid to escape from the uterus. Conversely, polyhydramnios consists of an increase in amniotic fluid volume.

The causes can be idiopathic, maternal diabetes, fetal (congenital abnormalities, genetic, aneuploidy, or other causes such as intestinal-type malformations such as atresia, cardiac, intrathoracic, renal as nephroma, dysplasia, neurological disorders such as anencephaly), or placental (infections such as cytomegalovirus, toxoplasmosis; multiple pregnancies, twin-twin transfusion syndrome) (Lord et al. 2022; Moore 2017; Ogunyemi and Friedman 2018).

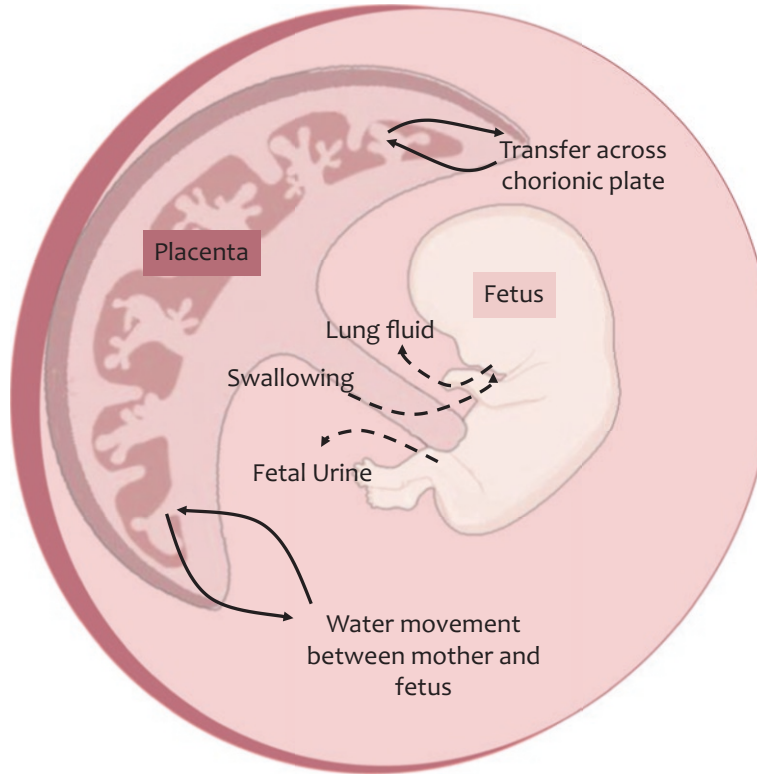

Fig. 1.3 Amniotic fluid production. Early gestation: amniotic fluid is similar to maternal plasma with 90% water (continuous arrow). Late gestation: amniotic fluid contains fetal urine, pulmonary secretions, and other fetal fluids (dashed arrow)

The presence of inflammatory cells in the amniotic fluid is associated with adverse pregnancy outcomes, causing premature delivery and, in some cases, rupture of membranes. Inflammation can be caused by infection, but there are also cases in the absence of pathogenic microorganisms that generally occur in the first weeks of pregnancy (Myntti et al. 2017).

Another associated disorder is maternal embolism syndrome, which, despite presenting with a low incidence in the population, shows a fatality rate of approximately 20–60%. Therefore, maternal embolism syndrome is considered to be potentially fatal. It is associated with hypoxia, hypotension, convulsions, and disseminated intravascular coagulopathy during labor or near the end of labor due to the entry of amniotic fluid into the maternal circulation (Hell et al. 2017; Tamura et al. 2017).

1.2.3 Placental Pathologies

The placenta is an adaptive organ that allows the embryo's development by facilitating multiple functions and establishing an adequate environment (Tamura et al. 2017). Therefore, placental malfunction or misdevelopment can affect both the mother and the fetus, and this condition can also appear in subsequent pregnancies (Redline 2015).

Various placental alterations can be identified, such as alterations at the site of implantation associated with the degree of uterine invasion (placenta accreta, increta, or percreta), gestational trophoblastic, and maternal and fetal infectious diseases. Most of these alterations can be diagnosed during gestation, but others require a histopathological evaluation to determine the diagnosis (Kulkarni et al. 2017; Stevens et al. 2015).

1.2.3.1 Abnormally Invasive Placenta

Abnormal placenta invasion occurs when the trophoblast migrates and invades beyond the site of normal adhesion (decidua and the inner third of the myometrium). Among the abnormal types of placental invasion that occur are an accreted placenta (placenta accreta) with trophoblast invasion into the myometrium but without infringement of the decidua (<50% of the myometrium) (Martimucci et al. 2019; Piñas Carrillo and Chandraharan 2019). The deep invasion (placenta increta) occurs when the trophoblast partially penetrates the myometrium (>50% of the myometrium) (Piñas Carrillo and Chandraharan 2019), and the most severe type of placental invasion, called placenta percreta, takes place when the invasion reaches the uterine serosa or even pelvic organs (DaSilva-Arnold et al. 2018; Drăgușin et al. 2018). Placenta accreta is the most common type of placental invasion, occurring in approximately 75% of cases, whereas placenta increta and placenta percreta correspond to about 20% of all cases (Bartels et al. 2018; Drăgușin et al. 2018). Placental invasion can cause irreparable uterine damage and invasion of organs beyond the uterus, such as the bladder, intestines, pelvic sidewall, and other nearby structures, in addition to causing postpartum hemorrhage (DaSilva-Arnold et al. 2018).

The diagnosis and confirmation of abnormal invasion of the placenta are made through histopathological examination, which evaluates the trophoblast's adhesion degree. Although it is impossible to identify abnormal invasion at the macroscopic level, it can be suspected from imaging tests (transvaginal ultrasound) and clinical history (Bartels et al. 2018; Berhan and Urgie 2020; Shainker et al. 2020). In addition, biomarkers for the diagnosis of this pathological condition have been investigated through proteomic research. For example, one study demonstrated that patients with placenta accreta had a different plasma protein profile than control cases. An increase in 37 proteins and a decrease in 19 proteins were detected. In addition, SMAD4, PDGF, VEGF, and SNCA stood out in the analyses as regulators of ADAMTS1 and TIMP3 signaling pathways related to the epithelium-mesenchyme transition, angiogenesis, and invasion, processes involved in this type of pathology (Shainker et al. 2020).

1.2.3.2 Gestational Trophoblastic Disease

This disease category comprises the pathologies that arise from abnormal trophoblastic tissue. The common denominator is proliferation, sometimes leading to local invasion and at other times to the development of metastasis. Among these pathologies are molar pregnancy (90%), invasive mole (5–8%), choriocarcinoma (1–2%), placental site trophoblastic tumor, and epithelioid trophoblastic tumor (1–2%) (Kathpalia et al. 2018; Moussa et al. 2018)

Molar pregnancy, or a hydatidiform mole, is characterized by variable trophoblastic proliferation and hydropic changes in the villi. Two forms of this pathology have been differentiated: complete and partial hydatidiform moles (Candelier 2016; Nickkho-Amiry et al. 2019). Molar pregnancy is generally diploid, composed of edematous villi, cytologic atypia, moderate to marked hyperplasia, trophoblastic inclusions, hypercellular myxoid villous stroma, canalicular vascular structures, and there is no formation of embryonic tissue or fetal membranes. On the other hand, the partial hydatidiform mole is mostly triploid and contains large villi due to hyperplasia, some of which are irregularly shaped with scalloped edges and trophoblastic inclusions. The partial hydatidiform mole can also have hydropic villi or other smaller and immature fibrotic villi and nonviable fetal tissue, whereby complete development of a normal or altered fetus can be found (Drăgușin et al. 2018; Moein-Vaziri et al. 2018; Ronnett 2018). Molar pregnancy is usually benign but can also become malignant, invasive, or metastatic (DaSilva-

Arnold et al. 2018). One major complication of partial and complete molar pregnancy is the risk of persistent or invasive disease and miscarriages (Cole and Kramer 2016).

An invasive mole is a malignant tumor that invades the myometrium, causing hemorrhagic necrosis and/or distant metastasis (Zhang et al. 2019). It is diagnosed by a high and sustained serum concentration of human chorionic gonadotropin hormone and subsequent histological analysis (Shen et al. 2017). Early diagnosis and treatment of invasive moles are of great importance since their occurrence can lead to a poor prognosis through the development of choriocarcinoma, thus increasing morbidity and mortality (Zhang et al. 2019).

Choriocarcinoma is an aggressive, metastatic, malignant tumor of generalized dissemination (Drăgușin et al. 2018). It presents with cytotrophoblast and syncytiotrophoblast proliferation and a biphasic to a triphasic organizational pattern of the tumor cells in sheets or cords. But can also be observed in a random focal arrangement with pleomorphism, increased nuclear size, and mitotic activity (Hui 2018; Lazare et al. 2019). At the macroscopic level, a central mass exhibiting hemorrhage and extensive necrosis is observed infiltrating and increasing the size of the uterus, with invasion into the myometrium and adjacent tissue. There is also an increase in the levels of the human chorionic gonadotropin hormone, which causes the formation of cysts and increases the size of the ovaries (Drăgușin et al. 2018; Hui 2018).

Finally, there are placental site trophoblastic and epithelioid trophoblastic tumors, which typically arise after a full-term pregnancy or a nonmolar miscarriage. Even though they have the lowest incidence of occurrence within gestational trophoblastic tumors, they have the highest mortality rate, resistance to chemotherapy, and usually require a complete hysterectomy (Hui 2018). Placental site trophoblastic tumors are generally present as solid nodular masses, deep myometrial invasion, local bleeding, necrosis, and infiltrative growth forming cords, nests, and sheets. On the other hand, the epithelioid trophoblastic tumor develops from malignant trophoblast cells of the chorionic layer, present in the cervix or lower uterine segment in 50% of cases. Epithelioid trophoblastic tumors form nodules or cystic hemorrhagic masses, and their cells are arranged in nests or cords (Hui 2018).

1.2.3.3 Infectious Processes

The placenta, amniotic fluid, membranes, and fetus have traditionally been considered sterile, and this idea has been questioned due to the presence of commensal microorganisms in the reproductive tract (Lactobacillus). However, there are challenges in determining the appropriate controls and demonstrating these microorganisms' viability (Franasiak and Scott 2017; Heerema-McKenney 2018). Furthermore, when maternal blood reaches the intervillous space, the placenta is exposed to various pathogens present in the maternal circulation that could cause infection and inflammation (Ander et al. 2019), leading to infertility, miscarriage, fetal death, growth retardation, developmental abnormalities, premature delivery, neonatal morbidity, and maternal mortality (Heerema-McKenney 2018)

Viral infections have been associated with adverse gestational results and alteration to placental function, although viruses rarely cross the placental barrier. There are different mechanisms of transmission (Racicot and Mor 2017), such as through the maternal endothelium to the extravillous trophoblast, via macrophages to trophoblasts, by ascending or vertical infection (prenatal, perinatal, or postnatal) of the urogenital tract or by paracellular routes to the fetal capillaries (Arora et al. 2017; León-Juárez et al. 2017). Successful infection of trophoblastic cells may result in a reduced invasion, increased apoptosis, increased proinflammatory cytokines, and reduced expression of HLA-G, among other alterations that depend on the type of virus (Racicot and Mor 2017). One of the main obstacles to viral infection is entry into the host cell. This process is achieved via two main mechanisms, direct fusion with the plasma membrane or internalization within endosomes and subsequent release into the cytoplasm (León-Juárez et al. 2017). There is a growing list of viral agents shown to be transmitted from the mother to fetus,

for example, herpes simplex virus (type I and II), varicella-zoster, cytomegalovirus, rubella, HIV, hepatitis (A, B, C, and E), Ebola fever virus, and Lassa, among others (Silasi et al. 2015).

The presence of pathogenic bacteria in fetal tissue through hematogenous dissemination or via ascending infection through the cervix and an inflammatory immune response is often related to premature delivery, usually caused by gram-positive, oral bacteria of the abnormal vaginal microbiota or reproductive tract (Keelan et al. 2016). The most common bacterial infections are caused by *Listeria monocytogenes* (listeriosis) (Vázquez-Boland et al. 2017), *Ureaplasma* spp., *Mycoplasma hominis* (Keelan et al. 2016), *Treponema pallidum* (syphilis) (Vázquez-Boland et al. 2017), *Escherichia coli* (sepsis), *Mycobacterium tuberculosis* (tuberculosis) and *Gardnerella* spp. (bacterial vaginosis), among others (Heerema-McKenney 2018). One of the mechanisms of infection via bacteria is pathogen-associated molecular patterns (PAMPs), which are recognized by the immune system. In addition, it triggers the inflammatory process by increasing pro-inflammatory cytokines and chemokines (Humann et al. 2016; Ilekis et al. 2016).

Protozoa are unicellular organisms considered essential factors in fetal death (Shiadeh et al. 2016). The suggested mechanism of infection is through the release of proteases that allow collagen breakdown and entrance into the cell through receptors, especially in cytotrophoblast cells (Capellini et al. 2015), causing chorioamnionitis, villitis, or intervillositis (Redline 2015). Among the most common protozoa associated with abortion are *Plasmodium falciparum* (malaria), *Toxoplasma gondii* (toxoplasmosis) (Vermillion and Klein 2018), *Neospora caninum* (neosporosis), *Sarcocystis sp* (sarcosis), etc. (Shaapan 2016).

Finally, although rarely occurring, fungal infections present factors that increase the risk of infection, such as immunological changes, alterations in estrogen levels, and glycogen production. Among these, the most frequent are vulvovaginal candidiasis and systemic fungal infections such as blastomycosis or histoplasmosis (Aguin and Sobel 2015; Tsega and Mekonnen 2019), which can be distinguished from infections with *Candida* spp. (chorioamnionitis) (Maki et al. 2017), *Blastomyces dermatitidis*, and *B. gilchristii,* among others (McBride et al. 2017). In addition, in premature births, amniotic fluid containing *Candida* spp. has been isolated and shown to be linked to one major complication: the spread via the blood to the spinal cord, leading to fungal meningitis (Chan and Smith 2018).

The syncytiotrophoblast is the main protective barrier to infection (Kaminski et al. 2019). The ability of microorganisms to cause infection depends on the gestational age of the fetuses due to changes in trophoblast cell receptors, which induce a response to lower or decreased doses of PAMPs, causing the cytotrophoblast layer to become increasingly thinner after the first trimester (Hui 2018; Ronnett 2018).

1.3 Maternal-Fetal-Placental Unit

Pregnancy depends on multiple endocrine, paracrine, and juxtacrine factors between the mother, fetus, and the placenta, an endocrine organ that allows an optimal intrauterine space, molecular interactions, and immunomodulation (Castillo-Castrejon et al. 2018, p.; Vlahos et al. 2019). The maternal-fetal unit is an environment controlled both hormonally and immunologically for the proper development of the placenta and the embryo. Controlling processes like proliferation, migration, invasion, differentiation, and remodeling occur during gestation (Sun et al. 2019).

The maternal-fetal-placental unit refers to the interaction between the uterus (decidua and inner third of myometrium), the cells forming the placenta, and the fetus (Lash 2015), as well as endocrine and metabolic exchanges (Pasqualini and Chetrite 2016). In addition, the interaction and exchange of endogenous or exogenous substances is a critical and essential process during development (Dellschaft et al. 2020; Mirbod 2018).

Traditionally, histological photomicrographs of the placenta show that the terminal villous is filled by various numbers of fetal capillaries. However, in the last decade, 3D image recon-

struction techniques have revealed that there is only one branched capillary at the top of the terminal villi, which is tortuous and irregular, generating loops and localized dilations (sinusoids) (Jirkovská et al. 2008).

The capacity of the placenta for nutrient exchange with fetal blood depends on several factors, such as (1) the total capillary surface area within a villous branch, (2) the diffusion distance across villous tissue (vasculo-syncytial membrane), (3) the flow resistance found into the capillary network, and (iv) the metabolic demands of the villous itself (Jensen and Chernyavsky 2019). In pathological pregnancies, the architecture of the villous tree changes dramatically. For example, in gestational diabetes, the terminal villi are characterized by chorangiosis (an increment of terminal villi capillary branching), villous immaturity, and an increased number of cytotrophoblasts, syncytiotrophoblasts, and syncytial knots (Carrasco-Wong et al. 2020). In preeclampsia, histological findings include infarcts, increased syncytial knots, cytotrophoblastic proliferation, thickening of the trophoblastic basement membrane, hypovascularity of the villi, and obliterative enlarged endothelial cells in the fetal capillaries (Soma et al. 1982). Among these findings, one of the most potentially important defects is the thickening of the vasculo-syncytial membrane, which is minimal in the normal placenta, altering the proper diffusion of nutrients from maternal to fetal blood.

1.3.1 Oxygen

Proper oxygenation is crucial for fetal and placental development (Schneider 1996), and this has been demonstrated in environmental or pathological hypoxic conditions (Soares et al. 2017). Traditionally, it is considered that oxygen diffuses across the vasculo-syncytial membrane, which is 2–3 μm in thickness at the term of pregnancy (Sibley et al. 1998). It is proposed that oxygen transfer depends partially on the differences in blood pressure observed between the maternal blood, in the IVS (intervillous space), and the fetal blood (Nye et al. 2018). The latter is reinforced by the Bohr-Haldane effect, which indicates that as the maternal blood takes up carbon dioxide, it becomes acidotic, and the release of maternal oxygen is favored; the contrary effect occurs in fetal blood, whereby an increase in carbon dioxide release is associated with an increase in oxygen capture (Nye et al. 2018). Oxyhemoglobin also helps in the maternal-fetal flow of oxygen since fetal hemoglobin sub-units α/γ have a higher affinity to oxygen than the α/β sub-unit of maternal hemoglobin (Mushambi and Jones 2009) (Fig. 1.4).

1.3.2 Influence of Placental Architecture on Oxygen Transport

Understanding oxygen transfer through the placenta and its clinical implications is an open and unsolved field. A comprehensive review from Nye et al. suggests that for a proper study of this issue, two properties of the experimental approach are necessary: (1) the accuracy of oxygen measurement and (2) the resolution of the placental structure must be linked by mathematical modeling (Nye et al. 2018). The focus of the following discussion is not the mathematical approaches but their architectural and histological implications (Figs. 1.5, 1.6, and 1.7).

By solving the convection-diffusion equation via 2D + 1D geometry, based on histological photomicrographs, Serov et al. propose that the optimal villi density of a healthy placenta is 0.47 ± 0.06 (arbitrary units), which is required for a maximal oxygen uptake of 1.6 ± 0.8 cm^3/min (Serov et al. 2015). Interestingly, less or more villi density, commonly found in preeclampsia and diabetes, respectively, reduces the oxygen uptake (Mushambi and Jones 2009). The latter suggests a direct impact of the placental architecture on the performance of the organ itself. In a second paper, the same group of authors indicated that (i) the effective villous radius, (ii) the maximal oxygen inflow into the placenta and the ratio of the transit time of maternal blood, and (iii) the oxygen extraction time also influence the proper oxygen uptake (Serov et al. 2015). However, the latter

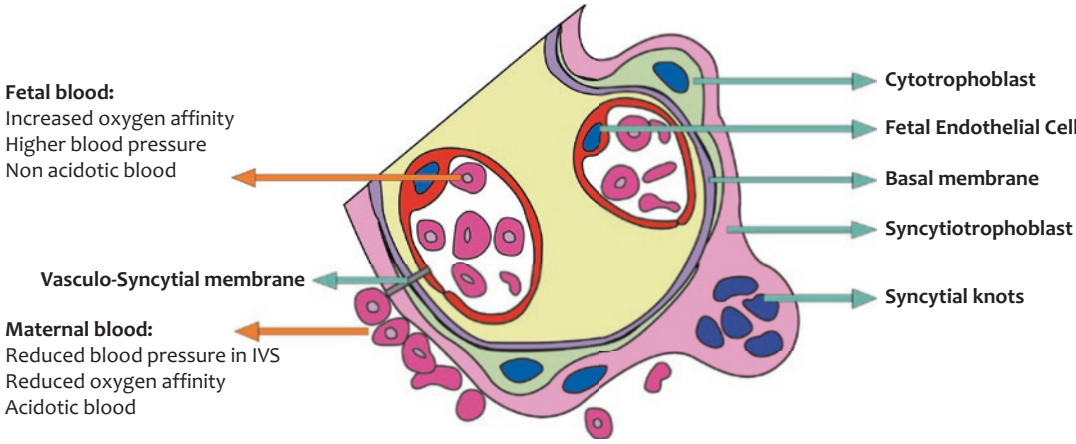

Fig. 1.4 Physico-chemical features that allow maximum gas interchange. The flow of gasses occurs through the vasculo-syncytial membrane (VSM) barrier, composed by cytoplasm of syncytiotrophoblast and endothelial cells. Thus, the narrowest VSM barrier allows the highest gasses diffusion. The differences between the maternal and fetal blood pressure, being low in maternal in comparison to fetal, induce the diffusion of oxygen to fetal blood. The affinity of fetal hemoglobin sub-units α/γ for oxygen is higher than the affinity of maternal α/β sub-units, increasing the ability to capture oxygen. Along with the fetal CO_2 flows to maternal blood, this becomes acidotic, which favors the release of maternal oxygen

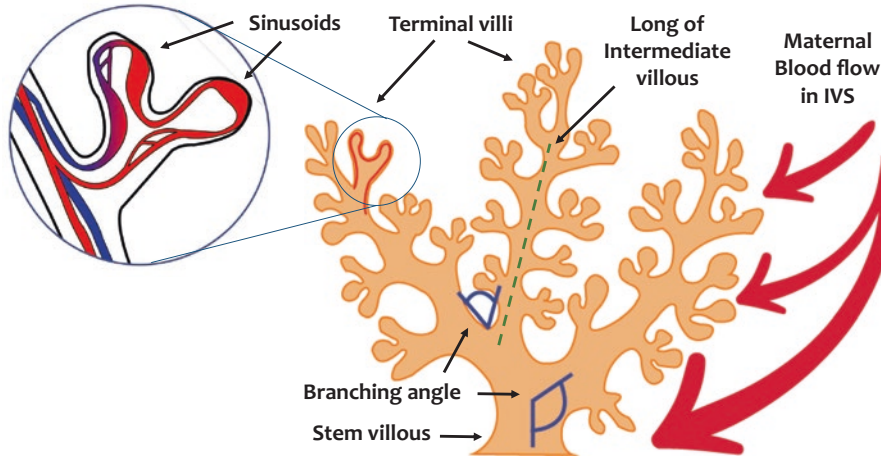

Fig. 1.5 Structural features of the villus tree that favor optimal oxygen delivery. The proper development of villus trees is crucial to reaching a maximum oxygen diffusion into fetal blood. One is the angle of villi branching, where wider is better (pointed out in the cartoon by a blue angle symbol). Also, a long intermediate villous is reacted with an increment of oxygen exchange (denoted by a green straight line). Together, both features allow the maternal blood in intervillous space (IVS) to flow deeper into villus trees. Finally, at the end of pregnancy, the elongation of fetal capillaries produces the formation of sinusoids (observed in the circular offset), which minimizes the broadness of the vasculo-syncytial membrane

study bases its calculation on a simplified homogeneous geometry for intervillous space (IVS), which does not consider the possible implications of the 3D geometry of the villous tree. Lin et al. further explored this issue and developed a mathematical model to evaluate the influence of villous tree architecture, from the stem villi to the terminal villi level, on the oxygen exchange. They found that maternal blood is allowed to flow deep in the IVS in cases when the stem and intermedi-

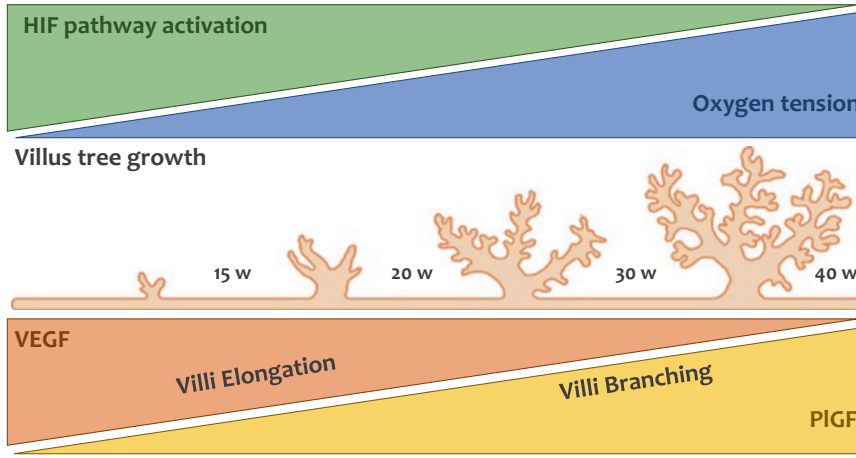

Fig. 1.6 The interplay between oxygen and HIF pathway activation controls the villus tree growth. At early placentation, low oxygen tension is found in the placenta (categorized as hypoxic placenta). The latter induces the activation of HIF pathway, which in turn transactivates several target genes, being the most important VEGF. VEGF is responsible for inducing branching angiogenesis (observed between the 20th and 30th week of gestation) Along with the villus tree development, more maternally oxygenated blood goes into the placenta, increasing the oxygen tension. Then, the HIF pathway is gradually turned off, reducing VEGF expression and, now, allowing the expression of PlGF. Since the 30th week of gestation, the action of PlGF induces the elongation of the already branched capillary into the villi, thus generating a mature villous tree

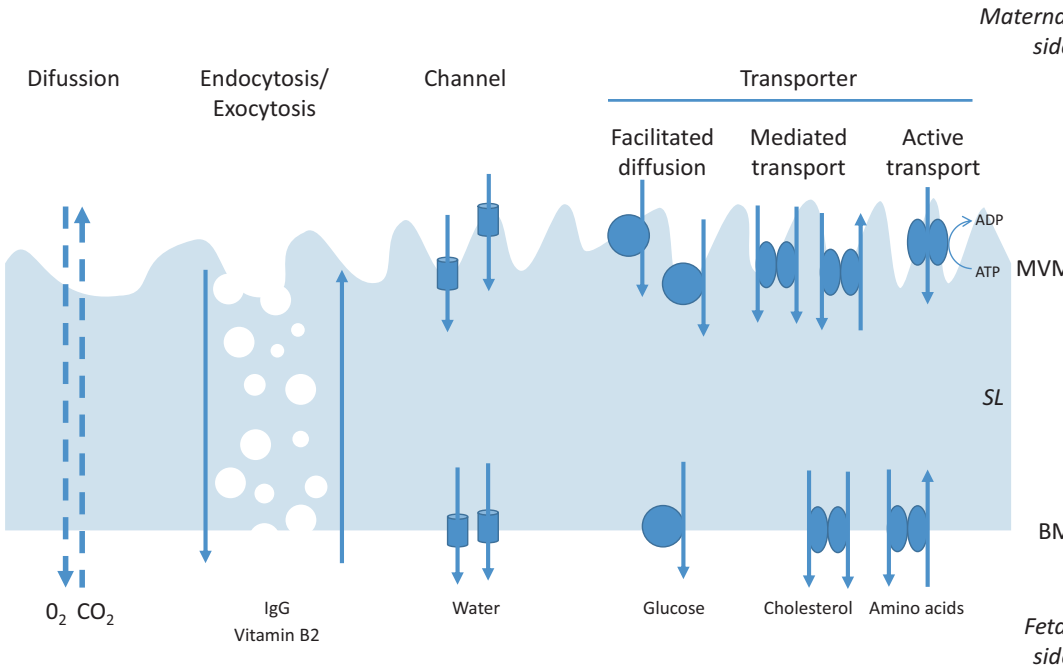

Fig. 1.7 Mechanisms involve placental transportations. Placental transportation from the mother-to-fetus includes diffusion, observed on gases such as oxygen (O_2) and carbon dioxide (CO_2); endocytosis/exocytosis, described for maternal IgG and some nutrients (vitamin B2); channel, described mainly for water; and transporter-mediated, including facilitated diffusion (i.e., glucose) and mediated and active transport (i.e., cholesterol and amino acids). All the mechanism components described before have been observed in both the microvillous membrane (MVM) and basal membrane (BM) of the syncytiotrophoblast layer (SL)

ate villi are longer or more separated from each other. In contrast, an obstruction to maternal blood flow occurs when the villous branches are short in length and exhibit smaller branch angles without affecting the number of branching generations (Lin et al. 2016).

The shape of the terminal villi is vital for proper oxygen exchange and crucial to the fetal capillary net structure within each terminal villi. Using an axisymmetric model on a 3D reconstructed model, Pearce et al. described sinusoids, or regions of dilated capillaries, that increase oxygen transfer by up to 15% (Pearce et al. 2016). The latter point is significant since, in a normal-term placenta, 35% of the terminal villi are sinusoids (Benirschke's Pathology of the Human Placenta 2012b). The author asserts that the improvement in oxygen transfer could be explained by the increased degree of capillary dilation found in placentas that develop at high altitudes (Burton et al. 1996). The beneficial effect of the sinusoid is proposed to be due to the formation of a vasculo-syncytial membrane in a full-term pregnancy, made of the juxtaposition of thin layers of the plasma membrane of syncytiotrophoblast and endothelial cells; generated by the pressure created by the fetal capillaries against the syncytiotrophoblast (Benirschke's Pathology of the Human Placenta 2012b). The engrossment of the vasculo-syncytial membrane could be part of the hypoxic condition found in preeclampsia (Soma et al. 1982).

1.3.3 Oxygen-Induced Molecular Control of Placental Architecture/Performance

1.3.3.1 Hypoxia-Induced Factor (HIF)

Hypoxia-induced factors 1α, 2α, and 3α are transcription factors that translocate to the nucleus during hypoxia (Semenza and Wang 1992). Among these, the transcription factor HIF-1α is one of the most studied. HIF-1α is consistently produced and degraded in the proteasome during normoxia. The protein degradation is induced via the hydroxylation of proline residues (Pro402 and Pro564) by oxygen-dependent enzymes HIF-prolyl hydroxylases (HPH 1, 2, and 3) (Epstein et al. 2001; Yu et al. 2001). During hypoxia, it is proposed that the low oxygen level could inhibit the posttranslational modification of HIF1α, preventing its degradation and keeping its activity (Hayashi et al. 2019). However, HIF-1α also can be stabilized in normoxia by signaling growth factors such as insulin, insulin-like growth factors 1 and 2, epidermal growth factor, fibroblast growth factor 2, interleukin 1β, tumor necrosis factor α, angiotensin II, thrombin, transforming growth factor β1, platelet-derived growth factor, and hepatocyte growth factor (Wenger 2002)

After translocation to the nucleus, HIF-1α interacts with its co-activator dioxin receptor/aryl hydrocarbon receptor (AhR) nuclear translocator (ARNT), also known as HIF-1β (Hoffman et al. 1991). Together they bind to Hypoxia Response Element (HRE) located in the promoter or the enhancers of target genes (Gassmann et al. 1997; Wood et al. 1996) related to erythropoiesis, iron metabolism, vascular function regulation, and anaerobic energy, among others (Wenger 2002). Indeed, HIF regulates more than 1000 genes (Semenza 2013).

1.3.3.2 HIF Is Necessary for Placental Development

Several studies have reported the importance of the HIF pathway for proper placental development. For example, murine models of global and maternal loss-of-function (LOF) of Arnt and Hif1α showed a dramatic effect on placentation, such as (i) impaired labyrinthine vascularization, (ii) a decrease in junctional zone trophoblast progenitor cells (being mild in Hif1α compared to Arnt LOF models), (iii) disruption of both decidual natural killer cell expansion and trophoblast invasion into the interstitium, and mid-gestation lethality (9.5–10.5 dpf) (Abbott and Buckalew 2000; Adelman et al. 2000; Cowden Dahl et al. 2005; Kenchegowda et al. 2017; Kotch et al. 1999; Kozak et al. 1997; Maltepe et al. 1997). The number of pathways altered during placentation due to the lack of HIF is broad, including loss of proper cellular differentiation and possibly a failure of placental vasculogenesis and angiogenesis, as discussed below.

1.3.3.3 HIF Controls the Placental Cell Fate

In vivo and *in vitro* studies evidenced that the HIF pathway is essential for the proper development of the mammalian placenta via regulation of cell differentiation. The knockout of Arnt ($Arnt^{-/-}$) in mice has demonstrated the importance of the HIF pathway in preventing the differentiation of the diploid spongiotrophoblast into giant trophoblast cells, specifically during the transition of E8.5 to E9.5 (Adelman et al. 2000). Also, $Arnt^{-/-}$ placentas and E9.5 were smaller than WT or hemizygous placentas, showing no fetal vessels and a classical spongiotrophoblast region filled with trophoblast giant cells (TGCs) (Adelman et al. 2000).

As Arnt is a co-activator of either HIF1α or HIF2α, Dahl et al. evaluated their involvement in placental cell differentiation. The results showed that a lack of HIF1α without a shortage of HIF2α resulted in the same alterations observed in the $Arnt^{-/-}$ placentas described above (Cowden Dahl et al. 2005). Furthermore, Wakeland et al. demonstrated that HIF exerts the same influence on placental cell differentiation.

Primary cell culture of villous cytotrophoblasts from early pregnancy showed that the differentiation to proximal-column extravillous trophoblast (pcEVT) is induced by high oxygen levels (20%). In contrast, low oxygen levels (2%) favored differentiation to invasive extravillous trophoblasts (EVT). HIF-1β (ARNT) was necessary for both processes (Wakeland et al. 2017).

1.3.3.4 HIF Requirement for Angiogenesis in the Placenta

Placenta vasculogenesis and angiogenesis are complex events governed by different factors that act at specific moments during pregnancy. Several proteins are indicated as crucial for proper capillary development, including vascular endothelial growth factor (VEGF), placental growth factor (PlGF), angiopoietins, basic fibroblast growth factor, and transforming growth factor (Cerdeira and Karumanchi 2012). Among them, VEGF and PLGF, both members of the VEGF family, are shown to be controlled by oxygen tension, as described below.

HIF1α induces VEGF expression during the response, found during the first stages of placentation, as a response to the hypoxic environment (Forsythe et al. 1996). VEGF is highly expressed during the first trimester of pregnancy by trophoblast cells and Hofbauer cells (Demir et al. 2004; Geva et al. 2002). The VEGF expression is associated with the branching angiogenesis by the proliferation of endothelial cells and tube formation induced by the activation of VEGF-receptor-2 and VEGF-receptor-1, respectively (Breier 2000). After the first trimester, as the oxygen tension increases, the expression of VEGF is reduced (Kaufmann et al. 2004). Conversely, PlGF concentration increases during pregnancy, especially at 28 and 32 weeks (Levine et al. 2004).

PlGF has pro-angiogenic functions but can inhibit branching angiogenesis (Björndahl et al. 2004; Fischer et al. 2008). The change in the kind of angiogenesis is referred to as the "angiogenesis switch," whereby branching angiogenesis switches to non-branching angiogenesis, favoring the growth of existing branched fetal capillaries in the terminal villi (Kaufmann et al. 2004). In addition, high oxygen levels are associated with PlGF expression (Nye et al. 2018). For this reason, it is possible to propose that reducing HIF signaling due to hyperoxia allows for PlGF expression.

The latter idea is supported by findings reported by Fujii et al. They showed that in hypoxic conditions, PlGF expression was reduced in BeWo cells and primary cytotrophoblasts when high levels of HIF1a and HIF2α are concurrent (Fujii et al. 2017). The authors also demonstrated that knockdown of HIF2α but not of HIF1α prevented PlGF reduction (Fujii et al. 2017). In contrast, other studies have indicated that hypoxia activates PlGF expression via HIF1α in cultured endothelial cells (Tudisco et al. 2014).

In conclusion, the placenta's primary function, which is the transport of oxygen from the mother to the fetus, is regulated not only by oxy-

gen tension but also by the placental architecture, which modulates the proper transfer of oxygen.

The proper function of the placenta is regulated by complex processes beginning in the early stages of placentation and ending with a full-term pregnancy or until a full-term pregnancy is reached. Critical events occur throughout this process at specific points.

1.4 Placental Transport

Since the placenta forms a physical barrier between the mother and the fetus, it is necessary to transport the nutrients from the maternal side to the fetus through the syncytial vascular membranes. The critical component is the syncytiotrophoblast layer, which transports a selection of the required nutrients for fetal growth by several transporters and solute carriers, which are involved in carbohydrates, amino acids, lipids, vitamins, and ions transfer (Kallol et al. 2018). Also, different mechanisms of transportation are present in these cells. These mechanisms provide optimal communication of the external medium with the growing fetus and prepares the newborn to be maternally independent. In this stage, maternal and fetal interaction is crucial since it will affect later life. The mechanisms observed include passive diffusion, endocytosis and exocytosis, channels, facilitated diffusion, and active transport.

1.4.1 Endocytosis and Exocytosis

The machinery of the exocytosis present on the apical membrane on the syncytiotrophoblast layer has been described, relating this as secretion function, in placenta at term and in a cell line (Gonzalez et al. 2014). Furthermore, on the microvillous membrane (MVM), a clathrin-dependent pathway for endocytosis as a regulator of proliferation signaling pathways from the maternal side has also been observed in the first-trimester placental (Karolczak-Bayatti et al. 2019). Endocytosis in the MVM is also related to receptor-mediated endocytosis (RME) to internalize specific ligands (*i.e.*, vitamin B2), nutrients (*i.e.*, HDL-cholesterol), and some drugs (Akour et al. 2013; Foraker et al. 2007; Kallol and Albrecht 2020). Several of these mechanisms involve the internal liberation of the molecules through transport on the other side of the membrane, not only for nutrients but also for first immunity (maternal IgG) (Aye et al. 2010; Fuenzalida et al. 2020; Palmeira et al. 2011). Nevertheless, these mechanisms are not only involved in normal fetal growth but could also play a role in pregnancy pathology, such as preeclampsia, through the internalization of antiphospholipid antibodies (aPL), producing cell death and thereby contributing to the pathogenesis of preeclampsia (Viall et al. 2013).

1.4.2 Channels

Described mainly on the transportation of water (Damiano 2011; Pérez-Pérez et al. 2020). Even when fetal water recruitment is high throughout pregnancy, the underlying mechanism is not fully understood. Isoform 3 and 9 of Aquaporin are expressed in the placenta, which transfers water, glycerol, and urea. They may play a role in water transport to the fetus since an alteration in this expression is related to several complications, such as idiopathic polyhydramnios, oligohydramnios, preeclampsia, and gestational diabetes (Szpilbarg and Damiano 2017; Zhang et al. 2020; Zhu et al. 2009, 2010).

1.4.2.1 Transporters

Several highly studied transporter mechanisms involve the same at MVM and basal membrane (BM), but others include different transporters on the MVM and BM.

Glucose transport is included in this group and involves the expression of seven isoforms of glucose transporters (GLUTs) in the placenta (Illsley and Baumann 2020; Murphy et al. 2006). GLUT1 is the major glucose transporter

expressed in the whole placenta, mainly on the syncytiotrophoblast and significantly higher on MVM than BM (Wolf and Desoye 1993). It has been observed that GLUT1 on MVM remains unaltered during pregnancy; meanwhile, GLUT1 expression is increased on the BM during pregnancy until at least the 30th week of gestation (Sakata et al. 1995). On the other hand, GLUT4 expression has been observed in the first-trimester syncytiotrophoblast but not in the third-trimester syncytiotrophoblast. Furthermore, it has been described that insulin increases glucose uptake in the first-trimester placenta but not in term placentas (Sakata et al. 1995). Thus, it could be concluded that glucose transport in the placenta is facilitated by an insulin-independent mechanism, mainly mediated by GLUT1. Moreover, it could be due to the asymmetrical distribution of GLUT1 expression on both sides, the BM limits glucose transport to the fetus (Illsley and Baumann 2020).

Cholesterol is another major nutrient that is especially important for the growing fetus. Cholesterol is transported through the placenta in the form of high-density lipoprotein (HDL)-, low-density lipoprotein (LDL)-, or very-low-density lipoprotein (VLDL)-cholesterol, and the scavenger receptor class B type 1 (SRB1) and LDL receptor (LDLR), which are expressed on MVM and BM of the syncytiotrophoblast (Akour et al. 2013). Furthermore, the ATP-binding cassette (ABC)-transporter A1 and ABCG1 expression on BM are observed, where cholesterol is effluxed to the fetal circulation mediated by apolipoproteins (Fuenzalida et al. 2020). This mechanism involves various transporters on either side of the syncytiotrophoblast.

It has been observed that amino acid concentration increases on the fetal side more than on the maternal side; thus, the transfer of amino acids in the placenta should be mediated by active transport (Vaughan et al. 2017). On MVM, an accumulative transport is present via active transport for amino acids. This mechanism produces a high concentration of intracellular amino acids that, through facilitated diffusion on BM, transfer amino acids to the fetal side (Cleal et al. 2011).

In light of this data, it is possible to demonstrate that the syncytiotrophoblast layer is a highly specialized structure responsible for select nutrient transport following the fetus or fetal requirements. Furthermore, the polarized characteristics of the syncytiotrophoblast membrane can transport different nutrients or a variety of nutrients to the fetus, as well as either accelerate or reduce the entry into the fetal circulation (Cleal et al. 2011; Fuenzalida et al. 2020; Illsley and Baumann 2020), even when some molecules have facility transport (i.e., IgG and drugs) (Palmeira et al. 2011; Viall et al. 2013). Indeed, while it is described that nutrient transport is constantly adapting to the growing fetus' needs, stabilizing a dynamic phenomenon (Wolf and Desoye 1993), further investigations are necessary to further understand the highly regulated transport of the nutrients to the fetus for acknowledgement and to discover possible treatments to improve neonatal outcome.

1.5 Conclusion

Recently, we have learned much more about this intriguing organ called the placenta. However, there are many questions regarding its cell differentiation without an answer yet. The new techniques used to study human placental development could overcome these challenges and address a better understanding of the human placental development in healthy pregnancies. Furthermore, this knowledge may be used to treat gestational diseases due to placental pathology.

Oxygen and HIF pathways are essential for the proper development of the mammalian placenta by regulating cell differentiation. Besides, the mother's oxygen transport to the fetus is regulated by oxygen tension and placental architecture. However, much more information lacks the fine-tuning, at a molecular and cellular level of the oxygen-placenta relationship, especially in gestational pathologies.

The primary function of the placenta is to provide nutrients to the embryo. Any alteration in nutrient transport may contribute to gestational diseases. Understanding the mechanism involved

in normal fetal development and nutrient transport in the syncytial vascular membrane could help understand pregnancy pathology.

Understanding the normal human placental development and function through gestation is necessary to unveil and treat the problems observed during abnormal pregnancies.

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2 Role of Hormones During Gestation and Early Development: Pathways Involved in Developmental Programming

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Abstract

Accumulating evidence suggests that an altered maternal milieu and environmental insults during the intrauterine and perinatal periods of life affect the developing organism, leading to detrimental long-term outcomes and often to adult pathologies through programming effects. Hormones, together with growth factors, play critical roles in the regulation of maternal-fetal and maternal-neonate interfaces, and alterations in any of them may lead to programming effects on the developing organism. In this chapter, we will review the role of sex steroids, thyroid hormones, and insulin-like growth factors, as crucial factors involved in physiological processes during pregnancy and lactation, and their role in developmental programming effects during fetal and early neonatal life. Also, we will consider epidemiological evidence and data from animal models of altered maternal hormonal environments and focus on the role of different tissues in the establishment of maternal and fetus/infant interaction. Finally, we will identify unresolved questions and discuss potential future research directions.

Keywords

Developmental programming · Steroids · IGFs · Thyroid hormones · Gestation · Lactation

2.1 Introduction

Gestation and lactation are complex processes that involve the action of several hormones and metabolites. The management of pregnant women and neonates is a significant concern of healthcare around the world. In the last few years, great awareness about the health and well-being of developing fetuses, babies, infants, and pregnant and lactating women has been taking relevance in health research and public health policies. The first thousand days of life, the time spanning roughly between conception and the

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first two years of life, are unique windows of opportunity to support child development and long-term health, and alterations during these moments of life may have short- and long-term impact on health (Gluckman et al. 2010; Koletzko et al. 2017). During intrauterine and perinatal life, an inadequate intake of nutrients and changes in maternal hormones may permanently influence fetal development and organ function. This association is described as a consequence of cell and tissue plasticity in their interaction and response to the changing environment (Langley-Evans 2015). These events are known as "programming," a term that was initially used by Lucas (1991) and is currently included in the Developmental Origins of Health and Disease (DOHaD) hypothesis. This hypothesis describes the process by which exposure to environmental stimuli or insults during critical periods of development can trigger adaptations that result in permanent changes to the physiology of the organism, thus playing an essential role in the presence of non-transmissible diseases throughout the lifespan (Barker 2004; Gluckman et al. 2010). In this context, several projects have focused on the assessment of the intrauterine and neonatal environments and their influence on the developing organism. Furthermore, many pathologies are associated with hormonal changes during gestation and lactation, such as polycystic ovary syndrome (PCOS), metabolic syndrome, cardiovascular disease, neurological disorders, and growth defects (Abbott et al. 2002; Abruzzese et al. 2018; Hanson and Gluckman 2014).

The fetus is not only influenced by endogenous hormones but also by maternal hormones and other bioactive compounds, whose transport is modulated by the placenta. Among hormones, thyroid hormones (THs), sex steroids, and even growth factors, such as insulin-like growth factors (IGFs), are of great importance as their pathways influence embryonic and neonatal development. Although failures in the placenta may affect the developing organism, other tissues and organs are involved in this process; among them, adipose tissue plays a significant role in the regulation of pregnancy. The first stages of postnatal life are described as another window of susceptibility for developmental programming (Hanson and Gluckman 2014); mainly, lactation is another process that establishes maternal-neonate interaction and could be implicated in programming effects.

In this chapter, we will review the role of sex steroids, THs, and IGFs, as crucial factors involved in developmental programming effects during fetal and early neonatal life. We will also consider unresolved questions and propose new directions for research in line with recent findings in the field.

2.2 Steroids

Steroids participate in the regulation and establishment of the hypothalamic-pituitary-gonadal axis, as is the case with sex steroids (such as testosterone, progesterone, and 17 b-estradiol), as well as in the hypothalamic-pituitary-adrenal axis, as glucocorticoids do. The principal steroidogenic tissues are the gonads and the adrenal gland. However, during gestation and lactation, the placenta and the mammary glands (MGs) are also involved in steroid synthesis.

Steroids derived from cholesterol and their synthesis are strictly regulated and depend on the signaling of different pituitary hormones. These compounds share a typical basic structure (Fig. 2.1), and because of their lipophilic nature, they can cross the blood-brain and the milk-blood barriers as well as the placenta.

Androgens, progesterone, estrogens, and also glucocorticoids are involved in the regulation and maintenance of pregnancy from early implantation to parturition and also in the regulation of maternal milk production, secretion, and lactation (Carlsen et al. 2010; Chida et al. 2011; Kochenour 1980). Their biosynthesis and metabolism change during these processes and result from complex pathways involving the fetus, the neonate, the placenta, and the mother.

During adult female life, gonadal steroid secretion is cyclic and regulated by luteinizing hormone (LH) and follicle-stimulating hormone (FSH), which promote ovulation and stimulate the ovaries to produce androgens, estrogens, and

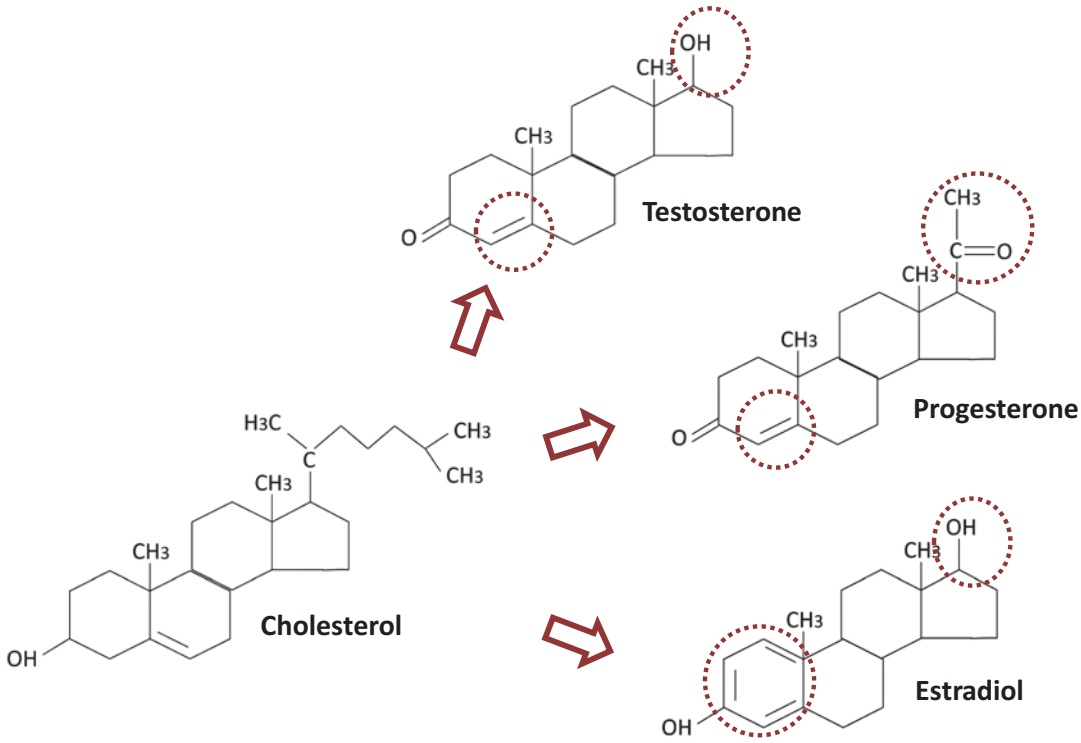

Fig. 2.1 Structure of the main sex steroids. Sex steroids, such as testosterone, progesterone, and estradiol, are derived from cholesterol and share a common basic structure. Red circles indicate differences in each of the hormones as compared to cholesterol structure

progesterone (Gougeon 1996). The adrenal glands can also synthesize some steroids, such as androgens and estradiol. Although the concentration of adrenal hormones is relatively constant, follicular development and growth, and the formation of corpus luteum after ovulation lead to fluctuations in sex hormones produced by the ovaries across the menstrual cycle (Gibson et al. 2016b). Besides, these sources of sex steroids, peripheral tissues, such as adipose tissue, also contribute to steroid production through steroid conversion.

All of these steroids are involved in female fertility regulation. Androgens play essential roles not only as estrogen precursors but also in normal follicular development and oocyte maturation (Walters 2015), and recently, they have been proposed as regulators of endometrial function (Simitsidellis et al. 2018). Estradiol also participates in folliculogenesis and, together with progesterone, regulates ovulation and prepares the uterus and breasts for possible fertilization (Madero et al. 2016; Simon et al. 2003; Vannuccini et al. 2016).

Once fertilization occurs, maternal hormones play essential roles in blastocyst implantation. After implantation, during the first trimester of a human pregnancy, the corpus luteum and the placenta regulate the production and secretion of progesterone and estradiol. Remarkably, human chorionic gonadotropin (hCG) is produced immediately after fertilization by syncytiotrophoblastic cells of the forming placenta and stimulates the corpus luteum to secrete progesterone and estrogens. As pregnancy advances, between 6 and 7 weeks of gestation, the corpus luteum function begins to decline, and the placenta takes the lead in the production and secretion of progesterone and estrogen (Mishell et al. 1973; Schindler 2005).

There are two different pathways for steroid action: the genomic or classical signaling path-

way and the nongenomic pathway (Lösel et al. 2003). The genomic pathway requires that steroid molecules enter the cell and interact with the corresponding cytosolic receptor, leading to a conformational change and migration to the nucleus, where the complex steroid-receptor influences the transcription of genes into mRNA. In this pathway, steroids exert their action through their correspondent receptors: the androgen receptor (AR), estrogen receptors alpha and beta (ERa and ERb), and progesterone receptors alpha and beta (PRa and PRb). In contrast to the genomic pathway, nongenomic signaling often occurs with a very short lag time and may involve membrane receptors.

2.2.1 Androgens

Several studies have demonstrated that androgens are essential for both uterine functions, pregnancy, and parturition (see Makieva et al. 2014). Furthermore, they exert a critical role in gestational development and maintenance. They act not only as substrates for estrogens biosynthesis during gestation but are also crucial for the development of the male reproductive tract during fetal life (MacLeod et al. 2010) and have growth and differentiation-promoting actions in the uterus (Makieva et al. 2014).

It is known that the endometrium is an androgen target tissue and that ARs are expressed in the endometrium throughout the menstrual cycle (Gibson et al. 2016a). Moreover, it has been shown that in vitro, human and rat myometrium and endometrium can convert androstenedione (A4) to testosterone and dihydrotestosterone (DHT) (Jasonni et al. 1982; Rose et al. 1978). Also, in the pig, the myometrium, derived from the non-pregnant and early pregnant pig uterus, can synthesize A4 and testosterone (Franczak 2008). During early pregnancy, androgens are present in the uterine environment among mammals, including humans. Castracane and colleagues reported that A4 and testosterone levels rise around the time of implantation (Castracane et al. 1998) and that the levels of DHT, a non-aromatizable androgen, rise in conception cycles since the luteal phase (Dawood and Saxena 1976). It is also important to highlight that, although apparently the placenta lacks the capacity for de novo androgens synthesis, recent studies suggest that the placental syncytiotrophoblast expresses both mRNA and protein of cytochrome P450 17A1 (CYP17), a limiting enzyme in testosterone synthesis (Escobar et al. 2011; Escobar and Carr 2011). Moreover, during mice gestation, there are two peaks of testosterone, one around day 9, on half of gestation, and the other around day 14 (Barkley et al. 1977), while in humans, androgens rise during the 2nd and 3rd trimesters of pregnancy (Makieva et al. 2014). Besides, androgens may also coordinate decidual-trophoblast interactions in early pregnancy (Diao et al. 2008; Wongwananuruk et al. 2016).

Androgens are involved in endometrial regulation and decidualization. It has also been shown that AR is expressed in human endometrial stromal cells during decidualization (Mertens et al. 1996) and remains expressed in the decidua during early pregnancy (Kajihara et al. 2012). However, whether androgens play a positive or negative effect on endometrium functions and implantation is still a matter of debate. In vivo studies in AR knockout mice showed that the lack of AR activation affected uterine development and proliferation (Nantermet et al. 2005; Walters et al. 2009). Also, in vitro studies reported that during decidualization, AR activation participates in the regulation of genes involved in cytoskeletal organization, cell motility, and cell cycle progression (Cloke et al. 2008). Moreover, DHT enhances decidualization of endometrial stromal cells and increases Insulin-Like Growth Factor Binding Protein-1 (IGFBP-1) expression (Wongwananuruk et al. 2016). IGFBP-1 is a well-known biochemical marker for decidualized endometrial stromal cells (Fowler et al. 1999) and stimulates cytoplasmic expansion, lipid droplets formation, and gap junction formation in decidualized human endometrial stromal cells (Kajihara et al. 2014).

An excess production of androgen in women, as is the case with PCOS, leads to decreased fertility, poor reproductive outcomes, and an increased incidence of miscarriage

(Kamalanathan et al. 2013). The observations in women with PCOS suggest an adverse role of androgen excess in the endometrium and the implantation process (Li et al. 2015). Thus, taking all the evidence together, androgens in adequate concentrations could be positive for endometrial functions and pregnancy maintenance, but in excess, they may exert detrimental effects. Nevertheless, further investigation is needed to find out the mechanisms of androgens during uterine development and function, and particularly of impaired endometrial function related to androgen excess.

Androgens are also involved in fetal development regulation, particularly in sex differentiation. This process refers to the development of the internal and external male or female structures and leads to the somatic sex phenotype (Welsh et al. 2014). Gonadal differentiation takes place in early gestation in both humans and rodents. It has been considered that the development of a female phenotype is a default state resulting from the absence of the testis-determining factors, such as Anti-Mullerian Hormone (AMH) and testosterone (Gustafson and Donahoe 1994). However, some factors are required for a functional female gonadal differentiation, such as the expression of Dosage-sensitive sex reversal-adrenal hypoplasia congenital critical region on the X chromosome, gene 1 (DAX 1), and wingless-type MMTV integration site family, member 4 (WNT-4) genes (Jordan et al. 2001; Vainio et al. 1999). In the case of the development of a male phenotype, it requires the action of testicular hormones to masculinize the fetus. Once male fetal gonads start to secrete hormones during early gestation, sex differentiation can take place. This process depends mainly on androgens and AMH. Together, these factors exert a masculinizing effect by promoting the development of the male reproductive tract and male external genitalia, and in the case of AMH, by suppressing female reproductive tract development (Morris et al. 2004; Welsh et al. 2014). During embryogenesis, androgens determine the morphogenesis processes of male-specific organs, such as the penis, prostate, and epididymis, that continue during neonatal and prepubertal periods and are complete before the onset of sexual maturity (Forest 1983; Murashima et al. 2015).

In males, fetal testis acts as a source of testosterone. However, in female fetuses, fetal ovaries do not synthesize androgens, and it is still unknown where testosterone is produced. Therefore, the androgens would have a maternal-placental origin (Baum et al. 1991; Hotchkiss et al. 2007).

To achieve a complete sex differentiation, the brain should also differentiate. This process depends on a surge of gonadal steroids that occurs primarily during embryonic development in primates and humans, followed by a second surge after birth, which could influence the sexual behavior, and a last one during adulthood, which leads to structural and functional changes in the brain (Clarkson and Herbison 2016; McCarthy and McCarthy 2011). While in humans and primates, a significant part of brain sexual differentiation is established by prenatal androgen exposure, in rodents, a neonatal testosterone surge that occurs in the hours following birth is responsible for establishing a sexually dimorphic brain circuitry that controls sexually differentiated behaviors and reproductive physiological processes (Clarkson and Herbison 2016; Lenz and McCarthy 2010).

Moreover, in mammals, androgens also influence gonadotropins' secretion. Differences in the gonadotropins' secretion are established during fetal and neonatal life. Testis androgens modulate the male brain during development, making it incapable of producing pre-ovulatory gonadotropin peaks during adulthood (Foecking et al. 2008).

2.2.2 Estrogens

Estrogens are essential not only for several reproductive functions but also for fetal development. They are involved in uterine development and function and play a key role in gestation and uterine contractility during parturition (Vannuccini et al. 2016). In a normal estrous cycle, estrogens are produced primarily by the ovaries and induce proliferative changes in the endometrium.

Estrogen levels during the estrous or menstrual cycle are a key determinant of the duration of uterine receptivity for embryo implantation (Simon et al. 2003). In humans, the window of receptivity starts around day 19 of the menstrual cycle, after a peak of estradiol, and lasts for 4–5 days (Vannuccini et al. 2016). Stromal decidualization occurs as part of the menstrual cycle and is independent of the presence of the blastocyst. Contrary, in rodents, decidualization initiates at the site of blastocyst attachment.

Ovarian steroid hormones govern uterine receptivity via their receptors, mainly through estrogen and progesterone receptors. In assisted reproductive technologies (ART), the administration of exogenous estrogens is necessary for endometrial preparation in cases in which frozen embryos or donated oocytes are used (Madero et al. 2016). Estrogen levels are high during pregnancy, and their concentrations rise in maternal circulation with the increasing gestational age.

Estrogens are also essential in endometrial angiogenesis during both the reproductive cycle (Lai et al. 2015; Shifren et al. 1996) and placental angiogenesis, contributing to the establishment of the blood vessel network that supports fetal growth in human and primate pregnancy (Albrecht and Pepe 2010). Estrogens, through estrogen receptor action, exert a positive regulation in vascular endothelial growth factor (VEGF) expression, a potent mitogen known to stimulate angiogenesis in humans, rats, baboons, and sheep (Ahmed et al. 1995; Albrecht and Pepe 2010; Cullinan-Bove and Koos 1993; Reynolds et al. 1998). During gestation, estrogens are formed in the placenta using fetal and maternal androgens and diffuse to the maternal and fetal compartments. The placenta does not have all the necessary enzymes to synthesize estrogens from cholesterol or progesterone. Moreover, the human trophoblast lacks the enzyme 17-hydroxylase and cannot produce the precursors of estrogen. Therefore, dehydroepiandrosterone sulfate (DHEA-S) produced in the fetal adrenal or maternal androgens is used as a substrate for estradiol production by trophoblasts (Kumar and Magon 2012).

Fetuses and newborns are exposed to estradiol derived from their mother and their gonads and brains (McCarthy 2008). In particular, estrogens are essential in primate ovarian development and primordial follicle formation (Fowler et al. 2011). For example, women born prematurely, not exposed to the increasing concentrations of estrogen in advanced pregnancy, show alterations in reproductive functions (Swamy et al. 2008). However, this signaling seems to be species-specific. In baboons, bovines, and humans, estrogen signaling acts in promoting primordial follicle pool formation, whereas in rodents, estrogens inhibit primordial follicle formation (Chen et al. 2009).

Estrogens are also important in organ development, not only in females but also in males. In male mice, ER expression is present during fetal life in the mesenchyme of all reproductive organs, such as the urogenital sinus, which forms the male prostate and bulbourethral gland, and also in Wolffian ducts, which form the epididymis, ductus deferens, and seminal vesicles (Cooke et al. 2017). Moreover, early estrogen exposure leads to male reproductive abnormalities, thus showing that male reproductive organs during fetal stages are estrogen targets (Cooke et al. 2017).

Estrogens, and mainly estradiol, have significant effects on the developing brain. They participate in the establishment of sex differences and can exert pervasive trophic and neuroprotective effects (McCarthy 2008). Moreover, estradiol participates in neural differentiation as it promotes neurite outgrowth from fetal hypothalamic explants of both sexes (Bakker and Brock 2010). During development, estradiol plays an important role in the brain's sexual differentiation, as do androgens. The classic Organization and Activation hypothesis states that hormone effects in early life (pre- and peri-natal periods, depending on the species) specify sex differences in the brain (Gegenhuber and Tollkuhn 2019; Phoenix et al. 1959). There is some evidence that indicates that normal sexual differentiation of the female brain requires estradiol (Bakker and Brock 2010). In rodents, ovines, and bovines, the expression of both ERs is present in the fetal brain (Friedman et al. 1983; Panin et al. 2015; Schaub et al. 2008), and particularly in bovines, it has been shown that ERs expression increases through gestation, showing no differences in the mRNA expression

of ERa or ERb between sexes (Panin et al. 2015). In rodents, sexual differentiation occurs in the neonatal period. Estradiol contributes to masculinization, particularly at birth when male mice and rats experience a dramatic increase in circulating testosterone that is converted to estradiol in the brain by aromatase (Gegenhuber and Tollkuhn 2019). It is through both ERa and ERb that masculinization and defeminization of sexual behaviors occur in rodents (Bodo et al. 2006; Kudwa et al. 2006).

2.2.3 Progesterone

Progesterone is an essential regulator of female reproduction. It is a critical player in uterine remodeling, implantation, and pregnancy maintenance. In humans, in early pregnancy, progesterone is produced by the corpus luteum, and then, around the 7th–9th week of gestation, the placenta takes over its production (Schindler 2005). In contrast, in the rat, the corpus luteum is the primary source of progesterone throughout the entire pregnancy (Sanyal 1978).

Progesterone is crucial from implantation to parturition. This steroid is involved in preventing maternal rejection of the trophoblast as it mediates the suppression of the maternal immunological response to the fetus (Kumar and Magon 2012). Furthermore, by the end of gestation, placental progesterone secretion increases, preventing preterm labor (Xiao-xue Wang et al. 2019a, b). Moreover, progesterone, together with prolactin, growth factors, and estrogen, are also involved in MGs development during pregnancy and postpartum (Conneely et al. 2007; Shyamala 1997). Besides, in the last years, it has become clear that progesterone is involved in neural development and also in brain sexual differentiation, as progesterone receptor is expressed in some regions of the developing brain at particular stages (around birth) and is differentially expressed between sexes (González-Orozco and Camacho-Arroyo 2019; Princy S. Quadros et al. 2002a, b; Wagner and Quadros-Mennella 2017). Yet, little is known about progesterone's effects on the developing brain.

In women that undergo ART treatments, progesterone and hCG are low, so the luteal phase is supported with progesterone between other therapies (*e.g.*, hCG or gonadotropin-releasing hormone administration), allowing implantation and improving pregnancy rates in in-vitro fertilization (IVF) treatment (Conforti et al. 2017; Griesinger et al. 2018; van der Linden et al. 2015). Moreover, it is also used, usually during the first trimester of pregnancy, to prevent spontaneous miscarriages (Xiao-xue Wang et al. 2019a, b). However, evidence of the possible effects of progesterone and progestagen administration in preventing miscarriages and in fetal development is controversial. Some studies have reported that exposure to progesterone or progestagen during pregnancy can affect embryonic development and differentiation (Ai et al. 2014) and lead to fetal malformations (Harlap et al. 1975). For example, it has been reported that progesterone administration has an inhibitory effect on cardiac development and cardiomyocyte differentiation (Kang et al. 2016) if administered in high concentrations and that the administration of exogenous progesterone could lead to abnormal blood flow patterns and subsequently to nuchal translucency (Giorlandino et al. 2015).

2.3 Thyroid Hormones

THs (triiodothyronine (T3) and thyroxine (T4)) (Fig. 2.2) are crucial for tissue development and differentiation in many species. In fact, many investigations have shown that they can interact with steroid hormones, growth hormones, and prolactin signaling (Ambrosio et al. 2017; Parikh Shan S. et al., 2017).

Pregnancy presents challenges to the maternal organism physiology and on the THs dynamics. Many aspects need to be considered to deepen the role of THs during pregnancy. In this regard, three principal research areas are proposed to approach THs action, which include the pregnancy-related metabolic demand, the fetus' metabolic demand, and also the pregnant mother's metabolic demand.

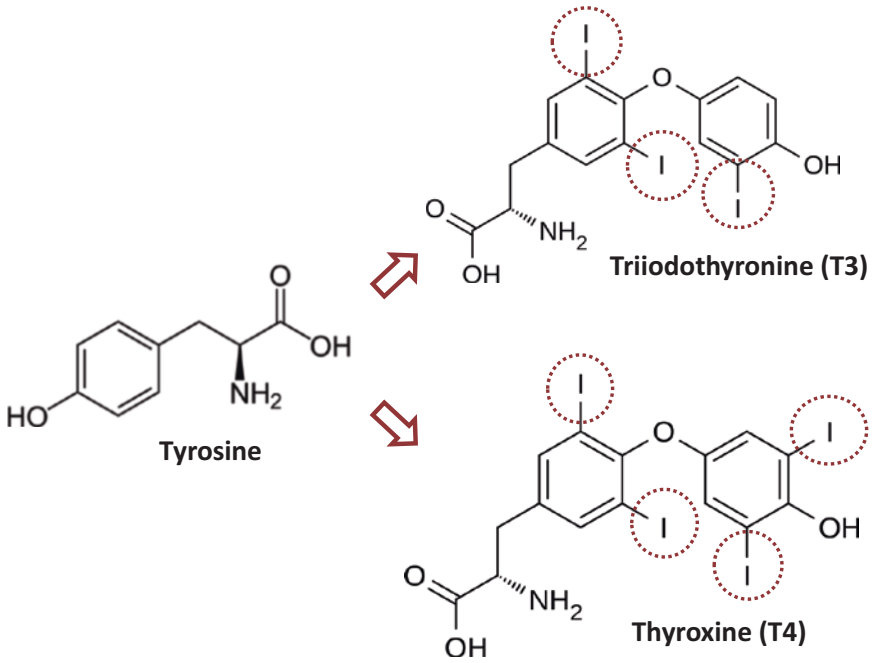

Fig. 2.2 Structure of the main thyroid hormones. Triiodothyronine (T3) and thyroxine (T4) are the main thyroid hormones. They share a common structure, derived from the amino acid tyrosine, and have iodine in their structure (red circles). The position and number of iodines are important. Although other iodinated molecules could be generated, they show little or no biological activity

The first weeks of pregnancy involve the change of THs balance towards a new physiological state (Silva et al. 2018). The chorionic gonadotropin's alpha subunit stimulates the thyroid gland and leads to an increase in the T4 release and a decrease in thyroid-stimulating hormone (TSH) serum level. At the same time, the estrogen level rises, and the hepatic synthesis of thyroglobulin binding protein (TBP) increases, thus leading to a decrease in the free THs serum levels. All of these events take place in an organism with high placental deiodinase type 3 expression and activity, which converts the T4 circulating in reverse T3, an inactive form of thyroid hormone. The increase in estrogen and TBP levels and the action of chorionic gonadotropin, acting as a thyroid gland stimulator, are evidence of the change in the thyroid axis dynamics in maternal organisms. Although in this period the metabolic priority is the fetoplacental unit, the maternal organism's metabolism maintains a high demand of THs that can sustain both vital functions and breast tissue development and differentiation, necessary to support future breastfeeding (Silva et al. 2018).

Despite the importance of THs during pregnancy, laboratory THs analysis through gestation does not achieve precision standards (Alcázar Lázaro et al. 2019; Castillo et al. 2018; Kianpour et al. 2019; Magri et al. 2019; Zhou et al. 2018). Years ago, many studies tried to reach the old biochemical goal of achieving global benchmarks that allowed the determination of optimal THs levels during pregnancy. However, in recent years, research has been focusing on personalized medicine and precision diagnosis based on custom and individual parameters.

Several studies support the fact that THs analysis through pregnancy currently has some technical limitations. Given the fact that THs concentration changes during pregnancy, the reference intervals should be determined not only in each trimester of pregnancy but also attending to the ethnic and geographical variability (Gao et al. 2018; Gunapalasingham et al. 2019; Kim et al. 2015, 2018; Kostecka-Matyja et al. 2017; Maraka

et al. 2017; Moon et al. 2015). Regarding TSH levels, although there is currently a reference interval published by the American Thyroid Association, several authors have shown significant discrepancies between their reference intervals and the international references proposed, due to differences in the techniques used, the ethnicities, or the geographical region analyzed (Gao et al. 2018; Gunapalasingham et al. 2019; Kim et al. 2015, 2018; Kostecka-Matyja et al. 2017; Maraka et al. 2017; Moon et al. 2015). This evidence supports the postulate that the use of international parameters could generate a misunderstanding of results that can lead to unnecessary treatment. Therefore, special care must be taken in the interpretation of the THs and TSH results, especially considering the critical role that THs have through pregnancy.

In the last decade, a link between the hypothalamic-pituitary-gonadal and thyroid axes has been demonstrated. In 2012, Marlatt and colleagues proved that in the goldfish, the ERs expression is tissue-specific and THs-dependent, with the gonads and brain especially sensitive to this regulation (Marlatt et al. 2012). Although the mechanism is not yet determined, it has been established that it is related to thyroid hormone receptor (TR) expression. This ER and TR cross-regulation would be central in the peripartum period, when the TR expression pattern is under variation in different tissues (Anguiano et al. 2004). These studies highlight that in tissue differentiation, THs respond depending on the stage of development (Keijzer et al. 2007). An example of this was provided by Gagne et al. (2013), who demonstrated that TRb-DNA binding depends on the developmental stage of the mouse cerebellum (Gagne et al. 2013), proving that the THs have a regulatory mechanism that guarantees to meet the tissue demand of each stage (Gagne et al. 2013).

TR expression is tissue- and time-dependent. This fact arises from their functions; although they are not redundant, the absence of one of the isoforms can be partially compensated in the absence of the other. In 2010, Cheng et al. summarized this expression pattern, highlighting that TRa1 is constitutively expressed during embryonic development, while TRb1 does so at later developmental stages in the kidney, liver, brain, heart, and thyroid gland (Cheng et al. 2010).

The TR chemical nature gives them repressive gene transcription activity in the absence of THs. The TR action mechanism was described some decades ago and proved that without binding to THs, the TR binds to the DNA response elements (TRE) and heterodimerizes with transcriptional repressors as the nuclear receptor co-repressor (NCor). The THs binding induces a conformational change, allowing the release of repressors and the recruitment of transcriptional co-activators. These last ones are acyltransferase enzymes that act by adding acyl groups to histones, relaxing chromatin and increasing mRNA transcription. Although this mechanism is common to all TR, the TRa1 isoform lacks its TH binding domain, whereby some authors attributed transcriptional inhibitory actions exclusively to it (Mendoza and Hollenberg 2017).

TH genomic signaling is widely studied. However, in the last few years, a non-genomic mechanism of action has been described for THs. In the last decade, it was shown that in the cell membrane of erythrocytes, lymphocytes, and hepatocytes, there are binding sites analogous to those of THs (Blondeau 1986; Kostrouch et al. 1987) linked to intracellular calcium transport. Moreover, the membrane integrin, avb3, has THs binding sites that can activate the Mitogen-Activated Protein Kinases (MAPK) signaling pathway and trigger non-genomic events such as mitosis and angiogenesis, and that this activation is T4-dependent (Cheng et al. 2010).

The THs roles in mammalian differentiation and development have been especially highlighted for their function in neural development; however, in recent years, it has been shown that they also have a central role in the development of several tissues. In mammals, during prenatal life, the THs stimulate hepatocyte and pancreatic beta-cell precursor proliferation. In the neonatal period, THs promote myocardial cell maturation, while in the postnatal period, they encourage the intestinal epithelium precursor cell proliferation (Pascual and Aranda 2013). Although mature organs express both isoforms of TR, during the perinatal period of life, the expression of the alpha isoform,

TRa, is critical for tissue differentiation (López-Fontal et al. 2010; Perra et al. 2009).

This evidence proves the THs role in the differentiation and perinatal development of many tissues, including the differentiation of myoblasts, the contraction of skeletal muscle, and the epidermal proliferation and hair follicle growth (Pascual and Aranda 2013). All these actions respond to both genomic and non-genomic mechanisms of action. However, fetal development studies emphasize on THs action through epigenetic mechanisms. In this context, Van Rooji and Olso (2007a, b) showed that T3 regulates the miR-208 expression, a microRNA that is expressed in cardiac cells of species such as rats, mice, and humans, and that in turn inhibits the Major histocompatibility complex beta (MHCb) expression, which expression represses the incidence of cardiac hypertrophy (van Rooij and Olson 2007a, b). This epigenetic perspective in the role of THs in fetal programming has been supported by many researchers, who have shown that, for example, TRb fetal expression in cardiomyocytes is regulated by miR-27a (Nishi et al. 2011) and that THs are involved in the establishment of the epigenome of hypothalamic neurons, thus emphasizing their role in fetal neurological development (Martinez et al. 2018). Moreover, a recent study has suggested that the establishment of fetal neuronal epigenome depends not only on circulating THs levels during fetal life but also on the THs levels to which gametes were exposed (Martinez et al. 2018).

2.4 Insulin-Like Growth Factors System

The IGF axis is one of the most important systems that regulates fetal and placental growth and is of particular interest in fetal development because it influences growth during gestation as well as its sensitivity to the early nutritional environment (Gicquel and Bouc 2006; Switkowski et al. 2017). In mammals, the IGF system is comprised of two ligands: IGF-I and IGF-II, two receptors: the IGF type 1 and 2 receptors (IGF1R and IGF2R), and six binding proteins: IGFBP1-6 that bind IGF-I and IGF-II with different affinity and regulate their bioavailability (Gicquel and Bouc 2006). Moreover, their expression is different depending on the tissue and development stage. IGF-I and IGF-II signaling involve mitogenic and anti-apoptotic effects and the differentiation of some tissues, acting mainly through IGF1R (Gicquel and Bouc 2006). These two growth factors, together with insulin, are key players in the regulation of fetal growth and development in all mammals.

IGF-I and IGF-II are both expressed in fetal tissues and bloodstream throughout gestation and stimulate fetal and placental growth in response to nutrient availability (Switkowski et al. 2017). IGF-II is the primary regulator of placental and embryonic growth and is also involved in the nutrient transfer. It is expressed at high levels in several somatic tissues during fetal development and declines within days following birth. In adulthood, its expression occurs mainly in the liver and some regions of the brain (Bergman et al. 2013; Gluckman and Butler 1983). Fetal levels of IGF-I start to increase in late gestation. In fetal and perinatal development, its concentrations respond to the nutritional environment, while in adult life, its levels are regulated by growth hormone (GH) (Gicquel and Bouc 2006; Switkowski et al. 2017). At birth, there is a shift from IGF-II to IGF-I predominance, which becomes dependent on GH. IGF-II levels reach their highest expression during fetal life, while IGF-I levels rise in infant and juvenile life and decline after puberty (Sara et al. 1983).

The IGF-II gene is an imprinted gene that shows paternal expression in the fetus and placenta (Constância et al. 2002). The deletion of the paternal allele of IGF-II leads to intrauterine growth retardation (IUGR) (DeChiara et al. 1991). Both IGFs participate in placental growth and development (Forbes et al. 2008). IGF-II exerts effects on both placental structure development and functions. It engages in promoting trophoblast migration and invasion into decidua and myometrium and also in the remodeling of the uterine spiral arteries that contribute to the delivery of maternal blood to the placenta (Hamilton et al. 1998; Harris et al. 2011). Therefore, IGF-II can affect fetal growth by acting in the placenta

to influence the supply of and the genetic demand for maternal nutrients in the fetus (Constância et al. 2002; Reik et al. 2001).

IGF-I is an important determinant of fetal and postnatal growth. It plays different roles depending on the developmental stage, source, and target cell type (Hellström et al. 2016). Although IGF-II is abundantly expressed during fetal life, IGF-I is essential in the regulation of fetal growth in majority of species (Le Stunff et al. 2018). During gestation, IGF-I originates from the fetus and placenta, and its expression correlates with gestational age and fetal weight, increasing in the last trimester of human pregnancy (Muhammad et al. 2017; Shang and Wen 2018). Furthermore, IGF-I concentrations in fetal and cord serum correlate with birth weight, being increased in neonates born large for gestational age and decreased in fetuses and neonates that display IUGR (Klammt et al. 2008). The IGF-I knockout mice show a dramatic growth deficiency (Liu et al. 1993). In the placenta, IGF-I participates in the stimulation of the placental transfer of essential nutrients from the mother to the fetus (Hellström et al. 2016). Like other growth factors, IGF-I participates in morphogenesis, cellular differentiation, proliferation, and maturation of fetal organs, glands, and bones, and it is also involved in protein and glucose synthesis (Shang and Wen 2018; Vatten et al. 2002; Yakar and Adamo 2012).

Breast milk contains several nutrients, hormonal factors, and vitamins that are relevant for the neonatal development of the newborn. Among other factors, many of the beneficial effects of breast milk are attributed to IGFs (Elmlinger et al. 2007). Both IGFs and IGFBPs are present in human milk, particularly in colostrum (Baxter et al. 1984; Elmlinger et al. 2007; Savino et al. 2009), and also in other species such as rats (Donovan et al. 1991), pigs (Simmen et al. 1988), bovines (Francis et al. 1986), and rhesus monkeys (Wilson et al. 1991). Newborns, besides receiving IGF-I in milk, also express and secrete it in saliva, biliary fluid, and pancreatic juice (Chaurasia et al. 1994). Other reports revealed that the stomach and intestine express IGF1R, which suggests that this growth factor is also involved in gastrointestinal tract growth (Burrin 1997; Chaurasia et al. 1994).

Formula-fed infants show higher levels of IGF-I if compared to those that were breastfed (Savino et al. 2005), which suggests that it is related to the amount of protein intake that is higher in formula milk than in breast milk (Hoppe et al. 2004; Savino et al. 2005). Concerning this, high protein intake stimulates IGF-I secretion and hence triggers growth and mitogenic activities, particularly those of the adipose tissue, thus predisposing to adiposity and the risk of obesity in later life in formula-fed infants (Wabitsch et al. 1995). Nevertheless, it has been shown that in preterm infants, breastfeeding leads to an increase in IGF-I serum levels. In these cases, as IGF-I has mitogenic activities and participates in tissue maturation, differentiation, and growth, it has been proposed that early breastfeeding in premature babies acts positively in their development, as IGF-I may act positively on general growth, reducing metabolic disorders, lung and retinal immaturity, and brain developmental abnormalities (Alzaree et al. 2019; Lenhartova et al. 2017).

2.5 Developmental Programming

As we have already described in the preceding sections, several hormones, including androgens, estrogens, progesterone, TH, and IGFs, regulate the growth of the developing organism and its metabolism during pregnancy by controlling the supply of nutrients through the placenta (Barker 2004; Gluckman et al. 2008) (Fig. 2.3). Hormonal and growth factors' balance is crucial for morphogenesis, tissue differentiation, embryo development and growth, parturition, brain development, and sexual differentiation. Therefore, alterations in these mediators may lead to long-term consequences that, in turn, may affect postnatal and adult health (Baud and Berkane 2019). The environment in which a fetus develops is essential for its growth and maturation. The DOHaD hypothesis postulates that exposure to particular environmental influences during some windows of

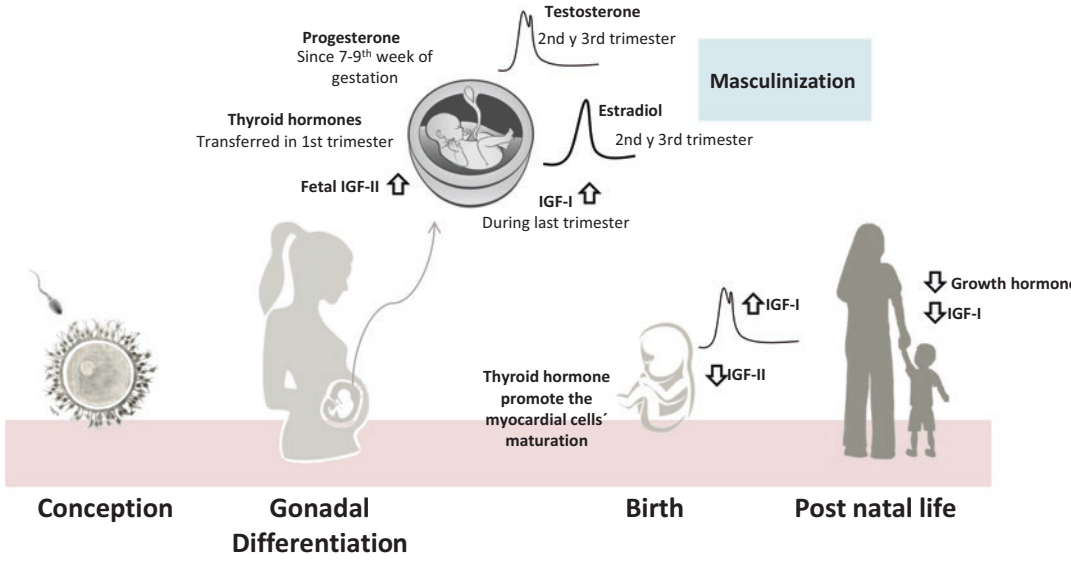

Fig. 2.3 Hormonal changes from fetal development to postnatal life in humans. IGF-II is the predominant form of IGFs in fetal life. It starts to decline by the end of gestation, and at birth, IGF-II continues its gradual decrease. During the last trimester of fetal development, IGF-I concentration starts to increase and participates in the stimulation of the placental transfer of essential nutrients from the mother to the fetus. IGF-I reaches its highest levels during neonatal life, and, together with the growth hormone, the concentrations gradually decrease until adulthood. In the first trimester of pregnancy, the thyroid hormone is transferred from the mother to the fetus that expresses thyroid hormone receptors. During the neonatal period, thyroid hormones are essential for promoting myocardial cells´ maturation. Progesterone is first produced by the maternal corpus luteum, and around the 7–9th week of gestation, it is produced by the placenta. Its production increases since this period, contributing to gestational maintenance. During the second and third trimesters of pregnancy, there exists a peak of estradiol and testosterone that participate in sex differentiation. Androgens determine the morphogenesis processes of male-specific organs, and estrogens participate in fetal organ development and are crucial in gonadal and brain development

susceptibility during fetal and postnatal development may have long-term and permanent effects (Hanson and Gluckman 2014). Several detrimental outcomes and complex and non-communicable chronic diseases have been described as having fetal or perinatal origins, emphasizing a developmental programming effect in their etiology (see Table 2.1).

2.5.1 Androgens and Estrogens in Developmental Programming

Androgens and estrogens play critical roles in fetal and neonatal development, not only in modulating reproductive but also neuroendocrine and metabolic functions. Deficit or excess of any of these hormones may affect several systems, leading to sex-dependent effects. In this regard, Phoenix et al. (1959) showed that prenatal treatment of female guinea pigs with testosterone had a long-term impact on behavior as well as on phenotypic reproductive outcomes, but on the contrary, their male siblings showed neither structural abnormalities nor behavioral changes. Based on these experiments, many other studies in different species have shown that prenatal testosterone excess exposure in females is related to several alterations. Among them, one of the most important is the development of PCOS-like features.

One of the main effects described in female rodents, monkeys, and sheep exposed to prenatal androgen excess is reproductive alterations that involve the development of ovarian cysts, altered follicle development, anovulation, reduced fertil-

Table 2.1 Main outcomes in the offspring due to alterations in maternal hormonal balance or in the uterine environment

Hormonal or maternal environment imbalance	Specie	Principal offspring outcomes	References
Androgen excess (Fetal life)	Human (PCOS women)	Females Hirsutism Increased ovarian volume Increased LH High androgen levels High insulin High AMH Decreased adiponectin levels Males High cholesterol Increased LDL	Females Sir-Petermann et al. (2006, 2007, 2009) and Crisosto et al. (2007, 2019) Males Crisosto et al. (2017)
	Rat	Females Ovarian cysts Altered estrous cycle or anovulation Uterine hyperplasia Increased serum testosterone Altered ovarian steroidogenesis Impaired gonadotrophin secretion Insulin resistance Hyperglycemia Dyslipidemia Hepatic steatosis Males Altered lipid profile Decreased sperm count Reduced testis size Hormonal alterations Decreased body weight at birth Glucose intolerance Hyperinsulinemia	Females Wolf (2002), Demissie et al. (2008), Ramezani Tehrani et al. (2014), Yan et al. (2014), Abruzzese et al. (2016, 2019) and Ferreira et al. (2019) Males Wolf (2002), Ramezani Tehrani et al. (2013) and More et al. (2016)
	Monkey	Increased testosterone Increased fat depots Altered ovarian and adrenal steroidogenesis External genital masculinization Obliteration of the external vaginal orifice Masculinized behavior Impaired AMH levels Impaired ovarian reserve Mild to moderate glucose intolerance	Eisner et al. (2002, 2003), Dumesic et al. (2009), Abbott et al. (2010)
	Sheep	Females Follicular persistence Insulin resistance Hyperinsulinemia Altered ovarian steroidogenesis Deficits in the feedback control of GnRH/LH Peripheral insulin resistance Insulin resistance Disruption of adipose tissue mass Decreased adipocyte size Males Altered lipid profile Reduced sperm count Decreased sperm motility Increased AMH levels Reduced body weight Reduced testis weight	Females Ortega et al. (2010), Veiga-Lopez et al. (2013), Padmanabhan et al. (2014), Ahn et al. (2015), Lu et al. (2016) and Puttabyatappa et al. (2017) Males Recabarren et al. (2008, 2017) and Scully et al. (2018)

(continued)

Table 2.1 (continued)

Hormonal or maternal environment imbalance	Specie	Principal offspring outcomes	References
	Mouse	High LH levels Increased testosterone Altered estrous cycle Glucose intolerance Impaired pancreatic function Increased adipocyte size	Roland et al. (2010)
Estradiol excess (Neonatal life)	Rat	Decreased the number of primordial follicles Impaired ovarian follicular development Ovarian cysts Altered expression of several neurotrasmitters, such as dopamin and serotonin, in the ventromedial-arcuate nucleus Increased AMH expression	Sotomayor-Zárate et al. (2011) and Martinez-Pinto et al. (2018)
Maternal hypothyroidism	Human	Reduced birth weight High levels of TSH and free T4 in newborns Increased scores for attention-deficit/hyperactivity disorder symptoms in children	Blazer et al. (2003) and Modesto et al. (2015)
Maternal hyperthyroidism	Rat	Slightly elevated fetal brain Altered expression of fetal brain cytoskeletal proteins	Evans et al. (2002)
Maternal obesity	Human	High fetal birth weight Increased cord plasma insulin at birth Low concentrations of IGFB1-4 in cord plasma Increased Fetal-placental weight ratio	Ferraro et al. (2012) and Lappas (2015)
	Sheep	Decreased IGF-I serum levels Low IGF-I expression in liver Decreased GH mRNA expression in the pituitary Reduced expression of leptin receptor in the pituitary Increased percentage of body fat	Tuersunjiang et al. (2017)
Gestational diabetes	Human	Low concentrations of IGFB1-3 in cord plasma	Lappas (2015)
	Rat	Increased fetal weight Fetal hyperinsulinemia Fetal hyperglycemia Fetal triglyceridemia High fetal IGF-I serum levels Impaired expression of IGFs axis in several organs	Martínez et al. (2008) and White et al. (2015)

Evidence based on human studies and animal models of developmental programming effects: *IGF-I* Insulin growth factor I, *AMH* antimullerian hormone, *GnRH* gonadotropin releasing hormone, *LH* luteinizing hormone, *LDL* low density lipoprotein, *TSH* Thyroid-Stimulating Hormone, *T4* thyroxine, *IGFBP* insulin growth factor binding protein

ity, uterine hyperplasia, altered ovarian reserve and oocyte quality, hormonal imbalance in puberty and adulthood, altered ovarian steroidogenesis and gonadotropin signaling (Abruzzese et al. 2019; Dumesic et al. 2009; Eisner et al. 2002; Ferreira et al. 2019; Padmanabhan et al. 2014; Ramezani Tehrani et al. 2014; Wolf 2002), and also the neuroendocrine regulation of these (Ahn et al. 2015; Yan et al. 2014). Together with these findings, it has been described that androgen exposure during prenatal life in females also leads to several metabolic disturbances that involve impaired insulin sensitivity and metabolic syndrome features and affect not only reproductive organs but also other insulin target tissues (Abbott et al. 2010; Abruzzese et al. 2016; Demissie et al. 2008; Puttabyatappa et al., 2017; Roland et al. 2010). In that context, the liver of prenatally androgen-exposed animals shows an altered insulin signaling response (Lu et al. 2016), impaired lipid metabolism (Abruzzese et al. 2016), and a tendency to develop hepatic steatosis (Demissie et al. 2008; Puttabyatappa et al., 2017) from puberty to adulthood.

Adipose tissue function is also altered after exposure to prenatal androgen excess. In rhesus monkeys, the prenatal androgen excess leads to an increase in total abdominal and intra-abdominal fat depots (Eisner et al. 2003). In sheep, it was also shown to disrupt adipose tissue mass and distribution, leading to a decrease in adipocyte size and visceral adiposity (Veiga-Lopez et al. 2013), while in mice, prenatal androgen treatment with DHT increased adipocyte size without affecting fat mass (Roland et al. 2010). Taking all of this evidence, prenatal androgen exposure triggers programming effects on different organs. These effects vary depending on the time of androgen exposure and also among the species under study, as these differ in the time and way in which each organ differentiates and organizes.

Although the effects of prenatal androgen excess on females are widely studied, little is known about the consequences of androgen excess on males. In the last few years, increasing attention has come to this point. Recent studies suggest that prenatal androgen exposure may also impair endocrine and reproductive functions in males but that the impact is different from that found in females (Domonkos et al. 2017; Ramezani Tehrani et al., 2013; Recabarren et al. 2017, 2008b; Scully et al. 2018). In this regard, it has been reported that prenatal androgen excess leads to reduced testis size, decreased sperm count, reduced sperm motility, and hormonal alterations in several species (Bormann et al. 2011; Domonkos et al. 2017; Ramezani Tehrani et al., 2013; Recabarren et al. 2017; Scully et al. 2018). In rats, it was also shown that testosterone exposure during prenatal life leads to pups with a decrease in body weight at birth, hyperinsulinemia, and glucose intolerance (More et al. 2016).

The mechanisms by which prenatal androgen excess affects the developing embryos are still a matter of study. Although it has been reported that androgen excess in the uterus affects placental homeostasis (Sun et al. 2012), fetal androgen exposure can also have intergenerational and transgenerational effects (Risal et al. 2019). Probably through permanent effects that alter the metabolism and hormonal balance, thus recreating, in the following generations, an altered maternal environment and also involving mechanisms of epigenetic inheritance.

Estrogen also plays an essential role during development. Most of the studies have shown that estradiol has a potent action in rodents and sheep during the first few days of postnatal life and that an increase in estradiol levels could lead to reproductive alterations and PCOS-like features in adult life (Martinez-Pinto et al. 2018; Sotomayor-Zárate et al., 2011). In the last decades, increasing attention has been given to synthetic compounds that have an estrogenic effect, such as bisphenol A and parabens. These compounds are ubiquitously present in the environment and can mimic estrogenic actions as they interact with ERs. Particular attention has been given to bisphenol A, as it can be found in many products, including plastic food and drink containers and toys. Gestational exposure to bisphenol A has been related to several effects, such as altered reproductive and metabolic functions, behavioral abnormalities, and also cancer (Abruzzese et al. 2018; Giulivo et al. 2016).

Moreover, neonatal exposure to this compound has been found to alter the hypothalamic-pituitary-gonadal and thyroid axes in female rats (Fernández et al. 2010; Fernandez et al. 2018).

2.5.1.1 PCOS as a Case

PCOS is a common endocrine and reproductive disorder that is associated with metabolic syndrome, cardiovascular risk, and insulin resistance (Apridonidze et al. 2005; Cussons et al. 2008). According to the Rotterdam criteria, PCOS is defined by the presence of at least two out of the following three criteria: oligo- or anovulatory cycles, hyperandrogenism (clinical and/or biochemical), and/or morphological polycystic ovaries on ultrasound (Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group 2004). These criteria recognize four different phenotypes: three of them are hyperandrogenic forms of PCOS, whereas the fourth phenotype is a normoandrogenic form (Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group 2004).

PCOS etiology has been proposed to be multifactorial, with genetic, environmental, and intrauterine factors contributing to its development. However, in the last few years, increasing evidence supports fetal origins in the development of the syndrome (Abbott et al. 2002; Melo et al. 2015). Prenatal androgen exposure has been shown to lead to PCOS-like features and related metabolic alterations in several species, such as rats (Abruzzese et al. 2016, 2019; Demissie et al. 2008), mice (Roland et al. 2010), sheep (Ortega et al. 2010; Padmanabhan et al. 2014), and rhesus monkeys (Abbott et al. 2010; Dumesic et al. 2009). Furthermore, recent studies have reported that during pregnancy, women with PCOS display signs of hormonal imbalance, such as high androgen levels and increased AMH (Glintborg et al. 2018; Piltonen et al. 2019). In this context, daughters born from PCOS mothers show hirsutism, increased ovarian volumes, LH, androgen, insulin, triglycerides, and AMH levels, and decreased adiponectin levels in the prepubertal stage (Crisosto et al. 2007, 2019; Sir-Petermann et al. 2006, 2007, 2009). Also, a recent study has shown that sons of women with PCOS display metabolic alterations, showing increased total cholesterol and LDL levels during puberty (Crisosto et al. 2017).

2.5.2 THs in Developmental Programming

The role of THs in the maternal-fetal interface establishment has been extensively investigated in the last few years. THs are expressed in women's reproductive tract. THs are present in human follicular fluid, and also that granulosa cells express TR (Silva et al. 2018; Wakim et al. 1993). Moreover, human embryos express TR, with this expression being different in trophectoderm cells and embryonic inner cell mass (Silva et al. 2018).

Studies conducted in human embryos demonstrated that TH, and particularly T3, affect mitochondrial activity, either by regulating mitochondrial DNA transcription or by regulating the transcription of the mitochondrial transcription factor A (TFAM) or other regulators of mitochondrial biogenesis (Das et al. 2009; Noli et al. 2019). In addition to this, it was shown that the presence of T3, in the culture medium, induces a switch in the cells energetic metabolism, which allows achieving a more efficient oxidative phosphorylation in the inner cell mass. These findings highlight the role of T3 in embryonic cell development and suggest that supplementation of culture media with T3 may improve outcomes for women undergoing in vitro fertilization (Noli et al. 2019).

Recent studies show that for a correct establishment of the maternal-fetal interface, it is essential that embryo implantation occurs in an anti-inflammatory environment where the Th2 lymphocytes immune response predominates (Koga et al. 2009; Toder et al. 2003). In this regard, THs increase the interleukin 10 (IL-10) and nitric oxide synthase 2 (NOS2) expression and decrease tumor necrosis factor-alpha (TNFa) placental expression, thus contributing to the generation of an anti-inflammatory environment (Silva et al. 2018). Moreover, THs participate in several other events, such as (1) regulation of VEGF expression and endometrial vascularize-

tion, (2) metalloproteinases regulation and decidualization, and (3) FAS/FASL inhibition and trophoblasts survival and differentiation control (Silva et al. 2018). All of these events are central to the establishment of the maternal-fetal interface. Therefore, alterations in THs levels can lead to fertility problems and pregnancy complications, as seen in women with thyroid pathologies.

THs disorders are common in women of reproductive age. Both hyperthyroidism and hypothyroidism have been related to adverse pregnancy outcomes, such as an increased risk of abortion, premature delivery, intrauterine fetal death, fetal growth restriction, and congenital anomalies (Blazer et al. 2003; Boroumand Rezazadeh 2015; Tudosa et al. 2010). Moreover, given the importance of THs on neuronal and brain development, it has been proposed that maternal thyroid disorders may interfere with fetal brain development. Although these effects are not well defined, alterations in neuronal differentiation and several affections in cognitive and motor functions have been described (de Escobar et al. 2004; Evans et al. 2002; Modesto et al. 2015; Smallridge and Ladenson 2001).

The thyroid axis has an important role not only during intrauterine life but also in the peripartum period. In this regard, it has been shown that THs levels and their regulation are central in the establishment of the breastfeeding onset. In this context, Anguiano et al. (2004) showed that the MG has regulatory mechanisms that protect it from THs peripartum fluctuations establishment (Campo Verde Arboccó et al. 2015, 2016, 2017; Hapon et al. 2003; Pennacchio et al. 2017; Varas et al. 1999, 2001, 2002; Varas and Jahn 2005).

Hypothyroidism is one of the leading causes of breastfeeding failure. For several years, this issue has been addressed by adjusting THs levels until achieving milk production. Nevertheless, this strategy ignores that, although breastfeeding may be achieved even without reaching THs optimal levels, milk may lack macro- and micronutrients. In this regard, in a rat model of hypothyroidism, Hapon et al. (2003) showed that exclusive breastfeeding could be achieved, but milk quality was reduced in terms of its macronutrients (lactose and triglycerides). This deficit in lactation was reflected in the reduced growth rate of the offspring (Hapon et al. 2003).

Given THs molecular nature and their role in gene regulation, THs demand will be higher in periods of active milk synthesis. During these periods, the need for THs is controlled by the alveoli metabolic requirements and the alveolar emptying cycles. In healthy mothers, this THs demand is supplied by the thyroid gland. Nevertheless, in hypothyroid mothers, THs levels may be sufficient to accomplish breastfeeding but not to respond to synthesis cycles dynamic fluctuations. Therefore, milk could be produced but may not achieve an optimal macronutrient balance, as suggested by Hapon et al. (2003).

THs not only participate in breastfeeding and lactation onset but also in their maintenance. In cases of THs deficit, the MG can trigger compensatory mechanisms to counterbalance it. However, these mechanisms are neither constant nor can they achieve the transcriptional profile of a regular MG (B Anguiano et al. 2004; Campo Verde Arboccó et al. 2015, 2016, 2017).

2.5.3 IGFs and Developmental Programming

The GH-IGF axis is a crucial component underpinning early life developmental programming. During fetal development, placental GH is the principal regulator of maternal IGF-I. In the fetus, IGF controls its growth directly, independently of GH. It has been reported that in cases of IUGR, this system is impaired. Human mutations in IGF-I and its receptor lead to growth restriction (Klammt et al. 2008). Moreover, imprinting defects in the IGF-II gene have also been associated with impaired fetal and postnatal growth (Butler 2002).

It has been proposed that the GH-IGF axis is a target of intrauterine programming in growth retardation cases caused by altered maternal nutrition, in both undernutrition or overweight. Some studies have shown that fetal cord blood concentrations of IGF-I and IGF-II are reduced in pregnancies with IUGR (Lee et al. 2010) and

that children born with IUGR display low circulating levels of IGF-I (Verkauskiene et al. 2005). In the case of maternal obesity and excessive gestational weight gain, there are no alterations in maternal and cord blood IGF-I and IGF-II levels (Ferraro et al. 2012, 2013). Still, it has been described as changes in maternal (Ferraro et al. 2013) and cord levels of some of the IGFBPs (Ferraro et al. 2012), which regulate IGFs bioavailability. Moreover, animal studies of maternal undernutrition or high-fat diet have also shown alterations in the IGFs axis. Rat models of maternal malnutrition reveal that IGF-I levels are reduced in their offspring at adulthood (Smith et al. 2014). In sheep, male offspring of obese mothers display decreased levels of serum IGF-I together with low IGF-I mRNA and protein liver expression during adult life (Tuersunjiang et al. 2017).

Another pathology related to maternal nutrient alteration is gestational diabetes, in which fetuses are exposed to a hyperglycemic environment during pregnancy. The diabetic environment in the offspring leads to weight increase (Kc et al. 2015). The IGFs axis is affected by gestational diabetes. In this regard, a study has shown that maternal plasma levels of IGFBP1 and IGFBP6 and cord plasma levels of IGFBP1-3 were lower in non-obese women with gestational diabetes when compared with non-obese women with normal glucose tolerance. Furthermore, obesity did not affect IGFBP1-7 levels in women with gestational diabetes (Lappas 2015). Thus, suggesting that the IGFs axis, and particularly changes in IGFs availability in pregnant women, may present a contributing mechanism to alterations in birth weight and neonatal body composition. Together with these findings, in a neonatal-induced streptozotocin rat model of mild diabetes during pregnancy, it was reported that the IGF axis is impaired and that placental, embryonic, and fetal development are altered (Martínez et al. 2008; White et al. 2015). Moreover, in this animal model, the authors found elevated levels of fetal IGF-I without changes in maternal IGF-I plasma levels. Besides, they reported not only an increase in fetal weight in the offspring of diabetic rats but also in the weight of the fetal organs, together with an impairment in IGFs axis components (such as IGFs, IGF receptors, and IGFBPs), predominantly in the placenta and in fetal tissues such as heart and liver in an organ-specific dependent manner (White et al. 2015). This evidence shows that this system is involved in the mechanisms related to the growth-promoting effect of the diabetic environment during intrauterine development.

2.5.4 Adipokines and Hormonal Regulation: Contribution of Adipose Tissue to Developmental Programming Effects

Several authors have focused their attention on the role of the placenta in developmental programming, as factors that may perturb the fetus must be transmitted across the placenta to affect the developing organism. However, in the last few years, some attention has been given to the role of adipose tissue and the endocrine factors secreted by it, such as adipokines, during pregnancy, lactation, and early development.

The adipose tissue has an essential role in reproduction and survival in females (McNamara and Huber 2018). Moreover, it has been described that it is crucial in the regulation of pregnancy, fetal, and neonatal development (Fig. 2.4). It has the ability to rapidly respond to fluctuations in nutrient and energy supply through adipocyte hypertrophy and hyperplasia (Sun et al. 2011). The accelerated expansion and remodeling of the adipose tissue leads to a metabolic phenotype that promotes metabolic alterations and cardiovascular disease (Sun et al. 2011). In human health, adipose tissue dysregulation contributes to the development of insulin resistance, obesity, and metabolic syndrome (McNamara and Huber 2018).

There are two types of adipose tissue in mammals: white adipose tissue (WAT) and brown adipose tissue (BAT) (Mathew et al. 2018). In neonatal life, BAT is abundant, but in postnatal life (adult puberty), WAT predominates over BAT (Saely et al. 2012). Adipose tissue plays an essen-

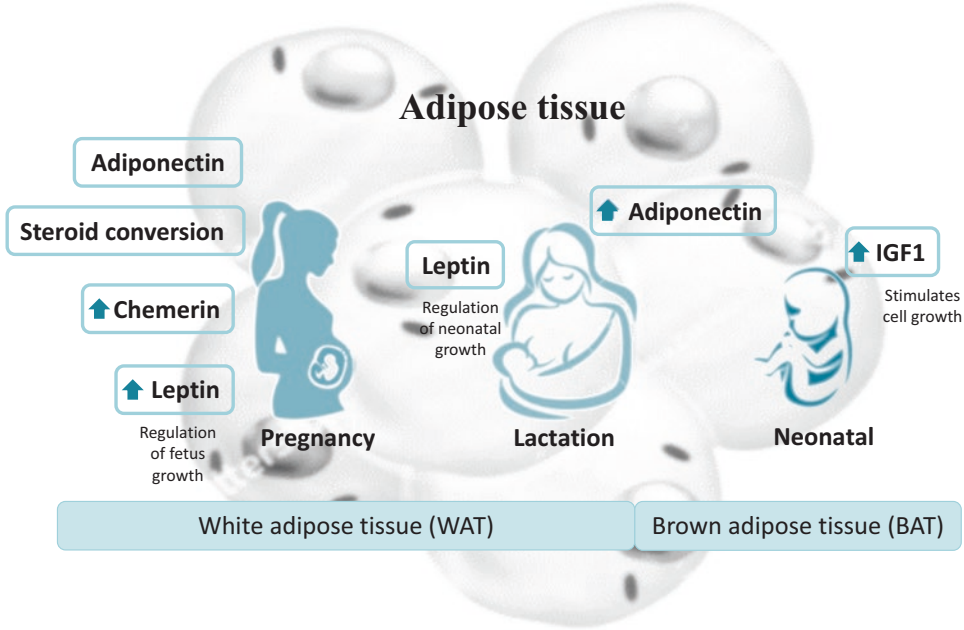

Fig. 2.4 Role of adipokines and secreted hormones by the adipose tissue during pregnancy, lactation, and neonatal life. In pregnancy, maternal white adipose tissue secretes chemerin, adiponectin, and leptin (which regulate the fetus growth), among others. Moreover, it contributes to the maintenance of the maternal hormonal environment through steroid conversion. In turn, hormones regulate adipose tissue development. During lactation, adiponectin increases and leptin declines. This last adipokine is known to participate in the regulation of neonatal growth and the support of the maternal energy demand. In neonatal life, brown adipose tissue is the most abundant form. It contributes to the maintenance of newborn body temperature and to the secretion of IGF-I that stimulates cell growth. IGF-I is also produced by the white adipose tissue during postnatal life when it becomes the predominant form

tial role in neonates. BAT develops during the third trimester of pregnancy and expands in the first postnatal period (Merkestein et al. 2014). This tissue helps the newborn to survive during the temperature change period between the intrauterine and the external environments. Therefore, a decrease in BAT levels can lead to hypothermia or neonatal death (Argentato et al. 2018). On the other hand, WAT is involved in energy storage, and it is distributed throughout the body, including subcutaneous, retroperitoneal, abdominal, and gonadal areas (McNamara and Huber 2018). The energy balance in this tissue is mainly modulated by non-esterified fatty acid suppression and the increased clearance of triacylglycerol (TAG). The visceral adipose tissue (VAT), in adults, especially appears to be more endocrinologically active than other adipose depots (Hajer et al. 2008; McNamara and Huber 2018). In many mammalian species, including humans, the endocrine function of VAT is essential for the regulation of energy metabolism and the adaptation to lactation. Furthermore, endocrine signals from this tissue contribute to the modulation of fertility and inflammatory response (McNamara and Huber 2018).

During pregnancy and lactation, the nutritional situation of mothers is considered to be important for the health of the mother as well as the offspring, and therefore adequate dietary recommendations are important (Lomas-Soria et al. 2018; Marangoni et al. 2016). Maternal body fat mass increases throughout the pregnancy with the accumulation of fat mainly on the trunk and thighs (Jayabalan et al. 2017), and the fat is retained during pregnancy (Sohlström and Forsum 1995) but between 12 and 37 weeks of human pregnancy, subcutaneous adipose tissue decreases. In pregnant women, the expansion of preperitoneal and abdominal adipose tissue is

necessary to support nutrient supply from the mother to the fetus (Jayabalan et al. 2017). Also, adipose tissue is not only involved in the regulation of maternal and fetal metabolism during gestation but is also implicated in post-partum maternal metabolism (Sohlström and Forsum 1995). It has been reported that changes in body composition during lactation are responses to complex neuroendocrine and biochemical stimuli that are susceptible to modifications by environmental factors (Butte and Hopkinson 1998). Following this, during lactation, adipose tissue is redistributed and contributes to the regulation and adaptation of maternal metabolism to high energetic demand. Alterations in adipose tissue during pregnancy can affect the offspring. In this regard, it has been described that the offspring of obese mothers usually have a higher risk of congenital anomalies (Jayabalan et al. 2017), particularly those who are born large for gestational age are prone to develop obesity and metabolic syndrome (Drake and Reynolds 2010).

Adipose tissue was traditionally considered as an energy storage organ, but currently, its role as an endocrine organ has been emphasized. It can synthesize different bioactive compounds that regulate metabolic homeostasis and have effects on several systems, mainly on the insulin sensibility and metabolic homeostasis (Kershaw and Flier 2004). It produces and secretes many signaling molecules known as adipokines, such as chemerin, adiponectin, and leptin, that act as hormones and modulate the immune, endocrine, and reproductive systems (Hajer et al. 2008). Besides its role in the secretion of adipokines, adipose tissue is also involved in the regulation of sex-hormone levels. Steroidogenesis conversion occurs in this tissue, but adipose tissue can de novo synthesize sex hormones as well (Corrales et al. 2018). The expression of estrogen, progesterone, and prolactin helps to develop adipose tissue to provide specific depots that will later be used to support fetal and mammary growth (Morel et al. 2016). As mentioned before, these hormones are involved in pregnancy from implantation to parturition, and particularly, progesterone directs the storage of TAG during most of the gestational period (Morel et al. 2016).

Recent evidence suggests a role for the adipokines in human pregnancy and development, and alterations in any of these molecules have been associated with different pathologies. Chemerin is a newly discovered adipokine that acts as a chemoattractant for innate immune cells and as an important player in the initiation of immune responses (Yang et al. 2018b). Besides, a role for chemerin has been described in several metabolic and reproductive disorders, such as PCOS, obesity, and preeclampsia (Cetin et al. 2017; Tan et al. 2009; Yang et al. 2018a). It was suggested that chemerin is crucial in the maintenance of early pregnancy and the prevention of miscarriage caused by abnormal immunity (Yang et al. 2018b). However, its role during gestation remains unclear. Chemerin levels increase during pregnancy, and low expression of chemerin in decidua is related to a higher risk of early spontaneous abortion (Yang et al. 2018b). On the other hand, recent studies have reported that, as compared with healthy pregnant women, PCOS patients (Yang et al. 2018a) and women with preeclampsia (Cetin et al. 2017) show higher levels of circulating chemerin. Although in the last year great attention has been given to chemerin and its role in reproduction, further research is needed to understand the role of this adipokine in gestational establishment and maintenance.

Another important adipokine is leptin. During pregnancy, leptin is involved in the regulation of fetal growth (Kiess et al. 1998). The maternal peripheral leptin levels are increased during pregnancy, suggesting that in the second trimester, leptin concentrations reach a peak and remain elevated until parturition (Henson and Castracane 2000). During pregnancy, high leptin concentrations promote the shift of nutrients from the mother to the fetus and do not inhibit food intake (McNamara and Huber 2018). Leptin is not only secreted by adipocytes; placenta and fetus also synthesize leptin that affects growth by modulating GH secretion (Henson and Castracane 2000).

Besides their functions in pregnancy, the adipokines secreted by adipocytes also play important roles during parturition and lactation. Three to four weeks before parturition, adiponectin starts to decrease and rises again during lactation

(McNamara and Huber 2018). Before parturition, the concentration of leptin starts decreasing shortly and remains low during the entire lactation period (Henson and Castracane 2000). The rapid decline of leptin is common in mammalians and may serve to support the maternal energy demand during lactation.

Another important molecule secreted by adipose tissue is IGF-I. As mentioned before, together with GH, IGFs provide a signal to control embryonic and postnatal development (Hamilton et al. 1998). Although the majority of circulating IGF-I comes from the liver, several studies in the past decade have suggested that IGF-I modulates adipocyte development (Stephens 2016). In adipose tissue, there exists a robust regulatory system that modulates IGF-I levels, including its production and secretion (Gunawardana and Piston 2015; Stephens 2016). In humans, elimination of IGF-I and its receptor (IGF1R) results in retardation of growth in stature (Arends et al. 2002; Solomon-Zemler et al. 2017).

All of this evidence highlights that not only the distribution of adipose tissue but also its functioning have a significant effect throughout pregnancy, lactation, and fetal development.

2.6 Epigenetic and Developmental Programming

Organisms interact with the changing environment. Prenatal and neonatal periods of life are known as windows of susceptibility in which developing organisms respond in an adaptive way to the stimuli or insults. Nutritional and hormonal signals from the mother to the developing organisms permit it to know the environment beyond the mother, allowing the organism to adjust its phenotype.

This plastic response to the environment leads to consequences in the adult phenotype and, in some cases, to the development of diseases. The mechanisms involved in developmental programming effects are still unclear but involve adaptive responses, changes in organ physiology and structure and changes in gene expression, and particularly in the last few years, the role of epigenetic mechanisms has been highlighted (Fig. 2.5) (Bianco-Miotto et al. 2017; Godfrey et al. 2007; Hanson and Gluckman 2014). Epigenetic mechanisms have also been related to the intergenerational and transgenerational reported effects of programming. Among these phenomena are changes in DNA methylation, histone modification, and non-coding RNA expression and regulation. However, the specific mechanisms by which developing organisms sense the environment and induce epigenetic changes leading to long-term effects are still a matter of study.

2.6.1 Epigenetic Regulation During Gestation

It has been proposed that during early development, environmental factors, nutritional status, and hormonal variations can induce alterations to epigenetic marks together with long-term changes in gene expression, potentially conditioning the adult phenotype (Godfrey et al. 2007). The proposed mechanism suggests that early stressors or environmental cues in pregnant females induce epigenetic alterations not only in the developing embryos but also in their germline. The changes in the epigenome of the gametes could be transmitted to the future generations that were not exposed to the insult, thus leading to transgenerational effects. Evidence in humans and animal models of developmental programming has shown changes in epigenetic marks in different maternal and fetal tissues, as well as changes in metabolic, developmental, and endocrine pathways (Table 2.2). Nonetheless, the studies are limited, and most of the findings are studies of association, but whether the epigenetic changes are causes or consequences of developmental programming is still a matter of debate. Moreover, it has to be taken into consideration when analyzing different tissues that epigenetic marks are tissue-specific and that they may change at different stages of development. Thus, marks found at early stages, for example, in

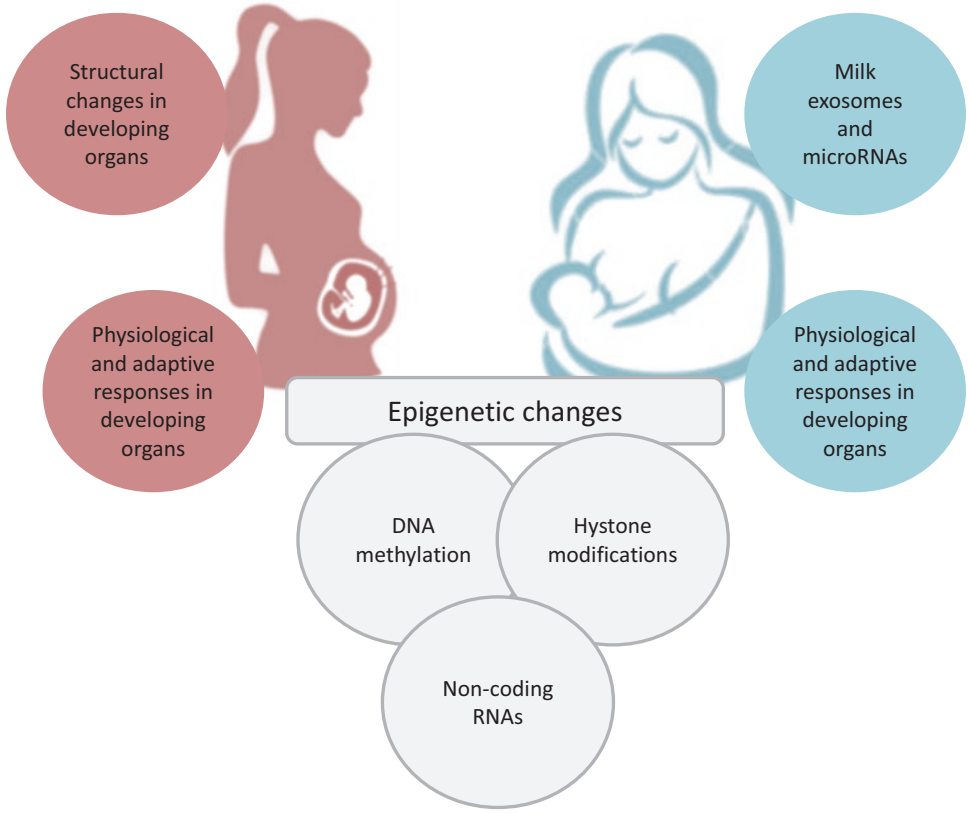

Fig. 2.5 Mechanisms involved in developmental programming effects. Epigenetic changes in both prenatal and neonatal periods of life have been described as the predominant mechanisms involved in programming effects. Moreover, physiological changes and adaptive responses have also been suggested as one of the mechanisms affecting development. During fetal life, while organs are developing, structural changes take place due to environmental insults and maternal hormonal changes. Moreover, in the last few years, special attention has been given to lactation and milk composition as involved in developmental programming effects. Maternal milk exosomes and microRNAs also participate in programming effects

infants, may not be concordant with those found at adulthood, as epigenome marks may be influenced by postnatal modifications from aging, environmental factors, and consequences of earlier life experience (Bjornsson et al. 2008).

In this regard, studies in rhesus monkeys have shown that prenatal androgen exposure affects the visceral adipose tissue methylation profile of infant and adult female monkeys. While exhibiting no overlap in terms of specific genes differentially methylated, the infant and adult methylation patterns pointed to common pathways; in both cases, the affected genes were involved in transforming growth factor b (TGF-b) signaling and cell membrane function and structure (Xu et al. 2011). Together with these findings, a pilot study in PCOS patients who present high levels of androgens during pregnancy showed changes in umbilical cord blood methylation pattern as compared with control women. The reported changes were in genes involved in glucotoxic, lipotoxic, and inflammatory pathways (Lambertini et al. 2017). In cases of mild maternal hypothyroidism, it has been shown that the THs deficit induces impaired cognitive capacity in the offspring and DNA hypermethylation changes in the Bdnf gene (a member of the neurotrophin growth factor) (Kawahori et al. 2018). Together, these findings suggest that an altered hormonal environment in utero, such as androgen excess or THs deficit, influences changes in methylation patterns.

Table 2.2 Main epigenetic modifications in the offspring due to alterations in maternal hormonal balance or in the uterine environment

Hormonal or maternal environment imbalance	Specie	Sample used	Gene	Epigenetic modification	References
Androgen excess	Human (anovulatory PCOS women)	Umbilical cord blood	918 genes conforming the PCOS "superpathway" (genes controlling hormonal regulation (e.g., ER), mitochondrial activity (e.g., APP),and glucose metabolism (e.g., INS))	Hypo/hyper-methylation	Lambertini et al. (2017)
	Rat	Theca cell (ovary)	Steroidogenic genes (GATA6 and StAR promoter)	Demethylation	Salehi Jahromi et al. (2018)
		Peripheral blood cells and ovary	AR and Cyp11A1	Hypomethylation	Xia et al. (2015)
	Monkey	Visceral adipose tissue	163 loci in infant and 325 loci in adult, affecting several significant pathways (including the antiproliferative role of TOB in T-cell signalingand TGF-b signaling)	Methylation	Xu et al. (2011)
	Sheep	Ovary	SERPINA5, HSD3B1, HSD17B14, TNK1, LIPG, and others	Histone methylation and acetylation	Sinha et al. (2020)
	Mouse	Ovary	LHR	Demethylation	Zhu et al. (2010)
Maternal hypothyroidism	Rat	Cortical and embryonic neocortical tissues	Gfap	Methylation and hystone acetylation	Kumar et al. (2018)
	Mouse	Hippocampus	Promoter region of Bdnf	Hypermethylation	Kawahori et al. (2018)
Maternal hyperthyroidism	Mouse	Sperm	Genes involved in the early development of the central nervous system	Hypomethylation	Martinez et al. (2018)
IUGR	Human	Placenta	sFLT-1 and VEGF	Hypo/hyper-methylation	Selcen Cebe et al. (2019)
	Rat	Placenta	Wnt2	Hypomethylation	Reamon-Buettner et al. (2014)
		Liver	IGF-I	Hystoneand DNA methylation	Fu et al. (2014)

(continued)

Table 2.2 (continued)

Hormonal or maternal environment imbalance	Specie	Sample used	Gene	Epigenetic modification	References
Gestational diabetes	Human	Blood	Genes involved in metabolic pathways	CpG methylation	Hjort et al. (2018)
	Rat	Placenta and liver	Functional gene pathways involved in endocrine function, metabolism, and insulin responses	Hypermethylation	Petropoulos et al. (2015)
	Mouse	Pancreas	Genes involved in glycolipids metabolism and related signaling pathways	Hypermethylation	Zhu et al. (2019)
Maternal obesity	Human	Blood	Genes involved in metabolic pathways	CpG methylation	Hjort et al. (2018)
	Rat	White adipose tissue	Developmentally genes	CpG methylation	Borengasser et al. (2013)
		Liver	PPARa	Histone methylation	Borengasser et al. (2014)
	Monkey	Liver	Npas2	Acetylation of fetal histone H3	Suter et al. (2011)
	Mouse	Placenta and liver	Epigenetic machinery genes	Altered expression of epigenetic machinery genes	Panchenko et al. (2016)

Evidence based on human studies and animal models of developmental programming effects *ER* estrogen receptor, *APP* Amyloid Beta Precursor Protein, *INS* insulin, *GATA6* GATA-binding protein, *StAR* Stroidogenic acute regulatory protein. *SERPINA5* Serpin Family A Member 5, *HSD3B1* ydroxy-Delta-5-Steroid Dehydrogenase, *HSD17B14* Hydroxysteroid 17-Beta Dehydrogenase 14, *TNK1* Tyrosine Kinase Non Receptor 1, *LIPG* Lipase G, endothelial type, *AR* androgen receptor, *Cyp11a1* cytochrome P450 family 11, subfamily A, polypeptide 1, *TOB* anti-proliferative protein, *TGF-b* transforming growth factor-b, *LHR* luteinizing hormone receptor, *Gfap*: Glial fibrillary acidic protein, *Bdnf* Brain Derived Neurotrophic Factor, *sFLT-1* soluble fms-like tyrosine kinase-1, *VEGF* vascular endothelial growth factor, *Wnt2* wingless-type MMTV integration site family member 2, *IGF-I* Insulin growth factor I, *PPARa* peroxisome proliferator-activated receptors alpha, *Npas2* Neuronal PAS domain protein 2

Furthermore, in IUGR, maternal obesity, and gestational diabetes, epigenetic changes have also been reported. In a widely used rat model of IUGR, it has been shown that changes in the pattern of DNA methylation and histone modifications on the rat hepatic IGF-I gene occur in a sex-specific manner at prepubertal age (Fu et al. 2014). Moreover, in other models of mild IUGR, there were found changes in the placental methylation pattern, particularly hypomethylation of the Wnt2 gene promoter (involved in placental and embryonic development) in IUGR animals as compared to controls (Reamon-Buettner et al., 2014). Human studies in the offspring of cases of gestational diabetes and maternal obesity have also reported changes in the epigenome. For example, alterations in the methylation pattern of genes involved in metabolic pathways and cell differentiation and proliferation, such as Prkcz and Prkar1b, have been reported using peripheral blood samples (Hjort et al., n.d.), while alterations in genes involved in cell adhesion, chemokine signalling, ligand–receptor interactions, and in growth and metabolic control were reported using cord blood and placenta samples (Finer et al. 2015). Together with this evidence, studies in animal models have shown that the gene expression of the epigenetic machinery is sensitive to maternal obesity, at least in the fetal liver and the placenta (Panchenko et al. 2016). In this regard, it was shown that, in IUGR rats, the hyperacetylation of histone H3 in the liver occurs in association with decreased protein expression of histone deacetylase 1 (HDAC1) and HDAC activity (Liguori et al. 2010). Thereby, these studies suggest that the maternal environment influences the epigenetic machinery expression and activity and leads to epigenetic changes in the developing organism.

2.6.2 Milk MicroRNAs in Lactation

It has been widely suggested that breastfed vs. formula-fed infants have different growth patterns (Lind et al. 2018). This evidence has led to exploring how mammalian lactation and milk composition are related to neonatal development. Moreover, it has been studied the association between breastfeeding and later obesity and other metabolic alterations and non-communicable diseases (Lind et al. 2018), thus suggesting that lactation influences development and susceptibility to adult diseases.

It has been shown that during gestation, epigenetic differentiation occurs not only in the fetus but also in maternal tissues. Among these tissues, the MG begins an epigenetic remodeling program that starts in gestation and culminates during breastfeeding. This process is called "mammary epigenetic landscape," and its objective is to unlock the transcription of both milk synthesis machinery genes and milk macro- and micronutrient genes (Rijnkels et al. 2010). It has been shown that the micronutrients present in breast milk are not only minerals and vitamins but are also milk exosomes and microRNAs (Melnik and Schmitz 2017a, b).

Several studies have shown that breast milk exosomes can be synthesized by mammary epithelial cells and mammary stem cells or by immune cells that infiltrate the gland during lactation. Milk exosome concentration and their miRNA composition vary throughout lactation, being modified by the suction intensity and frequency (Melnik and Schmitz 2017a, b, 2019), and even more, it has been shown that pre-term milk has exosomes and miRNA composition different from that of term milk (Kahn et al. 2018; Xingyun Wang et al. 2019a, b). In 2014, it was shown that the miRNA concentration in milk does not present a linear correlation with the miRNAs present in the mammary cells, therefore suggesting that these miRNAs are not a subproduct of the mammary cell (Izumi et al. 2014).

Livestock industry, dairy industry, and laboratory rodents' researches have shown that the exosomes' physical and chemical characteristics allow them to act as short- and long-distance messengers. Exosome-mediated tissue communication is a well-described process in many organs. Exosomes present different properties that allow them to act as messengers between tissues. Some of these, such as their digestive resistance, intestinal absorption, and their ability to circulate through blood flow, also allow milk exosomes to act as messengers beyond maternal tissues, transporting mammary cell genetic messages to infant's cells (Lönnerdal 2019).

Exosomes transport miRNAs that act as signals between tissues. MiRNA-148a-3p and miRNA-21 are abundant in human milk and can regulate the expression of DNA methyltransferases. It has been proposed that miRNAs can regulate gene expression by decreasing the levels of the enzymes involved in methylation and also by indirect effects as they also participate in the recruitment of enzymes that bind to unmethylated CpG islands and promote gene expression. (Melnik and Schmitz 2017a, b). Among milk miRNAs, targets are FTO (FTO: Fat mass- and obesity-associated-protein) and mTOR genes (mTOR1: mechanistic target of rapamycin), which demethylation promotes the expression of genes involved in post-natal growth and development (Melnik 2015a, b). Moreover, miRNA-29 has recently been related to post-prandial insulin secretion increase, confirming that milk miRNAs would play a central role in cellular anabolism (Dahlmans et al. 2017; Melnik and Schmitz 2019). It has been demonstrated that milk miRNAs regulate several genes involved in postnatal development and growth. However, recent data suggest that they also control other genes, such as genes implicated in adipocyte differentiation, muscle and bone differentiation and development, and even genes that regulate the central control of appetite (Melnik and Schmitz 2019). Through in vitro studies, it has also been shown that milk exosomes can inhibit the expression of pro-inflammatory interleukins in human necrotizing enterocolitis, proving that they could mediate the short-term breast milk protective effects. All this evidence shows that milk miRNAs have a central role in the establishment of infant epigenome and also in the prevention of both, childhood and adult diseases (Melnik and Schmitz 2017a, b). It is important to highlight that breast milk quality not only relies on milk macro- and micronutrient composition but also on the exosomes and miRNAs content. The mammary epithelial cell is responsible for the synthesis of milk compounds, and partially for milk exosome synthesis. The exosome synthesis depends on the mTOR signaling pathway (Zou et al. 2019) that acts as an internal node where intra- and extracellular information converges. In this context, it has been shown that THs and oxidative stress are involved in exosome synthesis, acting through the mTOR pathway (Mishra et al. 2019; Yau et al. 2019). This evidence suggests that THs are therefore involved in the infant epigenome establishment.

2.7 Conclusions

The role of hormones and growth factors in organogenesis and maternal and fetal homeostasis is well studied. For several years, attention has focused on their physiological actions without exploring their role as messengers in the establishment of the maternal-fetal interface and the long-term consequences if this system is altered. In this chapter, according to the accumulated evidence, we highlight their role in developmental programming. Considering that hormones and growth factors play a crucial role in this phenomenon, it is essential to update the conceptual framework and deepen the knowledge of the effects of hormonal imbalance on fetal and neonatal development. Hormones exert their action together with adipokines and growth factors.

Hormones, together with adipokines and growth factors, are implicated in the etiopathology of complex diseases. However, the mechanism of action and the interaction between these factors remains unclear. Studies from animal models and human populations have deepened on these points. Although some aspects have been clarified, in other cases, new questions have emerged. The different results among studies could be due to the type of hormone used, the animal species elected, and the time of exposure (related to the developmental stages of the species chosen). Nevertheless, all the studies agree that hormonal alterations during fetal and neonatal life lead to long-term consequences. Despite all the evidence that indicates that hormonal balance and homeostasis should be strictly regulated, clinical interventions during pregnancy and lactation still fail at this point. Moreover, it

remains unclear how and when to measure hormonal levels during pregnancy, and their accuracy and significance as biomarkers related to metabolic and complex diseases in the offspring are still a matter of debate.

To deepen the study of the hormonal system and its action during gestation and neonatal life, it is essential to understand the effects of hormone-like compounds on developing organisms. In this regard, given the increasing exposure of the population to endocrine disruptors, it is crucial to comprehend their role in the programming of different metabolic and reproductive alterations, and the particular mechanism of actions of these disruptors. The understanding of developmental programming molecular mechanisms could help promote public health policies and develop clinical therapeutic and prevention approaches.

It is necessary to highlight that the interaction between the mother and the child is not only given during gestation and fetal development but also during lactation. Epidemiological data have revealed an increased incidence of non-communicable diseases, which are related to maternal and infant health, developing environments, and wellbeing. In Latin American populations and developing countries, these numbers are even higher than in developed countries. Therefore, it is crucial to establish new paradigms and perspectives for the approach, diagnosis, and treatment of these disorders in order to take action in public health affairs. In this context, it is necessary to highlight the role of lactation in maternal and newborn health. The relevance of maternal milk production and breastfeeding needs to be considered when developing public health policies and medical interventions.

The field of epigenetics has gained relevance in the last few years. Identification of epigenetic marks, mainly at earlier stages of life, could allow identifying individuals at risk of developing complex and metabolic diseases and enable preventive therapies. Furthermore, these epigenetic marks may be a target of future pharmacological interventions. Further research is needed to elucidate the possible molecular targets to allow pharmacological actions.

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3 Physiological and Pathophysiological Role of Large-Conductance Calcium-Activated Potassium Channels (BKCa) in HUVECs and Placenta

Fernanda Neira, Nataly Neira, Javier Torres, and Marcelo González-Ortiz

Abstract

BKCa channels (large-conductance Ca^{2+}-activated K^+ channels) play a critical role in regulating vascular tone and blood pressure. These channels are present in the smooth muscle cells of blood vessels and are activated by voltage and increased intracellular Ca^{2+} concentration. More recently, the expression and activity of BKCa have been proposed to be relevant in endothelial cells, too, specifically in human umbilical vein endothelial cells (HUVECs), the more studied cell type in the fetoplacental circulation. The role of BKCa in endothelial cells is not well understood, but in HUVECs or placental endothelium, these channels could be crucial for vascular tone regulation during pregnancy as part of endothelium-derived hyperpolarization (EDH), a key mechanism for an organ that lacks nervous system innervation like the placenta.

In this review, we will discuss the evidence about the role of BKCa (and other Ca^{2+}-activated K^+ channels) in HUVECs and the placenta to propose a physiological mechanism for fetoplacental vascular regulation and a pathophysiological role of BKCa, mainly associated with pregnancy pathologies that present maternal hypertension and/or placental hypoxia, like preeclampsia.

Keywords

Placenta · Potassium channels · BKCa · HUVECs · Preeclampsia · Endothelial cells

3.1 Placental Function

The human placenta is responsible for the fetus's nutrition during development. It is formed by a blood vessel network that maintains an adequate fetal supply of oxygen and nutrients and supports

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the proper biochemical connection with the mother (Wang and Zhao 2010).

Early placental development creates a cellular, molecular, biochemical, and rheological microenvironment susceptible to maternal and fetal factors and immunological, genetic, and epigenetic mechanisms, which can cause pathophysiological changes that lead to adverse gestational outcomes (Apaza Valencia 2014).

The mature placenta results from implantation, proliferation, and differentiation of the trophoblast, which is the leading process for establishing maternal-fetal flow. This organ is vital for the survival, growth, and development of the embryo and fetus (Prieto-Gómez et al. 2018). After implantation, the extraembryonic mesoderm appears between the trophoblast and the blastocyst cavity. This extraembryonic mesoderm, along with the trophoblast, will constitute the chorion. The placental villi, formed from the chorion, will absorb nutrients and oxygen from the maternal blood transported to the embryo. These villi are constituted by syncytiotrophoblast and cytotrophoblast and will receive the name of primary chorionic villi. The cotyledons of the placenta are lobules composed of the union of several chorionic villi and are separated by a septa structure derived from the basal decidua (Lecarpentier et al. 2015; Turco and Moffett 2019).

The chorionic plate of the placenta contains vessels that derivate from the bifurcation of the umbilical cord vessels. Placental circulation development is the result of the contribution of maternal and fetal circulation. Fetal blood is carried into the placenta through the two umbilical arteries, creating networks of capillaries in the chorionic villi. After the exchange occurs, capillaries converge in larger veins until they form the umbilical vein. The umbilical vein is the vessel responsible for delivering all nutrients and other substances from the maternal blood to the fetus. It also obtains oxygenated blood, whereas the blood with carbon dioxide and the waste passes through the umbilical arteries (Lecarpentier et al. 2015; Prieto-Gómez et al. 2018; Turco and Moffett 2019).

Prieto-Gómez and colleagues described:

> *The placenta will present a total of 20 to 35 cotyledons at the end of the pregnancy and each of these will present from 1 to 4 stem villi. Approximately 150 mL of maternal blood per minute is exchanged between the decidua and the villi, so that the uterine spiral arteries carry maternal blood to the villi, diffusing the components transported by the blood through the placental barrier and reaching the chorionic vessels, where the return blood will circulate to the embryo. This blood arrives at the villi with low pressure, but it manages to reach the villi. The route of the blood follows the villi until arriving at the capillary networks of the fetal vessels* (Prieto-Gómez et al. 2018).

The placenta is the organ responsible for ensuring the exchange of metabolic and gaseous substances between mother and fetus, becoming a functional barrier between fetal and maternal circulation. In addition, the syncytiotrophoblast is the structure that acts in the human placenta as a transporting barrier, regulating the transference of nutrients, solutes, and water between maternal and fetal blood (Lecarpentier et al. 2015; Prieto-Gómez et al. 2018; Turco and Moffett 2019; Wang and Zhao 2010).

3.2 Fetoplacental Circulation

Fetoplacental circulation differs from systemic circulation because it lacks nervous system innervation (Marzioni et al. 2004). As a result, the endocrine and paracrine regulation mechanisms generate a low-resistance vascular bed to maintain a high flow of oxygenated blood to the fetus (Mills et al. 2009). The blood flow between mother and fetus is maintained approximately from the end of the first trimester until 40 weeks of gestation or term. The volume of placental blood flow is about 600–700 ml/min (80% of the uterine perfusion) at term (Wang and Zhao 2010).

Anatomically, the fetoplacental circulation comprises two umbilical arteries that branch across the placental disc. These chorionic plate arteries, which range from ~2 mm to 100μm in diameter, eventually penetrate the chorionic plate, where each vessel, now termed a stem vil-

lus artery, supplies an individual placental cotyledon. Continual branching through intermediate villi eventually leads to terminal villi, containing a convoluted mass of capillary loops, closely associated with the syncytiotrophoblast, bathed by maternal blood in the intervillous space. Finally, blood returns to the fetus via stem villus veins and chorionic plate veins, forming a single vein within the umbilical vein (Castellucci and Kaufmann 2000; Wang and Zhao 2010).

Two critical factors for vasculature development in the placenta are vascular endothelial growth factor (VEGF) and placental growth factor (PlGF). VEGF levels are high at the beginning of pregnancy and promote the de novo vessel formation for developing fetal-placental vasculature. Conversely, low levels of VEGF correlate with preeclampsia, spontaneous preterm delivery, and recurrent pregnancy loss. Later in pregnancy, PlGF promotes angiogenesis and vasculogenesis during embryonic development and regulates VEGF-mediated angiogenesis. A third essential factor is the soluble form of VEGF receptor 1, sFlt-1, an anti-angiogenic factor that can competitively bind to PlGF and VEGF and prevent the physiological actions of these pro-angiogenic factors. In a healthy pregnancy, the level of sFlt-1 increases at the end of the third trimester. However, if sFlt-1 levels are pathologically increased, it will inhibit blood vessel development early in pregnancy, leading to preeclampsia (Chen and Zheng 2014).

In the placenta, the regulation of vascular tone is maintained by locally synthesized endothelial-derived relaxing [EDRFs; e.g., nitric oxide (NO)], contracting (EDCFs), and hyperpolarizing (EDHFs) factors. NO is involved in several functions in the placental vascular system, including regulating fetal nutrient and oxygen supply, mother artery pressure, and mother-fetus signal-mediated communication (González and Rivas 2020; Rodríguez and González 2014). NO is a free radical synthesized by endothelial NOS (eNOS) from L-arginine in human placental vasculature (González et al. 2004, 2011; Sobrevia et al. 1996). On the other hand, the expression and activity of calcium-activated potassium (K^+) channels (KCa) have a role in human placental vascular reactivity regarding local regulation of stimuli, such as oxygen or circulating hormones (Wareing 2014). KCa activity associates with myo-endothelial hyperpolarization and lower resistance in the placental vasculature (González and Rivas 2020). Activation of KCa channels leads to an efflux of K^+, which in turn causes hyperpolarization of the smooth muscle cells, reducing their contractility and resulting in vasodilation.

The propagation of hyperpolarization from endothelial cells to vascular smooth muscle cells and the bioavailability of NO would be the leading mechanisms in regulating blood flow in macrocirculation and microcirculation of the placenta. Regulation of KCa channels has been linked with vascular smooth muscle cell (VSMC) hyperpolarization, leading to the relaxation of blood vessels. Because potassium ion is part of the chemical signals that arise within endothelial cells, the single EDHF concept evolved into the more inclusive, endothelium-dependent hyperpolarization (EDH) signaling (Garland and Dora 2021). The KCa channels are related to regulating plasma membrane potential and have been shown to be the key proteins for EDH, a central mechanism for local regulation of vascular relaxation related to intracellular calcium mobilization in endothelial cells. New evidence shows that endothelial and smooth muscle potassium channels are critical regulators of placental vascular physiology, which will be described in the following paragraphs.

3.3 Regulation of Placental Circulation by Potassium Channels

A successful pregnancy depends on the adequate contribution of blood flow from the mother to the fetus. In the placenta, the regulation of vascular tone depends on the correct balance between vasoconstrictor and vasodilator substances since, as we mentioned previously, the placenta does not present nervous innervation. K^+ is the principal intracellular cation, and its equilibrium and diffusion across the plasma membrane are crucial

for several cell functions. Therefore, the K^+ regulation mechanisms are finely controlled. In addition, K^+ channels are vascular regulators of the proliferation, angiogenesis, and secretion of vasoactive factors, and their dysfunction could be involved in vascular diseases related to pregnancy, like preeclampsia (Boeldt and Bird 2017).

K^+ channels have been described in all cell types. Their functions include regulating the cell volume, the membrane potential, and the hormonal secretion of excitable and non-excitable cells, which explains their fundamental role in the placental vessel tissues (Martín et al. 2014). The placental chorionic plate arteries lack autonomic neuronal innervation (Mills et al. 2009); therefore, the control of vascular tone must rely predominantly on local and humoral factors, suggesting that local physical (e.g., rheological properties), paracrine (e.g., endothelial-derived factors), and circulating (e.g., insulin, angiotensin II) factors will contribute to blood flow regulation in small fetoplacental vessels. The cells of blood vessel walls detect and respond to these local environmental changes, such as oxygenation and hydrostatic pressure (Wareing 2014). Some authors indicate that *"chorionic plate arteries have the size characteristics of resistance vessels and may contribute significantly to determining overall placental vascular resistance and blood flow"* (He et al. 2018). The human fetoplacental circulation is a low-resistance, high-flow vascular system with deoxygenated arterial relative to venous blood. K^+ channels have an essential role in maintaining the plasma membrane potential and smooth muscle tone, and a variety of agonists can modify the vascular tone by regulating K^+ channel activity. Several K^+ channel subtypes have been identified in vascular smooth muscle cells, and altered K^+ channel activity has been associated with pregnancy disease (Brereton et al. 2013).

K^+ channels are the largest and most diverse ion channel family proteins, known to play essential roles in the physiological activity of endothelial and smooth muscle cells in different vascular beds. K^+ channels are tetramers comprising four pore-forming alpha subunits, each coupled to a regulatory beta subunit. K^+ channels are categorized by their predicted transmembrane structure or gating properties. Structurally, K^+ channel subfamilies are based on the alpha subunit transmembrane domain predicted from their amino acid sequences. Functionally, four main types of K^+ channels have been classified and described in vascular smooth muscle cells by their gating properties and include voltage-gated (K_V) channels, Ca^{2+}-activated K^+ (KCa) channels, two-pore domain (K_2P) channels, and inward rectifying potassium (IRK) channels. Due to diversity and the ability of K^+ channels to influence cell membrane potential, these channels play a central role in many cells for cell survival and regulation of specialized cell functions. In addition, the K^+ channel-dependent hyperpolarization regulates plasma membrane potential in endothelial and vascular smooth muscle cells. Therefore, K^+ channel expression is relevant for endothelial and smooth muscle cell interaction for physiological vascular function (Wareing 2014; Wareing and Greenwood 2011).

Calcium-activated potassium (KCa) channels play an essential role in regulating the membrane potential of arterial smooth muscle cells and are also involved in regulating vascular tone. Changes in plasma membrane voltage activate the KCa, and their particularity is that they open with the increase of intracellular Ca^{2+} concentrations. KCa channels are subclassified by large conductance (BKCa), intermediate conductance (IKCa), and small conductance (SKCa) capacity. The human placenta expresses BKCa, mainly in vascular smooth muscle cells, and SKCa and IKCa are predominantly expressed in the endothelium (Hu and Zhang 2012, 2023).

K^+ channels are critical regulators of vascular smooth muscle dilation, proliferation, and angiogenesis and may play a role in pregnancy-induced vascularization (Rada et al. 2014). In addition, BKCa channel expression in fetoplacental vascular tissues has been demonstrated by RT-PCR, western blot, and immunohistochemistry (Wareing and Greenwood 2011).

3.4 The Large Conductance Potassium Channels (BKCa)

The BKCa channel (also known as MaxiK, Slo1, or BK) is formed by numerous isoforms of pore-forming α subunit and accessory subunits β and γ, which can affect the kinetics and pharmacological properties since it regulates the calcium sensitivity of the channel. They are found in groups of 20–100 units and are activated by Ca^{2+} currents released from the sarcoplasmic reticulum after the opening of ryanodine receptors (RyRs) (Félétou 2009). The Ca^{2+} release is known as "Ca^{2+} sparks" and induces the activation of BKCa channels, allowing the output of K^+ and generating what is known as "spontaneous transient outward currents" (STOCs) (Benham and Bolton 1986).

Three different subunits form the BKCa channels: alpha (α), beta (β), and gamma (γ) subunits. The pore-forming α subunit is composed of seven transmembrane domains and an extracellular N-terminal domain, and some domains (S2-S3-S4) have the role of adjusting the voltage sensitivity, and a C-terminal has two RCK domains (K^+ conductance regulation), which provide a site for Ca^{2+} concentration sensitivity. β subunits (β1–β4) are formed by two transmembrane domains and regulate the channel's voltage dependence, kinetics, and pharmacological properties. Also, they have a role in the regulation of calcium sensitivity. Lastly, **γ** subunits (γ1–γ3) provide regulation of the channel by responding to changes in the redox state of the cell (Kaczmarek et al. 2017), and there is evidence that shows a role in voltage gating activity (Chen et al. 2022). However, the specific role and properties of γ subunits are still underinvestigated.

Different vasoactive factors will carry out their vasodilator functions by activating these channels. For example, nitric oxide (NO) can activate the BKCa channel through protein kinase G (PKG)-dependent phosphorylation at three specific sites, an effect that results in the enhancement of channel activity (Francis et al. 2010; Gamper and Ooi 2015). The cytochrome P450 (CYP) arachidonic acid metabolites, 20-hydroxyeicosatetranoic acid (20-HETE), and epoxyeicosatrienoic acids (EETs) are EDRFs that induce vascular smooth muscle hyperpolarization through the activation of BKCa (Imig 2016). Carbon monoxide (CO), synthesized by heme oxygenase, activates BKCa through NO via PKG, PKA, and S-nitrosylation pathways in human cardiac fibroblast (Bae et al. 2021). On the other hand, hydrogen peroxide (H_2O_2) is related to endothelium-dependent relaxations in response to agonists or flow, and its activation compensates for the decrease in NO production. It can activate BKCa directly or through stimulation by soluble guanylyl cyclase (Félétou 2009).

Also, EDCFs interact with the BKCa channel. For example, endothelin 1 (ET-1) increases the open probability of BKCa in human umbilical vein endothelial cells (HUVECs) (Kuhlmann et al. 2005) but also inhibits the BKCa activity in renal smooth muscle cells (Betts and Kozlowski 2000). On the other hand, 20-HETE can induce vasoconstriction in some vascular beds by inhibiting BKCa in vascular smooth muscle cells, contributing to the onset of hypertension and endothelial dysfunction (Félétou 2009).

This evidence demonstrates that the endothelium-derived factors regulate the BKCa activity to induce relaxation or contraction, depending on the molecular scenario, vascular bed, and physiological demands. In the following paragraphs, we will discuss the relevance of BKCa for fetoplacental vascular physiology and pathophysiology.

3.5 The Expression and Activity of BKCa in Human Umbilical Vein Endothelial Cells (HUVECs)

Much published evidence attributes the BKCa to a primary role in vascular smooth muscle cell (VSMC) physiology. Despite this, emerging evidence shows that BKCa channels express in endothelial cells, specifically in HUVECs. The first pieces of evidence were published in the 1990s. Nilius and Riemann (1990) used the patch clamp technique in the cell-attached mode to determine the presence of high-conductance

potassium currents in HUVECs, which was confirmed by Kestler and colleagues (1998), who demonstrated the dependence of these currents on the plasma membrane potential with a conductance of 172.9 picosiemens (pS) and 262.1 pS (at 60 mV) in cell-attached and in outside-out patches, respectively. The identity of this current was confirmed by Dong and colleagues (2007) because neither apamin (a specific blocker of the SKCa channel) nor TRAM34 (a selective blocker of the IKCa channel) affected the channel activity. In contrast, the KCa channel activity was inhibited by tetraethylammonium (TEA), a non-specific BKCa channel inhibitor. TEA decreased NPo (a product of channel number [N] and open probability [Po]) and apparent channel current amplitude by 80%. Iberiotoxin, a specific BKCa channel inhibitor, decreased NPo without changing the current amplitude. Furthermore, the inhibitory effect of TEA and iberiotoxin was fully reversible because washout restored the channel activity (Dong et al. 2007). Thus, the data confirmed that the BKCa channel is functional in endothelial cells.

Wiecha and colleagues (1998) showed that insulin (100 microU/ml) caused a significant increase in BKCa open-state probability in HUVEC, which indicates that extracellular stimuli could modulate the channel activity. Like insulin, another growth factor, the vascular endothelial growth factor-A (VEGF-A), strongly stimulated BKCa activity after 8 min of incubation (Faehling et al. 2001). The flavonoid quercetin and sildenafil (phosphodiesterase type 5 inhibitor) caused hyperpolarization of HUVECs associated with increased intracellular Ca^{2+} and cGMP. The effects of quercetin and sildenafil were blocked by iberiotoxin (IBTX), the specific inhibitor of BKCa (Kuhlmann et al. 2005a, b; Luedders et al. 2006). In the case of Kaempferol, a flavonoid with vasodilatory properties, the mechanism of BKCa-dependent hyperpolarization in HUVECs involves the cAMP/PKA pathway (Xu et al. 2008). Another endothelial vasodilator, CO, gradually increased BKCa channel activity in HUVECs. Applying CO and CORM3 (CO donor) increased the BKCa channel activity to 322% and 392% of the control value, respectively (Dong et al. 2007). CO could activate the BKCa channels through NO release and cGMP formation in endothelial cells or by directly modifying the BKCa channel protein. In addition, CO may activate the BKCa channels by binding to channel-associated heme (Jaggar et al. 2005) (see Fig. 3.1).

In the opposite direction, angiotensin II (Ang II) inhibits the BKCa current associated with increased superoxide and hydrogen peroxide levels in the control HUVECs, an effect blocked by the antagonist of Ang II type 1 receptor (Park et al. 2008). In pathological models, as HUVECs incubated with interleukin-1b (IL-1b) or lipopolysaccharide (LPS), the studies show activation of BKCa (Burgazli et al. 2014; Li et al. 2012). In the case of IL-1b, the BKCa activation induces the synthesis of NO and ROS and monocyte adhesion to HUVECs (Burgazli et al. 2014).

The expression of BKCa (α subunit) in this cell type has been demonstrated by immunofluorescence, being stimulated this expression by insulin after 30 min or 8 h of incubation. Insulin stimulation induces plasma membrane hyperpolarization and NO synthesis. IBTX and Tram34 (IKCa inhibitor) blocked the insulin-induced NO synthesis, so the endothelium-dependent vasodilatory mechanism depends on BKCa and IKCa activity (Rojas et al. 2020). Indeed, the insulin-induced vasodilatation of placental vessels was totally blocked by the co-incubation with IBTX (Cabrera et al. 2016; Rojas et al. 2020). Interestingly, the incubation of control placental vessels with IBTX induces a quick contraction and, later, a significant relaxation (Rojas et al. 2020), which could be attributed to a compensatory mechanism against the reduction of EDH involving a higher release of NO (IBTX increased the endothelium-dependent NO). In placental vessels, the incubation with IBTX reduces the H_2O_2-induced contraction but not the contraction induced by U466619 (thromboxane A2 analog) (Cabrera et al. 2016). Despite H_2O_2 increasing the activity of BKCa in HUVECs (Bychkov et al. 1999; Park et al. 2008), the final effect of H_2O_2 in placental vessels is a contraction countered by

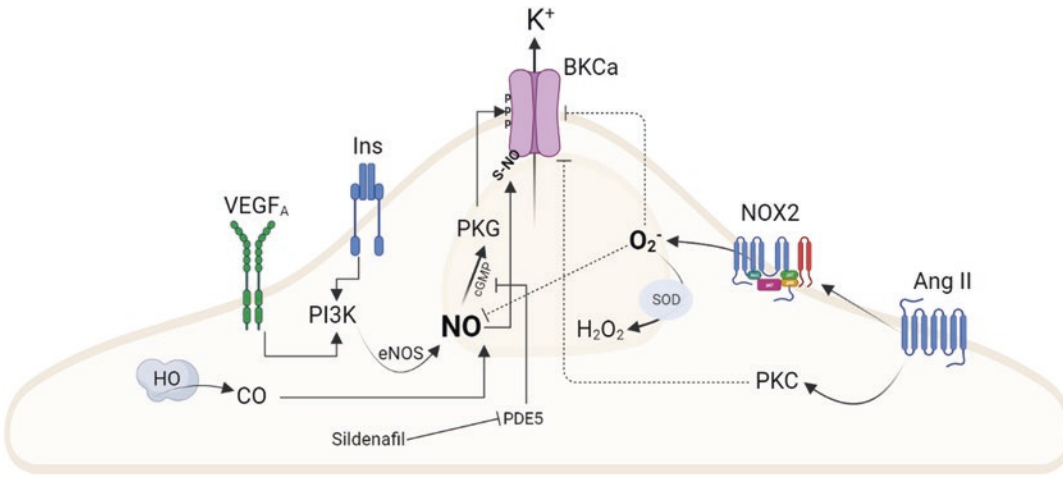

Fig. 3.1 Regulation of BKCa in human umbilical vein endothelial cells (HUVECs). The expression (α and β1 subunits) and activity of BKCa have been demonstrated in HUVEC. Agonists like insulin and vascular endothelial growth factor A ($VEGF_A$) activate its receptors and then PI3K (phosphoinositide 3-kinases) to increase the activity of eNOS (endothelial NO synthase). The increase of NO (nitric oxide) activates the soluble guanylyl cyclase (sGC) to increase the levels of cGMP and then PKG (protein kinase G). The PKG can phosphorylate BKCa at three sites for activation. Also, NO could directly activate BKCa through S-nitrosylation (S-NO). The heme oxygenase (HO) 1 enzyme can be induced in HUVECs and enhance the levels of CO (carbon monoxide). This vasodilator molecule can increase the levels and activity of NO and cGMP/PKG signaling to activate BKCa. Additionally, sildenafil acts on the inhibition of PDE5 (phosphodiesterase-5) and increases the levels of cGMP. On the other hand, a primary inhibitory mechanism for BKCa in HUVECs is the activation of NOX2 (NADPH oxidase 2) by angiotensin 2 (Ang II), which increases the intracellular levels of O_2^- (superoxide). The O_2^- could inhibit BKCa directly through oxidation or indirectly to reduce NO bioavailability. Also, the activation of the AT1 receptor of angiotensin II increases the activity of PKC (protein kinase C), which inhibits the BKCa activity. (Created with BioRender.com)

IBTX, which is associated with the depolarization induced by lower concentrations (<1 mM) of H_2O_2 in HUVECs (Bychkov et al. 1999). It is important to note that the presence of multiple cysteines and methionines within Slo1 (α subunits) proteins results in a complex response to oxidizers and cysteine-modifying reagents. The main evidence shows that heterologously expressed Slo1 channels are inhibited by H_2O_2 (Gamper and Ooi 2015) (see Fig. 3.1). Further research is necessary to solve this discrepancy and consider the effects of H_2O_2 on β1 and γ subunits.

On the other hand, to analyze the discrepancies between data from HUVECs and from the placental vessels, it is essential to consider that in the vascular reactivity data of Cabrera and colleagues (2016), there is a combination of the activity or inhibition of channels in both endothelium and smooth muscle is obtained.

3.6 Potential Roles of BKCa in Placental Pathologies

Preeclampsia is characterized by the new onset of hypertension and proteinuria during the last trimester of pregnancy, usually associated with edema and hyperuricemia. In severe preeclampsia, there is the presence of systemic endothelial dysfunction and microangiopathy, in which the target organ may be the brain (seizures or eclampsia), the liver [the hemolysis, elevated liver function tests, and low platelet count (HELLP) syndrome], or the kidney (glomerular endotheliosis and proteinuria) (Karumanchi et al. 2005). Although efforts have been made to understand preeclampsia in the last decades, still, pathophysiological mechanisms are not well known, especially the endothelial dysfunction mechanism.

In chorionic plate arteries (CPAs) from women with preeclampsia, a decrease in the expression

of β1 subunit of BKCa (mRNA and protein) associated with pathologic vascular remodeling enhanced the vasoconstrictor response and decreased sensitivity to vasoactive substances in CPAs. The BKCa-dependent relaxation is significantly reduced in preeclamptic CPAs, as is the effect of sodium nitroprusside (SNP, NO donor). The maximal SNP-induced relaxation percentages were smaller in the preeclamptic subjects than in the controls, indicating that the sensitivity of CPAs to SNP-induced relaxation was reduced in the preeclamptic group. Treating CPAs with TEA or IBTX significantly decreased the relaxation in the control group, but no differences were observed in the preeclamptic CPAs. The vascular alterations in CPAs in response to the BKCa channel opener NS1619 and BKCa blockers demonstrated that the expression or activity of BKCa channels was remarkably impaired in preeclampsia (He et al. 2018). Similarly, a study with HUVECs from preeclamptic patients determined a decreased expression of β1 subunit of BKCa (mRNA and protein), which was associated with reduced IBTX-sensitive outward K^+ currents. The alterations in the BKCa currents were partially reversed by incubation with apocynin (NOX2 inhibitor), so NOX2-mediated O_2^- production is involved in regulating KCa channels in the preeclamptic endothelium (Chen et al. 2017). In summary, the decreased β1 subunit in placental or umbilical vessels may impede BKCa function and contribute to vascular resistance in preeclampsia (see Fig. 3.2).

Additionally, the expression of IKCa and SKCa is reduced in CPAs from preeclampsia. The intensity of KCa-mediated vasodilatation responses to NS309 (opener of IKCa and SKCa) in the preeclampsia group was only about half of that in normal pregnancy (Li et al. 2017). Interestingly, Li and colleagues observed that the pre-constricted endothelium-denuded CPAs' relaxation in response to NS309 was reduced by 86% compared with the control, comparable to the response of preeclamptic vessels. So, IKCa and SKCa channels in the endothelium may contribute more significantly to maintaining vascular function than those in smooth muscle. In normal CPAs, endogenous NO's blockage suppresses vasodilatory responses to NS309, implicating a direct involvement of NO in IKCa- and SKCa-mediated vascular function on CPAs. In a NO deficiency status, endothelial IKCa- and SKCa-mediated vasodilatation can serve as a compensatory mechanism, which was aberrant in preeclampsia (Li et al. 2017) (see Fig. 3.2).

A study with plasma samples of preeclamptic patients determined that treating HUVECs with this conditioned media decreased the K^+ inward currents, which were identified as inward rectifier K^+ channel (IRK) currents. On the other hand, preeclampsia-treated cells could have a higher proportion of outward current, mainly associated with KCa channel activity. These changes could increase the driving force for Ca^{2+} during Ca^{2+} influx or be a compensatory manifestation of the lower proportion of IRK currents (Watanapa et al. 2012). In normal HUVECs, the incubation with H_2O_2 induces depolarization (Bychkov et al. 1999) and contraction in chorionic plate veins (Cabrera et al. 2016). Also, oxidized LDL (oxLDL) increases the KCa activity in normal HUVECs (Kuhlmann et al. 2003), and both factors (H_2O_2 and oxLDL) are part of the pathophysiology of preeclampsia (Aouache et al. 2018). However, the effects of these molecules on normal HUVECs or normal placental vessels could not be directly associated with the pathophysiology of preeclampsia because the early alterations of placentation could modify the response of endothelial or smooth muscle cells to specific molecules or stimuli. The modulation of BKCa channels by oxidizers is incredibly complex, and the outcome of such modulation is likely to depend on the nature of the redox changes and the local environment (Gamper and Ooi 2015). Significantly, as we mentioned before, the studies of He and Li (He et al. 2018; Li et al. 2017) reporting lower expression of KCa in placental vessels from preeclampsia correlate with the recent study of Karadas and colleagues (2023), who showed that human umbilical arteries from preeclamptic patients have a significantly reduced relaxation in response to the specific opener of BKCa, NS11021. Together, these results show that the BKCa channels may contribute at least partially to increased vascular resistance due to

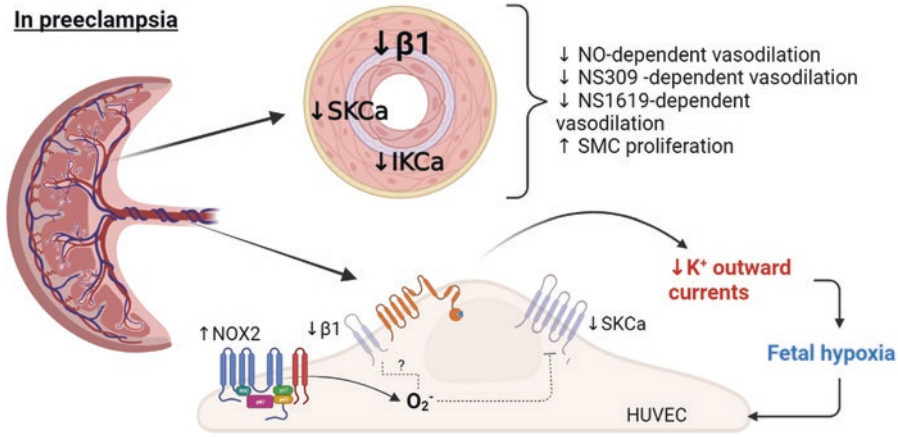

Fig. 3.2 Impairment of KCa in preeclampsia. In the chorionic plate, arteries from preeclamptic patients has been shown that there is a significant reduction of $\beta 1$ subunit of BKCa, IKCa, and SKCa. These alterations are directly associated with lower vasodilatation in response to NO, NS309 (IKCa and SKCa opener) or NS1619 (BKCa opener), and higher proliferation of vascular smooth muscle cells (VSMCs) in the human placenta. The studies in preeclamptic HUVECs show that there is a lower expression of $\beta 1$ subunit of BKCa and SKCa. Part of these reduced expressions could be due to the higher activity of NOX2 and increment of oxidative stress (O_2^-, mainly). The total effect of the KCa impairment is the reduction of outward K^+ currents, which is associated with higher vascular resistance and fetal hypoxia. This mechanism could be a positive feedback, in which the fetoplacental hypoxia increases the oxidative stress in endothelial cells and worsens the KCa function and plasma membrane regulation. (Created with BioRender.com)

(or contributing to) fetal hypoxia. Supporting this hypothesis, chronic hypoxia in pregnant animals significantly attenuates the NS1619 (BKCa opener) relaxation of uterine arteries, and the treatment of vessels with the free radicals scavenger, N-acetylcysteine, enhances the activity of BKCa (Zhu et al. 2014) (see mechanism proposed in Fig. 3.2). Additionally, in human myometrial arteries from pregnant women who live at high altitudes (>2500 m or 8200 ft), there is a reduced expression of α subunit of BKCa in smooth muscle cells and a lack of TEA-sensitive vasodilation in response to NS11021 (Fallahi et al. 2022).

Finally, epigenetic mechanisms have been proposed to be relevant for regulating BKCa in preeclampsia or gestational hypoxia. Thereby, the hypermethylation of BKCa β1 subunit-encoding gene KCNMB1 promoter is associated with increased uterine vascular tone in hypoxic animals (Hu et al. 2018). The hypermethylation of KCNMB1 is attributed to the downregulation of ten-eleven translocation methylcytosine dioxygenase 1 (TET1) (Hu et al. 2017), an enzyme that catalyzes the conversion of the modified DNA base 5-methylcytosine (5-mC) to 5-hydroxymethylcytosine (5-hmC). This reaction has been proposed as the initial step of active DNA demethylation (Ito et al. 2011). Recently, it has been demonstrated that inhibiting microRNA 210 (miR-210) could eliminate hypoxia-mediated downregulation of TET1 expression in uterine arteries and restore β1 subunit expression (Hu et al. 2018). Notably, the studies of non-coding RNAs in preeclampsia suggest that hypoxia-inducible miR-210 is highly expressed in the human placenta during early pregnancy (Ogoyama et al. 2022). Thus, the suppression of miR-210 could be a potential mechanism to restore the β1 subunit expression in the preeclamptic placenta, which needs to be elucidated in further studies.

3.7 Concluding Remarks

The endothelium-derived hyperpolarization (EDH) is a central mechanism for regulating the vascular tone in the human placenta, and KCa channels, particularly the BKCa channels, are

key proteins involved in this mechanism. The expression and activity of BKCa subunits α and $\beta 1$ have been described in HUVECs, the main endothelial cell type studied in the fetoplacental circulation. The BKCa currents can be regulated in HUVECs by several mechanisms through plasma membrane receptors like insulin, VEGF-A, angiotensin II, or LPS; or intracellular signaling like NO, CO, quercetin, or sildenafil (Fig. 3.1). The aberrant expression or regulation of BKCa in HUVECs and the placenta could be part of the etiology of pregnancy diseases like preeclampsia or fetal growth restriction, which present with endothelial dysfunction from the early stages of pregnancy (Fig. 3.2). Further research is necessary to understand the role of all types and subunits of KCa channels in the different cell types that constitutes the fetoplacental unit, both in physiological and pathophysiological conditions.

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4 Metabolic Interaction Between Folate, Vitamin B12, and Polyunsaturated Fatty Acids in Pregnancy

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Abstract

Fetal growth and development are influenced by maternal nutrition and gestational weight gain. Adequate intake of nutrients such as folate, vitamin B12, and docosahexaenoic acid (DHA) is essential for healthy fetal and placental development. Many countries have a national flour fortification program with folic acid (FA), together with pre-pregnancy supplementation of FA (400 μg/day) during the first trimester of pregnancy. The latter has been recommended by the WHO and adapted to local requirements by perinatal guidelines. On the other hand, in population studies, many women of childbearing age have vitamin B12 deficiency (<148 pmol/L), which can be additionally masked by high FA intake and maternal pregestational obesity. Under these conditions, these patients could be having pregnancies in a folate/vitamin B12 imbalance, which is associated with higher adiposity, insulin resistance, altered lipid metabolism, and low DHA levels in their offspring. However, if these neonatal consequences of maternal pregestational obesity and folate/vitamin B12 imbalance can be reverted by DHA supplementation during pregnancy has not been addressed. This chapter reviews the literature and exposes the current gaps in knowledge and challenges in maternal nutrition with a life-course perspective.

4.1 Introduction

Growth and fetal development are complex processes influenced by genetics and environmental factors, including maternal nutrition, weight gain during pregnancy, maternal and fetal hormones,

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placental function, and maternal stress, among others. Both fetal growth restriction and overgrowth are associated with an increased risk of developing phenotypes that compromise the offspring's health in the neonatal period and later in life (Sibley et al. 2010). The Developmental Origins of Health and Disease (DOHaD) concept, named Barker's hypothesis, first reported the association of low birth weight and the risk of Type 2 Diabetes Mellitus (T2DM) and cardiovascular disease. DOHaD proposes that environmental exposures of the mother prior to or during gestation induce changes in the metabolism, functionality, and growth of systems and organs of the developing fetus by regulating the expression patterns of specific genes whose effects will be revealed in the early stages of the extrauterine life (Gluckman et al. 2007; Godfrey et al. 2007). In adverse intrauterine conditions, the fetus and the placenta adapt to limit their growth and prioritize the development of essential tissues like the brain, heart, and pancreas (Sandovici et al. 2012; Zhang et al. 2015).

According to studies of survivors of the Dutch famine, where a population of the north Netherlands was exposed to profound food deprivation due to the Second World War, maternal pregestational nutritional status and weight gain during pregnancy are essential to ensure adequate fetal growth and development (Barker 2007; De Boo and Harding 2006). The food shortage lasted nearly 6 months, affected the whole population of that country, and was called "The Dutch Hunger Winter Study" (1944–1945) (De Boo and Harding 2006; Jiménez-Chillarón et al. 2012; Schulz 2010; Yajnik 2014). The children conceived and gestated during the hunger months have been followed up in several cohort studies. As a result, many chronic health problems were significantly increased in this population, including type 2 diabetes, obesity, lung disease, and altered coagulation. In addition, several epigenetic mechanisms (DNA methylation) changes were observed in those exposed in fetal life to these severe nutrient restriction conditions. These studies have concluded that there are critical windows of sensitivity during human development to establish metabolic syndrome and systems physiology later in life (Rinaudo and Wang 2012).

In an epidemiological study in rural Gambia, Waterland et al. evaluated that mothers' periconceptional nutritional status influences their offspring's epigenome (Waterland et al. 2010). Maternal food intake during the rainy season in rural Gambia is characterized by reduced nutrient availability, whereas during the dry season, it is characterized by high nutrient availability. For the first time in humans, this study evidenced that epigenetic regulation occurs at specific genomic loci, resulting in epigenetic modifications that affect gene expression in tissues and persist into adulthood (Waterland et al. 2010). The increased risk of disease later in time induced by adverse environments during periconceptional and intrauterine development may be explained by epigenetic mechanisms affecting the expression of specific genes through DNA methylation, histone modifications, and non-coding RNAs (Bianco-Miotto et al. 2017).

Several studies have evaluated the effects of maternal nutrition, including macronutrients and micronutrients, on the offspring, emphasizing their role in DOHaD (Jiménez-Chillarón et al. 2012). Adequate nutrient intake (in quantity and diversity) during pregnancy is essential; among the critical micronutrients, B complex vitamins are essential. The bioavailability of micronutrients like methionine, choline, betaine, and vitamins B (folate, B2, B6, and B12) may influence DNA methylation by modifying the activity of the one-carbon cycle and the production of S-adenosyl methionine (SAM) (Jiménez-Chillarón et al. 2012). The critical role of vitamin B has been extensively described in the prospective Pune Maternal Nutrition Study. The small and thin Indian babies presented a higher fat mass at birth than the larger English babies. The latter showed lower adiposity at birth (Yajnik 2014). Furthermore, Indian babies had an increased risk of developing diabetes later in life due to higher insulin and leptin and lower adiponectin levels at birth. These effects were associated with high maternal folate and low vitamin B12 (Yajnik 2014).

4.2 Maternal and Fetal Folates and Vitamin B12 Requirements During Gestation

Folate requirements during pregnancy are 600 μg DFE/day (Institute of Medicine (US) Standing Committee on the Scientific Evaluation of Dietary Reference Intakes and its Panel on Folate, Other B Vitamins, and Choline 1998). This vitamin has been strongly associated with preventing neural tube defects (NTD) (Czeizel and Dudás 1992). Therefore, the World Health Organization (WHO) and the Centers for Disease Control and Prevention (CDC) recommended food fortification with 1.4 mg of folic acid per kg of product (Bailey et al. 2015). Furthermore, women of childbearing age should consume 400 μg/day of folic acid (supplements or fortified foods) in addition to natural dietary folate to prevent NTD and 5 mg/day if they have a previous history of NTD (Institute of Medicine (US) Standing Committee on the Scientific Evaluation of Dietary Reference Intakes and its Panel on Folate, Other B Vitamins, and Choline 1998; Organización Mundial de la Salud 2014). Based on this, in 2000, Chile started the "Program of Flour Fortification" with 1.8 mg/kg of FA (accepted range: 1.0–2.6 mg/kg) according to the last report (Subsecretaría de Salud Pública. Ministerio de Salud. Instituto de Salud Pública de Chile 2011). Additionally to the food fortification, the perinatal guide of the Chilean Ministry of Health (Ministerio de Salud – Gobierno de Chile, Subsecretaría de Salud Pública, División Prevención y Control de Enfermedades, Departamento de Ciclo Vital, Programa Nacional Salud de la Mujer 2015) recommends prenatal supplementation with 1000 μg/day of folic acid, 3 months prior to pregnancy and until the first trimester of pregnancy, to decrease NTD rates (Ministerio de Salud – Gobierno de Chile, Subsecretaría de Salud Pública, División Prevención y Control de Enfermedades, Departamento de Ciclo Vital, Programa Nacional Salud de la Mujer 2015).

Despite the WHO recommendation and according to the last report about the food fortification program, Chile has the highest folic acid fortification in wheat flour (3.4 mg/kg) (Subsecretaría de Salud Pública. Ministerio de Salud. Instituto de Salud Pública de Chile 2011). In other Latin American countries, the folic acid levels in fortified food are below 1.8 mg/kg of product (David 2004). On the other hand, the Chilean National Survey of Food Consumption (Ministerio de Salud, Gobierno de Chile 2009) showed that women (14–64 years) consume 427 μg/day (CI 95% 416–438) of dietary folate equivalent (DFE). The DFE is the folate plus folic acid, reaching the intake recommendation for adult people of 400 μg DFE/day from all foods. In Chile, bread intake is between 73 and 184 g bread per day, contributing 128–323 μg of folic acid per day, without considering the intake of other foods fortified or supplementation with folic acid (1000 μg/day). This analysis is relevant since the Food and Agricultural Organization of the United States (FAO) and the Institute of Medicine (IOM) established a maximum tolerable level (upper level, UL) for folic acid of 1000 μg/day (considering only fortified foods and supplements). Intakes over this concentration (1000 μg/day) can mask the deficiency of vitamin B12, correcting the hematological symptoms without improving the neurological symptoms distinctive of vitamin B12 deficiency (Institute of Medicine (US) Standing Committee on the Scientific Evaluation of Dietary Reference Intakes and its Panel on Folate, Other B Vitamins, and Choline 1998). In 2003, 3 years post-fortification with FA in a sample of 751 women of childbearing age, Hertrampf et al. found a 300% increase in plasma folate concentrations (from 9.7 ± 4.3 to 37.2 ± 9.5) in red blood cells (from 290 ± 102 nmol/L to 707 ± 179 nmol/L) and the absence of folate deficiency (Hertrampf et al. 2003).

The requirements of vitamin B12 increase from 2.4 to 2.6 μg/day during pregnancy. According to the National Survey of Food Consumption (ENCA 2010), vitamin B12 intake by Chilean women (14–64 years) was 1.6 μg/day (CI 95% 0.7–3.0) (Ministerio de Salud, Gobierno de Chile 2009). The study previously described showed that after folic acid fortification, 10% of

these women presented a vitamin B12 deficiency (<148 pmol), and 13% had depleted (148–221 pmol) vitamin B12 (Hertrampf et al. 2003). Unfortunately, there are no data on folate or vitamin B12 intake or blood levels in Chilean pregnant women. Recently, the National Health Survey (ENS 2016–2017) reported the folate status in a small sample of women of childbearing age in the metropolitan area of Chile (n = 222), showing that only 0.9% of women presented folate deficiency and 7% of them had supra-physiological levels (>45 nmol/L) (Busso et al. 2021). Therefore, it is likely that pregnant women are consuming much higher folic acid quantities than the recommended (600 μg DFE/day) and even more than the tolerable UL (1000 μg FA/day), with unknown consequences for themselves and their children. This condition is paralleled with the prediction of low vitamin B12 plasma levels in women of reproductive age (Hertrampf et al. 2003).

4.3 Biological Functions of Folate and Vitamin B12

Folates are transported from the mother to the fetus through the placenta by three specific transporters (RFC, FOLR1, and PCFT/HCP1) (Solanky et al. 2010) and other non-specific transporters belonging to the ABC superfamily (Keating et al. 2011). In a previous study from our group, differential expression of the placental FOLR1 receptor was reported according to birth weight (Caviedes et al. 2016) and gestational age (Castaño et al. 2017). Vitamin B12 needs to be bound to proteins to be transported in the blood. Transcobalamin (TC) and haptocorrin are the primary transporters of vitamin B12 in plasma; TC binds over 70% of vitamin B12 transported across the placenta (Layden et al. 2016). Vitamin B12 bound to TC constitutes the HoloTC complex (Hughes et al. 2013). All maternal vitamin B12 is transported through the placenta by the specific transcobalamin receptor (TCblR/CD320) that recognizes HoloTC but not haptocorrin (Abuyaman et al. 2013; Quadros et al. 2009; Schneider and Miller 2010). Both folate and vitamin B12 are taken up by placental cells to participate in the synergic metabolic pathways and be transferred to the fetus (Fig. 4.1).

The metabolism of folate and vitamin B12 is present in most organs, including the placenta (Shin et al. 2014). Folates uptake occurs in the syncytiotrophoblast (the specialized cell of the placenta) in the form of 5-methyltetrahydrofolate (5-MTHF), the main circulating form of folate in the body (Scott 1999), or like folic acid. First, folic acid is converted into dihydrofolate (DHF) and 5-MTHF. Folate and vitamin B12 participate in the remethylation of homocysteine (Hcy) to methionine (transmethylation pathways) through methionine synthetase (MS), with vitamin B12 (in the form of methylcobalamin–MetCbl) as a co-factor and 5-MTHF as the methyl donor (Hoffbrand 2014; Scaglione and Panzavolta 2014). SAM is produced in the transmethylation pathways after the previous methionine synthesis and ATP (Hoffbrand 2014). SAM is the primary methyl group donor in the methylation reactions in the body and, consequently, participates in epigenetic mechanisms that include DNA and histone methylation (Anderson et al. 2012; Molloy 2012). Moreover, SAM is an allosteric activator of cystathionine β synthase (CβS) (Ereño-Orbea et al. 2014), which requires vitamin B6 as a co-factor in the transsulfuration pathway of Hcy, where cysteine, an important precursor of glutathione (γ glutamyl-cysteinyl-glycine, GSH), is produced (Hoffman 2011). The transsulfuration pathway constitutes an essential pathway for Hcy degradation (Scaglione and Panzavolta 2014) (Fig. 4.2).

On the other hand, vitamin B12, or cobalamin (Cbl), in the adenosylcobalamin form (AdoCbl or AdoB12), is a co-factor of the methyl malonyl-CoA mutase (MMCoAM) in the mitochondria (Obeid et al. 2015). The enzyme MMCoAM converts methyl malonyl CoA (MMCoA) to succinyl-CoA to enter the Krebs cycle (Adaikalakoteswari et al. 2016). The MMCoA and MMCoAM are regulators of the enzyme carnitine palmitoyl transferase-1 (CPT-1) (López-Viñas et al. 2007; Takahashi-Iñiguez et al. 2012), which is responsible for transporting fatty acids into the mitochondria to be β-oxidized

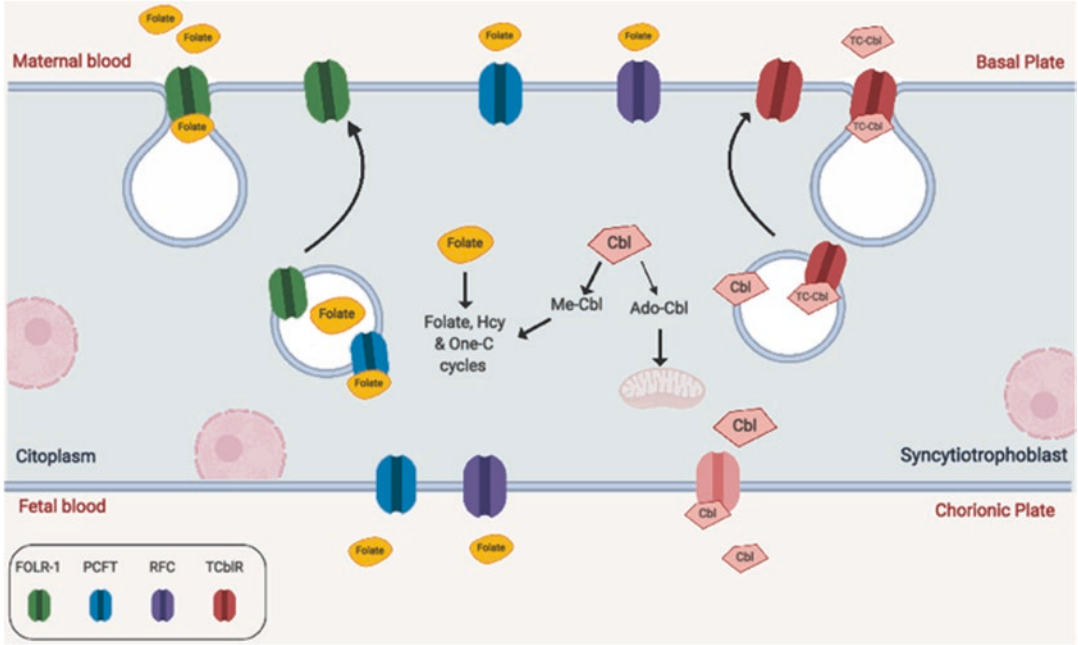

Fig. 4.1 Placental transport of folate and vitamin B12 from mother to fetus Folates are transported from the mother to the fetus through the placenta by three specific transporters (RFC, FOLR1, and PCFT/HCP1). Vitamin B12 bound to TC is called HoloTC. Maternal vitamin B12 is transported through the placenta by the specific transcobalamin receptor (TCblR/CD320)

(Nsiah-Sefaa and McKenzie 2016). In this way, an imbalance of folates (excess or deficit) and vitamin B12 (deficit) may conduce to Hcy levels above normal values, decreasing the synthesis of SAM and methionine and altering the energetic balance by inhibiting the CPT-1 and then affecting the β-oxidation. Consequently, these vitamins are related indirectly to lipid metabolism (Fig. 4.2).

4.4 Folate and Vitamin B12 Imbalance During Pregnancy

The imbalance of folate and vitamin B12 means that circulating folate concentrations (in plasma or erythrocytes) are higher and vitamin B12 levels (in plasma) are lower when analyzed as a ratio (folate/vitamin B12) regarding normal levels. The WHO established the cut-off points for the adult non-pregnant population: for serum folate as a hematological indicator, concentrations are classified as high >45.3 nmol/L, normal 13.5–45.3 nmol/L, and deficit <6.8 nmol/L; in erythrocytes: depletion <362 nmol/L and anemia 226 nmol/L (Organización Mundial de la Salud 2012); for plasma vitamin B12 levels as: normal >221 pmol/L, depletion 148–221 pmol/L, and deficit <148 pmol/L (Allen 2009). Some authors indicated that elevated circulating Hcy (>13 μmol/L) levels indicate a metabolic alteration 16 and that MMA above 0.37 μmol/L and Hcy above 21 μmol/L are indicators of vitamin B12 deficit (Green et al. 2017). There is no consensus about the cut-off points for these vitamins and their relationship with metabolic alterations during pregnancy. In the National Health and Nutrition Examination Survey (NHANES) of 1991–1994 ($n = 4940$) and 1999–2002 ($n = 5473$) with the general population in the United States, aged 20 years and older, it was shown that a high folate/vitamin B12 ratio, such as high plasma folate (>45.3 nmol/L) and low vitamin B12 (<148 pmol/L) levels, was associated with higher

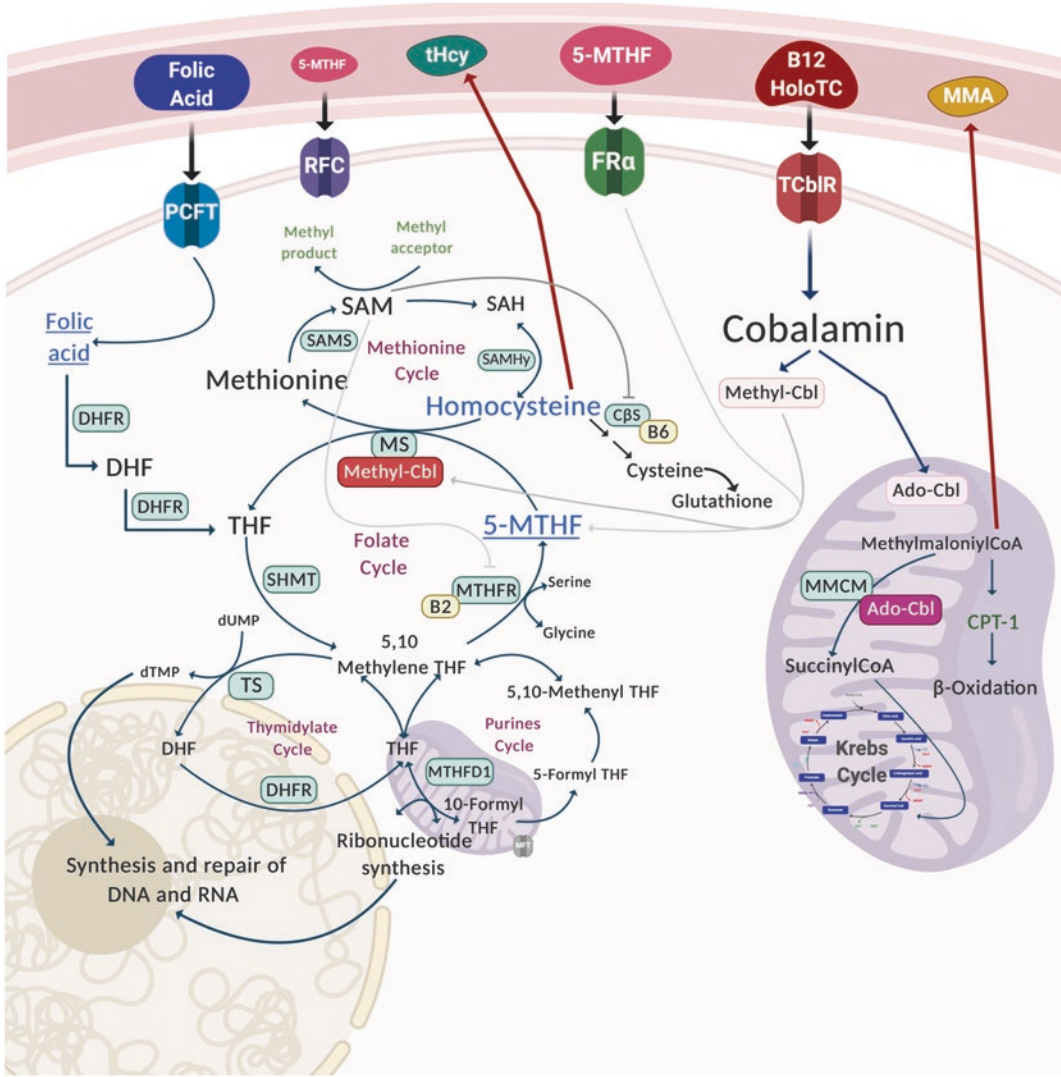

Fig. 4.2 Folate and vitamin B12 metabolism Folate uptake occurs in the syncytiotrophoblast in the form of 5-MTHF, or folic acid. Folate and vitamin B12 participate in the remethylation of homocysteine to methionine (transmethylation pathways) through methionine synthetase (MS), with vitamin B12 (in the form of MetCbl or MetB12) as a co-factor and 5-MTHF as a methyl group donor. SAM is produced in the transmethylation pathways after the previous methionine synthesis and with ATP. SAM is an allosteric activator of cystathionine β synthase (CβS). Vitamin B12, or cobalamin (Cbl), in the AdenosylCobalamin (AdoCbl) form is a co-factor of the methyl malonyl-CoA mutase (MMCoAM) in the mitochondria. The enzyme MMCoAM is responsible for the conversion of methylmalonil CoA (MMCoA) to succinyl-CoA to enter in the Krebs cycle. In turn of this, MMCoA and MMCoAM are regulators of the enzyme Carnitine Palmitoyl Transferase-1 (CPT-1)

concentrations of Hcy (>20 μmol/L) and MMA (>0.8 μmol/L) (Selhub and Rosenberg 2016).

In an observational study from India, a high maternal intake of folic acid and a low vitamin B12 level were related to small for gestational age (SGA) infants (Dwarkanath et al. 2013). A previous study found (median, 25th–75th centile) higher serum folate levels (66, 25–219 nmol/L) with low serum vitamin B12 (219, 97–868 pmol/L). Therefore, a more elevated serum folate/vitamin B12 ratio was found in the cord blood of preterm newborns (305, 181–428)

compared to term newborns (141, 86–216; $p = 0.002$) (Castaño et al. 2017). These results were unexpected because preterm births have been associated with low maternal folate levels in plasma or RBC folate (Tamura and Picciano 2006). Therefore, an explanation for a higher folate/vitamin B12 ratio in preterm newborns may be related to the high levels of folic acid intake in the Chilean population.

Similarly, in a cohort of pregnant women and their children in Pune, India, the authors found that children from mothers with high erythrocyte folate (>1144 nmol/L) along with low plasma vitamin B12 levels (<114 pmol/L) during pregnancy showed higher homeostasis model assessment for insulin resistance (HOMA-IR) at 6 years of age (Yajnik et al. 2008). Similar results were found in Indian children at 9.5 and 13.5 years (Krishnaveni et al. 2014). In summary, high folate and low vitamin B12 intake or blood levels have been related to adverse pregnancy outcomes and metabolic alterations.

On the other hand, increased cholesterol and Hcy levels were produced by the adipocyte cell line Chub-S7 and subcutaneous adipose tissue collected at the time of cesarean section, cultured under low or no B12 concentrations (Adaikalakoteswari et al. 2015). The induction of cholesterol biosynthesis was associated with a reduced SAM/SAH ratio, an indicator of the methylation potential. In the proximal promoter regions of SREBF1 and LDLR, the binding sites for PPARγ and C/EBPα were hypo-methylated, and their transcripts and cholesterol biosynthesis were significantly increased in vitamin B12-deficient conditions. Therefore, low B12 plasma levels (<148 pmol/L) increase cholesterol biosynthesis in human adipose tissue (Adaikalakoteswari et al. 2015).

Studies in non-pregnant (Arias et al. 2017) and pregnant women (McNulty et al. 2013) supplemented with folic acid have observed that vitamin B12 levels decreased after supplementation, suggesting that high blood levels of folic acid may affect the vitamin B12 metabolism. Selhub et al. indicated that a possible mechanism for this could be that folic acid can oxidize the cobalt of vitamin B12, which should be in a highly reduced state (Cob I) to accept the methyl group from 5-methyl tetrahydrofolate, and for this, the plasma vitamin B12 levels decrease (Selhub and Rosenberg 2016); however, this hypothesis has yet to be tested.

The effect of folate/vitamin B12 imbalance has also been studied in the human placenta. In BeWo and JEG-3 placental cell lines cultured with high folic acid (2000 ng/mL), the tumor necrosis factor-alpha (TNFα) gene transcript was overexpressed. Also, higher levels of Hcy and malondialdehyde (MDA-lipoperoxidation marker) were found. However, by treating these cells with the two active forms of vitamin B12 (adocobalamin and methylcobalamin), MDA, Hcy, and inflammation levels decreased significantly (Shah et al. 2016).

4.5 DHA in Pregnancy and Biological Functions

Unlike vitamins, the estimation of the requirements of polyunsaturated fatty acids (PUFAs) intake during pregnancy lacks sufficient studies to establish recommendations (Flock et al. 2013; Kris-Etherton et al. 2009). However, the WHO and the Food and Agriculture Organization of the United Nations (FAO) recommend 300 mg/day of EPA+DHA intake for pregnant and lactating women, of which 200 mg should be DHA (FAO 2010). The benefits of long-chain PUFAs in different metabolic diseases have been widely studied (Flock et al. 2013). Especially in pregnancy, LC-PUFAs positively affect women with gestational diabetes mellitus (GDM), preeclampsia, and intrauterine growth restriction (IUGR) (Wadhwani et al. 2018). From a fetal perspective, maternal DHA is the only supply source (Rogers et al. 2013).

PUFAs are classified into two main series (*n*-6 and *n*-3), considered essential fatty acids, since the body does not synthesize them. Linoleic acid (LA, 18: 2*n*-6) and alpha-linolenic acid (ALA, 18: 3*n*-3) are the main PUFAs in the diet. From these fatty acids, important LC-PUFAs such as arachidonic acid (AA 20: 4*n*-6), eicosapentaenoic acid (EPA, 20: 5*n*3), and docosahexaenoic acid (DHA, 22: 6*n*3) are derived (FAO 2010).

These LC-PUFAs have essential functions in developing the nervous system of the fetus and the child. These fatty acids are part of the structure of cell membranes, and their primary location is in the brain; therefore, they are related to cognitive development (Colombo et al. 2017; Jones et al. 2014; Scifres and Sadovsky 2011). In addition to their structural and energy source functions, these fatty acids are precursors of eicosanoids such as prostaglandins, prostacyclins, thromboxanes, and leukotrienes (FAO 2010). These molecules regulate physiological and pathological processes, gene expression, cell differentiation, immunity, and inflammation (FAO 2010; Scifres and Sadovsky 2011).

Circulating fatty acids are mainly bound to albumin, triglycerides, and phospholipids; therefore, different proteins participate in their transport and release. Lipoprotein lipases (LPL) or epithelial lipase (EL) participate in their release, as do other transport proteins in the uptake of fatty acids into the cytoplasm, like fatty acid translocases (FAT), fatty acid-binding proteins of the plasma membrane (FABPpm), and fatty acid transport proteins (FATP) (Jones et al. 2014; Wadhwani et al. 2018). The FABPs direct these fatty acids towards the nucleus, lipid droplets, or fetal circulation (Scifres and Sadovsky 2011). The placenta also expresses the same hepatic mechanisms of cholesterol transport and lipid metabolism genes (Scifres and Sadovsky 2011).

LC-PUFAs regulate the energy metabolism by acting as ligands of the nuclear receptor family of transcriptional regulators involved in lipid metabolism, such as peroxisome proliferator-activated receptors (PPARα, PPARγ, and PPARδ). PPARs form a heterodimer with retinol alpha receptor X (RXRα) and bind the peroxisome proliferator response element (PPRE) on target genes (Nakamura et al. 2014). PPARs also interact with hepatic alpha receptor X (LXRα) and protein binding of sterol regulatory elements (SREBP-1c) (Gil-Sánchez et al. 2011; Meher et al. 2014; Nakamura et al. 2014; Scifres and Sadovsky 2011); this interaction has also been described in the trophoblast (Scifres and Sadovsky 2011). PPARγ and RXRα participate in systemic and cellular metabolism, increasing lipid uptake and accumulation. LXRα is activated by oxysterols and derivatives of cholesterol and induces the transcription of genes required for reverse cholesterol transport and de novo lipogenesis in the liver, such as SREBP-1c, which responds to increased insulin levels. LC-PUFAs such as DHA inhibit the induction of SREBP-1c by the LXRα agonist and suppress de novo lipogenesis. PPARα is predominantly expressed in the liver and gastrointestinal tract and regulates the fatty acid β-oxidation by inducing genes like CPT-1, ETFDH, and HADHA that are involved in mitochondrial β-oxidation of PUFAs (Nakamura et al. 2014). However, little is known about lipid metabolism in the placenta of women with obesity or the effects of maternal DHA supplementation.

DHA has been mostly studied concerning child neurodevelopment since it is an essential nutrient for the central nervous system (Morse 2012). In a pregnant cohort from Canada, cord plasma fatty acid levels were lower in newborns of women with gestational diabetes compared to non-diabetic pregnancies, and a lower cord plasma DHA was associated with lower fetal insulin sensitivity, even after adjustment for maternal and newborn characteristics (Zhao et al. 2014). The Generation R Study showed a higher maternal *n*-6: *n*-3 PUFA ratio associated with higher total body and abdominal fat mass in childhood. Higher DHA was associated with a lower childhood total body fat percentage, without changes in BMI and abdominal fat mass (Vidakovic et al. 2016).

In the GUSTO cohort, maternal (26–28 w) plasma DHA levels were associated with a higher postnatal length/height ratio at 12 months and 5 years of age. Linoleic acid was positively associated with birth weight, body mass index, head circumference, and neonatal abdominal adipose tissue volume (Bernard et al. 2017). Additionally, in a randomized, triple-blind, placebo-controlled trial from Iran, participants received 1000 mg of fish oil (120 mg of DHA) from week 20 of gestation to birth. No significant differences in the maternal outcomes were found (Ostadrahimi et al. 2017). Maternal DHA is essential during

pregnancy, although the offspring's effects are inconclusive, suggesting that studies evaluating DHA interactions with other nutrients are required.

4.6 Interaction Between Folate, Vitamin B12, DHA, and Maternal Obesity

According to the WHO, the body mass index (BMI, kg/m^2) in adults is normal weight BMI: 18.5–24.9; overweight BMI: 25–29.9; and obesity BMI ≥ 30 (WHO 2000). The prevalence of obesity has increased worldwide and is currently considered a public health problem (Ng et al. 2014). In addition to this prevalence, insulin resistance (IR), type 2 diabetes, and cardiovascular diseases (CVD) are associated with obesity Chile is no stranger to this phenomenon, where adults presented an excess weight (BMI >25) prevalence of 74% in 2017, according to the National Health Survey (MINSAL 2017). This prevalence has been related to the nutritional transition, which began in the 1970s and increased the NCDs' prevalence (Atalah et al. 2014). In this scenario, women of childbearing age and pregnant women have a higher risk of developing this condition, affecting their offspring's health. In the country, more than 50% of women between 15 and 44 years of age are overweight (BMI > 25), and 23% of the pregnant population (classified by Atalah recommendations (Atalah et al. 1997)) are obese (BMI > 30) (Farías 2013). Pregnant women treated in the public health system have a prevalence of obesity of 32.4% (MINSAL 2016).

Maternal obesity represents an increased risk for the mother and her offspring in developing metabolic complications such as impaired lipid metabolism, inflammation, and oxidative stress, among others (Hrolfsdottir et al. 2016; Madan et al. 2009; Malti et al. 2014). Studies in pregnant women with obesity have found that more significant weight gain during pregnancy is related to an altered lipid profile in the mother. Nevertheless, obesity before pregnancy affects the mother, the offspring (Cinelli et al. 2016), the placental nutrient uptake, the metabolism, and the lipid profile (Segura et al. 2017). Furthermore, oxidative stress represents a risk factor in the decrease of maternal levels of DHA, and on the contrary, the supplementation of this fatty acid could improve the antioxidant response (Leghi and Muhlhausler 2016). According to this, if obesity before and during pregnancy alters micronutrients and lipids in the mother, it would be a risk factor in the fetus's adequate supply of these nutrients, affecting both placental and offspring metabolism.

Regarding the relationship between obesity and micronutrient levels, it has been found that folate and vitamin B12 levels in plasma are lower and red blood cell (RBC) folate levels are higher in women of reproductive age and pregnant women with obesity compared to normal-weight women (Berglund et al. 2016; Bird et al. 2015; Bjørke-Monsen et al. 2016; da Silva et al. 2013; Knight et al. 2015; Park et al. 2017; Shen et al. 2016; Sukumar et al. 2016; Tinker et al. 2012; Wang et al. 2016). However, it is unclear how obesity can affect folate and B12 levels. Some authors say that high or low folate levels in obesity may be due to a redistribution from plasma towards the other tissues, as evidenced by the higher folate levels in RBC (da Silva et al. 2013).

Few studies in humans have evaluated the effects of the interaction of these vitamins with DHA. In a prospective study, maternal folate, B12, and DHA levels were lower, and Hcy was higher in the last trimester than in the first trimester of pregnancy. A negative correlation between Hcy and DHA in the third trimester was found. A positive correlation between folate, DHA, and birth weight was observed in the third trimester of pregnancy (Wadhwani et al. 2015). This study provides evidence about the association of the levels of these nutrients with birth weight, suggesting that a balance in their intake could be beneficial for the mother and her offspring.

In the HELENA study with non-pregnant adolescents, a negative correlation between Hcy and DHA levels and a positive correlation between these vitamins' biomarkers, mainly folate and B12 with EPA and DHA, were described. An increase of 10 nmol/L of erythrocyte folate, or HTC, in adolescent women produced a rise of

15.85 μmol/L of EPA but not of DHA, while an increase of 10 nmol/L of Hcy in men had a decrease of 2.06 μmol/L of DHA (Iglesia et al. 2017). These results suggest that LC-PUFA levels can be affected by other nutrients, such as B-complex vitamins. Therefore, it is necessary to study this relationship between pregnant women and their offspring.

In addition, Wistar rats fed with diets containing different folate levels and low or deficient vitamin B12 showed decreased DHA levels in maternal plasma and placenta (Kulkarni et al. 2011; van Wijk et al. 2012). These tissues also had lower global methylation, and these values were restored by supplementing with *n*-3 (Kulkarni et al. 2011). Kumar et al. found that pregnant rats fed with a folate- and B12-deficient diet showed altered body weight and lipid profiles. Their offspring presented low birth weight, higher body fat at 3 months, altered lipid profile at 12 months, higher levels of TNFα, IL-6, leptin, and lower levels of adiponectin and IL-1β, higher activity of lipogenic enzymes like FAS and acetyl CoA-Carboxylase (ACC) (Kumar et al. 2013, 2014). Meher et al. found that pups from pregnant rats with a restrictive diet in folate and B12 showed lower DHA and ARA levels and lower expression of PPARα (β-oxidation marker) and PPARγ (lipogenesis marker) in the liver. When these mothers were supplemented with *n*-3, the expression of these transcription factors was normalized, and the expression of SREBP-1c, LXRα, and RXRα was reduced (Meher et al. 2014). In summary, low maternal B12 levels affect lipid metabolism by increasing cholesterol levels, lowering DHA levels, and a low mRNA expression for CPT-1 (β-oxidation marker) and ACC1 (lipogenesis marker in the liver of offspring). Notably, the supplementation with *n*-3 restored these effects (Khaire et al. 2015).

The mechanisms behind the imbalance of folate/vitamin B12 and DHA levels could be explained by two potential mechanisms: (1) folate and vitamin B12 are involved in synthesizing SAM, the substrate of methyl reactions. One of the main acceptors of methyl groups are phospholipids; phosphatidylethanolamine (PE) and PE methyltransferase (PEMT) catalyze the methylation of PE to phosphatidylcholine (PC). PE methylation to PC is essential for DHA mobilization from the liver to plasma and other tissues (Wadhwani et al. 2018); (2) the opposite effects of DHA supplementation over low B12 could be explained since DHA regulates lipid metabolism through PPARγ, SREBP-1c inhibition, and PPARα stimulation. In conclusion, imbalanced folate/vitamin B12 alters lipid metabolism due to greater lipogenesis and less β-oxidation. This interaction between folate/vitamin B12 and its effects on lipid metabolism remains unknown in pregnant women, their placentas, and their offspring.

Additionally, it has been shown that maternal obesity modulates the lipid metabolism in the placenta by modifying the expression of genes involved in the transport and storage of lipids (Hirschmugl et al. 2017). In a population-based prospective cohort study at 20 weeks of gestation, obese women had higher total saturated fatty acid concentrations and lower total *n*-3 PUFA concentrations than normal-weight women (Vidakovic et al. 2015). A similar study found that pre-pregnancy BMI was inversely associated with maternal MUFA, LA, and DHA and fetal *n*-6 and DHA after adjusting for maternal lipids (Cinelli et al. 2016).

In studies with maternal supplementation with DHA, total lipid content was significantly lower in the placentas of obese women supplemented with *n*-3 PUFAs (800 mg DHA). The mRNA expression of placental FAS and diacylglycerol O-acyltransferase 1 (DGAT1), two enzymes involved in the accumulation and esterification of fatty acids, was negatively correlated with maternal plasma enrichment in DHA and EPA (Calabuig-Navarro et al. 2016). Another study evaluating the effect of maternal DHA supplementation on child adiposity found a significant increase in erythrocyte DHA levels at 36 weeks of gestation but no significant differences in the neonate's adiposity at birth, 2, or 4 years (Foster et al. 2017). Maternal obesity influences lipid metabolism; however, the interaction of maternal obesity in the presence of folate/vitamin B12 imbalance and DHA supplementation is unknown (Fig. 4.3).

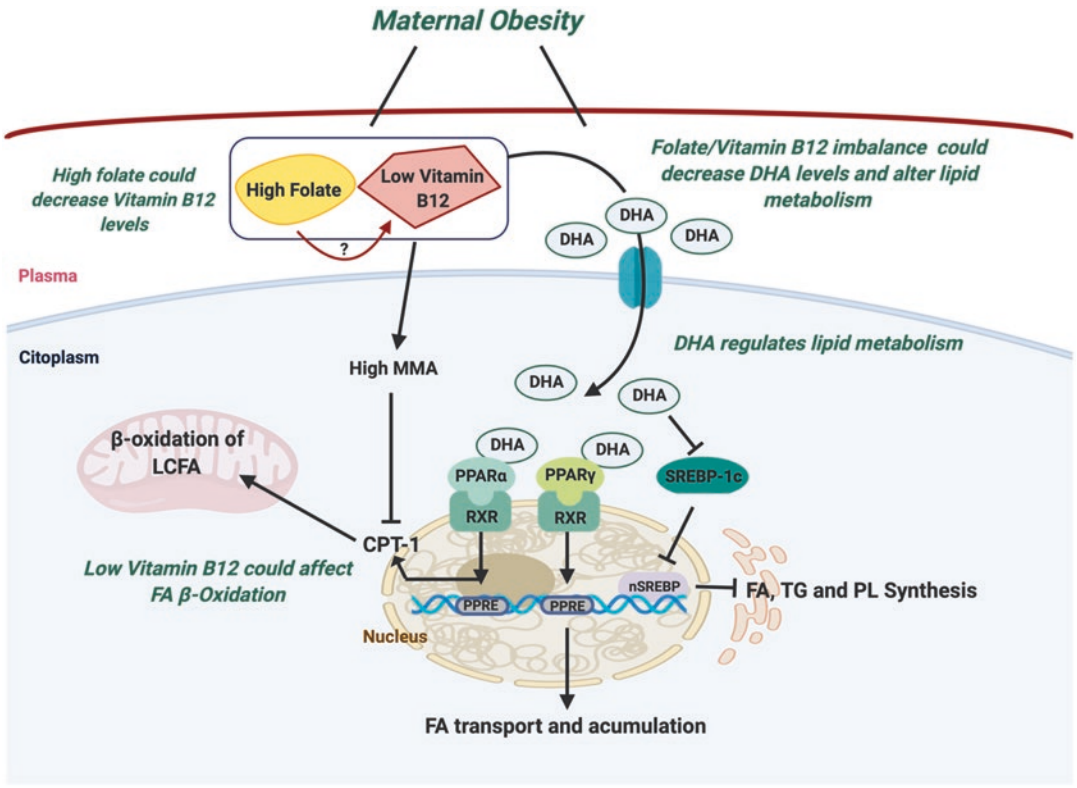

Fig. 4.3 Folate, vitamin B12, and DHA interactions in maternal obesity Maternal obesity increases folate/vitamin B12 imbalance, which increases methylmalonic acid (MMA), which downregulates the enzyme carnitine palmitoyltransferase 1 (CPT-1). Consequently, the β-oxidation is blocked. Maternal obesity decreases the transfer of DHA to the fetus. Lower DHA levels in the placenta may affect the fatty acid β-oxidation, transport, accumulation, and synthesis

In synthesis, it is clear that fetal programming related to altered maternal nutritional status is a risk factor for the early development of non-communicable chronic diseases, and some pregnant women, particularly in Chile, may be exposed to a high folic acid intake and, presumably, to low vitamin B12 levels. We have the following premises: (1) there is a relationship between maternal folate/vitamin B12 imbalance and low DHA levels over pregnancy outcomes and lipid metabolism in the offspring, both humans and animals; (2) maternal obesity influences folate and B12 levels; and (3) this interaction (folate, vitamin B12, and DHA) has not been thoroughly studied in the placentas of pregnant women with obesity.

The future is very challenging from the DOHaD point of view. As a broad mean, maternal and fetal exposome are the most critical factors that can be considered, modified, and optimized to improve the health of our next generation. Important moments of the life cycle need to be considered in the prevention strategies of the different countries, and the most relevant ones are those in the reproductive cycle. Starting with healthy adolescents with balanced nutrition and lifestyle practices, including physical activity, to optimize gamete biology and later pregnancy and the first 1000 days of life. Many knowledge gaps still need our best efforts to unravel the best choices and interventions to optimize development and health for future generations.

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5 Evidence of Nitric Oxide Impairment During Hypertensive Pregnancies

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Abstract

Hypertensive disorders of pregnancy complicate up to 10% of pregnancies worldwide, and they can be classified into (1) gestational hypertension, (2) preeclampsia, (3) chronic hypertension and (4) chronic hypertension with preeclampsia. Nitric oxide (NO) plays an essential role in the haemodynamic adaptations observed during pregnancy. It has been shown that the nitric oxide pathway's dysfunction during pregnancy is associated with placental- and vascular-related diseases such as hypertensive disorders of pregnancy. This review aims to present a brief definition of hypertensive disorders of pregnancy and physiological maternal cardiovascular adaptations during pregnancy. We also detail how NO signalling is altered in the (a) systemic vasculature, (b) uterine artery/spiral arteries, (c) implantation and (d) placenta of hypertensive disorders during pregnancy. We conclude by summarizing the anti-hypertensive therapy of hypertensive disorders of pregnancy as a specific management strategy.

Keywords

Nitric oxide · Gestational hypertension · Preeclampsia

5.1 Introduction

The discovery of nitric oxide (NO), as the major component synthesized from the vascular endothelium, part of the endothelium-derived relaxing factors (EDRF), was a remarkable event in the vascular field.

The pregnancy requires an extensive remodelling from the vasculature, favouring the augmented flow requirement into the uterus, allowing a healthy foetal-grow environment. With this regard, NO elicits vascular remodelling in the maternal uterine artery, a condition required for a healthy pregnancy outcome (Osol et al. 2019; Zullino et al. 2018).

The adaptations in the systemic vasculature are also essential, considering that the cardiac output and plasma volume may rise over 30%

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during a healthy pregnancy. In addition, the resistance arteries must favour vasodilation, allowing better pressure control. Therefore, NO production is augmented during pregnancy, favouring the maintenance of blood pressure (BP) and ideal conditions for a healthy pregnancy (Chang and Streitman 2012; Osol et al. 2019; Torgersen and Curran 2006; Zullino et al. 2018).

NO is also produced locally in the placenta and modulates implantation. NO elicits proliferation, apoptosis, gene expression and local vascular tone regulation, among other functions. All these events are essential players for trophoblast invasion and placental development, as well as placental vasculogenesis and angiogenesis (Krause et al. 2011).

In more than 30 years, EDRF/NO signalling pathway was deeply investigated, and the establishment of a condition called endothelial dysfunction was recognized as an earlier marker for several cardiovascular diseases, including hypertension and hypertensive disorders during pregnancy (Boeldt and Bird 2017; McLaughlin et al. 2018). Here, we discuss how alterations in the NO pathway may impact vascular, uterine and placental function during hypertensive disorders during pregnancy (Fig. 5.1).

5.2 Hypertension on Pregnancy

Hypertensive disorders during pregnancy represent the primary cause of maternal and infant mortality (Pinheiro et al. 2016). High blood pressure contributes to augmented undesirable outcomes during pregnancy, including growth restriction and stillbirth (Sutton et al. 2020; Tabatabaee et al. 2020). Additionally, children of mothers with hypertension display augmented early onset of cardiovascular disease from childhood and later life (Staley et al. 2015; von Ehr and von Versen-Höynck 2016). The children and the mothers have a higher chance of developing cardiovascular disease after pregnancy since preeclampsia (PE) is considered an independent risk factor for their development (Haukkamaa et al. 2009; Stuart et al. 2018; Tooher et al. 2017).

According to the American Heart Association Clinical Practice Guideline (Whelton et al. 2018), BP is currently categorized into normal, defined as systolic blood pressure (SBP) <120 and diastolic blood pressure (DBP) <80 mmHg; elevated, defined as an SBP of 120–129 and DBP of <80 mmHg; stage 1, set as an SBP of 130–139 or a DBP of 80–89 mmHg; and stage 2, defined as an SBP >140 or a DBP ≥90 mm Hg. However, this classification comprises human adults, and hypertension is classified differently during pregnancy, mainly based on the timeframe when hypertension is diagnosed.

Therefore, the term hypertension in pregnancy is commonly used to describe a broad spectrum of patients, including women with mild elevations in BP and those with severe hypertension, or even with dysfunction of various organs (Lindheimer et al. 2010). Concerning hypertensive disorders that affect pregnancy, four main types can be highlighted according to Table 5.1.

5.2.1 Gestational Hypertension

It is defined as transient hypertension developed after 20 weeks of gestation or chronic hyperten-

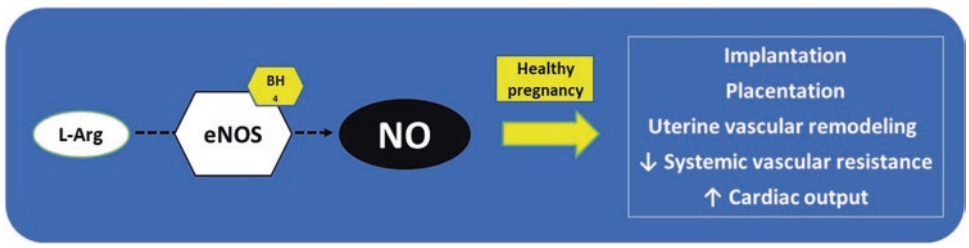

Fig. 5.1 The physiological impact of NO-pathway in vascular, uterine and placental function during pregnancy. Abbreviations: *BH_4* tetrahydrobiopterin, *eNOS* endothelial nitric oxide synthase, *L-Arg* L-Arginine, *NO* nitric oxide

Table 5.1 Diagnostic criteria for hypertensive disorders of pregnancy

Classification	Diagnostic criteria
Gestational hypertension	Early-onset hypertension (>20 weeks of gestational age); SBP of 140 mmHg or more and/or DBP of 90 mmHg or more, on two different occasions, with at least 4 h apart
Severe gestational hypertension	Early-onset hypertension (>20 weeks of gestational age); SBP of 160 mmHg and/or DPB of 110 mmHg
Preeclampsia	New-onset hypertension (>20 weeks of gestational age or near term); SBP of 140 mmHg or more and/or DBP of 90 mmHg or more, on two different occasions, with at least 4 h apart Proteinuria (≥300 mg/24 h urine); Thrombocytopenia (platelet <100,000 × 10^9/L); Renal insufficiency (serum creatinine >1.1 mg/dL); Impaired liver function (elevated blood concentrations of liver enzymes); Pulmonary edema
Chronic hypertension	Hypertension before pregnancy or before the 20th week; SBP of 140 mmHg or more and/or DBP of 90 mmHg or more, on two different occasions, with at least 4 h apart;
Superimposed PE	Chronic hypertension; Proteinuria (≥300 mg/24 h urine) after 20 weeks of gestation or an additional increase in those who already presented it

sion not identified until the last half of pregnancy (Leeman et al. 2016). This group of patients is characterized by the recent onset of hypertension and increased BP (>20 weeks of gestational age), with no proteinuria or other severe features ("ACOG Practice Bulletin No. 202" 2019). Pregnant who present SBP equal or greater than 140 mmHg and/or DBP equal or greater than 90 mmHg, identified on two different occasions, with at least 4 h apart ("Report of the National High Blood Pressure Education Program Working Group on High Blood Pressure in Pregnancy" 2000), are considered with gestational hypertension. When SBP reaches 160 mmHg, or DPB reaches 110 mmHg or both, it is severe gestational hypertension (Bernstein et al. 2017). In this case, it is expected that pregnancy outcomes may vary according to the disease severity (Leeman et al. 2016), where non-severe gestational hypertension is related to less harmful results. In contrast, severe gestational hypertension presents significant risks of morbidity for the mother and foetus, being responsible for preterm delivery at <37 weeks of gestation and small-for-gestational-age infants (Buchbinder et al. 2002; Cruz et al. 2011).

5.2.2 Preeclampsia

PE is a widespread clinical syndrome characterized by multisystem involvement and clinical laboratory abnormalities that affect 2–8% of all pregnancies, representing a leading cause of worldwide maternal, foetal and neonatal morbidity and mortality (Ghulmiyyah and Sibai 2012; Ornaghi and Paidas 2017). The diagnosis of PE is based on the presence of new-onset hypertension (SBP 140 mmHg and/or DBP 90 mmHg), associated with proteinuria, in a previously normotensive woman. These features are frequently observed after 20 weeks of gestation and in the near term ("Hypertension in pregnancy. Report of the American College of Obstetricians and Gynecologists' Task Force on Hypertension in Pregnancy" 2013). Proteinuria is no longer a required element for diagnosis. There are other signs and symptoms of PE, including maternal organ dysfunction and foetal growth restriction, in women without proteinuria (Homer et al. 2008). Since 2014, parameters including thrombocytopenia (platelet count <100,000 × 10^9/L), renal insufficiency (serum creatinine concentration > 1.1 mg/dL), impaired liver function (elevated blood concentrations of liver enzymes) and pulmonary oedema may be used as criteria for the diagnosis of PE (Duhig et al. 2018; Tranquilli et al. 2014).

The exact mechanism involved in the physiopathology of PE remains unknown. Evidence strongly supports the involvement of the placenta

(Cheng and Wang 2009; McMaster et al. 2004; Redman 1991) since normal placentation is required for a healthy pregnancy. Cytotrophoblast cells migrate and deeply invade the maternal spiral arteries to establish adequate uteroplacental blood flow (Carter et al. 2015). In PE, cytotrophoblast invasion of the uterus is shallow, and spiral artery invasion is incomplete (Fisher 2015).

Regarding its pathophysiology, PE can be described as two distinct forms of the disease: early- and late-onset PE (Raymond and Peterson 2011). The early-onset PE occurs when PE happens before 34 weeks of gestation. This condition is associated with severe complications in the mother and foetus, including impaired placentation, foetal growth restriction and organ dysfunction (Raymond and Peterson 2011; Wójtowicz et al. 2019). Moreover, early-onset PE has been related to persistent effects after pregnancy to mother and offspring like increased risk for maternal hypertension and future cardiovascular disease (Lazdam et al. 2012; Mol et al. 2016; Veerbeek et al. 2015) and, more recently, infant-elevated BP (Chourdakis et al. 2020). On the other hand, late-onset PE is the most common form of the disease but presents less severe clinical symptoms (Kenneth et al. 2010; Stergiotou et al. 2013). It develops after 34 weeks of gestation and is related to healthy placenta and appropriate foetal growth and birth weight (Erez et al. 2017; Eskild et al. 2009).

5.2.3 Chronic Hypertension

Chronic hypertension (CH) is a common comorbidity during pregnancy, affecting around 3–5% of all pregnancies (Sibai 2002). CH is characterized by SBP values ≥140 mmHg and/or DBP ≥90 mmHg diagnosed before pregnancy or before the 20th week of pregnancy, measured correctly on two occasions with at least 4 h apart (Ankumah and Sibai 2017; "Hypertension in pregnancy. Report of the American College of Obstetricians and Gynecologists' Task Force on Hypertension in Pregnancy" 2013). CH can be identified as an essential/primary or secondary in this scenery, necessary for managing the disease (Seely and Ecker 2011). In addition, women who present arterial hypertension for the first time during pregnancy but it does not reach expected levels in the postpartum period are also classified as CH. In some cases, the haemodynamic changes in pregnancy can hide CH diagnostic, where a decrease in systemic vascular resistance leads to BP normalization (American College of Obstetricians and Gynecologists' Committee on Practice Bulletins – Obstetrics 2019). Although many pregnancies affected by CH usually occur well, with the typical outcome, there are a few maternal and foetal complications that they are exposed to, such as superimposed PE, placenta abruption, foetal growth restriction and perinatal death (Seely and Ecker 2014).

5.2.4 Chronic Hypertension with Preeclampsia

The superimposed PE is the most prevalent complication affecting pregnant women with preexistent hypertension, with proteinuria (≥0.3 g/24 h) after 20 weeks of gestation, or an additional increase in proteinuria in those who already presented it ("Hypertension in pregnancy. Report of the American College of Obstetricians and Gynecologists' Task Force on Hypertension in Pregnancy" 2013). This type can also occur when there is a sudden increase in BP in women with previously controlled levels or other clinical alterations characteristic of PE such as thrombocytopenia, renal insufficiency and pulmonary oedema (Costa et al. 2016; Guedes-Martins 2017). In this case, PE tends to be severe (early-onset) and present worse maternal and foetal outcomes than in either condition alone (Chappell et al. 2008).

5.3 Maternal Cardiovascular Adaptations During a Normal Pregnancy

During pregnancy, the mother's body must undergo many changes to adapt to its inclusive environment to support a baby's growth. These

adaptations are necessary to guarantee the proper nutritional apport and oxygen supply to the conceptus in the uterus and later in lactation (Napso et al. 2018). Furthermore, since pregnancy is a precisely and dynamically regulated process, it requires crucial changes in the immunological (Edey et al. 2018), metabolic (Lain and Catalano 2007), pulmonary (LoMauro and Aliverti 2015) and cardiovascular (Li et al. 2012) systems of the mother.

Maternal cardiovascular adaptations during pregnancy include many modifications such as increased stroke and plasma volume and cardiac output, decreased peripheral resistance and arterial compliance, among others (Krause et al. 2011). These changes result in reduced mother arterial BP, observed during pregnancy, reaching normal levels by its end (Torgersen and Curran 2006). The maternal adaptations found in the cardiovascular and haemodynamic systems occur to guarantee a correct uteroplacental blood flow and are necessary for proper foetal growth and development. Besides that, it also prevents the mother from developing hypertension (Osol et al. 2019). On the other hand, failures in this process lead to increased risk for the mother of developing cardiovascular complications and can result in maternal and foetal morbidity such as gestational hypertension, PE and intrauterine growth restriction (IUGR)(Fu 2018). More recently, it has been shown that placental function, perfusion, and foetal weight are strongly associated with maternal haemodynamic responses (Garcia-Gonzalez et al. 2020).

The average cardiac output range in rest relies on 2.5–4.2 L/min/m^2 (Wannenburg and Little 2010). Still, it begins to change early in pregnancy, within 1–2 weeks after fertilization, increasing progressively 30–50% more than the observed in non-pregnant. Initially, this is mediated by increased stroke volume, in consequence of enhanced venous return and plasma volume expansion and, later, by increased heart rate (Bader et al. 1955; Chang and Streitman 2012). Moreover, a pregnancy-related cardiac remodelling is observed as left ventricular mass, and wall thickness slightly increases (De Haas et al. 2017). Regarding maternal BP, both SBP and DBP present a variation throughout pregnancy. Although there are some disagreements, most studies show that BP starts to fall gradually very early in pregnancy and rises again by the end (Ayala et al. 1997; Shen et al. 2017).

As a consequence of the increase in cardiac output and decrease in BP, systemic vascular resistance also changes during pregnancy, marked by a reduction in total peripheral resistance, was pulmonary and vascular resistance decrease with a range around 34% and 21%, respectively (Clark et al. 1989; Troiano 2018). Reduction in peripheral vascular resistance is associated with reduced arterial tone and augmented vasodilatation, plasma volume and vascular distensibility (Osol et al. 2019; Poppas et al. 1997). These changes seem to happen upon the actions of progesterone on vascular smooth muscle cells (Hall et al. 2011; Pang and Thomas 2018) and by the fact that this hormone presents modulatory effects on blood vessel contractibility (Barbagallo et al. 2001). Furthermore, the development of the uteroplacental vascular compartment also contributes to maternal vascular resistance reduction (Wang and Zhao 2010).

Following these alterations, the uterine vascular system also undergoes some changes to achieve coordinated uteroplacental blood flow. Uterine circulation adaptations are characterized by a significant decrease in uterine vascular resistance and dramatic structure alterations such as increased vessel diameter, luminal dilatation and trophoblast invasion, among others (D'Errico and Stapleton 2019; Osol and Mandala 2009). Such adaptations aim to support the subsequent development of the placental bed, which is crucial for maintaining a healthy pregnancy and baby growth.

Although the mechanisms responsible for these changes are still not fully understood, it is believed that NO plays an essential role in the haemodynamic adaptations observed during pregnancy. In this regard, little evidence shows that increased NO mediates dilatation in cardiovascular adaptations to pregnancy (Chu and Beilin 1993; Conrad et al. 1993), and the inhibition of NO synthesis attenuates the decreased peripheral resistance (Hall et al. 2011).

5.4 NO Pathway During Pregnancy

NO is produced from the oxidation of the essential amino acid L-arginine to L-citrulline. This reaction is catalysed by the enzyme nitric oxide synthase (NOS), which has three isoforms divided into two categories, (a) the constitutive NOS (cNOS), calcium/calmodulin-dependent, and (b) the inducible NOS (iNOS), calcium-independent, being activated in a variety of cell types and tissues such as endothelium, nerves and immune cells and in pregnancy-foetal trophoblast (Webster et al. 2008; Yang et al. 2015). Once induced, iNOS can produce NO for an extended period, which can result in pathological processes (Flora Filho and Zilberstein 2000). cNOS is subdivided into neural NOS (nNOS), ordinarily present in neurons, and endothelial NOS (eNOS), expressed constitutively in endothelial cells and platelets (Webster et al. 2008). NO production depends on eNOS dimerization, and this enzyme binds to co-factors such as tetrahydrobiopterin (BH4)(Osol et al. 2017).

The production and action of NO are performed due to the stimulation of chemical messengers in the endothelium cell. As soon as these chemicals bind to their receptor on the endothelium, NO is produced and diffused through vascular smooth muscle cells adjacent to the endothelium, producing cyclic guanosine monophosphate (cGMP), causing smooth muscle relaxation. In addition, phosphodiesterase (PDE) degrades cGMP, resulting in vasoconstriction (Furchgott and Zawadzki 1980; Murad 1994; Púzserová et al. 2008).

During pregnancy, progesterone levels are high since this hormone is crucial for the maintenance of pregnancy, and its receptors are associated with increased eNOS activity and NO production through the Akt signalling pathway (Pang and Thomas 2017, 2018). Further, NOS isoform expression is dynamically regulated in the placenta. The iNOS isoform is expressed mainly at the foeto-maternal interface in the first stages of pregnancy (Marinoni et al. 2004) and abundant peaks at mid-gestation (Baylis et al. 1999; Suzuki et al. 2009). On the other hand, eNOS is expressed in syncytiotrophoblast, early endothelium and extravillous trophoblast at the beginning of gestation (Ariel et al. 1998; Rossmanith et al. 1999). As pregnancy continues, there are an increase and redistribution of eNOS expression, mainly to syncytiotrophoblast and endothelial cells (Dötsch et al. 2001; Myatt et al. 1997; Rossmanith et al. 1999; Schiessl et al. 2005).

In agreement with what is seen in the placenta, during pregnancy, NO-dependent vasodilation is increased in the human uterine artery due to augmented eNOS activity and expression (Nelson et al. 1998, 2000).

5.4.1 Systemic Vasculature

A healthy pregnancy is characterized by remarkable physiological adaptations in the maternal circulation, such as higher intravascular volume and decreased vascular resistance, leading to a slightly reduced arterial BP compared to non-pregnant women (Christianson 1976; Mashini et al. 1987; Tomimatsu et al. 2017). However, during PE, peripheral vascular resistance (and thus arterial pressure) is increased due to augmented placental soluble fms-like tyrosine kinase (sFlt-1), counteracting vascular endothelial growth factor (VEGF)- and placenta growth factor (PlGF)-induced microvascular relaxation (Maynard et al. 2003).

During PE, the vascular adaptations necessary for healthy pregnancies are impaired. Altered vascular reactivity and endothelial dysfunction have been demonstrated before the subsequent onset of PE (Myatt and Webster 2009). In fact, at 22 weeks of gestation, women with PE show abnormal Doppler flow velocity waveforms, in a ratio of 30% (15:48) in healthy pregnancies versus 65% (12:19) in preeclamptic pregnancies (Myers et al. 2005). Flow-mediated dilation (FMD) in the brachial artery is significantly decreased in preeclamptic women, confirming endothelial dysfunction (Yoshida et al. 2000). Interestingly, endothelial dysfunction persists even three months after postpartum and is reversed by ascorbic acid administration, an anti-

oxidant therapy (Agatisa et al. 2004; Chambers et al. 2001). Moreover, a systematic review and meta-analysis reported lower FMD in women with PE, even three years postpartum (Weissgerber et al. 2016).

Conversely, arterial stiffness and microvascular endothelial function, measured by the reactive hyperemia index, are higher in PE (Mannaerts et al. 2019). Interestingly, women with PE display a progressive increase in endothelium-dependent and endothelium-independent vasodilation during pregnancy to levels over and above that seen in a healthy pregnancy. Significantly, these changes precede the onset of clinical disease by weeks or months and might be related to a compensatory increased sensitivity of the microcirculation to NO (Khan et al. 2005).

Incubation of myometrial vessels from normal pregnant women with plasma from women in whom PE develops collected weeks before the diagnosis can alter endothelial function. A wire myograph study resulted in decreased endothelium-dependent relaxation to bradykinin after 1-h and 18-h incubation (Myers et al. 2005), suggesting NO signalling plays a significant role during this pathogenesis.

Asymmetric dimethylarginine (ADMA), a natural NOS inhibitor, is elevated in PE, supporting the theory of reduced NO activity being a cause or result of the pathology observed in PE (Fickling et al. 1993; Myatt and Webster 2009; Possomato-Vieira and Khalil 2016). Further, single nucleotide polymorphisms in the gene encoding for dimethylarginine dimethylaminohydrolases (DDAHs), which stimulate ADMA degradation, have been noted in PE (Myatt et al. 1997). Both plasma and placental levels of DDAHs were reduced in preeclamptic patients (Akbar et al. 2005; Anderssohn et al. 2012; Ehsanipoor et al. 2013). Moreover, the transport of L-arginine to the platelets is reduced in PE and could, in turn, affect NO production in preeclamptic women (Pimentel et al. 2013). With this regard, a meta-analysis study suggests that the eNOS 894G > T polymorphism is associated with increased susceptibility to PE, specifically in Caucasians and mixed populations (Abbasi et al. 2021).

The umbilical cord of preeclamptic women displays decreased eNOS expression compared to a healthy pregnancy, and it is more significant as PE gets severe (Bhavina et al. 2014; Zawiejska et al. 2014). Rats and mice models of PE, using a synthetic inhibitor of NOS, L-NAME, display increased BP, foetal growth restriction. In these models, there is an impairment of acetylcholine-induced vasorelaxation in the aorta (Amaral et al. 2018; Buhimschi et al. 1995; Shu et al. 2018; Takiuti et al. 1999). Indeed, time-controlled L-NAME administration led to the development of early-onset PE phenotypes that mimic subtypes of PE in humans (Cushen and Goulopoulou 2017; Soobryan et al. 2017). eNOS knockout mice ($eNOS^{-/-}$) are hypertensive before and during pregnancy. This experimental model of hypertension also displays proteinuria, reduced cardiac output, uterine artery dysfunction, foetal growth restriction and placental hypoxia (Kulandavelu et al. 2012; Kusinski et al. 2012). Although eNOS ablation results in PE-like symptoms, they do not develop hypertension during pregnancy. Therefore, they may be a more suitable animal model for chronic hypertension, eliciting foetal growth restriction (Marshall et al. 2018).

Pregnancy-induced hypertension in response to chronic reduced uterine perfusion pressure (RUPP) in rats has been associated with no change in whole-body NO production but a decrease in renal protein expression of neuronal NO synthase (Alexander et al. 2001). Interestingly, offspring born after IUGR displayed in adulthood increased BP and impaired aorta vasodilation, with augmented eNOS uncoupling and arginase activity and decreased eNOS expression and NO production in this vascular bed. Moreover, aorta preincubation with L-arginine or arginase inhibitor restored all the outcomes shown in the aorta (Grandvuillemin et al. 2018).

L-NAME and RUPP rat models to PE treated with sildenafil, a drug that promotes vascular relaxation by the inactivation of the PDE, resulted in decreased BP and increased plasma concentration of NO (George et al. 2013; Motta et al. 2015). Further, in the L-NAME plus sildenafil

model, the resistance index and the pulsatility index of the umbilical artery blood flow were normalized after treatment with sildenafil (Stanley et al. 2012). In the same way, thoracic aorta rings display improvement in relaxation and cGMP concentration (Turgut et al. 2008).

Vascular function studies have shown reduced relaxation, eNOS expression and NO production in the mesenteric artery and aorta of the RUPP rat model (Amaral et al. 2015; Crews et al. 2000; Mazzuca et al. 2014). In the same way, hypertensive pregnancy induced by DOCA-salt displayed decreased NO-dependent relaxation in mesenteric vessels (Mitchell et al. 2007), supporting a specific reduction in NO synthesis in the vasculature.

The uteroplacental remodelling and vascular changes seen during pregnancy are modulated, at least partially by NO. Matrix metalloproteinases (MMPs), the gelatinases MMP-2 and MMP-9 are targets for NO modulation. In a hypertensive model induced by L-NAME-infusion, rats showed increased MMP-2 activity in the uterus, placenta and aorta during the pregnancy. Augmented MMP-9 activity in plasma and placenta coincided with reduced NO levels, suggesting that NO bioavailability may regulate MMP activation during hypertensive pregnancy (Nascimento et al. 2019).

Placental ischemia during pregnancy is associated with increased plasma cytokines, which may contribute to increased vascular resistance and hypertension during pregnancy. Studies have shown that cytokine infusion has been used to develop animal models of hypertensive pregnancy once endothelium-dependent vascular relaxation is reduced in tumour necrosis factor-alpha (TNF-α) and interleukin (IL)-6 infused pregnant animals, possibly due to the inhibition of the endothelium-dependent NO-cGMP pathway (Davis et al. 2002; Orshal and Khalil 2004).

5.4.2 Uterine Artery/Spiral Arteries

During pregnancy, there is increased activity of the NO vasodilatory mechanism in the maternal systemic vasculature in general, and this is even more pronounced in the uterine vasculature in particular (Boeldt et al. 2011). Blood flow to the pregnant uterus is supplied by four arteries: the right and left main uterine artery and the right and left uterine branches of the ovarian artery. These vessels, in turn, give rise to several arcuate arteries arching over the uterus, which branch centripetally into myometrial arteries that penetrate the myometrium and, at the level of the decidua, terminate in approximately 200 spiral arterioles supplying blood to the intervillous space (Browne et al. 2015).

Further analysis has demonstrated that the diameter of uterine and radial arteries increases by 55% and 30%, respectively, in early gestation (Rennie et al. 2016) and that the myogenic tone, the ability of vessels to maintain vasoconstriction under conditions of increased intravascular pressure, is raised by pregnancy (Morton et al. 2017).

The uterine artery plays an essential role during pregnancy since the entire process of placentation involves foetal trophoblastic cells that invade the uterus and become decidual for further development of the spiral arteries (Browne et al. 2015; Soares et al. 2014).

The uterine vasculature undergoes significant changes during pregnancy since it is responsible for adequate perfusion at the maternal-foetal interface. Thus, during pregnancy, the gestational growth of the uterine vasculature is accompanied by hypertrophy and hyperplasia of the arterial wall (Cooke and Davidge 2003), sympathetic denervation (Xiao et al. 2001), profound vasodilation (Goulopoulou et al. 2012; Wight et al. 2000) and reduced vascular tone (Everett and Lees 2012). In addition, during the early stages of pregnancy, the cytotrophoblasts invade the uterine spiral arteries, progressively replacing endothelial cells, medial elastic tissue, vascular smooth muscle and neural tissue (Tanbe and Khalil 2010). These changes culminate in a decrease in uterine vascular resistance, allowing a 20-fold increase in blood flow in this artery during pregnancy (Nelson et al. 2000), resulting in the supply of much of the blood flow to the uterus (Browne et al. 2015; Webster et al. 2008).

Uterine spiral arteries are the conduits for delivering maternal nutrients to the foetus and

undergo fundamental changes of their cellular (endothelial and smooth muscle cell) and extracellular constituents. Maternal blood directly bathes the trophoblast (functional units of the placenta), which requires restructuring of the uterine spiral arterial tree, which are targeted and structurally modified to create conduits with altered vascular regulation properties, maximizing the flow of maternal resources to the placenta (Soares et al. 2014).

A human uterine artery endothelial cell culture model showed that pregnancy augments vascular VEGF, which significantly stimulates angiogenesis and activation of the eNOS-NO pathway (increase Akt, eNOS phosphorylation and NO production) (Zhang et al. 2017). NO plays an essential role in the endometrial invasion, trophoblast apoptosis, neovascularization and angiogenesis, and its levels on the amniotic fluid compartment are positively correlated with uterine artery Doppler (Zafer et al. 2018). During pregnancy, wall shear stress is required for uterine artery growth/remodelling (via NO signalling) and the presence of an adjacent vein (Ko et al. 2018).

Similar to changes in vascular function observed in humans, rat pregnancy is associated with a blunted systemic vasoconstrictor response and enhanced endothelial-dependent vasodilation (Cooke and Davidge 2003), including NO/cGMP pathway through estrogen receptors (Tropea et al. 2015). In the same way, ovine uterine artery endothelial cells from pregnant ewes incubated with 17-β-estradiol's metabolites displayed increased total NO metabolites (NOx), which were blunted by incubation with estrogen receptor antagonists, demonstrating that these metabolites contribute to rise vasodilation and uterine blood flow during pregnancy (Landeros et al. 2019).

The uterine circulation displays decreased resistance and increased blood flow during pregnancy, leading to augmented shear stress and significant vasodilation to support the uteroplacental perfusion. A key mediator of these processes is the NO (Arishe et al. 2020; John et al. 2018). However, in PE, the uterine vascular remodelling is harmed, the invasion of spiral arteries by extravillous trophoblast is impaired and arteries remain as small resistance vessels (Arishe et al. 2020).

Moreover, vascular resistance is determined under physiological conditions by combining passive resistance artery structure and the ambient level of constriction or tone. L-NAME-induced hypertensive pregnancy developed impaired arterial remodelling and reduced tone, which were returned to control levels with hydralazine treatment (maintaining NO inhibition but lowering BP)(Barron et al. 2010).

During pregnancy, sexual hormones display an essential role. The steroid hormones, in particular testosterone levels, were upregulated in preeclamptic women's circulation, positively correlated with vascular dysfunction. The higher testosterone levels impaired endothelial-dependent relaxation in the uterine arteries in a pregnant rat model of elevated maternal testosterone (Kumar et al. 2018).

The healthy growth of the main uterine artery is impaired during chronic hypoxia pregnancy, leading to reduced uterine artery blood flow and contributing to an increased frequency of PE and IUGR. In fact, myometrial arteries from pregnant women living at high altitude (contributing to chronic hypoxia) demonstrate impaired NO signalling contributing to diminished uteroplacental perfusion (Lorca et al. 2019).

Piezo 1, a mechanoreceptor that leads to vasodilation via NO, was demonstrated to be upregulated during pregnancy, and its express ion is increased in the uterine artery (John et al. 2018). Preliminary data from Arise and co-authors showed that the uterine artery of hypertensive rats displayed decreased relaxation response to Yoda 1 (specific activator of Piezo 1) compared to non-pregnant rats, indicating a possible down-regulation of NO pathway through Piezo 1 in PE (Arishe et al. 2020).

A study with pregnant sheep demonstrated that occlusion of the uterine artery for 24 h did not change systemic arterial BP. However, it decreased around 22% of NO levels in both arterial and uterine venous during occlusion, without

altering eNOS expression. This data suggest that shear stress may increase endothelial-derived NO production to reduce vasomotor tone in pregnancy (Joyce et al. 2002).

Prolonged continuous intra-arterial infusions of L-NAME (72-h) decreased uteroplacental blood flow by 32%, uterine cGMP synthesis at 70% and increased uteroplacental vascularisation resistance around 68% during ovine pregnancy (Rosenfeld and Roy 2014).

A rat model of IUGR by administering a low-Na^+ diet reduces maternal blood volume expansion and uteroplacental perfusion. This model also indicates that the NO pathway is activated in radial uterine arteries as a compensatory mechanism for lowering blood uteroplacental perfusion (Bigonnesse et al. 2018).

Systemic endothelial dysfunction is thought to play a central role in the development of PE. A study shows that pregnant rat uterine arteries incubated with plasma from preeclamptic women altered its relaxation. Furthermore, circulating factors lead to endothelial dysfunction via oxidative stress and vasodilator pathways, and these alterations were observed exclusively in some of the vascular territories, once mesenteric arteries did not show any change (Kao et al. 2016).

Uterine arteries from a model of PE using pregnant female human angiotensinogen transgenic rat mated with male human renin transgenic rat displayed functional alterations at early gestation before the preeclamptic phenotype is established, probably related to the increased role of prostanoids, contributing to the development of a hypertensive pregnancy (Pulgar et al. 2015).

PE is associated with impaired uteroplacental adaptations during pregnancy and abnormalities in the eNOS. eNOS knockout mice present reduced uteroplacental blood flow, uterine artery diameter and spiral artery length in late pregnancy (Kulandavelu et al. 2012). Additionally, NOS genes have been associated with hypertensive pregnancy (Tanbe and Khalil 2010) as well as ACVR2A; STOX1; and those of VEGF, hypoxia-inducible factor (HIF), Fas, leptin and angiotensinogen; and cytokines such as TNF-α and IL-10 (Ali and Khalil 2015).

5.4.3 Implantation

During implantation, the endometrium undergoes remarkable changes such as proliferation and differentiation into a well-vascularized decidual tissue to provide an optimum environment for the embryo (Chwalisz and Garfield 2000).

NO production is essential for maintaining pregnancy from early stages, considering that embryonic development, implantation and placental perfusion are regulated by NO (Maul et al. 2003). This molecule acts to relax the myometrial and vascular smooth muscles, inhibiting platelet aggregation, and also plays a role in inflammation (Chwalisz and Garfield 2000).

Growing evidence shows that NO may play an important role in implantation and decidualization (Chwalisz and Garfield 2000; Maul et al. 2003; Norman and Cameron 1996). All three NOS isoforms are present within the mouse and rat implantation site, with iNOS and eNOS being the most prominent (Mara Suburo et al. 1995; Purcell et al. 1999; Schmidt et al. 1992). In the same way, NOS isoforms have also been found in the human endometrium (Ota et al. 1998; Telfer et al. 1997; Tseng et al. 1996) as even the fallopian tube (Shao et al. 2010). Interestingly, immunostaining for eNOS is more significant during the mid-secretory phase of the menstrual cycle (Ota et al. 1998).

In a healthy pregnancy, cytotrophoblast cells invade the decidua and the myometrium and migrate into maternal spiral arteries. However, this process is harmed during preeclamptic pregnancy, once cytotrophoblast invasion is shallow and limited to outer parts of the decidua. Maternal spiral arteries are narrow due to insufficient invasion by cytotrophoblast cells to form the vascular trophoblast (McMaster et al. 2004; Mohaupt 2007). L-NAME treatment during the peri-implantation phase demonstrates an inhibitory effect on mice implantation, suggesting that NO is involved in decidualization (Chwalisz et al. 1999; Purcell et al. 1999).

Degradation of the uterine epithelial and trophoblastic invasion requires specialized enzymes, such as the MMPs, which are expressed in tro-

phoblasts. In addition, NO and other types of reactive nitrogen oxides participate in connective tissue remodelling by controlling the activity of the MMPs (Trounson 1998). Indeed, placentas from pregnant women with severe forms of PE demonstrated decreased staining to VEGF (McMaster et al. 2004), which promotes phosphorylation of eNOS, thus increasing the MMP enzymes activity (Feliers et al. 2005).

Moreover, patients with PE and gestational hypertension without antihypertensive treatment showed higher levels and activity of myeloperoxidase, an enzyme responsible for producing reactive oxygen species and associated with consumption of the vasodilator NO. Inhibition of this enzyme activity in vitro improved NO bioavailability from the human umbilical vein endothelial cell line (Rocha-Penha et al. 2017).

A prospective case-controlled study on women with idiopathic recurrent spontaneous miscarriage (IRSM) demonstrated angiogenic and vasoactive factors associated with impaired endometrial perfusion, which could make the endometrium unreceptive and eventually cause early pregnancy loss. In this study, VEGF, eNOS and NO were found to be down-regulated in women with IRSM, where VEGF and eNOS were the major factors contributing towards vascular dysfunction, and they were strongly correlated with blood flow impairment (Banerjee et al. 2013).

Successful embryo implantation in assisted reproduction technology cycles depends on embryo quality, endometrial receptivity and a non-traumatic embryo transfer (Schoolcraft et al. 2001). The use of pharmacological agents (such as NO donors) that inhibit uterine contractions may improve implantation and pregnancy rates (Bisits et al. 2004).

A systematic review identified uterine relaxant agents administered in the peri-implantation period during assisted reproduction treatments that could improve pregnancy outcomes. The meta-analyses did not show a statistically significant benefit of any uterine relaxing agents on live birth rate, clinical pregnancy, spontaneous abortion, ectopic pregnancy and multiple pregnancy rate (Khairy et al. 2016). Two studies compared NO donors with no treatment (Farzi et al. 2005; Ohl et al. 2002) and showed a relative risk of 1.11 (95% CI 0.68–1.80) for live birth showing a non-significant effect.

Ectopic implantation in the first trimester of pregnancy is a common cause of human maternal morbidity and mortality (Corpa 2006). Of importance, NO production during fluctuating physiological conditions regulates the importance of fallopian tube functions, such as ciliary activity or contractility (Rosselli et al. 1998).

Studies have shown human and bovine fallopian tubes treated with L-NAME augmented contractility of the fallopian tubes (Ekerhovd et al. 1997; Rosselli et al. 1994), whereas the opposite was observed in vitro treatment with NO donors (Ekerhovd et al. 1999), demonstrating that NO plays a role during the transport of the embryo.

Moreover, infection with *Chlamydia trachomatis* induces an inflammatory response that eventually leads to tubal epithelial destruction and functional impairment, caused by a high NO output mediated by iNOS (Shao et al. 2010).

5.4.4 Placenta

The placenta is a complex organ that plays pleiotropic roles during foetal growth. In addition to being responsible for allowing the absorption of nutrients, elimination of waste and the gas exchange between the mother and the foetus, the placenta also has a function of the physiological and immunological barrier, separating the maternal and foetal circulation (Maltepe and Fisher 2015). Different cell-like surfaces make up this region of the maternal-foetal interface, such as the syncytiotrophoblast, which exposes the placenta to the side of the maternal circulation and the endothelium, which is in contact with the foetal blood (Desoye and Hauguel-de Mouzon 2007; Garcia-Ruíz et al. 2015). In addition, placental functioning is susceptible to regulatory hormones, cytokines, growth factors and substrates present in maternal and foetal circulation. Consequently, it can be affected by any change in these factors (Desoye and Hauguel-de Mouzon 2007; Huynh et al. 2015).

At the beginning of pregnancy, there are a series of critical processes of proliferation and differentiation, predominant in trophoblast cells, allowing the formation of villous and extravillous structures. Subsequently, the placenta anchors in the uterus and remodels the spiral arteries, making them vessels with low vascular resistance. Then, the villi are differentiated through several stages of maturation. Thus, during the first half of pregnancy, the trophoblast undergoes marked changes, while extensive angiogenesis and vascularization occur in the second half of pregnancy (Cross et al. 1994; Desoye and Hauguel-de Mouzon 2007; Soares et al. 2012).

The pathophysiology of PE involves abnormal placentation by defective trophoblastic invasion leading to decreased maternal uteroplacental blood flow, which could be a result of reduced bioavailability of NO (Guerby et al. 2019b; Sánchez-Aranguren et al. 2014). Syncytiotrophoblast and Hofbauer cells from the human placenta express eNOS and iNOS isoenzymes, and in this way, the placenta has a crucial role in the development of PE (Eis et al. 1995, 1997; Pánczél et al. 2019). A case-control study of placentas from women with gestational hypertension showed that eNOS and iNOS mRNA levels were significantly reduced and NO and peroxynitrite ($ONOO^-$) production were both considerably higher (Vignini et al. 2016).

Indeed, placentas from preeclamptic women presented a lower degree of eNOS expression in the syncytiotrophoblasts and reduced L-arginine levels in the serum (Kim et al. 2006; Shaheen et al. 2020). Syncytiotrophoblast extracellular vesicles, which carry signals from the syncytiotrophoblast to the mother, such as NO, are lower in preeclamptic placentas and might contribute to the overall decreased NO bioavailability seen in PE (Motta-Mejia et al. 2017).

Importantly, in this organ, eNOS expression is associated with cytotrophoblast to syncytiotrophoblast differentiation (Eis et al. 1995; Sánchez-Aranguren et al. 2014). Several post-translational modifications (PTM) may regulate eNOS activity (Heiss and Dirsch 2014), including lipid peroxidation products (LPP). Placentas from preeclamptic women displayed a stable change of eNOS by LPP, specifically 4-oxo-2(E)-nonenal (ONE). In fact, ONE incubation alters the production of NO in cultured HTR8 human trophoblasts under hypoxic conditions, leading to decreased expression and activation, thereby contributing to the process of PE (Guerby et al. 2019b).

Recent evidence indicates that S-glutathionylation may occur on the eNOS, leading to eNOS uncoupling, characterized by a decreased NO production and an increased generation of superoxide anion ($O_2^{\bullet -}$). Placentas from preeclamptic women present high levels of eNOS glutathionylation and reduced NO production, and this PTM was able to alter trophoblast migration, an important event occurring during early placentation (Guerby et al. 2019a).

L-Arginine levels of patients with PE are significantly lower than healthy controls, and its levels in preeclamptic villi are decreased. In contrast, no changes are observed in the decidua when compared to the control group (Pánczél et al. 2019). L-Arginine supplementation decreases BP in preeclamptic women and the incidence of high-risk PE (Camarena Pulido et al. 2016; Rytlewski et al. 2005). Moreover, elevated levels of arginase, an enzyme that competes with NOS for L-arginine, were found to decrease in both maternal vasculature and placenta from women who developed PE (Bernardi et al. 2015; Sankaralingam et al. 2010).

Nevertheless, measured results of eNOS expression/activity and NO production are still contradictory in the placenta. Indeed, it was demonstrated that NO synthase activity was not altered in villous tissue of the placenta from preeclamptic women compared to a healthy pregnancy (Conrad and Davis 1995). In the same way, preeclamptic and control placentas did not show any significant differences in their mRNA levels of NO metabolite or their NOS expression (Nishizawa et al. 2009). Importantly, BH4, a required co-factor to NO production, displayed a similar concentration in the preeclamptic placenta than normal (Kukor et al. 2000). Myatt and co-workers showed eNOS expression to be elevated in the endothelium of placental villi of preeclamptic women, and they suggest this could be

a compensatory mechanism (Ghabour et al. 1995). Moreover, eNOS, iNOS and estrogen receptor expression are significantly elevated in trophoblast cells of preeclamptic placentas compared with healthy controls (Schiessl et al. 2005).

Following, HELLP syndrome (haemolysis, elevated liver enzyme levels and low platelet count) is a dangerously severe complication of pregnancy characterized by multisystemic thrombotic microangiopathy and occurs in about 10–12% of cases with severe PE. Evidence shows a reduced eNOS and iNOS gene expression in placentas from women with HELLP syndrome, indicating a placental dysfunction. On the other hand, these placentas also present higher NO formation, which could be explained as a counteraction to the impaired fetoplacental perfusion, typical of the syndrome (Mazzanti et al. 2011).

In the preeclamptic placenta, due to impaired trophoblast invasion, a hypoxic environment is settled, favouring oxidative stress and inflammation, which leads to an endothelial dysfunction involving the release of the sFlt-1, which is a circulating anti-angiogenic protein and an endogenous inhibitor of VEGF (Sánchez-Aranguren et al. 2014). Of importance, VEGF–Flt-1 interaction induces the release of NO (Papapetropoulos et al. 1997), which in turn up-regulates the activity and expression of pro-invasive MMP (Trounson 1998) required for proper vascular adaptation placentation. Consequently, immunostaining of VEGF and sFlt-1 are reduced in preeclamptic placentas (Zhou et al. 2002).

A recent study showed that doxycycline, an MMP inhibitor, attenuated L-NAME-induced hypertension in pregnant rats, reduced activity of placental MMP-2 and MMP-9 and reversed anti-angiogenic/pro-angiogenic imbalance (Nascimento et al. 2018).

The human preeclamptic placenta presents an increased expression of androgen receptors compared to a healthy placenta. Further, genetic polymorphisms in the androgen receptor are associated with an increased risk of PE (Kumar et al. 2018). Finally, of importance, testosterone treatment is shown to decrease NO production (Motamer et al. 2019).

Hypertensive pregnancy induced by the DOCA-salt model in rats displays decreased NO levels in the placenta and reduced plasma PlGF with concomitant increased plasma sFlt-1, which are reversed by pravastatin treatment. In this regard, the pleiotropic effects of these statins are associated with NO signalling, leading to augmented levels of vasodilator NO (Chimini et al. 2019).

Dysregulation of hydrogen sulphide (H_2S)-producing enzymes has been related to hypertensive pregnancy, and H_2S may interact with NO, modulating its production. Treatment with hydrosulphide (NaHS), an H_2S donor, blunted the increases in systolic BP, improved foetal weight and restored placental efficiency in hypertensive pregnant rats (DOCA-salt model), suggesting that reestablishment of placental efficiency may be partially modulated by increases in placental NO formation (Possomato-Vieira et al. 2018).

Figure 5.2 brings the impact of hypertensive pregnancy in several systems and organs, where nitric oxide is impaired.

5.5 Reactive Oxygen Species (ROS) and NO Bioavailability During Hypertension

During pregnancy, NO plays a significant role in decreasing peripheral vascular resistance and maintaining proper placental oxygenation (Osol et al. 2017; Sánchez-Aranguren et al. 2014). Reactive oxygen species, including nitric oxide (•NO), nitrogen monoxide ($O_2•^-$), hydrogen peroxide (H_2O_2), hydroxyl radical (•OH) and $ONOO^-$ are molecules that regulate many functions in human physiology (Kalyanaraman 2013). In fact, during a healthy pregnancy, ROS generation is increased. It may be necessary for proper physiology functions of the ovary, including ovarian steroid genesis, oocyte maturation, ovulation, the formation of blastocysts, implantation, luteolysis and luteal maintenance in pregnancy (Lu et al. 2018; Mutinati et al. 2013; Sánchez-Aranguren et al. 2014; Yang et al. 2012).

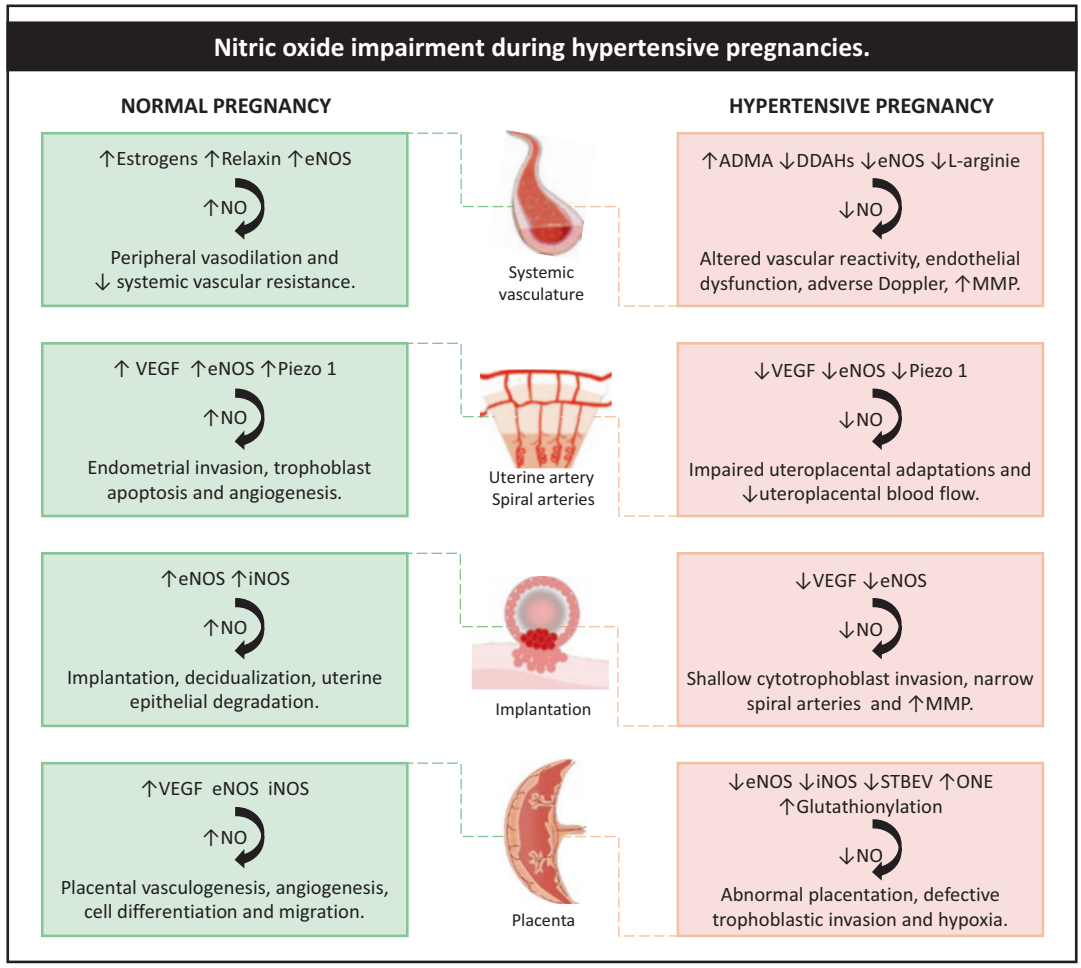

Fig. 5.2 Evidence of nitric oxide impairment during hypertensive pregnancies. During a normal pregnancy, estrogens modulate the systemic arterial vasodilation by upregulating eNOS. The peptide hormone relaxin stimulates the formation of endothelin, increasing NO-mediated arterial vasodilation and leading to systemic vascular resistance fall. In hypertensive pregnancy, these vascular adaptations are impaired. In PE, asymmetric dimethylarginine (ADMA), an NOS inhibitor, is elevated, and dimethylarginine dimethylaminohydrolases (DDAHs), which stimulate ADMA degradation, is reduced, leading to impaired NO bioavailability. Additionally, L-arginine transport into human platelets, which is important to NO production, is reduced in PE. Consistent with reduced NO levels, augmented MMP-9 activity was found during hypertensive pregnancy. Concomitantly, altered vascular reactivity, endothelial dysfunction and adverse Doppler are found in PE. Pregnancy increases vascular endothelial growth factor (VEGF) in the uterus and upregulates the mechanoreceptor Piezo 1. VEGF stimulates angiogenesis and activates the eNOS-NO pathway and Piezo 1 leads to vasodilation by increasing NO release. NO plays an essential role in the endometrial invasion, trophoblast apoptosis and angiogenesis, supporting uteroplacental perfusion. PE is associated with eNOS abnormalities and decreased VEGF levels. Recent studies found an indication of NO pathway downregulation through Piezo 1 in PE. The reduced NO levels have been related to reduced uteroplacental blood flow and impaired uteroplacental adaptations observed in PE. During the implantation, eNOS, iNOS and NO release are increased. Thus, NO is involved in implantation, decidualization and uterine epithelial degradation. During PE, VEGF levels and eNOS phosphorylation are reduced, increasing the MMPs' enzyme activity. In consequence, cytotrophoblast invasion is shallow, and maternal spiral arteries are narrow. In the placenta, eNOS and iNOS promote NO production, and VEGF induces eNOS-dependent NO synthesis. Further, placental vasculogenesis, angiogenesis, cell differentiation and endothelial cell migration depend on NO. Reduced eNOS and iNOS mRNA levels in gestational hypertension and placentas from preeclamptic women presented lower eNOS expression. Syncytiotrophoblast extracellular vesicles (STBEV) are lower and might contribute to decrease NO bioavailability in PE. A study has shown that placental eNOS is modified by the lipid peroxidation product, 4-oxo-2(E)-nonenal (ONE), in PE. Incubation with ONE reduced the NO production. Moreover, preeclamptic placentas present high levels of eNOS S-glutathionylation, leading to eNOS uncoupling and decreasing NO production. Thus, these events are related to impaired trophoblast migration, abnormal placentation and hypoxia

In this way, pregnancy represents a state of oxidative stress due to increased maternal metabolism and metabolic activity of the placenta (Myatt and Webster 2009). However, when the balance between pro-oxidants and antioxidants is harmed, and ROS production is much higher, oxidative stress is installed. This phenomenon has been shown in PE (Kalyanaraman 2013; Lappas et al. 2010; Matsubara et al. 2010; Sánchez-Aranguren et al. 2014).

Studies have shown increased $ONOO^-$ in the placentas and systemic blood vessels of women with PE (Bos et al. 2019; Matsubara et al. 2010; Myatt et al. 1996; Roggensack et al. 1999). Of importance, superoxide reacts with NO to form $ONOO^-$. Interestingly, nitrotyrosine (product of tyrosine nitration mediated by reactive nitrogen species, such as $ONOO^-$ anion) is increased in the placenta and the maternal vasculature of preeclamptic women (Myatt et al. 1996; Roggensack et al. 1999), leading to questions if it is either a cause or consequence of this condition. Placentas incubated with $ONOO^-$ resemble those from pregnancies complicated by PE (Kossenjans et al. 2000), suggesting that the presence of ROS could be a causative role in PE.

Importantly, NO bioavailability may also result from $ONOO^-$-mediated degradation of BH4, as described before, as an essential co-factor for NO production through eNOS. When BH4 is decreased or absent, eNOS becomes uncoupled, leading to more superoxide than NO, which further contributes to endothelial dysfunction (Milstien and Katusic 1999; Mitchell et al. 2007).

A rat model of pregnancy-induced hypertension (DOCA-salt) demonstrated augmented aortic superoxide and $ONOO^-$ levels compared to control, which was normalized by scavenging reactive oxygen species and increased levels of BH4, suggesting that ROS, as well as uncoupled eNOS, contributes to PE-like symptoms (Mitchell et al. 2007).

Mitochondrial dysfunction also contributes to ROS generation and oxidative stress. Corroborating with previous data, the RUPP rat model of PE displays increased mitochondrial ROS. However, treatment with mitochondrial-specific antioxidants (MitoQ/MitoTEMPO) for four days resulted in decreased BP. Moreover, incubation of endothelial cells with RUPP serum augmented mitochondrial ROS, which was attenuated in the presence of mitochondrial-antioxidant-treated RUPP rat serum (Vaka et al. 2018), demonstrating an essential role of ROS in the regulation of BP.

5.6 Treatment Targeting NO Pathway During Pathway

Several treatment strategies have been investigated to prevent or manage adverse pregnancy outcomes on the growing foetus and maternal health (Morton et al. 2017). Uterine and fetoplacental circulation of preeclamptic women were carried out. In this study, a long-term transdermal administration of isosorbide dinitrate, an NO donor, suppressed the BP, reduced the average pulsatility index in the uterine arteries and increased the size of the amniotic fluid pocket, improving fetoplacental circulation. This therapy may be a tool for avoiding maternal hypertension (Nakatsuka et al. 2002). In the same way, isosorbide mononitrate vaginally applied in a day pattern until delivery lowered the incidence of PE, preterm birth, IUGR and neonatal admission to the intensive care (Abdel Razik et al. 2016).

Contrary, a randomized, double-blind placebo-controlled trial with low-dose prophylactic isosorbide mononitrate in high-risk women receiving standard aspirin showed that there is no significant reduction in the incidence of hypertensive disorders of pregnancy neither substantial effect on the severity of the disease, gestational age at diagnosis of disease or maternal-perinatal morbidity (Ponmozhi et al. 2019).

Impaired placental function and, consequently, reduced placental perfusion is the leading cause of foetal growth restriction. Many treatments target to increase blood flow to the foetal/placental unit through the uterine artery. A randomized controlled pilot study demonstrated a reduced risk of 39% for the development of foetal growth restriction, by administering nitrate pentaerythrityl tetranitrate (PETN), an NO donor, to patients with impaired uterine artery Doppler at mid-gestation.

This substance is widely used for the treatment of cardiovascular disease and has been shown to have protective effects on human endothelial cells. The effects of PETN on adverse pregnancy outcome were investigated in a randomized, double-blinded, placebo-controlled, multi-centre trial (Groten et al. 2019). Notably, the significant decrease in the incidence of foetal growth restriction and preterm birth was confirmed and the efficacy and safety of PETN. The effectiveness of PETN for secondary prevention of IUGR, PE and preterm birth in pregnancies at risk was also tested. In this randomized, placebo-controlled, double-blinded study, PETN significantly decreased the risk for IUGR and/or perinatal death as well as preterm birth. However, PETN did not improve the risk of PE. Additionally, NO placental abruption occurred in the PETN group (Schleussner et al. 2014).

PDE inhibitors are used to improve cGMP availability, favouring vascular relaxation. A placebo-controlled randomized trial using sildenafil, a PDE type 5 inhibitor, increased uterine vasodilatation, improving intra-uterine foetal growth. However, this treatment has not prolonged pregnancy duration, even improved pregnancy outcomes in severe early-onset foetal growth restriction (Sharp et al. 2018). Studies using experimental models also showed improvement in uterine artery blood flow and foetal outcomes. In addition, treatment with sildenafil lowers maternal systolic BP, improved vascular reactivity, decreases protein excretion and foetal mortality and increased foetal growth in various hypertensive pregnancy models, such as L-NAME-induced hypertensive pregnancy and dams deficient in eNOS, a nonsevere hypertensive murine model (Gonçalves-Rizzi et al. 2018; Ramesar et al. 2010; Roberts et al. 2016).

In the same way, treatment with sodium nitrite blunts hypertension in pregnancy and restores the NO bioavailability, improving the number of viable and resorbed foetuses and antioxidant function. Concomitantly, sodium nitrite prevents the L-NAME-induced high-circulating sFlt-1 and VEGF levels (Gonçalves-Rizzi et al. 2016, 2018).

The NO donor 2-[[4-[(nitroxy)methyl]benzoyl]thio]-benzoic acid methyl ester (SE175) induced significant mouse uterine arteries relaxation and human placental arteries in vitro. $eNOS^{-/-}$ pregnant mice, which exhibit impaired uteroplacental blood flow and foetal growth restriction, were treated with SE175, resulting in increased foetal weight, improvement of the spiral artery diameter and decreased placental weight, indicating improvement of placental efficiency in $eNOS^{-/-}$ mice (Cureton et al. 2017).

As well known, arginine is an essential substrate for NO synthesis. Heifers, daily receiving an intraperitoneal infusion of arginine during all gestational periods, did not increase blood flow to the uterus. However, arginine-treatment favoured augmented maternal plasma concentration of arginine and progesterone, as also decreased heart hate, favouring a better gestational outcome (Yunta et al. 2015).

Vascular endothelial function can be achieved by regulating NO release, anti-apoptotic and antioxidant actions and activation of cell survival signalling, improving pregnancy-related disorders. The SOCS-JAK-STAT pathway plays an essential role in vascular smooth muscle cell proliferation, associated with endothelial cytokines. L-NAME-induced preeclamptic rats treated with cyano-(3-hydroxy) N-styrene benzylamine (AG490), a common JAK kinase inhibitor, to simulate the inhibition effect of SOCS on JAK-STAT in the signalling intervention, exhibited lower expressions of p/t-JAK2, p/t-STAT3 and SOSC1 as also decreased serum levels to IL-6 and TNF-α and increased NO and IL-10 (Luo et al. 2016).

Other hormones may also contribute to NO bioavailability. For example, in a healthy pregnancy, relaxin plays an important vasodilatory role in maintaining vascular compliance. This hormone is produced from both the ovary and placenta, with essential effects during pregnancy. In a RUPP-induced PE model, rats receiving recombinant human relaxin-2 lowered BP and improved sFlt-1, TNF-α and NO bioavailability. These data suggest a potential therapeutic role for relaxin in maintaining maternal health and prolonging pregnancy in the face of placental ischemia (Santiago-Font et al. 2016).

5.7 Conclusion

In summary, NO plays a significant role in the physiology of pregnancy, and a deficiency in the NO bioavailability is related to hypertensive disorders of pregnancy. In comparison, treatment with drugs that act on the NO pathway or NO donors shows promising results at this disease linked to NO dysfunction. However, future work is needed to understand mechanistic pathophysiology contributed to the alternations in NO bioavailability and/or NO signalling in hypertensive disorders during pregnancy.

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6

Pathophysiology of Preeclampsia and L-Arginine/L-Citrulline Supplementation as a Potential Strategy to Improve Birth Outcomes

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Abstract

In preeclampsia, the shallow invasion of cytotrophoblast cells to uterine spiral arteries, leading to a reduction in placental blood flow, is associated with an imbalance of proangiogenic/antiangiogenic factors to impaired nitric oxide (NO) production. Proangiogenic factors, such as vascular endothelial growth factor (VEGF) and placental growth factor (PlGF), require NO to induce angiogenesis through antioxidant regulation mechanisms. At the same time, there are increases in antiangiogenic factors in preeclampsia, such as soluble fms-like tyrosine kinase type 1 receptor (sFIt1) and toll-like receptor 9 (TLR9), which are mechanism derivates in the reduction of NO bioavailability and oxidative stress in placenta.

Different strategies have been proposed to prevent or alleviate the detrimental effects of preeclampsia. However, the only intervention to avoid the severe consequences of the disease is the interruption of pregnancy. In this scenario, different approaches have been analysed to treat preeclamptic pregnant women safely. The supplementation with amino acids is one of them, especially those associated with NO synthesis. In this review, we discuss emerging concepts in the pathogenesis of preeclampsia to highlight L-arginine and L-citrulline supplementation as potential strategies to improve birth outcomes. Clinical and experimental data concerning L-arginine and L-citrulline supplementation have shown benefits in improving NO availability in the placenta and uterine-placental circulation, prolonging pregnancy in

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patients with gestational hypertension and decreasing maternal blood pressure.

Keywords

Preeclampsia · Endothelial dysfunction · Nitric oxide · L-Arginine · L-Citrulline

6.1 Introduction

Preeclampsia is defined by gestational hypertension, new-onset proteinuria, and potentially other end-organ dysfunction after 20 weeks of pregnancy or new-onset preeclampsia-associated signs in the absence of proteinuria that causes a high maternal and foetus mortality (Brown et al. 2018). In a normal pregnancy, blood flow between the mother and foetus is supported by an adequate vascularization of the placenta. However, in preeclampsia, inadequate placentation is observed due to the poor trophoblastic invasion of the helical uterine arteries and a marked generalized inflammatory response triggered by oxidative and/or hypoxic stress in placentas (Magee et al. 2014). At the same time, the increased blood pressure described in patients may be a compensatory mechanism against the reduction of maternal–foetal blood flow (Barra et al. 2012).

According to the "National High Blood Pressure Education Program Working Group on High Blood Pressure in Pregnancy (NHBPEP)," chronic hypertension in pregnancy is defined as hypertension (systolic pressure ≥ 140 mmHg and/or diastolic pressure ≥ 90 mmHg) present before the 20th week of pregnancy or hypertension present before pregnancy (ACOG Committee on Obstetric Practice 2002; "Report of the National High Blood Pressure Education Program Working Group on High Blood Pressure in Pregnancy" 2000). Preeclampsia can be classified as early (<34 weeks) or late (≥34 weeks) according to gestational age at diagnosis or delivery (Chaiworapongsa et al. 2014). As preeclampsia is a multisystem disease, it can also present HELLP syndrome (defined as the presence of haemolysis, elevated liver enzymes, and a low platelet count) (Weinstein 2005) and renal dysfunction, functionally expressed as vascular constriction, a decline in glomerular filtration rate (GFR), and renal plasma flow (RPF) (Noris et al. 2005).

Worldwide, approximately 292,982 women die due to complications during pregnancy, childbirth, or postpartum, corresponding to a maternal mortality ratio (MMR) of 209 deaths per 100,000 live births in 2013 (Kassebaum et al. 2014). Between 2003 and 2009, haemorrhage and hypertensive disorders significantly contributed to maternal deaths (27.1% and 14%, respectively). Meanwhile, in Latin America and the Caribbean, hypertensive disorders are responsible for deaths with an average frequency of 22.1% (Say et al. 2014). Particularly in Chile, maternal deaths by hypertension decreased from 21 to 7 from 1990 to 2013, and overall MMR fell from 47.8 per 100,000 live births to 18.7 between 1990 and 2013 (Kassebaum et al. 2014). Despite this decrease, the leading causes of maternal death are hypertensive disorders (preeclampsia and eclampsia) (Donoso Siña 2011). The World Health Organization (WHO) has identified a reduction of 75% in maternal mortality between 1990 and 2015 as part of the Millennium Development Goals (Eiland et al. 2012). In Chile, reducing maternal mortality and morbidity is the primary goal of the Health Department's plan for 2011–2020 ("Estrategia Nacional de Salud 2011–2020" n.d.).

In this chapter, we have compiled a critical review of the pathophysiology of preeclampsia, giving special attention to the following mechanisms: alteration in trophoblastic invasion and placental ischaemia/hypoxia, proangiogenic and antiangiogenic factors, and the role of nitric oxide (NO). Finally, we will discuss L-arginine and L-citrulline supplementation as potential therapy for preventing or treating preeclampsia.

6.2 Pathophysiology of Preeclampsia

The aetiology of preeclampsia is unknown. This disease contributes significantly to maternal and neonatal morbidity and mortality because it

leads to several adverse pregnancy outcomes such as intrauterine growth restriction (IUGR), neonatal and perinatal mortality, premature birth, and severe neonatal morbidity (Backes et al. 2011) and predisposes women in later life to cardiovascular diseases (Rana et al. 2019). Importantly, there is evidence that this condition is a placental pathology. The early-onset preeclampsia arises from defective placentation, and late-onset preeclampsia is associated with maternal cardiovascular maladaptation to placental senescence (Burton et al. 2019). The placenta is the target organ in preeclampsia. Placenta dysfunction could be the disease's origin or a consequence, and the evidence shows different molecular mechanisms altered in the preeclamptic placenta. In Fig. 6.1, we summarize some of the molecular mechanisms we will review in this chapter, all related to the higher placental vascular resistance and the primary adverse outcomes of the disease, maternal hypertension, and IUGR.

The pathophysiology of preeclampsia consists of two stages: preclinical (first and second trimesters) and clinical (late second and third trimesters). The first stage consists of early abnormal placentation when there is a restricted invasion by a subtype of trophoblasts described as extravillous cytotrophoblasts. The clinical stage or maternal syndrome (second stage) is marked by a generalized inflammatory response triggered by oxidative stress and/or persistent placental hypoxia, which in turn promotes an increase of antiangiogenic factors, such as sFLT-1 (soluble fms-like tyrosine kinase 1) and sEng (soluble endoglin), and inflammatory mediators (Rana et al. 2019). These conditions could lead to endoplasmic reticulum (ER) stress, disturbing the physiological function of ER on post-translational modifications and protein folding.

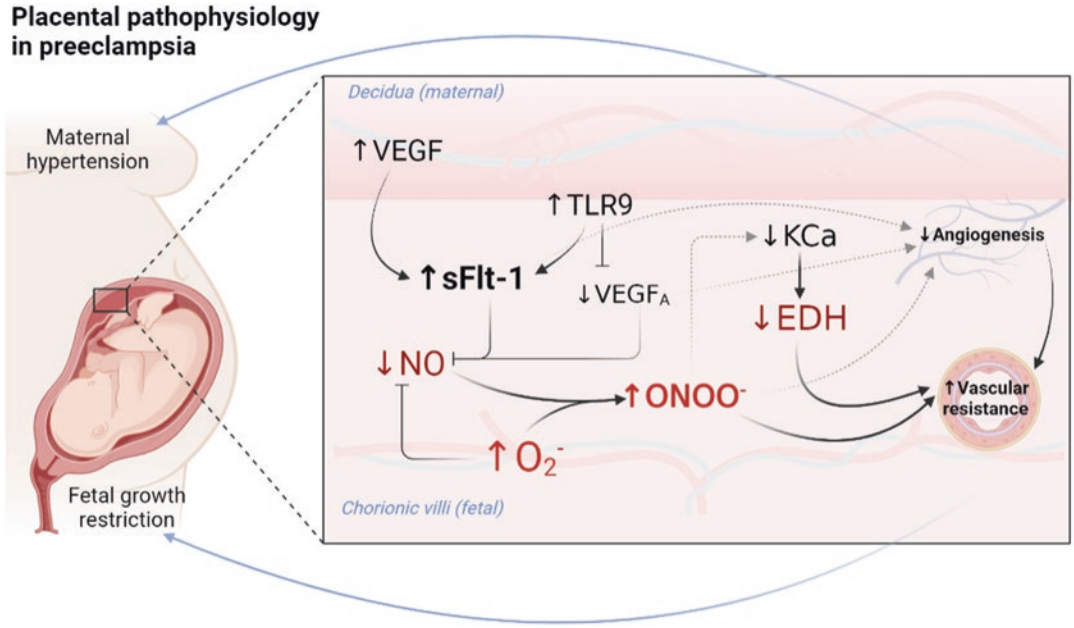

Fig. 6.1 Placental pathophysiology in preeclampsia. In decidual tissue of preeclamptic pregnancies, a higher expression of VEGF induces enhancement of soluble VEGF receptor 1 (sFlt-1) (Fan et al. 2014). The levels of sFlt-1 also increase by activation of toll-like receptor 9 (TLR9), a mechanism that decreases the levels of $VEGF_A$ in the foetoplacental interface (He et al. 2018a). The increase of sFlt-1 and decrease of $VEGF_A$ induce a direct decrease in NO synthesis and a reduction of NO bioavailability due to increased superoxide (O_2^-) accumulation and peroxynitrite ($ONOO^-$) synthesis (Amaral et al. 2018). Additionally, $ONOO^-$ could regulate the expression and activity of calcium-activated potassium (KCa) channels, which are downregulated in preeclampsia (He et al. 2018b; Li et al. 2017). All these placental alterations contribute to adverse maternal and foetal outcomes in preeclampsia (i.e. maternal hypertension and foetal growth restriction). (Created with BioRender.com)

The consequence of cellular stress (plus hypoxia) is the cessation of cell proliferation and trophoblast apoptosis. However, a multi-step model indicates that chronic maternal vascular inflammation may generate preeclampsia under normal placentation conditions (Staff et al. 2014). Staff et al. propose acute atherosis, a spiral artery lesion, as a late stage of preeclampsia, including pregnancies with dysregulated maternal tolerization to allogeneic trophoblasts (with abnormal or poor placentation) and pregnancies with preexisting maternal chronic vascular inflammation (with normal placentation). The alteration of flow resulting from vascular lesions promotes dysfunctional uteroplacental perfusion with ER stress and placental oxidative stress (Staff et al. 2013). As a result, arterial stiffness is significantly higher in preeclampsia versus normal pregnancy, and flow-mediated dilation deteriorates in preeclamptic patients (Mannaerts et al. 2017).

Oxidative stress may cause protein carboxylation, lipid peroxidation, and DNA oxidation, all of which have been observed in the placenta of patients with preeclampsia (Chaiworapongsa et al. 2014). In this sense, ultrastructure and histomorphometric studies of the human umbilical cord vessels showed that cells from preeclamptic pregnancies are highly disorganized, showing discontinuity of vascular endothelium and edematous spaced smooth muscle cells (Almasry et al. 2016). In addition, in the placenta from preeclampsia, there is a significantly altered expression of tight junction proteins (claudins and occludins) compared with control placentas (Zhang et al. 2019). The alteration of tight junctions of trophoblasts and the endothelial cell could affect the barrier properties of the placenta in preeclampsia, which is related to the findings that plasma from preeclamptic women alters the brain-blood barrier in an in vitro model with human brain endothelial cell line (Bergman et al. 2021).

Additionally, maternal immune system dysregulation has been described in the literature. Hence, the immune imbalance results in an upregulation of pro-inflammatory cytokines, and a decrease of anti-inflammatory cytokines like interleukin-10 (IL-10) (Cubro et al. 2018). On the other hand, an ischaemia/hypoxia environment contributes to raising the concentration of reactive oxygen species (ROS), reducing the bioavailability of NO and prostacyclin (PGI2), attenuating vasodilator responses, and increasing vasoconstrictors like thromboxane A_2 (Amaral et al. 2018; Bowen et al. 2005). Specifically, the placental oxidative stress leads to NO depletion through the quick reaction between the superoxide (O_2^-) and NO to peroxynitrite ($ONOO^-$) accumulation (González et al. 2011b). The $ONOO^-$ is a strong and relatively stable oxidant species that could downregulate the expression and activity of calcium-activated potassium channels (KCa) (Yang et al. 2012), proteins involved in endothelium-derived hyperpolarization (Fig. 6.1).

All these mechanisms can lead to abnormal placenta function and, consecutively, to preeclampsia. In addition, generalized vascular constriction is present in preeclampsia compared with physiologic vasodilation in normal pregnancy (Karumanchi et al. 2005). Another marker that mediates endothelial dysfunction, which has been suggested to contribute to the development of preeclampsia, is the presence of asymmetric dimethylarginine (ADMA). ADMA is an endogenous inhibitor of nitric oxide synthase (NOS) (Böger et al. 2010) that is increased in women with preeclampsia, even before the development of the disease (Yuan et al. 2017), suggesting a role in the development of endothelial dysfunction and exacerbated inflammatory response (Garg et al. 2018). Besides the reduction of NO bioavailability, the higher placental vascular resistance associated with preeclampsia could be related to the downregulation of expression of small (SKCa) and intermediate (IKCa) KCa in placental arteries from preeclamptic pregnant women (Li et al. 2017). Also, in placental arteries, He et al. (2018b) demonstrated that the expression of $\beta 1$ subunit of large-conductance KCa (BKCa) is decreased in preeclampsia, which correlates with impairment vasodilation induced by NO. The alterations in KCa expression could be due to higher levels of $ONOO^-$ in preeclamp-

sia (Yang et al. 2012). As is shown in Fig. 6.1, the impairment of KCa expression is directly related to the alteration of EDH, a physiological mechanism to regulate vasodilation and vascular resistance.

The development of endothelial dysfunction in preeclampsia, mediated by oxidative stress, could be reversed with the supplementation of antioxidant vitamins (Beazley et al. 2005; Chambers et al. 2001). However, recent reports indicate that vitamin C and vitamin E supplementation just not decreases the risk of preeclampsia and neonatal outcome but also increases the risk of gestational hypertension in women at risk of preeclampsia and low birth weight in neonates (Basaran et al. 2010; Rahimi et al. 2009). Due to this controversy, other strategies like L-arginine or L-citrulline supplementation (for enhancement of NO synthesis) could provide a feasible means to prevent the detrimental effects of preeclampsia (Weckman et al. 2019).

6.3 Alterations in Trophoblastic Invasion and Placental Ischaemia/Hypoxia

The adequate trophoblast invasion and the establishment of uteroplacental blood flow occur during early human pregnancy when the cytotrophoblast stem cells follow two different pathways (Genbacev et al. 1996). In the villous pathway, cytotrophoblast cells fuse to form multinucleated syncytiotrophoblast. In the extravillous pathway, cytotrophoblast cells differentiate in interstitial extravillous and endovascular extravillous trophoblasts (Ji et al. 2013), which extensively colonize the decidua and adjacent uterine myometrium, destroying and replacing the endothelium of maternal vessels forming a low-resistance arterial system (Davison et al. 2004; Granger et al. 2002).

It has been proposed that in preeclampsia, the invasion of the uterine spiral arteries is limited to the proximal decidua with 30–50% of the spiral arteries of the placental bed without endovascular trophoblast remodelling (Granger et al. 2002). Chaiworapongsa et al. reported in 2014 that in preeclampsia (and eclampsia), the myometrial segment of the spiral artery fails to undergo a physiological transformation during the second trimester, which could explain the uteroplacental ischaemia effect (Chaiworapongsa et al. 2014). In preeclamptic rat models, the depth of endovascular trophoblast invasion of spiral arteries (observed on day 18) and its effect on vascular remodelling show that the mesometrial triangle contained significantly more endovascular trophoblasts than normal rats. In contrast, uterine spiral arteries contained a higher content of fibrinoid and vascular smooth muscle (Geusens et al. 2008, 2010). In vitro studies have shown that oxygen tension may regulate trophoblast differentiation and regulate placental organization, which is associated with DNA methylation and mitochondrial dysfunction (Chakraborty et al. 2016; Novakovic et al. 2017).

Furthermore, in vitro placental barrier model cultured under low oxygen exposure (3–8%) has shown an increase in the expression of oxygen-sensitive transcription factors, such as hypoxia-inducible factor 1α (HIF1α), along with the decrease of barrier permeability (Wong et al. 2020). In experimental ischaemia induced in animal models through reduction of uterine perfusion pressure (RUPP) by ligation of ovarian or uterine vessels, it has been observed that it causes a 60–90% decline in the arterial blood flow along with elevated blood pressure, as well as an increase of urinary albumin excretion, severe endotheliosis, mesangial expansion, and premature delivery (Fushima et al. 2016). Also, other RUPP models lead to a hypertensive state, mitochondrial dysfunction, impaired vasodilation, decreased IL-10, and increased ROS and pro-inflammatory cytokines (Chen et al. 2019; Vaka et al. 2018). In preeclampsia, the reduction of uteroplacental perfusion as a consequence of an abnormal cytotrophoblast invasion of spiral arteries has been indicated as the first event in the disease's pathophysiological mechanism (Staff 2019). At the same time, the placenta develops adaptive mechanisms under pathologi-

cal conditions, including regulation of non-coding RNAs (ncRNAs) (Wang et al. 2019a) and mitochondrial adaptations (Holland et al. 2018), which reflects the complexity of the preeclamptic mechanisms.

6.4 Proangiogenic and Antiangiogenic Factors

Placental vascularization involves vasculogenesis, angiogenesis, and pseudo-vasculogenesis or maternal spiral artery remodelling. These processes require a delicate balance of proangiogenic and antiangiogenic factors (Jardim et al. 2015). Growth factors such as vascular endothelial growth factor (VEGF) and placental growth factor (PlGF) are secreted by both decidual and decidual natural killer cells (dNK), which along with trophoblasts, participate in the spiral artery remodelling during normal pregnancy (Jia and Li 2020). Therefore, they have been considered crucial for invasion and pseudo-vasculogenesis (Noris et al. 2005). PlGF is a member of the VEGF family, predominantly expressed in the placenta. However, it is also expressed at low levels in many other tissues. Its primary role is angiogenesis in response to pathological ischaemia or injury through enhancing the activity of VEGF by competitively binding to its receptors (Chau et al. 2017). A study in a pregnant population between 16 and 19 weeks of gestation revealed that the decrease of PlGF levels below the cut-off values (62.5 pg/ml) increases the risk of preeclampsia, independently of medical history (Dover et al. 2013). Additionally, Romero et al. (2008) reported in a longitudinal nested case-control study that patients destined to develop preeclampsia (term or preterm) and those who delivered a small gestational age neonate had lower plasma concentrations of PlGF than those with a normal pregnancy throughout gestation.

VEGF is a potent endothelial cell-specific mitogen which interacts with two high-affinity receptors: kinase insert domain receptor (KDR) (also known as VEGFR-2 or flk-1) and FMS-like tyrosine kinase 1 (Flt-1), also known as VEGFR-1 and its soluble variant sFLT-1 (soluble fms-like tyrosine kinase-1) (Chau et al. 2017; Davison et al. 2004). Flt-1, which has a higher affinity for VEGF, is required for endothelial cell morphogenesis (embryogenic organization of vasculature) (Krueger et al. 2011), whereas flk-1/KDR is involved in the mitogenesis (cell proliferation) (He et al. 1999; Roman et al. 2002). Alternative splicing of Flt-1 results in the production of an endogenously secreted protein referred to as sFlt-1 (also known as sVEGFR-1) that can bind and antagonize the action of circulating VEGF, preventing the interaction of this growth factor with its endogenous receptors (Davison et al. 2004).

There is enough evidence to support the hypothesis that the ischaemic placenta contributes to endothelial cell dysfunction in maternal vasculature by inducing an alteration in the balance of circulating levels of angiogenic factors (VEGF/PlGF) and antiangiogenic factors (sFlt-1) (Gilbert et al. 2008). This imbalance is found in overt preeclampsia, although an increase in the maternal plasma concentration of s-Eng between the first and second trimesters of pregnancy has been observed (Erez et al. 2008). Studies in villous explants from normal-term placenta exposed to 1% O_2 (hypoxia) showed a significant increase in sFlt-1 synthesis (Ahmad and Ahmed 2004; Groesch et al. 2011). Trophoblast cells from preeclamptic placenta exposed to lower oxygen levels secrete high levels of sEng and sFlt-1 but lower PlGF (Gu et al. 2008). In hypertension induced by RUPP in pregnant rats, maternal plasma and amniotic fluid concentrations of sFlt-1 increase; meanwhile, plasma concentrations of VEGF and PlGF decrease (Gilbert et al. 2007). Parchem et al. (2018) reported that the PlGF KO ($Pgf^{-/-}$) mice present an elevation of serum sFlt-1 and placental Flt-1 levels in the junctional zone spongiotrophoblasts, parietal trophoblast giant cells, and decidua, together with alterations in the placental architecture despite it did not develop the classic signs of preeclampsia. However, Maynard et al. (2003) showed that the administration of sFlt-1 to pregnant rats via adenovirus-mediated gene transfer induces hypertension, proteinuria and reduction of circu-

lating levels of free VEGF and PlGF in similar concentrations to those seen in preeclamptic women. In normal pregnant rats treated with infusion of sFlt-1, significant increases in mean arterial pressure, pre-proendothelin (a potent vasoconstrictor) expression in the renal cortex (Murphy et al. 2010, 2012), and superoxide levels in the placenta, renal cortex, and aorta were observed (Tam Tam et al. 2011).

Additionally, it has been described that toll-like receptor 9 (TLR9), a receptor that plays a crucial role in innate immunity, suppresses angiogenesis by differentially regulating the expression of VEGFA and sFlt-1 at the feto-maternal interface in pregnant rats treated with a TLR9 agonist. These findings correlate with higher expression of TLR9 in the placenta of pregnant women with severe preeclampsia (He et al. 2018a). In summary, the suppression of expression and/or activity of antiangiogenic receptors like sFlt-1 and TLR9 represents a novel and promising therapeutic strategy in preeclampsia (Fig. 6.1).

Tube formation assay is used to demonstrate the angiogenic activity of endothelial cells. In endothelial cells exposed to the sera of preeclamptic patients, tube formation is inhibited, causing the alteration of the angiogenic mechanism. The addition of exogenous VEGF and PlGF to preeclamptic patients' serum restores tube formation in endothelial cells (Maynard et al. 2003). Quantifying angiogenic and antiangiogenic factors in the placenta of women with severe preeclampsia and IUGR indicates a significant increase in the levels of Flt-1, sFlt-1, and total $VEGF_A$ mRNA, without differences between mRNA levels of PlGF and VEGFR-2 (Ahmad and Ahmed 2004; Tsatsaris et al. 2003). The serum sFlt-1 measured in 120 women with preeclampsia showed abrupt increases in week five before the onset of preeclampsia, along with a decrease in circulating levels of VEGF and PlGF (Levine et al. 2004). In an extensive study in 4099 nulliparous women, the positive sFlt-1:PlGF ratio (defined as a ratio > 38) predicts a high absolute risk of preterm or severe preeclampsia (Sovio et al. 2017). However, a study indicates that women with preeclampsia have increased VEGF expression, primarily in decidual cells. Also, they exhibit a dramatic increase in sFlt-1 expression in extravillous trophoblasts, suggesting that a local increase in VEGF can trigger placental overexpression of sFlt-1, potentially contributing to the development of preeclampsia (Fan et al. 2014) (Fig. 6.1).

VEGF ($VEGF_A$, mainly) (Tsatsaris et al. 2003) increases NO synthesis in human, rabbit, and bovine endothelial cells (Gélinas et al. 2002; Wang et al. 2019b). Similar effects have been found with $VEGF_E$, another variant of the VEGF family (Cudmore et al. 2006). NO is a potent vasodilator synthesized from the cationic amino acid L-arginine by the isoenzyme endothelial nitric oxide synthase (eNOS) (Noris et al. 2005). The release of NO is an essential mechanism for the angiogenic properties of VEGF (Bussolati et al. 2001; Wang et al. 2019b). Women with hypertensive disorders showed a decrease in plasma of PlGF and eNOS levels (Zawiejska et al. 2014) and higher circulating sFlt-1 and sEng concentrations (Sandrim et al. 2008). The increased availability of sFlt-1 might counteract the NO-induced vasodilatory effects of VEGF, resulting in hypertension and proteinuria by blocking the effects of $VEGF_A$ (Chaiworapongsa et al. 2014). So, NO levels inversely correlate with antiangiogenic factor levels in preeclampsia. Hence, a strategy to improve NO synthesis could impact antiangiogenic factor levels. Indeed, the L-arginine supplementation, like a precursor of NO, in a rat preeclampsia model regulates antiangiogenic factors (Oludare et al. 2018).

The addition of VEGF in the culture medium led to a concentration-dependent increase in cGMP levels, an indicator of NO production that was reduced by the eNOS inhibitor nitro-L-arginine methyl ester (L-NAME). Moreover, VEGF promoted the NO-dependent proliferation and arrangement of tube structures in HUVEC. The inhibition of NO synthesis by L-NAME resulted in a 67% decrease in VEGF functions. In addition, the chelators of intracellular calcium, calmodulin antagonists, and specific inhibitors of phosphoinositide-3-kinase (PI3K) led to an inhibition of the VEGF-induced NO release (Papapetropoulos et al. 1997). Subsequently, He

et al. in 1999 evaluated the response of bovine aortic endothelial cells (BAECs) in treatment with VEGF (10 ng/ml), confirming that there is an increase in the phosphorylation of phospholipase Cγ1 (PLCγ1) and higher inositol 1,4,5-trisphosphate (IP3) levels via the activity of members of the Src kinase family (He et al. 1999). The mechanism involved in VEGF effects on NO release in BAEC was determined using pharmacological inhibitors of NOS (L-NAME), protein kinase C (PKC) (calphostin C), and PLC (U73122), which completely blocked the release of NO stimulated by VEGF (Gélinas et al. 2002; He et al. 1999). Recent information confirms the angiogenic role of $VEGF_A$ and its action mechanism through downstream signalling molecules, PLCγ1 and PKC, in different disorders related to proliferation and angiogenesis (Matsushima et al. 2020; Shinya et al. 2015).

With the information provided, we propose that shallow invasion of cytotrophoblast cells to uterine spiral arteries leads to a reduction in placental blood flow and could be associated with an imbalance of proangiogenic/antiangiogenic factors and impaired production of NO in the development of preeclampsia. Furthermore, these observations suggest that excess sFlt-1 circulating contributes to the pathogenesis of preeclampsia by neutralization of VEGF and PlGF (Chau et al. 2017; Davison et al. 2004; Levine et al. 2004), preventing physiological vasorelaxation and angiogenic/proliferative response (Papapetropoulos et al. 1997) (see Fig. 6.1).

6.5 Role of Nitric Oxide in Preeclampsia

NO is a critical factor in maternal and foetal homeostasis during pregnancy, maintaining a proper placental blood flow, foetal nutrition, and oxygenation leading to normal foetal growth and development (Casanello and Sobrevia 2002; Zullino et al. 2018). During normal pregnancy in humans and other animal species, physiological vascular adaptations, such as increased blood volume, increased cardiac output, decreased vascular resistance, and increased GFR and RPF (Jeyabalan and Conrad 2007), are accompanied by an increase in endogenous NO (Hodžić et al. 2017; Poniedziałek-Czajkowska et al. 2011). In experiments with pregnant rats, NO inhibition induced hypertension and proteinuria (Karumanchi et al. 2005), while inhibition of NOS activity increases perfusion pressure and potentiates the vascular response to noradrenaline, electrical stimulation, and angiotensin (Chu and Beilin 1993). During pregnancy, the higher production of NO is associated with increased kidney expression of inducible (iNOS) and neuronal (nNOS) NO synthases (Alexander et al. 1999). In RUPP pregnant rats, there is a decrease in protein expression of both iNOS and nNOS at day 19 of gestation, which is associated with impaired kidney function (Alexander et al. 2001). So, kidney disease and proteinuria in preeclampsia could be directly related to the impairment of NO signalling in the kidney. In addition, women with preeclampsia showed impaired endothelium-dependent vasodilatation in arteries (Karthikeyan and Lip 2007) and lower levels of NO in plasma (Tashie et al. 2020). Although some authors have reported an increase in NO serum concentrations in preeclamptic patients compared to the healthy pregnant control group (Acauan Filho et al. 2016), the main conclusion from the evidence is that there is a decrease of NO bioavailability in preeclampsia (Fig. 6.1).

Several biological functions of NO are mediated by the activation of the soluble guanylyl cyclase (sGC) and the subsequent production of cyclic GMP (cGMP) (Montfort et al. 2017; Nishizawa et al. 2009). In normal pregnancy, circulating levels of cGMP (as a representation of increased NO) and nitrite/nitrate metabolites are elevated compared with no pregnancy state (Nishizawa et al. 2009; Tropea et al. 2015). These increases could be essential for vasodilatory responses during normal gestation (Conrad et al. 1993; Gilbert et al. 2008). Conspicuously, a study with preeclamptic patients showed higher plasma nitrate levels and cGMP than normotensive pregnant women. However, a refined analysis indicates no positive correlation between the serum NO metabolites or cGMP with blood pressure (Nishizawa et al. 2009). This information sug-

gests that high levels of circulating NO metabolites are an epiphenomenon resulting from vasoconstriction in preeclampsia.

Endothelial NO synthesis comes from converting L-arginine to L-citrulline by Ca^{2+}/calmodulin-dependent eNOS, a process associated with L-arginine transport via y^+/CATs system (cationic amino acid transporters). In HUVEC, L-arginine transport is preferentially mediated by y^+/CAT-1 system (Casanello and Sobrevia 2002; Poniedziałek-Czajkowska et al. 2011) and to a lesser extent by the y^+L system (González et al. 2004). Also, it has been established that insulin increases L-arginine transport via hCAT-1 due to a higher *SLC7A1* gene expression, a phenomenon proposed to be, at least in part, responsible for the increased NO synthesis (González et al. 2011a). In the preeclamptic placenta, the expression of mRNA for human cationic amino acid transporter (hCAT 1, 2, and 4) and 4F2hc (system y^+L) showed no significant difference compared with the normal placenta (Speake et al. 2003). However, studies in HUVEC from pregnancies complicated with IUGR have shown that 24 h of exposure to 1% or 2% O_2 (hypoxia) leads to a reduction of L-arginine transport and eNOS activity (Casanello et al. 2009), possibly due to reduced expression of hCAT-1 and hCAT-2B transporters (Casanello and Sobrevia 2002). Platelet studies from spontaneously hypertensive pregnant rats (SHR-P) also show a reduction in L-arginine transport, eNOS activity, and expression of sGC, limiting factors for the production and effect of NO (Mullershausen et al. 2003; Ognibene et al. 2010). In contrast, an increase in L-arginine transport activity has been reported, mainly caused by the high activity of the y^+ system in preeclampsia (Speake et al. 2003) and higher eNOS protein and eNOS mRNA levels in IUGR. These changes could be an adaptive response to decreased NO and L-arginine transport (Casanello and Sobrevia 2002).

The strong relationship between the L-arginine-NO-cGMP pathway and corticotropin-releasing hormone (CRH) and other CRH-like peptides, such as UCN and UCN II (Urocortin and Urocortin II, respectively), has been described (Chen et al. 2005; Lu et al. 2018). These proteins have an essential role in the endothelium for vascular relaxation and hyperpolarization (Seçilmiş et al. 2007) and appear to play a significant role in the mechanisms responsible for maintaining human pregnancy. Treating normal placental explants with CRH or UCN induces a 2- to 2.5-fold increase in eNOS mRNA and eNOS protein, increasing cGMP levels after 5 min. However, the evaluation of the same treatment in preeclamptic placental explants showed a significant reduction in CRH/CRH-related peptide-induced cGMP response without changes in the mRNA of eNOS, iNOS, or sGC. The placental CRH/CRH-R system is expressed almost exclusively in syncytiotrophoblast, associated with the pathological mechanisms leading to a poorly perfused foetoplacental unit and abnormal vascular resistance (Karteris et al. 2005; Roberts et al. 1989) and, therefore, contributing to obstetrical pathophysiologies, such as preeclampsia, endometrial growth retardation, and preterm delivery (Makrigiannakis et al. 2018). In addition, novel information on the inflammatory process suggests that CRH and UCN II in trophoblast cell lines act as inflammatory molecules via NFkB and MAPK, modulating the normal hormonal/immune mechanisms occurring during the embryo implantation and parturition (Novembri et al. 2015). However, in the second trimester of pregnant women, a higher amniotic CRH and UCN effect is associated with a lower foetal size but not with neonatal birth outcomes (La Marca-Ghaemmaghami et al. 2017).

The NO mechanisms that induce endothelial cell migration and neovascularization remain unclear and need further investigation. Studies dealing with endothelial cells from different vascular beds have shown that cells stimulated with NO donors, such as S-nitroso-L-glutathione (GSNO) and S-nitroso-N-acetylpenicillamine (SNAP), increase PI3K and protein kinase B (PKB/Akt) activities. Meanwhile, the co-treatment with the PI3K inhibitor, wortmannin, suppresses the induction of PKB/Akt phosphorylation and activation by GSNO (Kawasaki et al. 2003). In pregnant rats, the intrauterine injection of wortmannin or rapamycin (mTOR inhibitor)

inhibits embryo implantation. Furthermore, it is associated with decreased iNOS and eNOS uterine expression and a significant reduction of p-PKB and p-S6K1 (Zeng et al. 2013). Since sGC is a target of NO, the treatment with 8-bromo-cGMP (300μM), a stable cGMP analogue, increases PI3K activity and a specific sGC inhibitor (ODQ, 1μM) suppresses the induction of PI3K activity by NO (Kawasaki et al. 2003). SNAP and cGMP analogue presence in bovine aortic endothelial cells (BAEC) increases cell migration in a concentration-dependent manner. In contrast, NO-induced endothelial cell migration is significantly attenuated in cells pre-treated with PI3K or sGC inhibitors. These results demonstrate that NO promotes endothelial cell migration, neovascularization, and embryo implantation via cGMP and PI3K-PKB/Akt and mTOR pathway (Kawasaki et al. 2003; Zeng et al. 2013).

6.6 L-Arginine Supplementation in Preeclampsia

Several therapies have been proposed for treating preeclampsia: L-arginine, magnesium sulphate, aspirin, calcium supplements, antioxidant vitamins, NO donors (Eiland et al. 2012; Gui et al. 2014), anti-hypertensive drugs (Abalos et al. 2018), or maternal diet regulation (Milman et al. 2016). Diet supplementation has been explored, and some examples are the studies with omega-3 polyunsaturated fatty acids (PUFA) to limit placental inflammation and oxidative stress (Jones et al. 2014), with docosahexaenoic acid (DHA) that reduced the risk of preeclampsia and the risk of severe preeclampsia in a prospective cohort of 65,220 singleton pregnancies (Arvizu et al. 2019) or the vitamin D supplementation to decrease the probability of developing preeclampsia (Pilz et al. 2018; Serrano-Díaz et al. 2018). In a rat model, prenatal supplementation with vitamin D reduced blood pressure and normalized VEGF and Flt-1 (Nema et al. 2020). On the other hand, therapies based on L-arginine supplementation could mainly improve the vascular alterations associated with preeclampsia and IUGR. These studies will be reviewed in the following paragraphs.

The L-arginine supplementation showed positive results in endothelial NO synthesis in diabetic rats (Kohli et al. 2004) and increased NO and L-citrulline in plasma correlated with hypotensive effect in healthy males (Mehta et al. 1996), but still the evidence is contradictory. The study of Alvares et al. showed that acute L-arginine supplementation does not increase plasma concentration of NO in healthy volunteers with normal plasma concentrations of ADMA (Alvares et al. 2012), but the L-arginine plasma levels (Evans et al. 2004), the mixed expired NO, and L-citrulline plasma levels (Kharitonov et al. 1995; Mehta et al. 1996) could increase after orally administered L-arginine. Significantly, eNOS activity depends on extracellular L-arginine rather than intracellular L-arginine (MacKenzie and Wadsworth 2003; Shin et al. 2011). L-Arginine is the substrate for the synthesis of NO and L-citrulline, and the recycling of L-citrulline to L-arginine also contributes for NO production in endothelial cells (Flam et al. 2007). In rat aorta endothelial cells, concentrations of L-arginine from 60 to 200μmol/L increase cell viability and NOS activity (Suschek et al. 2003). In HUVECs, insulin increases NOS-dependent NO synthesis in cells incubated with 100μM L-arginine (Rojas et al. 2020). In addition, extracellular media containing 200μM L-arginine is necessary for umbilical vein relaxation induced by insulin (there is no effect on vein relaxation in the absence of extracellular L-arginine) (González et al. 2011a). These data provide functional evidence that supplementing L-arginine from the extracellular medium optimizes the synthesis from eNOS (MacKenzie and Wadsworth 2003).

In experiments with different species, oral or intravenous administration of L-arginine does not cause sickness or death in any animal. A 70-kg human subject should tolerate long-term parenteral and enteral supplemental doses of 6 and 15 g/days of L-arginine, respectively, and a basal amount of L-arginine (4–6 g/days) from regular diets (Wu et al. 2007a). In rats, supplementation of L-arginine in the diet during early gestation or

throughout pregnancy increases implantation sites, embryonic survival, and litter size in association with elevated levels of L-arginine and its metabolites (NO, L-ornithine, and L-proline) (Zeng et al. 2008). In RUPP rat models, supplementation with L-arginine reversed the MAP increase, proteinuria, alterations in kidney function (Alexander et al. 2004), and natriuresis (Granger and Alexander 2000). Moura et al. showed that in SHR models, the supplementation with α-methyldopa, α-methyldopa plus L-arginine 2%, and just L-arginine 2% generates a significant fall in mean blood pressure after 20 days of treatment (Moura et al. 2006). Normal pregnant rats infused with sFlt1 exhibit a significant increase in MAP, a reduction in NO production by ∼70% in isolated glomeruli, and an increase of pre-pro-ET-1 in the renal cortex. The supplementation with 2% L-arginine in drinking water restored these parameters (Murphy et al. 2012). L-Arginine supplementation lowered blood pressure and protein excretion in salt-induced hypertensive female Sprague-Dawley rats (Arikawe et al. 2019). In parallel, adding 200μmol/L L-arginine protects against hydrogen peroxide (H_2O_2)-induced cell death in rat aortic endothelial cells. Without L-arginine, these cells are more sensitive to H_2O_2-induced cell death. A NO donor emulates the protective effects of L-arginine, demonstrating that L-arginine-dependent cell survival is attributable to NO synthesis (Suschek et al. 2003).

In human pregnancy, L-arginine supplementation improves uterine-placental circulation, prolonging pregnancy in patients with gestational hypertension and decreasing maternal BP. Furthermore, L-arginine is associated with a significant reduction in PE risk and has therapeutic effects on other pregnancy complications (Gui et al. 2014; Hsu and Tain 2019; Weckman et al. 2019). Concerning the antihypertensive effect of L-arginine, a prospective and randomized placebo-controlled study determined whether 3 g/day of L-arginine for 3 weeks regulates BP in patients with PE. This study showed significant results showing lowered systolic blood pressure (SBP) and diastolic blood pressure (DBP) and decreased MAP in the L-arginine group compared with the placebo group. In addition, the nitrate/nitrite (NOx) concentration in 24-h urinary excretion was significantly higher in the L-arginine group, such as plasma levels of L-citrulline (Rytlewski et al. 2005). Other studies show that in patients with gestational hypertension and proteinuria treated with 20 g/500 mL per day of intravenously administrated L-arginine for 5 days, followed by 4 g/day orally administrated for 2 weeks, there is a decrease in SBP and DBP (compared to placebo) and a significant increase of the period of "latency," defined as the time in days from entry into study until the time at delivery (Facchinetti et al. 2007; Rytlewski et al. 2006). Also, the median value of gestational age at delivery was lower in the placebo versus the L-arginine group (Facchinetti et al. 2007). However, other studies have shown that administering 12 g of L-arginine per day in preeclamptic women for more than 5 days does not change DBP (Staff et al. 2004). Similar results were reported by Neri et al. in a study including 80 pregnant women that received 4 g/day oral supplementations of L-arginine or placebo; they found no changes in BP after 10–12 weeks of treatment. However, women treated with L-arginine gave birth to children with higher gestational age (38.2 vs 36.6 months) and higher birth weight (3094 g vs 2836 g) than the placebo group. Additionally, the rate of neonatal complications was more likely in a placebo group than in groups supplemented with L-arginine (15% vs 2.5%) (Neri et al. 2010).

Valdillo-Ortega et al. showed the effect of L-arginine supplementation on preventing PE in women at risk. In 228 pregnant women at high risk of PE, the intake of two cereal bars per day providing 6.6 g of L-arginine and antioxidant vitamins before 24 weeks of gestation showed a significant reduction in the incidence of PE/eclampsia, along with a decrease in the overall rate of preterm birth and decline in the SBP and DBP. The beneficial effects were not statistically significant in the women receiving bars containing just antioxidant vitamins (Vadillo-Ortega et al. 2011). In their systematic review, Gui et al. suggested that L-arginine is more effective in reducing PE or eclampsia incidence than a pla-

cebo (odds ratio 0.348; 95% confidence limits 0.25, 058), with a significative reduction in DBP (Gui et al. 2014). A recent meta-analysis involving 1452 complicated pregnancies across eight countries concludes that "L-arginine supplementation during pregnancy had beneficial effects on birth weight, gestational age, and small for gestational age risk of infants in hypertensive and IUGR pregnancies." The effects of L-arginine supplementation could be attributed to increased NO in plasma, but due to the heterogeneity in the dosage and interventions, there is difficulty in the clinical application of L-arginine supplementation (Xu et al. 2022).

6.7 L-Citrulline Supplementation in Pregnancy

Other studies have focused on the potential benefit of L-citrulline supplementation because L-citrulline recycles L-arginine and improves NO synthesis (Morita et al. 2014). Recently, watermelon ingestion, an L-citrulline-rich source, has improved endothelial function and blood pressure (Volino-Souza et al. 2022). Furthermore, oral L-citrulline supplementation raises plasma L-arginine concentration and augments NO-dependent signalling in a dose-dependent manner in healthy volunteers (Schwedhelm et al. 2008) and after exercise protocol (Valaei et al. 2022). This evidence highlights the potentiality of L-citrulline supplementation, alone or in combination with L-arginine.

Supporting the relevance of L-citrulline plasma levels during pregnancy, a study with adolescent pregnant women with preeclampsia reveals lower levels of L-citrulline in plasma compared with normotensive pregnant (Hidayat et al. 2022). L-Citrulline supplementation also shows positive results in a mouse model of preeclampsia, with beneficial effects on maternal vascular health during pregnancy and the postnatal period (Gemmel et al. 2021). In Dahl salt-sensitive rat, a model of superimposed preeclampsia, the L-citrulline supplementation (2.5 g/L in drinking water) from the day of mating to the end of the lactation period reduced gestational hypertension, proteinuria, and levels of circulating sFlt-1. L-Citrulline improves placentation and foetal growth in a mechanism that involves higher nitric oxide synthesis in the maternal aorta and improvement of endothelium-derived hyperpolarizing factor-mediated vasorelaxation in resistance arteries (Man et al. 2022). In a preeclampsia-like rat model, the L-citrulline-supplemented group showed a reduction of SBP, DBP, and MAP and a tendency to increase the pup weight and pup/placenta weight ratio. Also, the endothelium-dependent relaxation was improved in the supplemented group compared with the preeclampsia-like model (Gemmel et al. 2021). In a Dahl salt-sensitive rat, parents' L-citrulline supplementation improved placental insufficiency and foetal growth, associated with angiogenesis and reduced fibrosis and senescence in the placentas. L-Citrulline improves the NO synthesis in rat aorta, and endothelium-derived hyperpolarization vasodilation mechanism meanwhile downregulated genes associated with hypertension and maternal inflammation (Man et al. 2022). In a model of IUGR, L-citrulline and L-arginine increase foetal growth, but just L-citrulline improves foetal muscle protein synthesis. The authors conclude that "L-Citrulline increases fetal growth in a model of IUGR, and the effect may be mediated by enhanced fetal muscle protein synthesis and/or increased NO production" (Bourdon et al. 2016). The maternal L-citrulline supplementation increased foetal weight, the foetal weight/placental weight ratio, and index of placental efficiency, inducing a higher expression of placental genes involved in angiogenesis (Tran et al. 2017). From this evidence, Fig. 6.2 highlights the potential benefits of L-citrulline supplementation in complicated pregnancies. Additionally, L-citrulline has some advantages over L-arginine because it is a neutral amino acid, which improves its intestinal absorption when orally administrated, and is an allosteric inhibitor of arginase, improving the L-arginine bioavailability (Morita et al. 2014). Further studies on L-citrulline during human pregnancy are necessary to determine its safety and therapeutic potential.

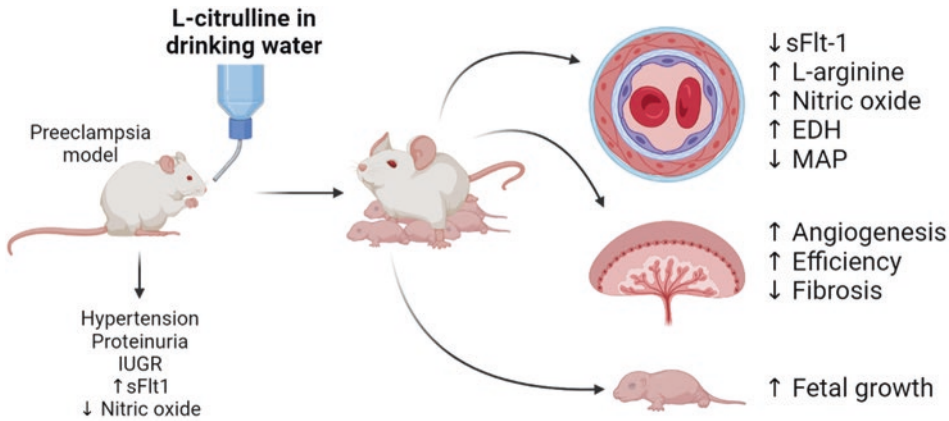

Fig. 6.2 L-Citrulline supplementation for preeclampsia treatment. In preeclampsia animal models (RUPP pregnant rats, L-NAME-treated pregnant rats, salt-induced hypertension), the oral L-citrulline supplementation improve several pregnancy parameters. In circulation and vascular system, there is reduction of soluble VEGF receptor 1 (sFlt-1) and mean arterial pressure (MAP), meanwhile increasing of plasma and placental levels of L-arginine and NO. The overall result of molecular and systemic changes induces by L-citrulline is the improvement of vascular relaxation mechanisms, like L-arginine/NO pathway and endothelium-derived hyperpolarization (EDH). For foetal well-being, the regulation of these mechanisms results in normalization of foetal growth. (Created with BioRender.com)

6.8 Conclusions and Perspectives

Food supplementation with L-arginine in other populations, for example, effects of watermelon supplementation (L-citrulline/L-arginine: 1.35 g/0.65 g two times per day) for 6 weeks in individuals with pre-hypertension (134/77 ± 5/3 mm Hg), resulted in an improvement in cardiovascular parameters such as brachial pulse pressure, aortic systolic blood pressure, aortic pulse pressure (Figueroa et al. 2011). Likewise, Zucker diabetic fatty rats were supplemented with watermelon pomace juice, whose contents of L-citrulline (2014 mg/L) and L-arginine (1150 mg/L), covering 71% of total free amino acids (4.5 g/L), showed an increased serum concentration of L-arginine, L-citrulline, L-ornithine, and NOx, and a decrease in serum concentrations of *D*-glucose and free fatty acids (Wu et al. 2007b). Therefore, we consider intervention in fortified foods for women with preeclampsia crucial. Unfortunately, there are currently few studies describing this topic. The L-arginine or L-citrulline supplementation has particular relevance considering that the prevalence of maternal undernutrition ranges from 10% to 19% in low- and middle-income countries (LMIC). Alongside, overweight and obesity are rising globally, especially in LMIC. The nutritional status of women at the time of conception and during pregnancy, along with nutritional status in the first 2 years of life, are important determinants of both undernutrition in childhood and obesity and related diseases in adulthood (Black et al. 2013). The studies with macronutrient food-based supplements in pregnancy show improvement rates of stillbirth, perinatal mortality, low birth weight, and small for gestational age in LMIC (Lassi et al. 2021). Although the required dose of L-arginine during pregnancy has not been determined, consumption has been estimated at ≤2–3 g/days in low-resource settings, compared with 4.3 g/day among pregnant women in the United States. So, supplemental L-arginine or L-citrulline in LMIC may reduce adverse birth outcomes associated with alterations of L-arginine-NO biosynthetic pathway (Weckman et al. 2019).

The findings about the role of NO in the pathophysiology of preeclampsia may indicate the potentiality of clinical use of L-arginine through

dietary intake or supplements. The treatment with L-arginine or L-citrulline seems promising in prolonging pregnancy and reducing BP, particularly in patients with gestational hypertension. However, there is still controversy about the antihypertensive action of exogenous L-arginine and its use as a prophylactic agent in women at high risk of preeclampsia. New studies indicate that using L-citrulline or the mix of L-citrulline plus L-arginine could have better results in preeclamptic pregnancies, mainly associated with improvement of angiogenesis, nitric oxide signalling, and placenta efficiency (Fig. 6.1). However, it is still necessary for further studies to conclude about the safety and efficacy of the treatment for human pregnancy.

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7

Maternal and Fetal Expression of ATP-Binding Cassette and Solute Carrier Transporters Involved in the Brain Disposition of Drugs

Pablo Torres-Vergara, Robin Rivera, Carlos Escudero, and Jeffrey Penny

Abstract

Evidence from preclinical and clinical studies demonstrate that pregnancy is a physiological state capable of modifying drug disposition. Factors including increased hepatic metabolism and renal excretion are responsible for impacting disposition, and the role of membrane transporters expressed in biological barriers, including the placental- and blood-brain barriers, has received considerable attention. In this regard, the brain disposition of drugs in the mother and fetus has been the subject of studies attempting to characterize the mechanisms by which pregnancy could alter the expression of ATP-binding cassette (ABC) and solute carrier (SLC) transporters. This chapter will summarize findings of the influence of pregnancy on the maternal and fetal expression of ABC and SLC transporters in the brain and the consequences of such changes on the disposition of therapeutic drugs.

Keywords

Pregnancy · Fetus · Mother · Brain · Drug disposition · Transporter · ATP-binding cassette · Solute carrier · Brain endothelial cell · Choroid plexus

Abbreviations

ABC	ATP-binding cassette
AZT	Zidovudine

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BBB	Blood-brain barrier
BCRP	Breast cancer resistance protein
BCSFB	Blood-cerebrospinal fluid barrier
CNT	Concentrative nucleoside transporter
CSF	Cerebrospinal fluid
ENT	Equilibrative nucleoside transporter
HIV	Human immunodeficiency virus
LAT	L-alpha-amino acid transporter
MCT	Monocarboxylate transporter
MRP	Multidrug resistance-associated protein
NRTI	nucleoside reverse-transcriptase inhibitors
OAT	Organic anion transporter
OATP	Organic anion transporting polypeptide
OCT	Organic cation transporter
P-GP	P-glycoprotein
Poly:IC	polyinosinic:polycytidylic acid, PXR, Pregnane X receptor
TGF	Transforming growth factor
TNF	Tumor necrosis factor alpha

7.1 Introduction

In recent years, medication usage in pregnancy has been continuously under revision. Although it is stated that any drug-based therapy should not be used unless the benefits for the mother overcome the risks to the fetus, the number of pregnant women who take medications is high (Bérard et al. 2019; Beyene and Beza 2018; Navaro et al. 2018; Viroga et al. 2013). Where pregnant women receive pharmacological treatment for pre-existing chronic disease, a dose adjustment may be required or selection of a therapeutic which presents a lower risk of teratogenicity and fetotoxicity (Leavitt et al. 2019; Millard et al. 2017; Veroniki et al. 2017). For example, in women receiving treatment for chronic hypertension with angiotensin-converting enzyme inhibitors or angiotensin receptor type 2 antagonists, safer alternatives, including methyldopa or labetalol, should be employed in pregnancy (Leavitt et al. 2019; Ringholm et al. 2019). Similarly, women infected with the human immunodeficiency virus (HIV) require antiretroviral therapy when pregnant to manage the progression of the disease and reduce the risk of perinatal infection (Fowler et al. 2016; Zash et al. 2017). According to the updated guidelines from the World Health Organization, pregnant women should receive a combined antiretroviral therapy based on three drugs. The first-line scheme is composed of tenofovir + lamivudine (or emtricitabine) + dolutegravir, and other drugs will be chosen depending on factors such as drug toxicity, hepatic comorbidities (hepatitis B or C virus), and previous use of antiretroviral drugs (Bailey et al. 2018; Irshad et al. 2021).

Drugs and endogenous mediators must cross several biological barriers to reach their target tissues. Furthermore, in pregnancy, the placenta is an additional barrier that functions to protect the developing fetus. Since the cell types that comprise these barriers express drug-metabolizing enzymes and uptake/efflux transporters with distinct phenotypes, the substrate transport characteristics at barrier sites depend on the abundance and cell localization of metabolic enzymes and transporter proteins.

In this regard, the characterization of transporters belonging to the ATP-binding cassette (ABC) and solute carrier (SLC) families, in terms of structure, substrate specificity, and expression profiles, in organs, including the intestine, liver, kidney, brain, and placenta, has led to increased understanding of the mechanisms involved in the disposition of drugs and endogenous mediators (Hillgren et al. 2013; Morris et al. 2017; Roth et al. 2012). Furthermore, in pregnancy, the placental expression of ABC and SLC transporters has been characterized at both the RNA and protein levels (Bloise et al. 2016; Joshi et al. 2016; Kallol et al. 2018; Nishimura and Naito 2005; Shuster et al. 2013). This knowledge has proved valuable in predicting the extent of fetal exposure to therapeutic drug substrates.

The physiology of pregnancy involves a series of changes mediated by endogenous factors that alter the expression of metabolizing enzymes and transporters, thereby modifying the hepatic and renal clearance and the distribution volume of drugs (Koren and Pariente 2018). Thus, although the plasma concentrations of the most commonly

used drugs are reduced in pregnancy (Koren and Pariente 2018; Pariente et al. 2016), their disposition in confined organs in both mother and fetus has been a matter of concern since the volume of distribution has increased.

One example of the above premise is the brain, an organ whose contact with the systemic circulation is tightly regulated by the blood-brain (BBB) and blood-cerebrospinal fluid (BCSF) barriers, two complex and dynamic interfaces. The restrictive nature of both barriers largely relies on the activity of ABC and SLC transporters (Morris et al. 2017), whose expression can be regulated by circulating factors from exogenous and endogenous sources (Qosa et al. 2015).

7.2 Expression of ABC and SLC Transporters in Biological Barriers Limits the Passage of Drugs to the Brain

7.2.1 Blood-Brain Barrier

The blood-brain barrier is a neurovascular unit that regulates the bidirectional passage of nutrients, endogenous mediators, xenobiotics, and their metabolites, thereby maintaining the integrity and homeostasis of the brain (Abbott 2013; Daneman and Prat 2015). The vascular bed of the BBB is composed of brain endothelial cells surrounded by a protein-based basement membrane (collagen, laminins). Cell types, including pericytes, glial cells, and neurons, are also closely associated and responsible for developing and maintaining a functional BBB phenotype (Cantrill et al. 2012; Sweeney et al. 2019).

Microvascular endothelial cells within the brain possess a phenotype that distinguishes them from the majority of peripheral endothelial cells, primarily due to the expression of highly restrictive tight junction proteins, metabolizing enzymes, SLC uptake transporters, and ABC efflux transporters (Cantrill et al. 2012). Consequently, the transcellular and paracellular transport of molecules at the BBB is tightly regulated.

The expression profile of ABC and SLC transporters involved in drug disposition in brain endothelial cells of several mammalian species, including humans, has been extensively studied. ABC efflux transporters can limit the movement of substrates from the systemic circulation into the brain while also facilitating the removal of drugs, mediators, and their metabolites from the brain parenchyma, thereby avoiding accumulation. Currently, there is consensus that the ABC transporters P-glycoprotein (P-GP, ABCB1), breast cancer resistance protein (BCRP, ABCG2), and members of the multidrug resistance-associated protein (MRP, ABCC) subfamily are highly expressed in brain endothelial cells, with phenotypical differences observed among species (Dauchy et al. 2008; Hoshi et al. 2013; Shawahna et al. 2011; Uchida et al. 2011; Warren et al. 2009). SLC transporters are primarily responsible for the uptake of molecules from the blood, and data from animal and human models have shown that members of the L-alpha-amino acid transporter (LAT), monocarboxylate transporter (MCT), concentrative nucleoside transporter (CNT), equilibrative nucleoside transporter (ENT), organic anion transporter (OAT), organic cation transporter (OCT), and organic anion transporting polypeptides (OATP) subfamilies are expressed in brain endothelial cells (Morris et al. 2017) (Fig. 7.1). Details of expression, localization, substrates, and inhibitors of mammalian brain endothelial cells ABC and SLC transporter are summarized in Table 7.1.

7.2.2 Blood-Cerebrospinal Fluid Barrier

The BCSFB constitutes part of the choroid plexus, an epithelial-endothelial structure in the brain ventricles responsible for CSF production (Hladky and Barrand 2016). Unlike the BBB, the BCSFB is composed of a monolayer of cuboid epithelial cells (with microvilli located at the ventricular domain) which surround the fenestrated stromal capillaries embedded in connective tissue (Solár et al. 2020). Although CECs express tight junction proteins, the BCSFB is

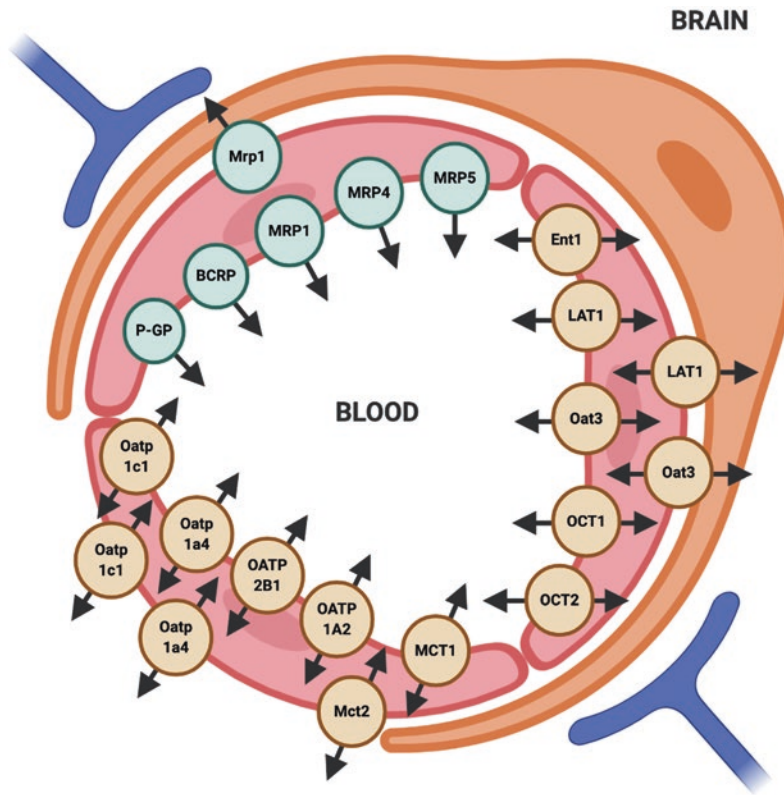

Fig. 7.1 ABC and SLC transporters involved in drug disposition expressed at the blood-brain barrier**.** Mammalian brain endothelial cells express high levels of efflux ABC transporters including P-GP, BCRP, and members of the MRP subfamily. SLC transporters are less characterized, but members of the MCT, OAT, OCT, and OATP subfamilies have been detected in animal and human brain endothelial cells

more permeable than the BBB, allowing the paracellular transport of larger molecules such as proteins from the blood into CSF (Pardridge 2011). Nonetheless, the polarized expression of ABC efflux and SLC uptake transporters compensates for the above limitation, providing more regulated transcellular passage of small molecules (Morris et al. 2017).

The expression profile of ABC and SLC transporters at the BCSFB has primarily been reported in animal models (Ho et al. 2012). In comparison to the BBB, the differences in expression profile among species (i.e., rodent/human) are more marked (Uchida et al. 2015); however, more studies are warranted to improve understanding of the complement of ABC and SLC transporters at the blood-cerebrospinal fluid barrier interface. The localization of ABC and SLC transporters within the BCSFB is presented in Table 7.1 and Fig. 7.2.

7.3 Expression Profile of ABC and SLC Transporters in the Maternal and Fetal Blood-Brain and Blood-Cerebrospinal Fluid Barriers

The developing fetus does not possess structurally robust BBB and BCSFB. Consequently, brain parenchyma is exposed to potentially harmful endogenous agents or xenobiotics capable of crossing the placental barrier. However, several studies conducted in animal models and with ex vivo human fetal brain tissue have demonstrated that the expression profile of ABC and SLC transporters appear to change throughout gestation, suggesting that, during development, the barrier properties of the BBB and BCSFB are highly dynamic. Furthermore, pregnancy-associated temporal changes in the expression profile and activities of ABC and SLC transport-

Table 7.1 ABC and SLC transporters expressed at the blood-brain and blood-cerebrospinal fluid barriers

Transporter	Gene	Molecular weight (kDa)	Level of expression	Localization		Drug substrates	Inhibitors	References
				Blood-brain barrier	Blood-cerebrospinal fluid barrier			
ATP-binding cassette								
P-GP	*ABCB1* (H) *abcb1a* (M, R) *abcb1b* (M, R)	170	mRNA (M, R, H) Protein (M, R, H)	Luminal (M, R, H)	Apical (H)	*Antibiotics*: Erythromycin, levofloxacin, ofloxacin *Anticancer drugs*: 5-fluorouracil, docetaxel, doxorubicin, etoposide, imatinib, paclitaxel, teniposide, vinblastine, vincristine *Antidepressant drugs*: amitriptyline, fluoxetine, paroxetine, sertraline Antiepileptic drugs: phenobarbital, phenytoin *Anti-HIV drugs*: Amprenavir, indinavir, nelfinavir, saquinavir, ritonavir *Antipsychotic drugs*: Chlorpromazine, trifluoperazine *Cardiovascular drugs*: carvedilol, celiprolol, digoxin, diltiazem, quinidine, talinolol, verapamil *Immunosuppressants*: Cyclosporin A, sirolimus, tacrolimus *Opioids*: methadone, morphine *Statins*: Atorvastatin, lovastatin, simvastatin	Cyclosporin A, elacridar, quinidine, tariquidar, valspodar, verapamil, zosquidar	Shawahna et al. (2011), Bauer et al. (2004), Warren et al. (2009), Uchida et al. (2011), Hoshi et al. (2013), Ohtsuki et al. (2013), Kamiie et al. (2008), Uchida et al. (2015), Dauchy et al. (2009), Chen et al. (2016) and Glaeser (2011)

(continued)

Table 7.1 (continued)

Transporter	Gene	Molecular weight (kDa)	Level of expression	Localization		Drug substrates	Inhibitors	References
				Blood-brain barrier	Blood-cerebrospinal fluid barrier			
BCRP	*ABCG2* (H) *abcg2* (M, R)	70 (monomer)	mRNA (M, R, H) Protein (M,R, H)	Luminal (M, R, H)	Apical (M, H)	*Antibiotics*: Ciprofloxacin, enrofloxacin, nitrofurantoin, norfloxacin, ofloxacin *Anticancer drugs*: Daunorubicin, doxorubicin, epirubicin, etoposide, gefitinib, imatinib, irinotecan, mitoxantrone, methotrexate, teniposide, topotecan *Cardiovascular drugs*: Azidopine, dihydropyridine, nicardipine, nitrendipine, reserpine *Anti-HIV drugs*: Delavirdine, lamivudine, lopinavir, nelfinavir, zidovudine *Immunosuppressants*: Cyclosporin A, leflunomide *Statins*: Atorvastatin, cerivastatin, pravastatin, pitavastatin, rosuvastatin *Others*: Cimetidine, glyburide, sulfasalazine	Abacavir, atazanavir, curcumin, cyclosporin A, efavirenz, fumitremorgin C, gefitinib, imatinib, Ko-143, quercetin, sirolimus	Shawahna et al. (2011), Warren et al. (2009), Uchida et al. (2011), Hoshi et al. (2013), Ohtsuki et al. (2013), Kamiie et al. (2008), Uchida et al. (2015), Dauchy et al. (2009), Tachikawa et al. (2005), Chen et al. (2016) and Meyer zu Schwabedissen and Kroemer (2011)
MRP1	*ABCC1* (H) *abcc1* (M, R)	190	mRNA (M, R, H) Protein	Luminal, abluminal (R)	Basolateral (M, R, H)	*Anticancer drugs*: Doxorubicin, etoposide, gefitinib, imatinib, methotrexate, vincristine *Anti-HIV drugs*: Indinavir, ritonavir, saquinavir	Cyclosporin A, ibrutinib, MK571, probenecid	Warren et al. (2009), Nies et al. (2004), Ohtsuki et al. (2013), Kusch-Poddar et al. (2005), Soontornmalai et al. (2006), Roberts et al. (2008a), Wijnholds et al. (2000), Zhang et al. (2015), Keppler (2011) and Choi and Yu (2014)

MRP2	*ABCC2* (H) *abcc2* (M, R)	> 200	mRNA (M, R, H) Protein	Luminal		*Antibiotics*: Ampicillin, azithromycin, cefodizime, ceftriaxone *Anticancer drugs*: Cisplatin, doxorubicin, epirubicin, etoposide, irinotecan, mitoxantrone, methotrexate, vinblastine, vincristine *Anti-HIV drugs*: Adefovir, cidofovir, indinavir, lopinavir, nelfinavir, ritonavir, saquinavir	Azithromycin, furosemide, genistein, MK571, probenecid	Warren et al. (2009), Ohtsuki et al. (2013), Kusch-Poddar et al. (2005), Bauer et al. (2008), Chen et al. (2016)
MRP4	*ABCC4* (H) *abcc4* (M, R)	160	mRNA (M, R, H) Protein (R,H)	Luminal, abluminal	Basolateral (M, R, H)	*Antibiotics*: Cefadroxil, cefazolin, cefmetazole, cefotaxime, ceftizoxime *Anticancer drugs*: Dasatinib, leucovorin, methotrexate, 6-thioguanine, 6-mercaptopurine, topotecan *Anti-HIV drugs*: Adefovir, nelfinavir, tenofovir *Cardiovascular drugs*: Enalaprilat, olmesartan *Diuretics*: Furosemide, hydrochlorothiazide	Ceefourin 1, dipyridamole, indomethacin, MK571, prazosin, probenecid, sildenafil, PU 23	Shawahna et al. (2011), Warren et al. (2009), Nies et al. (2004), Ohtsuki et al. (2013), Roberts et al. 2008a)(, Leggas et al. (2004), Chen et al. (2018), Russel et al. (2008) and Wen et al. (2015)
MRP5	*ABCC5* (H) *abcc5* (M, R)	170	mRNA (M, R, H) Protein (M, R, H)	Luminal	Basolateral (H)	6-thioguanine, 6-mercaptopurine, methotrexate	MK571	Warren et al. (2009), Nies et al. (2004), Roberts et al. (2008a), Karla et al. (2009) and Liu et al. (2010)
Solute carrier								
CNT2	*SLC28A2* (H) *slc28a2* (M, R)	72	mRNA (R) Protein (R, H)		Apical (R)	Mizoribine, ribavirin		Redzic et al. (2005), Mori et al. (2010), Kratzer et al. (2013) and Choudhuri et al. (2003)

(continued)

Table 7.1 (continued)

Transporter	Gene	Molecular weight (kDa)	Level of expression	Localization		Drug substrates	Inhibitors	References
				Blood-brain barrier	Blood-cerebrospinal fluid barrier			
ENT1	*SLC29A1* (H) *slc29a1* (M, R)	50	mRNA (R, H) Protein (M, R, H)	Luminal	Basolateral (R)	Entecavir, gemcitabine, fludarabine, trifluridine	Dilazep, dipyridamole, NBMPR, rapadocin	Uchida et al. (2011), Ito et al. (2011), Shawahna et al. (2011), Redzic et al. (2010), Uchida et al. (2015), Rehan et al. (2019), Playa et al. (2014), Ma et al. (2019), Takahashi et al. (2018a), Marce et al. (2006) and Pastor-Anglada et al. (2004)
ENT2	*SLC29A2* (H) *slc29a2* (M, R)	50	mRNA (R, H) Protein (R)		Apical (R), basolateral (R)	Fludarabine, gemcitabine	Dilazep, dipyridamole	Redzic et al. (2005), Redzic et al. (2010), Uchida et al. (2015), Playa et al. (2014), Shimada et al. (2015) and Pastor-Anglada et al. (2004)
LAT1	*SLC7A5* (H) *slc7a5* (M, R)	50	mRNA (M, R, H) Protein (M, R, H)	Luminal, abluminal		Gabapentin, L-dopa, melphalan, methyldopa, pregabalin	2-aminobicyclo-(2,2,1)-heptane-2-carboxylic acid (BCH), L-leucine, ESK242, JPH203	Uchida et al. (2011), Boado et al. (1999), Geier et al. (2013), Hoshi et al. (2013), Kamiie et al. (2008), Larsen et al. (2015), Wang and Holst (2015), Dickens et al. (2013), Takahashi et al. (2018b) and del Amo et al. (2008)
LAT2	*SLC7A8* (H) *slc7a8* (M, R)	60	mRNA (M, R, H) Protein (R)			Gabapentin, L-dopa, melphalan, methyldopa, pregabalin	BCH, L-leucine	Uchida et al. (2015), Muller and Heuer (2014) and del Amo et al. (2008)

MCT1	*SLC16A1* (H) *slc16a1* (M, R)	54	Protein (M, R, H)	Luminal, abluminal (R)	Apical (R), basolateral (R)	Gamma-hydroxybutyrate	AZD3965, AR-C155858, 7-(*N*-benzyl-*N*-methylamino)-2-oxo-2*H*-chromene-3-carboxylic acid, phloretin, quercetin	Kamiie et al. (2008), Uchida et al. (2011), Hoshi et al. (2013), Shawahna et al. (2011), Ito et al. (2011), Uchida et al. (2015) and Perez-Escuredo et al. (2016)
MCT3	*SLC16A8* (H) *slc16a8* (M, R)	50	Protein (R)		Basolateral (R)		DIDS, phloretin	Uchida et al. (2015), Bergersen et al. (1999) and Wilson et al. (1998)
MCT8	*SLC16A2* (H) *slc16a2* (M, R)	60	mRNA (M, R, H) Protein (M, R, H)	Luminal, abluminal (R)	Apical (M, R, H)	Thyroxine	Bosutinib, dasatinib, imatinib, silychristin, sunitinib	Roberts et al. (2008b), Heuer et al. (2005), Uchida et al. (2015), Johannes et al. (2016) and Braun et al. (2012)
OAT1	*SLC22A6* (H) *slc22a6* (M, R)	60	mRNA (M) Protein (R, H)		Apical (R, H)	*Antibiotics*: cefazolin, cefotiam, cefalexin, penicillin G, tetracycline *Anticancer drugs*: Leucovorin, methotrexate *Anti-HIV drugs*: Adefovir, cidofovir, didanosine, stavudine, tenofovir, zalcitabine, zidovudine *Cardiovascular drugs*: Captopril, furosemide, quinalaprilat, temocaprilat *NSAIDs*: Ibuprofen, indomethacin, ketoprofen *Other drugs*: Acyclovir, cimetidine, edaravone, para-aminohippurate, ranitidine, salicylate, silybin	Cabotegravir, candesartan, diflunisal, fluvastatin, furosemide, hydrochlorothiazide, ibuprofen, indomethacin, losartan, novobiocin, probenecid, rifampicin, simvastatin, telmisartan, valsartan	Alebouyeh et al. (2003), Nagle et al. (2013), Sweet et al. (2002), Burckhardt and Burckhardt (2011), VanWert et al. (2010), Reese et al. (2016) and Burckhardt (2012)

(continued)

Table 7.1 (continued)

Transporter	Gene	Molecular weight (kDa)	Level of expression	Localization		Drug substrates	Inhibitors	References
				Blood-brain barrier	Blood-cerebrospinal fluid barrier			
OAT3	*SLC22A8* (H) *slc22a8* (M, R)	62	mRNA (M) Protein (R, H)	Basolateral (R)	Apical (R, H)	*Antibiotics*: Cefaclor, cefazolin, cefdinir, cefotiam, ceftibuten, ceftizoxime, ciprofloxacin, penicillin G, tetracycline *Anticancer drugs*: 5-fluorouracil, 6-mercaptopurine, leucovorin, methotrexate *Anti-HIV drugs*: Adefovir, cidofovir, tenofovir, zidovudine *Antiviral drugs*: Acyclovir, valacyclovir *Cardiovascular drugs*: Captopril, quinalaprilat, temocaprilat *Diuretics*: Bumetanide, furosemide *Gastrointestinal drugs*: Famotidine, ranitidine *NSAIDs*: Ibuprofen, indomethacin, ketoprofen *Statins*: pravastatin, rosuvastatin *Other drugs*: Cimetidine, cortisol, edaravone, fexofenadine, para-aminohippurate, salicylate	Atorvastatin, candesartan, diflunisal, enalapril, ethacrynic acid, furosemide, indomethacin, losartan, mefenamic acid, pravastatin, novobiocin, oxaprozin, probenecid, valsartan	Alebouyeh et al. (2003), Uchida et al. (2015), Nagata et al. (2002), Nagle et al. (2013), Burckhardt and Burckhardt (2011), VanWert et al. (2010), Hoshi et al. (2013), Mori et al. (2003), Duan et al. (2012) and Burckhardt (2012)
OAT4	*SLC22A11* (H) *slc22a11* (M, R)	60	mRNA (H)			Ketoprofen, methotrexate, para-aminohippurate, pravastatin, salicylate	Benzylpenicillin, candesartan, losartan, probenecid, sulfobromophthalein, valsartan	VanWert et al. (2010), Kusch-Poddar et al. (2005), Cha et al. (2000), Yamashita et al. (2006) and Burckhardt (2012)

OCT1	*SLC22A1* (H) *slc22a1* (M, R)	56		Luminal (R, H)		Acyclovir, lamivudine, metformin, oxaliplatin, zalcitabine	Prazosin, procainamide, ritonavir, saquinavir, verapamil	Lin et al. (2010), Thomas et al. (2004), Jung et al. (2013) and Nies et al. (2011)
OCT2	*SLC22A2* (H) *slc22a2* (M, R)	63	mRNA (R) Protein (R)	Luminal (R, H)	Apical (R)	Amantadine, cimetidine, cisplatin, debrisoquine, famotidine, lamivudine, metformin, oxaliplatin, ranitidine, zalcitabine	Cimetidine, procainamide, ritonavir, saquinavir	Lin et al. (2010), Sweet et al. (2001), Thomas et al. (2004), Jung et al. (2013) and Nies et al. (2011)
OCT3	*SLC22A3* (H) *slc22a3* (M, R)	53	mRNA (M, R) Protein (R, H)			Lamivudine. lidocaine, metformin, oxaliplatin	Corticosterone	Geier et al. (2013), Dahlin et al. (2009), Sweet et al. (2001), Thomas et al. (2004), Wagner et al. (2016) and Nies et al. (2011)
OCTN2	*SLC22A5* (H) *slc22a5* (M, R)	63	mRNA (M, R, H)			Allopurinol, amisulpride, cephaloridine, cefoselis, cefepime, cefluprenam, colistin, entecavir, sulpiride	Camptothecin, cediranib, colistin, sulpiride	Shawahna et al. (2011), Kido et al. (2001), Pochini et al. (2019) and Ganapathy et al. (2000)
MATE1	*SLC47A1* (H) *slc47a1* (M, R)	62				Acyclovir, cefalexin, cefradine, cimetidine, cisplatin, ganciclovir, metformin, oxaliplatin, procainamide, quinine, tenofovir, topotecan	Cimetidine, imatinib, pyrimethamine	Uchida et al. (2015), Ito et al. (2012), Tanihara et al. (2009), Nakamura et al. (2010) and Nies et al. (2011)
PMAT	*SLC29A4* (H) *slc29a4* (M, R)	58				Atenolol, buformin, metformin, phenformin	Amprenavir, atazanavir, citalopram, decynium-22, fluvoxamine, lopinavir, nelfinavir, phenylethylamine, phenylbutylamine, quinine, saquinavir, tipranavir	Uchida et al. (2015), Duan et al. (2015), Zhou et al. (2007), Mimura et al. (2017) and Wang (2016)

(continued)

Table 7.1 (continued)

Transporter	Gene	Molecular weight (kDa)	Level of expression	Localization		Drug substrates	Inhibitors	References
				Blood-brain barrier	Blood-cerebrospinal fluid barrier			
OATP1A2 Oatp1a1[a] Oatp1a4[a]	*SLCO1A2* (H) *slco1a1* (M, R) *slco1a4* (M, R)	70	OATP1A2: Protein Oatp1a1: mRNA (M) Protein Oatp1a4: Protein (R)	OATP1A2: Luminal Oatp1a4: Luminal (R), abluminal (R)	Oatp1a4: Basolateral (R)	Erythromycin, fexofenadine, imatinib, ouabain, pitavastatin, rocuronium, rosuvastatin, saquinavir, thyroxine	Hesperidin, naringin, rifampicin, verapamil	Lee et al. (2005), Uchida et al. (2015), Kalliokoski and Niemi (2009), Bronger et al. (2005), Ose et al. (2010), Gao et al. (2015), Roberts et al. (2008a), Daneman et al. (2010)
OATP2B1	*SLCO2B1* (H) *Slco2b1* (M, R)	77	Protein (H)	Luminal (H)	Apical (R)	Benzylpenicillin, bosentan, fexofenadine, glibenclamide, statins (atorvastatin, fluvastatin, pravastatin, rosuvastatin)	Cyclosporin A, gemfibrozil	Bronger et al. (2005), Kalliokoski and Niemi (2009) and Roberts et al. (2008a)
OATP3A1	*SLCO3A1* (H) *Slco3a1* (M, R)	76	Protein (R, H)		Basolateral (H)	Benzylpenicillin		Uchida et al. (2015), Huber et al. (2007), Kalliokoski and Niemi (2009) and Roth et al. (2012)
OATP1C1	*SLCO1C1* (H) *Slco1c1* (M, R)	79	Protein (H)	Luminal (R), abluminal (R)	Apical (H, M, R), basolateral (H, M, R)	Bromosulfophthalein, thyroxin	Diclofenac, fenamic acid, meclofenamic acid, indocyanine green, phenytoin	Roberts et al. (2008b), Pizzagalli et al. (2002), Westholm et al. (2009)
PEPT2	*SLC15A2* (H) *Slc15a2* (M, R)	82	Protein (R) mRNA (R) Protein (M, R)		Apical (M, R)	5-aminolevulinic acid, bestatin, cefadroxil		Shu et al. (2002), Shen et al. (2004), Choudhuri et al. (2003)and Shen et al. (2007)

Abbreviations: *M* mouse, *R* rat, *H* human, *NSAID* non-steroidal anti-inflammatory drug
[a]Orthologs

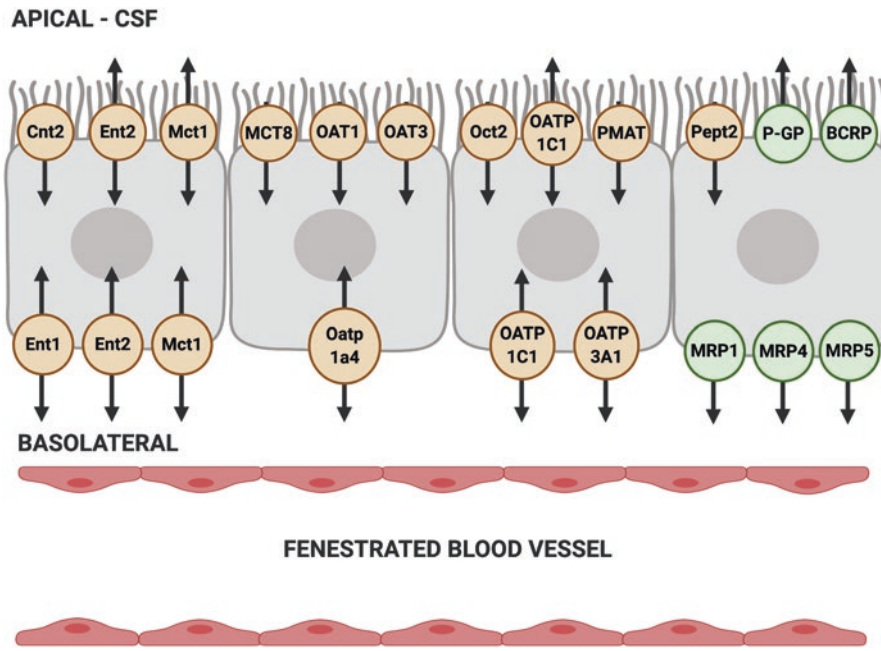

Fig. 7.2 ABC and SLC transporters involved in drug disposition expressed at the blood-cerebrospinal fluid barrier**.** Cuboid epithelial cells of the BCSFB also express ABC and SLC transporters, but the expression profile differs from that observed in BBB endothelial cells. Furthermore, the interspecies differences are more evident

ers within the maternal BBB and BCSFB have been reported, raising questions about the brain disposition of drugs during pregnancy. The expression of ABC and SLC transporters in the BBB and BSCFB of the developing fetus and the mother are shown in Table 7.2.

7.3.1 Transporter Expression in the Fetal Blood-Brain Interfaces

7.3.1.1 Blood-Brain Barrier

Expression of P-GP in human endothelial cells of the fetal brain increases with gestational age (Daood et al. 2008; Schumacher and Mollgård 1997; Virgintino et al. 2008). However, gestational-associated changes in expression are transporter specific. For example, in brain endothelial cells of human fetuses between 22 and 42 weeks of gestation, BCRP immunoreactivity at all gestational ages was high, with little variability; conversely, MRP1 was not detected at any stage (Daood et al. 2008). A later study found expression of MRP1 and BCRP, but not P-GP, in parenchymal brain vessels of embryos at 5 weeks' gestation. However, at 13 weeks' gestation, expression of all three transporters was observed (Møllgård et al. 2017).

In the fetal rodent BBB, the findings of several studies generally agree that the expression of P-GP increases with maturation. In mice, expression of *abcb1a* gene in brain endothelial cells at the neural tube of embryos at gestational day 10.5 (Qin and Sato 1995) was reported, while protein P-GP expression was detected in brain microvessels at gestational day 16 (Tsai et al. 2002). Later studies found that the brain expression of *abcb1a* mRNA increased from gestational days 15.5 to 18, with a similar outcome in protein P-GP as analyzed by immunohistochemistry of brain microvessels (Petropoulos et al. 2010). In rats, expression of protein P-GP in membrane and cytosolic fractions of fetal brains collected at gestational day 19 was negligible but increased at postnatal stages (Matsuoka et al. 1999). Immunohistochemistry studies confirmed the above findings and showed positive staining of

Table 7.2 Expression profile of ABC and SLC transporters in the fetal blood-brain and blood-cerebrospinal barriers

	Species	Model	ABC transporters	SLC transporters	References
Blood-brain barrier	Human	Embryos and fetuses obtained from ectopic pregnancies, between 7 and 18 weeks of gestation	P-GP: immunoreactivity in brain microvessels of 30 mm crown rump length (CRL) embryos. Increased immunoreactivity in brain microvessels of 123 mm CRL fetuses	No available data	Schumacher and Mollgård (1997)
		Brains extracted from spontaneously aborted fetuses at 12, 18, and 22 weeks of gestation	P-GP: Positive immunoreactivity from 12 weeks of gestation, with changes in the staining pattern at 18 and 22 weeks	No available data	Virgintino et al. (2008)
		Post-mortem analysis of brain from neonates collected between 22 and 42 weeks of gestation	P-GP: Increased expression from early to late gestational ages MRP1: No immunoreactivity BCRP: High immunoreactivity at all gestational ages	No available data	Daood et al. (2008)
		Brains extracted from legally aborted embryos and fetuses, between 5- and 21-weeks post-conception	P-GP: Positive immunostaining in parenchymal vessels, increasing from 5- to 13-weeks post-conception MRP1: Positive immunostaining in all studied gestational ages BCRP: Positive immunostaining in all studied gestational ages	No available data	Møllgård et al. (2017)
	Rodent	Non-specified mouse strain	P-gp: *mdr1a/3* gene expression in endothelial cells of the neural tube at gestational day 10.5	No available data	Qin and Sato (1995)
			P-gp: Increased brain *abcb1a* mRNA expression and immunostaining in brain microvessels from gestational day 15.5–18	No available data	Petropoulos et al. (2010)
		Wild-type FVB mice	P-gp: Increase in protein P-gp expression in brain microvessels from gestational day 16 to adult. Negative or weak immunoreactivity in brain at gestational day 16 and day of delivery	No available data	Tsai et al. (2002)
		C57BL/6 J mice	Bcrp: Positive immunoreactivity in the luminal side of cortical capillaries of brains from gestational day 15	No available data	Tachikawa et al. (2005)
		Non-specified rat strain	P-gp: Increase in mRNA levels at the forebrain from gestational day 13 to adult stages. Weak immunostaining in vessels at gestational day 13, which increase in postnatal and adult stages Mrp: In forebrain, mRNA expression of *abcc1* increases from gestational day 18 and *abcc4* remains constant at all gestational ages. No immunostaining for Mrp1 in brain vessels at all ages Bcrp: No changes in mRNA levels at the forebrain in all gestational ages. Positive immunoreactivity in cortical and pial brain vessels at gestational day 13	No available data	Ek et al. (2010)
		Wistar rats	P-gp: No expression of protein P-gp at gestational day 18 in membrane and cytosolic fractions of the brain but increased throughout development until adulthood. Positive immunostaining in microvessels from post-natal day 7	No available data	Matsuoka et al. (1999)
		Brain endothelial cells isolated from guinea pigs	P-gp: Increased protein P-gp levels in brain endothelial cells from gestational day 40 to postnatal day 14	No available data	Iqbal et al. (2011)

Blood-cerebrospinal fluid barrier	Human	Post-mortem analysis of brain from neonates collected between 22 and 42 weeks of gestation	P-GP: Positive immunostaining in choroid plexus at 22 weeks, with higher intensity at increasing gestational age BCRP: No immunoreactivity in choroid plexus MRP1: Positive immunoreactivity in choroid plexus at all gestational ages	No available data	Daood et al. (2008)
		Brains extracted from legally aborted embryos and fetuses, between 5- and 21-weeks post-conception	P-GP: No immunoreactivity at 5 weeks. Increased immunoreactivity in epithelial cells at 13 weeks, which decreases at 19 weeks MRP: Positive immunostaining BCRP: Positive immunostaining	No available data	Møllgård et al. (2017)
	Rodent	C57BL/6 J mice	Bcrp: Positive immunoreactivity at gestational day 15	No available data	Tachikawa et al. (2005)
		Non-specified rat strain	P-gp: Increase in *abcb1* mRNA levels from embryonic to adult ages Mrp: Increase in *abcc1* mRNA levels from embryonic to adult ages. Weak basolateral immunoreactivity in choroid plexus epithelial cells at gestational day 15, which increases at postnatal day 1 and adult Bcrp: Decrease in *abcg2* levels from embryonic to adult ages. Basolateral immunoreactivity in choroid plexus epithelial cells at gestational day 15 and postnatal day 1	No available data	Ek et al. (2010)
		Sprague-Dawley rats	P-gp: Increase in *abcb1a* mRNA levels from embryonic to postnatal day 2. Decrease in adulthood Mrp: Increase in *abcc1* mRNA levels from embryonic to adult ages Bcrp: Decrease in *abcg2* levels from embryonic to adult ages	Increase in mRNA levels of *slc15a2* (Pept2), *slc22a5* (Octn2), *slc22a8* (Oat3), *slco1a4* (Oatp1a4), and *slco1a5* (Oatp1a5) from embryonic to adult ages	Kratzer et al. (2013)

P-GP from postnatal day 7. Another study has shown that the mRNA expression of *abcb1a* in the forebrain and brain stem of rat fetuses is low from gestational days 13 to 18 and increased with maturation (Ek et al. 2010). The immunohistochemistry analysis of the striatum in fetuses at gestational day 13 showed faint staining for P-GP, which becomes more intense at postnatal stages.

BCRP protein expression has been reported in mouse and rat brain vessels at embryonic, postnatal, and adult stages, with no significant changes in *abcg2* mRNA expression reported throughout development (Ek et al. 2010; Tachikawa et al. 2005). Little is known of the expression profile of the MRP transporters in the fetal rodent BBB. Although one study has reported *Abcc1* and *Abcc4* mRNA expression in the forebrain of rat fetuses at different gestational days, no immunostaining was observed in brain vessels (Ek et al. 2010).

There is a lack of studies characterizing the effect of fetal development on the expression of SLC transporters involved in brain drug disposition at the fetal BBB. The only evidence of developmental changes is those reported by Harati et al. (Harati et al. 2013), who demonstrated increases in *slco1a4* mRNA expression and Oatp1a4 (an ortholog for human OATP1A2) protein expression in brain endothelial cells isolated from newborn and juvenile Wistar rats.

7.3.1.2 Blood-Cerebrospinal Fluid Barrier

P-GP and MRP1 expression, as detected by immunostaining, has been reported in the choroid plexus of human embryos and fetuses between 5 and 19 weeks of gestation (Daood et al. 2008; Møllgård et al. 2017). Furthermore, the expression of ABCB1 and ABCC1 decreases with increasing gestational age, with temporal changes in cellular localization (Møllgård et al. 2017). For BCRP, the above studies showed contrasting findings, with Daood et al. (2008) reporting no transporter expression, while Mollgard et al. (Møllgård et al. 2017) showed expression from the seventh week of gestation.

In rats, two reports consistently found that *abcb1* and *abcg2* mRNA expression in the BCSFB is higher than the observed for *abcc1* and *abcc4* at early gestational ages (Ek et al. 2010; Kratzer et al. 2013). The expression of *abcg2* mRNA decreased with increasing gestational age until adulthood, while the expression of *abcc1* and *abcc4* increased slightly throughout development in both studies. However, in the study of Kratzer et al. (2013), *abcb1* mRNA increased until postnatal day 2, and Ek et al. (2010) reported that the expression of *abcb1* mRNA showed no significant changes at any ages. Furthermore, this study demonstrated the protein expression of BCRP and MRP1 in the basolateral membrane of epithelial cells at gestational day 15, postnatal day one, and adults (Ek et al. 2010).

Transcriptomic analyses of the developmental changes in SLC transporter expression at the BCSFB of Sprague-Dawley rats report that the levels of mRNA transcripts for highly expressed transporters, including *Slc15a2* (Pept2), *Slc22a5* (Octn2), *Slc22a8* (Oat3), *Slco1a4* (Oatp1a4), and *Slco1a5* (Oatp1a5) increase from embryonic stages to adult (Kratzer et al. 2013).

7.3.2 Transporter Expression in the Maternal Blood-Brain Interfaces

Although the effect of pregnancy on the expression of ABC transporters in the maternal brain has been investigated, it is worth noting that the experimental approach is primarily based on studies carried out in the whole brain. Consequently, it is impossible to determine if the observed changes in transporter expression occur at the BBB or BSCFB. Furthermore, there is a lack of data reporting the effect of pregnancy on SLC transporter expression in the maternal brain.

In pregnant mice, expression of P-GP and MRP1 at the protein level in the BBB is highest at mid-gestation and decreases in later stages of development (Coles et al. 2009). Furthermore, a recent proteomics study demonstrated that the level of P-GP and BCRP expression in pregnant mice (gestational day 15) was not significantly different compared to their non-pregnant counterparts (Liao et al. 2018).

In higher species, such as macaques, transporter functional analyses, measured by positron

emission tomography (PET), showed that in pregnant individuals, the brain accumulation of the radiolabeled P-GP substrate [^{11}C]-verapamil decreased from mid-gestation to late gestation (Chung et al. 2010). In another series of experiments, treatment with cyclosporin A, a first-generation P-GP inhibitor, increased brain accumulation of ^{11}C-verapamil, with this change being more marked in late gestation. These results suggest that P-GP activity is increased in the later stages of pregnancy (Chung et al. 2010).

7.4 Regulation of ABC and SLC Transporter Expression in the Maternal and Fetal Brain

Evidence suggests that in pregnancy, the expression level of ABC and SLC transporters, both in the maternal and fetal brain, is a function of gestational age. However, few studies have provided evidence of the underlying mechanisms by which the expression of transporters is altered in the context of pregnancy and its associated conditions. This section will describe findings related to the influence of endogenous and exogenous factors on the expression and activity of ABC and SLC transporters in the brain during pregnancy.

7.4.1 Effect of Endogenous Mediators on the Expression of Brain ABC and SLC Transporters

Pregnancy is a physiological condition associated with marked changes in the levels of endogenous mediators, including hormones, growth factors, and cytokines, among others. In this regard, pro-inflammatory and anti-inflammatory cytokines are important mediators that ensure a healthy pregnancy (Palm et al. 2013; Yockey and Iwasaki 2018). However, in pregnancy-related diseases, including preeclampsia, the pro-inflammatory phenotype is exacerbated in the form of high levels of cytokines including interleukin-1β (IL-1β), interleukin-6 (IL-6), and tumor necrosis factor α (TNFα) (Aggarwal et al. 2019; Ma et al. 2019). Interestingly, IL-1β, IL-6, and TNFα have been demonstrated to decrease P-GP activity, in a time- and concentration-dependent manner, in brain endothelial cells of guinea pig fetuses at different gestational stages and pups at different postnatal ages (Iqbal et al. 2012). The effects of IL-1β, IL-6, and TNFα on P-GP activity were more marked at later stages of gestation and postnatal ages.

Transforming growth factor β (TGFβ), which is strongly involved in several female reproduction processes, including pregnancy (Li 2014), modulates the expression and activity of P-GP at the BBB. Treatment of brain endothelial cells, isolated from male fetuses and postnatal guinea pig pups, with TGFβ1 upregulated *abcb1* mRNA expression and P-GP activity (Baello et al. 2014), with changes mediated through activation of the TGFβ receptor type 1 isoforms, ALK1 and ALK5.

It is well-known that pregnancy is associated with dramatic changes in circulating sex hormone levels, but their influence on the expression of maternal and fetal BBB transporters has not been studied to date. However, 17β-estradiol treatment has been reported to downregulate BCRP mRNA expression and activity in brain capillaries isolated from female rats (Mahringer and Fricker 2010). Furthermore, using estrogen receptor knockout mice, the same study demonstrated that the actions of 17β-estradiol in BBB endothelial cells are mediated by ligand-based activation of the estradiol receptor subtype β.

7.4.2 Effect of Pathological Conditions on the Expression of Transporters During Pregnancy

In pregnancy, the influence of disease states on the expression and activity of ABC and SLC transporters involved in drug disposition in the brain is not well understood. To date, only two studies have attempted to address the effects of pathological conditions, namely, viral infection and maternal malnutrition, on the expression and activity of BBB P-GP. In the first study, pregnant C57BL6 mice dams were pre-treated with polyinosinic:polycytidylic acid (Poly:IC) (Bloise et al. 2017). This synthetic molecule emulates the

immune response to viral infection through activation of the toll-like receptor 3. The results demonstrated that the effects of Poly:IC on the activity of P-GP were time-dependent, since a 4 h, but not a 24 h, treatment increased the maternal levels of IL-6 and the accumulation of the P-GP substrate [^{3}H]-digoxin in the fetal, but not the maternal, brain.

The effect of maternal malnutrition on the expression of P-GP in the maternal and fetal brain has been studied in pregnant IMVS/Dunkin Hartley guinea pigs (Soo et al. 2012). The results showed that in late gestation, fetuses from mothers who received 85% of the average daily food intake possessed lower weight and increased brain capillary density. Furthermore, P-GP expression at the protein level in the brain was downregulated, with no changes in the *abcb1* mRNA expression observed. However, malnourished dams did not show changes in *abcb1*/P-GP expression at mRNA or protein levels compared to controls.

7.4.3 Effect of Therapeutic Drugs on the Expression of Brain ABC and SLC Transporters During Pregnancy

The ability of therapeutic drugs to regulate the expression of ABC and SLC transporters in the maternal and fetal brain is not well studied. To date, glucocorticoids are some of the most widely studied therapeutics, with consistent findings reported in the literature. In guinea pigs, hydrocortisone and dexamethasone upregulated the activity of P-GP in brain endothelial cells isolated from late-gestational fetuses and postnatal pups (Iqbal et al. 2011). Furthermore, a later study conducted by the same research group demonstrated that dexamethasone potentiated the IL-1β, IL-6, and TNFα-mediated inhibition of P-GP activity (Iqbal et al. 2016), likely via dexamethasone-mediated upregulation of a cytokine receptor.

The effects of glucocorticoids on the expression of BCRP in the fetal brain have also been studied. In pregnant FVB mice exposed to increasing doses of dexamethasone, while expression of the *abcb1a/abcb1b* and *abcg2* genes at the mRNA level was modified in the fetal brain at different stages of development, no significant changes in the expression of P-GP and BCRP protein, or the accumulation of the selective P-GP substrate digoxin and BCRP substrate mitoxantrone, were observed (Petropoulos et al. 2010, 2011). However, an interesting feature of both studies is that the dexamethasone-mediated effects on the expression of abcb1a/abcb1b and abcg2 mRNA were dose- and sex-dependent.

The pregnane X receptor (PXR), an orphan nuclear receptor known for its ubiquitous expression pattern, broad ligand specificity, and species-dependent ligand-based activation, regulates xenobiotic-mediated cellular events (Bauer et al. 2004; Yan and Xie 2016). Studies have revealed that chronic dosing of pregnant Wistar rats with the human PXR (hPXR) ligand rifampicin increased the mRNA and protein expression of P-GP in the brain of both dams and fetuses (Saljé et al. 2012). In the same study, treatment with the rodent Pxr ligand, dexamethasone, upregulated P-GP mRNA expression in the brain of fetuses, but not the dams, while chronic treatment with the herb product St. John's wort, whose active compound hyperforin is an hPXR ligand, failed to exert any effect on P-GP mRNA and protein expression in dams or fetuses (Saljé et al. 2012). The authors supported their findings, mentioning that despite being an hPXR ligand, rifampicin has been employed with success in rat models but acknowledged that the species-dependent ligand-based activation of PXR might influence the outcome of the other drugs tested.

7.5 ABC and SLC Transporter-Mediated Disposition of Drugs in the Maternal and Fetal Brain

Although it is well-known that drug pharmacokinetics are altered during pregnancy due to changes in intestinal absorption and hepatic/renal clearance, there is a scarcity of reliable data in humans due to ethical issues (Koren and Pariente 2018; Pariente et al. 2016). Therefore, rodent models have proved valuable for characterizing the influence of ABC and SLC transporters on the brain disposition of drugs during pregnancy. In

Table 7.3 Pharmacokinetic studies in preclinical models of pregnancy

Drug	Model	Transporter involved	Outcome	References
Opioids				
Buprenorphine	*Abcb1a* $^{-/-}$/*1b*$^{-/-}$ and *Abcb1a* $^{-/-}$/*1b*$^{-/-}$/*Abcg2*$^{-/-}$ FVB mice	P-gp	Increased maternal brain norbuprenorphine (P-gp substrate) levels in *Abcb1a* $^{-/-}$/*1b*$^{-/-}$ and *Abcb1a* $^{-/-}$/*1b*$^{-/-}$/*Abcg2*$^{-/-}$ mice	Liao et al. (2017)
Methadone	CD-1 mice	P-gp	Increased maternal brain/plasma ratio in late gestation, associated to down-regulation of P-gp levels	Coles et al. (2009)
Inhalable corticosteroids				
Budesonide and fluticasone	C57BL/6 J mice	P-gp	Decreased maternal brain/plasma ratio of budesonide in comparison to fluticasone, at gestational day 14.5. Co-treatment with zosuquidar did not increase brain maternal budesonide levels	Zaidi et al. (2019)
Anti-HIV drugs				
Emtricitabine and tenofovir	Wistar rats	P-gp and Bcrp	No effect on pharmacokinetics of emtricitabine and tenofovir	Cerveny et al. (2016)
Saquinavir	*Abcb1a/b*$^{-/-}$ knockout mice	P-gp	Increased maternal brain saquinavir levels in *Abcb1a/b*$^{-/-}$ knockout mice	(Huisman et al. 2001)
	CD-1 mice	P-gp, Mrp1	Increased maternal brain saquinavir levels in late gestation (gestational day 18), compared to mid gestation (gestational day 13). Correlation with down-regulation of P-gp and Mrp1 in late gestation	Coles et al. (2009)
Zidovudine	Sprague-Dawley rats	Bcrp	Decreased fetal brain concentrations of AZT in pregnant rats pre-exposed. Correlation with AZT-mediated increase in fetal brain Bcrp levels	Filia et al. (2017)
Anticancer drugs				
Paclitaxel	Sprague-Dawley rats	P-gp	Decreased maternal brain uptake of paclitaxel, compared to non-pregnant controls	Lee et al. (2014)

this section and Table 7.3, findings of pharmacokinetic studies carried out in preclinical models are presented.

7.5.1 Opioids

In the context of pregnancy, opioids are frequently employed for pain management (Fishman et al. 2019) since they effectively cross the BBB and exert their pain-relieving effects at the CNS level. However, as many opioids are substrates of ABC transporters expressed in brain endothelial cells, any change in transporter expression could impact the pharmacological outcome. Indeed, studies conducted in *Abcb1a*$^{-/-}$/*1b*$^{-/-}$ mice and in rats exposed to inhibitors have demonstrated that both brain bioavailability and the effects of opioids are increased when P-GP expression and activity are inhibited (Letrent et al. 1999; Thompson et al. 2000). Furthermore, studies also report that the maternal brain disposition of nor-

buprenorphine, the active metabolite of the opioid buprenorphine and P-GP substrate (Brown et al. 2012), was markedly increased in *Abcb1a*$^{-/-}$/*1b*$^{-/-}$ and *Abcb1a*$^{-/-}$/*1b*$^{-/-}$/*Abcg2*$^{-/-}$ FVB pregnant mice, when compared to the wild-type controls (Liao et al. 2017). However, by employing pregnant *Abcb1a*$^{-/-}$/*1b*$^{-/-}$/*Abcg2*$^{-/-}$ mice, the study showed the contribution of BCRP to the brain disposition of norbuprenorphine in dams is negligible.

Furthermore, it has been demonstrated that the maternal brain/plasma ratio of the opioid P-GP substrate methadone was increased in late-gestation CD-1 mice, compared to their mid-gestation counterparts (Coles et al. 2009). In these studies, the authors attributed this result to decreased P-GP expression levels in late gestation, leading to increased CNS penetration.

7.5.2 Inhalable Corticosteroids

Pregnant asthmatic women often require inhaled corticosteroids (Bjørn et al. 2015; Smy et al. 2014). However, inhalable corticosteroids' maternal and fetal brain disposition, including the P-GP substrate budesonide, and fluticasone, among others, are not well understood. A study carried out in pregnant C57BL/6 J mice (gestational day 14.5) showed that the maternal brain/plasma ratio of free radiolabeled budesonide was significantly lower than was observed for fluticasone propionate (Zaidi et al. 2019), suggesting that the brain disposition of the former is, at least, partly influenced by P-GP. The role of P-GP was further assessed in experiments using the selective P-GP inhibitor zosuquidar. These studies showed that zosuquidar did not significantly change the budesonide's maternal brain/plasma ratio but exerted a marked increase in the fetus/plasma ratio. Regarding the latter finding, the authors did not demonstrate if the inhibition of P-GP increased the fetal brain concentrations of the drug.

7.5.3 Anti-HIV Drugs

The influence of pregnancy on the transporter-mediated pharmacokinetics of drugs, including HIV-protease inhibitors and nucleoside reverse-transcriptase inhibitors (NRTI), has been investigated in animal models. Studies conducted in rodents demonstrated that the expression of P-GP has a marked effect on the maternal brain disposition of the protease inhibitor saquinavir.

In *Abcb1a*$^{-/-}$/*b*$^{-/-}$ knockout mice, the brain concentrations of [^{14}C]-saquinavir were 18.7-fold higher when compared to the wild-type controls, even when control animals were treated with ritonavir, another protease inhibitor co-administered to enhance the effects of saquinavir (Huisman et al. 2001). Furthermore, the maternal brain accumulation of saquinavir has been reported to increase with gestational age, with this outcome associated with a reduction of cerebral P-GP expression in late gestation (Coles et al. 2009).

Regarding the effect of anti-HIV drugs administered in pregnancy on P-GP expression, studies in Wistar rats have demonstrated that treatment with the NRTIs tenofovir and emtricitabine did not modify mRNA expression levels of *abcb1a*/*abcb1b* nor *abcg2* in the maternal and fetal brain (Cerveny et al. 2016).

The BCRP transporter plays a significant role in the fetal brain disposition of the NRTI drug zidovudine (AZT). Studies report that the brain concentrations of AZT in fetuses of Sprague-Dawley rats subjected to chronic oral administration of AZT were lower than in control animals where a single dose of the drug was administered intravenously (Filia et al. 2017). However, in studies where intraperitoneal gefitinib, a BCRP inhibitor, was co-administered with chronic oral AZT, fetal brain concentrations of AZT were similar to control animals. Thus, the authors suggested that AZT-mediated upregulation of BCRP in the fetal brain is responsible for the reduced accumulation of AZT within the brain.

7.5.4 Anticancer Drugs

Cancer in pregnancy is not a common event, so the pharmacokinetics of anticancer drugs, including chemotherapeutics, have not been extensively studied (Hepner et al. 2019). However, the brain disposition of paclitaxel,

a taxane used to treat cervical cancer in pregnancy (Zagouri et al. 2019), has been assessed in pregnant Sprague-Dawley rats. These studies report the cerebral uptake of the drug is low (Lee et al. 2014). However, cerebral uptake of paclitaxel in pregnant rats was twofold lower than the uptake reported in non-pregnant rats. These findings were attributed to gestational changes in the expression of P-GP and decreased plasma AUC. However, supporting evidence of changes in P-GP expression and AUC were not provided.

7.5.5 Other Drug Substrates

An array of other studies has attempted to assess the effect of pregnancy on the brain disposition of ABC transporter drug substrates.

Digoxin, a P-GP substrate, is frequently used in pharmacokinetic studies due to its sensitivity to the effects of both P-GP inducers and inhibitors. In fetuses from FVB mice, the brain accumulation of digoxin decreased from gestational day 15.5 to 18.5. This outcome is associated with upregulation of P-gp expression, at the mRNA and protein levels, at gestational day 18.5 (Petropoulos et al. 2010). Another study conducted in Sprague-Dawley rats showed that, after acute or chronic administration of digoxin, the brain/plasma and CSF/plasma ratios of the drug in pups and dams were lower than the observed in fetuses at gestational day 19, suggesting lower P-GP activity in the fetal BBB and BCSFB (Koehn et al. 2019). However, as the brain concentrations of digoxin were higher than those observed in CSF, the authors proposed that the P-GP-mediated efflux of digoxin at the BCSFB is more efficient than in the BBB. Furthermore, although the authors attribute lower brain/plasma and CSF/plasma ratios to a digoxin-mediated increase in *abcb1* mRNA expression in adults, the study did not analyze the changes in P-GP expression as a function of gestational age.

The effect of pregnancy on the pharmacokinetics of cimetidine, a prototypical antagonist of histamine H2 receptors and BCRP substrate, has been studied in pregnant Wistar rats infused with the drug. The fetal exposure to cimetidine decreased with increasing gestational age, and this outcome coincided with the upregulation of fetal *abcg2* mRNA expression until gestational day 18 (Cygalova et al. 2008). At gestational day 21, fetal *abcg2* mRNA levels were drastically reduced, but the fetus/plasma ratio of cimetidine in the brain was lower than observed at gestational day 18, suggesting the participation of other protective mechanisms.

A further study showed that the brain/plasma and CSF/plasma ratios of cimetidine are lower in postnatal pups and adults than in the fetus (Koehn et al. 2019). The authors noted that neither acute nor chronic exposure to cimetidine altered brain *abcg2* mRNA expression.

7.6 Concluding Remarks

The characterization of ABC and SLC transporter expression profiles in biological barriers represents a significant step toward a better understanding of the mechanisms involved in the disposition of therapeutic drugs and endogenous mediators. However, in the context of pregnancy, while there is a basic understanding of the gestational age-dependent changes in the expression of ABC and SLC transporters at the BBB and BCSFB, which reflect the dynamic nature of these barriers, further studies are required to understand better fetal and maternal brain drug disposition in healthy and complicated pregnancies.

The difficulties associated with the execution of clinical studies in pregnant women means animal models are the gold standard for preclinical pharmacokinetic studies in pregnancy. However, despite their lack of physiological relevance to humans, animal models allow exploration at tissue and cellular levels that would not be possible in humans. Furthermore, the use of appropriate models of pregnancy-related diseases, particularly employing imaging techniques with selective probe substrates of ABC and SLC transporters, would improve our understanding of the role of transporters in drug disposition during pregnancy.

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8 Development of the Placenta and Brain Are Affected by Selective Serotonin Reuptake Inhibitor Exposure During Critical Periods

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Abstract

Selective serotonin reuptake inhibitors (SSRIs) are usually prescribed to treat major depression and anxiety disorders. Fetal brain development exhibits dependency on serotonin (5-hydroxytryptamine, 5-HT) from maternal, placental, and fetal brain sources. At very early fetal stages, fetal serotonin is provided by maternal and placental sources. However, in later fetal stages, brain sources are indispensable for the appropriate development of neural circuitry and the rise of emergent functions implied in behavior acquisition. Thus, susceptible serotonin-related critical periods are recognized, involving the early maternal and placental 5-HT synthesis and the later endogenous 5-HT synthesis in the fetal brain. Acute and chronic exposure to SSRIs during these critical periods may result in short- and long-term placental and brain dysfunctions affecting intrauterine and postnatal life. Maternal and fetal cells express serotonin receptors which make them susceptible to changes in serotonin levels influenced by SSRIs. SSRIs block the serotonin transporter (SERT), which is required for 5-HT reuptake from the synaptic cleft into the presynaptic neuron. Chronic SSRI administration leads to pre- and postsynaptic 5-HT receptor rearrangement. In this review, we focus on the

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effects of SSRIs administered during critical periods upon placentation and brain development to be considered in evaluating the risk-safety balance in the clinical use of SSRIs.

8.1 Introduction

Anxiety, depression, and mood-related disorders often affect women's mental health during pregnancy and postpartum, with a prevalence ranging from 6% to 20% (Gaynes et al. 2005; McLean et al. 2019). The most prescribed antidepressants are the subtypes of selective serotonin reuptake inhibitors (SSRIs). The use of SSRIs in pregnant women reaches about 13% (Weisskopf et al. 2015; Edvinsson et al. 2019; Rosenfeld 2020). However, whereas SSRIs benefit the mother's mental health, their use has been related to detrimental effects on the offspring. In addition to neonatal withdrawal syndrome after intrauterine exposure to SSRIs (Zeskind and Stephens 2004; Alwan and Friedman 2009; Gentile and Galbally 2011; Hayes et al. 2012), several studies report an increased incidence of preterm birth, low birth weight, congenital cardiac disease (Källén and Otterblad Olausson 2006; Bar-Oz et al. 2007; Oberlander et al. 2008), psychomotor deficits (Casper et al. 2003), dysfunction in hypothalamic-pituitary-adrenal (HPA) axis stress responses (Oberlander et al. 2008), reduced somatosensory system development dysfunctional pain reactivity (Oberlander et al. 2005), increased autism like-symptoms like social avoidance and anxiety (Oberlander et al. 2010; Klinger et al. 2011; Grieb and Ragan 2019), and – although controversial (Clements et al. 2015) – increased risk for autism (Croen et al. 2011; Gentile and Galbally 2011; Harrington et al. 2013; Rai et al. 2013; Gentile 2015; Edvinsson et al. 2019).

The specific mechanisms through which SSRI treatments of maternal depression or anxiety-related syndromes are linked to impairment of normal embryonic development have not been determined. However, SSRI intake during pregnancy increases extracellular 5-HT in the mother and the amount of 5-HT maternal transferred into the developing embryo (Gentile and Galbally 2011).

The placenta is a transient vascular organ that develops and implants in the maternal uterus. The placenta receives blood supplies from the uteroplacental and fetoplacental blood circulations. SSRIs, such as citalopram and fluoxetine (brand name Prozac), can cross the human and rodent placenta exhibiting detectable concentrations in plasma, fetal cord blood, and amniotic fluid (Rampono et al. 2009; Velasquez et al. 2019). In the human placenta, there are detectable traces of 5-HT in trophoblast cells but strong stain for 5-HT in chorionic villus blood vessels (presumably by the presence of platelets in the embryonic and fetal circulation) at the first and second trimesters of gestation. At term, the 5-HT is detectable in the platelets in the maternal intervillous space without detection in trophoblast or chorionic blood vessels (Kliman et al. 2018).

Serotonin has a classical role as a neurotransmitter in the central nervous system and, in addition, it plays a neurotrophic role regulating the genesis, proliferation, differentiation, and maintenance of neurons in the brain as in the gastrointestinal tract (Gershon 2013; Popova et al. 2017; Teissier et al. 2017). Furthermore, 5-HT is also a regulator of craniofacial morphogenesis (Shuey et al. 1992; Moiseiwitsch 2000) and regulates the migration of neural crest cells (Moiseiwitsch and Lauder 1995).

The 5-HT brain phenotype results from a finely controlled sequential expression of transcription factors during fetal brain development and early postnatal life (see Fig. 8.1). In addition, 5-HT modulates the respiratory network in postnatal and adult life. Interestingly, perinatal fluoxetine exposure impairs central respiratory chemoreception during postnatal life (Bravo et al. 2016, 2017).

5-HT has broad physiological functions, and a perturbation of the 5-HT system will impact health, leading to multiple disorders across the lifespan. Here, we will discuss the critical periods of exposure to SSRIs in light of the appearance of 5-HT neurons in brain development, their temporal course related to fetus development, 5-HT metabolism of molecules during placentation, and how all these processes are related in time.

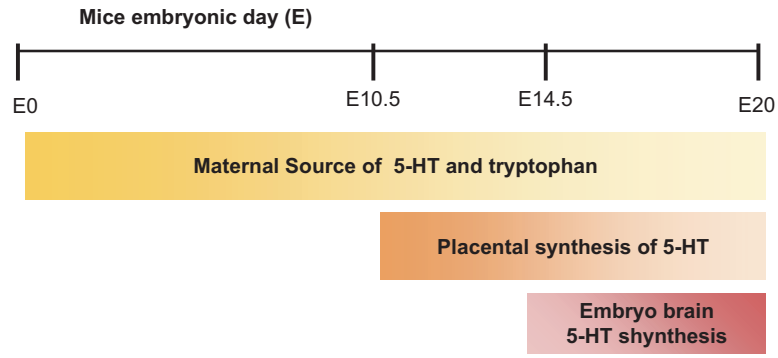

Fig. 8.1 Schematic representation of the source of serotonin (5-HT) in the temporal course of mice embryonic development. During gestation, the maternal plasma is the initial source of 5-HT and tryptophan. Platelets in maternal circulation actively sequester 5-HT and could release their content by controlled degranulation during early placentation. From studies in fetuses from dams that lack 5-HT in plasma, it is possible to determine that the primary source of 5-HT, from E10.5, is the placenta, which synthetic capacity begins to decline with the start of 5-HT synthesis in the fetal brain at E14.5

8.2 Ontogeny of the Serotonin System

The early appearance of 5-HT neurons in the hindbrain suggests a principal role of 5-HT in fetal brain development (Bonnin and Levitt 2011). The raphe 5-HT neurons are segregated into two clusters, a pontine rostral cluster, originated from the r1–r3 rhombomeres and subdivided into the dorsal raphe and median raphe (B6–B9), and a medullary caudal cluster, originated from the rhombomeres r5–r7, containing the raphe *pontis*, *pallidus*, and *obscurus* (B1–B5) (Kiyasova and Gaspar 2011).

The gene mutations or the null mice of transcription factors that regulated the 5-HT phenotype have been used to study the dysfunction of 5-HT during mice development (Deneris 2011). In mice, fetal raphe neurons' emergence is strictly regulated by the sequential expression of transcription factors that control a region-specific differentiation. First, they are under inhibitory control by the transcription factor "paired like homeobox 2b" (Phox2b) that impairs their differentiation to 5-HT neurons from embryonic day (E) 6.5 (E6.5). The "Sonic hedgehog" (Shh) morphogen expression leads to the activation of "forkhead box a2" (Foxa2) and "NK2 Homeobox 2" (Nkx2.2). At E10.5, Phox2b is switched off by the expression of Nkx2.2 (Briscoe et al. 1999; Pattyn et al. 2003). Also, Nkx2.2 induces the expression of transcription factors "LIM homeobox transcription factor 1 beta" (lmx1b) at E10.5 and "ETS domain transcription factor" (Pet-1) at E11 (Hendricks et al. 1999, 2003; Cordes 2005), both essentials for terminal differentiation of post-mitotic neurons and the beginning of serotonin synthesis (Cheng et al. 2003; Ding et al. 2003; Edvinsson et al. 2019). Pet-1 activates the transcription of genes for proteins involved in synthesizing 5-HT, tryptophan hydroxylase (TPH), aromatic L-amino acids decarboxylase (AADC), and serotonin transporter (SERT) from E12 (Hendricks et al. 1999, 2003; Gaspar et al. 2003).

In the central nervous system of mice, 5-HT neurons result from two waves of differentiation: a first involving bilateral rostral clusters at E10 and a second for the bilateral caudal clusters at E11 (Kiyasova and Gaspar 2011). The spatial differential expression of Shh, released along the ventral neural tube, and the fibroblast growth factor 8 (FGF8), secreted from the mid/hindbrain boundary and the rostral forebrain, and the fibroblast growth factor 4 (FGF4), which is produced in the primitive streak and paraxial mesoderm, determine the apparition of rostral and caudal 5-HT clusters. The coordinated expression of Shh and Fgf8 induces the rostral cluster of 5-HT neurons. Since the gradient of Fgf8 is low cau-

dally, whereas that of Fgf4 is high, the caudal cluster arises from the cooperative induction of Shh and Fgf4 (Ye et al. 1998; Kiyasova and Gaspar 2011).

The 5-HT neurons of the rostral cluster in the mid-hindbrain boundary that express FGF8 and "NK6 homeobox 1" (Nkx6.1) transcription factor differentiate into DR B7 neurons. The expression of "Achaete-Scute Family BHLH Transcription Factor 1" (Ascl1 or Mash1) and "GATA binding protein 2" (Gata2) transcription factors induce the final differentiation to B8 and B9 medial raphe cells (Craven et al. 2004). Shh as a morphogen signalling associated with the FGF4 and Nkx2.2, Gata2, "GATA binding protein 3" (Gata3), Ascl1/Mash1 origin the caudal cluster B2 and B3 cells. The rhombomere r4 is under the control of Nkx2.2; this transcription factor induces the differentiation of ventral motor neurons and 5-HT neurons (Craven et al. 2004; Kiyasova and Gaspar 2011; Gaspar and Lillesaar 2012).

B1–B5 cells project into the brainstem's ventral and the rostral area from the caudal cluster, whereas B6–B9 cells of the medial and rostral cluster send axonal projections to the ventral forebrain through the medial forebrain bundle to the rostral brain areas (Buznikov et al. 2001).

5-HT synthesis in neurons occurs via hydroxylation of the precursor tryptophan by TPH, the rate-limiting enzyme involved in the biosynthesis of serotonin, and decarboxylation of the intermediate molecule by AADC enzyme (Lauder et al. 1981). Two isoforms of the TPH are expressed differentially in tissues. TPH1 is expressed in non-neuronal tissues such as the pineal gland, the intestine, and the placenta (Badawy 2015). TPH1 expression in the pineal gland is detected at E14.5 (Côté et al. 2007), and in the TPH1$^{-/-}$ embryo, its development depends more on the maternal genotype than the embryo genotype (Côté et al. 2003, 2007). TPH2 isoform is mainly found in the raphe (Walther and Bader 2003; Gutknecht et al. 2008) with an initial Tph2 gene expression in E10–E10.5 (Gutknecht et al. 2009). TPH2$^{-/-}$ *knockout* mice showed raphe neurons with undetectable levels of 5-HT without evident morphological abnormalities. TPH2 *knockout* mice have altered growing and body control temperature and diminished arterial pressure and heart rate (Gutknecht et al. 2008). In rodent embryos, TPH1 or TPH2 are expressed until after E9.5, corresponding to gestational day 27–34 in humans (to see the conversion of embryonic age between species, visit www.translatingtime.org).

The machinery for 5-HT degradation is mainly represented by the enzyme monoamine oxidase A (MAO-A), which appears in the early gestational stages in mice during gastrulation and neurulation, having a crucial role in the control of apoptosis (Wang et al. 2011). 5-HT is stored in vesicles through the vesicular monoamines transporter 2 (VMAT2) in mice and rats, the preferentially expressed isoform in the central nervous system, the enterochromaffin, and enterochromaffin-like cells (Peter et al. 1995; Erickson et al. 1996). VMAT2 mRNA expression is detected in monoamine neurons of the brainstem from E13 (Hansson et al. 1998). By contrast, the VMAT1 isoform is the predominant transporter in the peripheral nervous system and neuroendocrine cells (Liu et al. 1994; Peter et al. 1995).

The SERT-mediated uptake of 5-HT regulates levels of 5-HT in the synaptic cleft. SERT can already be detected by autoradiographic localization of [^{3}H] citalopram binding from E12 (Brüning et al. 1997; Zhuang et al. 2005) and at an early stage, at E10.5 by b-gal staining in the outflow tract in SERTCre/1;R26R embryos in the mice hindbrain (Pavone et al. 2007).

The complete description of the developmental expression pattern of 5-HT receptors is still uncertain in rodents. 5-HT1A receptors are expressed in neocortical proliferative zones from E12 to E14.5 (Hillion et al. 1993) and microglia cells (Krabbe et al. 2012; Kolodziejczak et al. 2015). In post-mitotic cells, 5-HT2A, 5-HT2C, 5-HT3A, and 5-HT6 receptors are subsequently expressed in 5-HT neurons and pyramidal neurons in brain rats (Tecott et al. 1993; Johnson and Heinemann 1995; Vucurovic et al. 2010; Riccio et al. 2012; Dayer et al. 2015).

8.3 Sources of 5-HT Acting on Fetal Tissues

During fetal development, 5-HT receptors are expressed in brain areas days before neurons acquire the capacity for 5-HT synthesis and release, even prior future serotonergic axons from dorsal raphe could reach brain areas like the rostral forebrain (Gaspar et al. 2003; Bonnin et al. 2006; Muller et al. 2017). Furthermore, at early embryonic stages, 5-HT receptors can be detected in peripheral tissues like craniofacial regions and the heart (Buznikov et al. 2001). Then, it was proposed that 5-HT from sources outside the brain, craniofacial areas, or heart were responsible for activating these receptors. Thus, 5-HT from the maternal-embryonic circulation appears to be a good candidate for activating these 5-HT receptors to control proliferation, migration, and gene expression and hence, regulate the brain, heart, and craniofacial morphogenesis (Moiseiwitsch 2000; Nebigil et al. 2000; Lambert et al. 2001; Rosenfeld 2020).

8.3.1 Maternal 5-HT Source

The role of the maternal source of 5-HT in murine embryonic development and brain morphogenesis (before the embryonic stage in which neurons can synthesize 5-HT) has been shown by studies comparing the phenotype of pups born from homozygous and heterozygous mothers with respect to the knockdown tph1 gene (Côté et al. 2007; Staud and Karahoda 2018). TPH catalyzes the rate-limiting reaction in the biosynthesis of 5-HT. Expression of TPH2 isoform predominates in the brain, whereas the expression of TPH1 isoform is restricted to the pineal gland and the periphery at early developmental stages (Nakamura and Hasegawa 2007). E12.5 embryos from $tph1^{-/-}$ (null) mothers showed an overall size reduction compared to wild-type, heterozygous, and homozygous embryos developed in $tph1^{+/+}$ mothers. The longitudinal section of whole tph1 embryos revealed flattening of the head region at the level of the IV ventricle only in the embryos obtained from a null mother. Furthermore, 5′-bromo-2′-deoxyuridine (BrdU) pulse revealed that heterozygous embryos obtained from a null mother showed a 24% and 30% reduction in the number of labeled cells in the roof of the neopallial cortex and the ventricular zone, respectively, compared with the number of cells labeled in a null embryo developed in a heterozygous mother (Côté et al. 2007). These results strongly suggest that 5-HT derived from the maternal circulation influences peripheral organ morphogenesis, like in the heart (Côté et al. 2003), and controls brain morphogenesis during fetal development (Côté et al. 2003, 2007; Bonnin and Levitt 2011).

In the early placentation, the platelet could be a maternal source of 5-HT. Part of platelets' physiological role involves releasing 5-HT from dense granules to enhance plug formation and vasoconstriction at sites of vascular damage. Interestingly, platelets are the main source of 5-HT for immune cells following tissue damage and activation by other stimuli (Schoenichen et al. 2019). It is well-known that pregnancy is a state of active immunological regulation.

Although maternal platelets are not an essential component of the human placentation process, they could have a role in the 5-HT signalling in the early stage of pregnancy (Sato et al. 2010). Platelets in maternal circulation actively sequester 5-HT and could release their content by controlled degranulation during early placentation. Furthermore, the maternal 5-HT is uptake from the intervillous space by SERT in syncytiotrophoblast, a mechanism that could be inhibited by escitalopram (Kliman et al. 2018).

8.3.2 Placental Source of 5-HT

In mammals, the initial event of placental development is the segregation of trophectoderm concurrently with blastocoele fluid production as the morula develops into a blastocyst (Knott and Paul 2014). Evidence points to a specific role of pla-

cental 5-HT source in providing appropriate 5-HT supply to the forebrain, in contrast to the hindbrain. High-pressure liquid chromatography (HPLC) for assessing 5-HT levels at the hindbrain during embryogenesis revealed that Pet-1 *knockout* embryos show a consistent and massive reduction in 5-HT throughout fetal development. By contrast, in Pet-1 *knockout* forebrain, normal 5-HT levels are observed at E10.5–E15.5, and, only from E16.5 on, there is a significant reduction of 5-HT as expected for an exclusive provision by dorsal raphe neuronal axons, which at that stage are reaching in large numbers that brain area (Bonnin et al. 2011). These results are compatible with the notion that the forebrain switches from an early exogenous to a later endogenous 5-HT source. The early exogenous source could be the placenta because this organ has the necessary machinery to synthesize 5-HT, and the placental 5-HT synthesis capacity is greater at E14.5 than at E18.5. The synthetic capacity is not unique to mice, as human placental fetal villi at 11 weeks of gestation showed robust 5-HT synthesis (Bonnin et al. 2011).

Besides the placenta's 5-HT synthetic capacity, there is evidence that maternal blood would not be the 5-HT source in early embryonic development. The blood of SERT *knockout* ($SERT^{-/-}$) dams does not contain 5-HT. The forebrains of E12.5 $SERT^{+/-}$ fetuses born from $SERT^{-/-}$ dams have similar content of 5-HT to that observed in wild types (Bonnin et al. 2011). These data indicate that maternal blood 5-HT is not the primary source of fetal blood and forebrain 5-HT at the early stages of development. Accordingly, experiments in the murine ex vivo isolated placenta, in which independent catheterization and perfusion of maternal uterine artery and fetal umbilical vein can be accomplished, demonstrate that administration of tryptophan in the maternal circulation resulted in increased newly synthesized 5-HT in the fetal circulation. On the other hand, less than 1% of the amount of 5-HT administered via the uterine artery was recovered from the umbilical vein, suggesting that the placenta can synthesize and release 5-HT and is a barrier for the transference of 5-HT from the maternal to the fetal circulation (Bonnin et al. 2011). Intrauterine microinjection of the TPH inhibitor, *p*-chlorophenylalanine (PCPA), into the labyrinth zone of E14.5 placenta reduced the 5-HT content in the placenta and fetal forebrain but not in the fetal hindbrain. Therefore, the placental source of 5-HT allows the maintenance of normal levels of forebrain 5-HT during early fetal stages.

Several studies demonstrate 5-HT synthesis in the placenta in humans and rodents (Bonnin and Levitt 2011; Bonnin et al. 2011; Muller et al. 2017; Rosenfeld 2020). The trophoblast cells line the implantation site and invade the maternal decidual cells establishing the maternal artery blood flow to the fetus. Through immunocytochemistry, TPH1 and AADC proteins were detected in the syncytiotrophoblastic cell layer of the placenta already at E10.5–E14.5 in mice (Bonnin et al. 2011; John and Hemberger 2012; Badawy 2015). In humans, 5-HT synthesis from L-tryptophan starts at 11 weeks of gestation with the expression of TPH1 and TPH2 from the first trimester (Laurent et al. 2017), and 5-HT immunoreactivity is detected in syncytiotrophoblast, stroma cells, endothelium cells of placental villi, and trophoblast from the culture of placental trophoblast cells (Huang et al. 1998). Still, there is controversy about the placenta's capacity to synthesize 5-HT because the study of Kliman et al. (2018) did not detect the expression of TPH1 in any week of gestation analyzed. However, Laurent et al. (2017) and Ranzil et al. (2019) showed TPH1 expression in the first-trimester placenta. Also, TPH2 could have a role in the placental synthesis of 5-HT. Further studies are needed to confirm the early expression of TPH in the placenta.

SERT mRNA detection by northern blot revealed this transporter is expressed in mice placenta from E12.5 with a significant increase at E18.5. In human pregnant women, SERT mRNA has been found in syncytiotrophoblast by in situ hybridization and northern blot assay (Viau et al. 2009). The SERT protein expresses strongly and lightly in cytotrophoblast and syncytiotrophoblast, respectively, between 7 and 12 weeks of gestation. At term, there is an apical expression of SERT in syncytiotrophoblast, and intense immunoreaction in the interface between cytotrophoblast with syncytiotrophoblasts was observed

(Kliman et al. 2018). This evidence demonstrates that the placenta can uptake 5-HT from the first trimester of gestation until term.

On the other hand, MAO-A is a crucial component of 5-HT degradation in the placenta. MAO-A mRNA expression declined with embryo developmental age from E10.5 to E12.5, followed by a maximal expression at E15.5 (Verhaagh et al. 2001; Wang et al. 2011). MAO-A metabolizes the 5-HT in the cytotrophoblast cells (Yavarone et al. 1993; Hansson et al. 2009) and is highly expressed in syncytiotrophoblast from 8 weeks of gestation (at least) until the end of pregnancy (Kliman et al. 2018). The placental mechanism for 5-HT synthesis is graphically represented in Fig. 8.2, with emphasis on the effects of SSRIs on this mechanism.

Finally, placenta cells themselves are targeted by 5-HT; 5-HT1A receptors mRNA has been detected in cytotrophoblast and syncytiotropho-

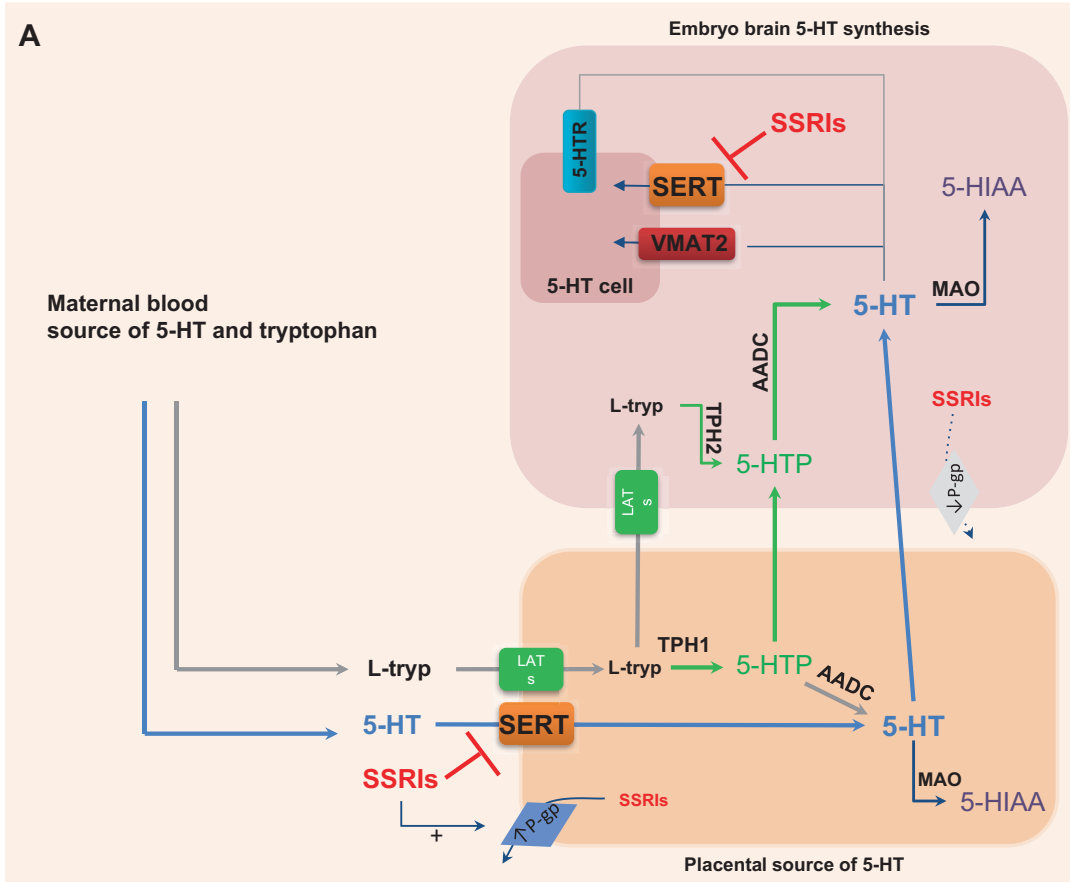

Fig. 8.2 Effects of SSRI exposition on the placental 5-HT synthesis and brain homeostasis. In normal conditions (**a**) the treatment with SSRIs reduces the SERT transport in the placenta and increases the placenta capacity for SSRI exclusion though P-gp expression. However, SSRIs can still cross the placental barrier and accumulate in the embryo's brain because of decreased P-gp expression. The placenta expresses an early capacity to uptake 5-HT from maternal blood and to synthesize 5-HT from L-tryptophan, evidenced by early expression of TPH1 and AADC. After E15, the embryonic brain can synthesize 5-HT, and the fetus is less dependent on maternal and placental sources. In (**b**), it is shown that in pathological conditions that induce maternal immune activation, the treatment with SSRIs can increase placental inflammation (mediated by IL-6) and decrease the capacity for 5-HT uptake. Albeit the reduction of placental 5-HT levels, there is an accumulation of 5-HT in the embryonic brain that could potentially have lasting consequences on offspring neurobiology. These are key areas for new investigations in animals, in vitro models, and clinics to understand and elucidate the SSRI safety-risk balance

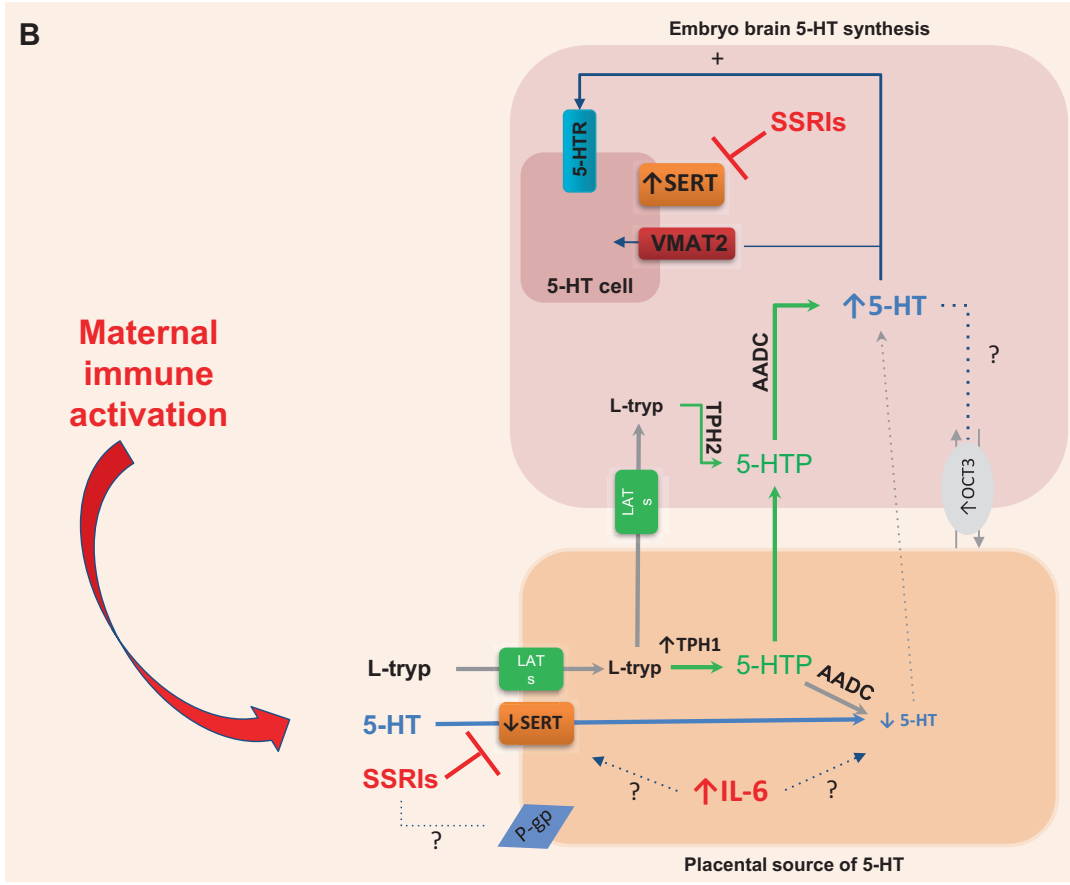

Fig. 8.2 (continued)

blast (Huang et al. 1998), whereas 5-HT2A receptors are widely distributed in human trophoblast (Viau et al. 2009).

8.3.3 Fetal Source of 5-HT

The appearance of raphe serotonergic neurons at E10.5 is the starting point at which maternal and placental sources cease to be the unique providers of fetal 5-HT; instead, the fetal brain becomes a new source for endogenous 5-HT (see Fig. 8.1). TPH2 in the raphe nucleus is initially detected at E10–E11, and its postnatal expression increases exponentially up to 3 weeks of age (Côté et al. 2007; Gutknecht et al. 2008). Although less studied in the context of fetal brain development, other peripheral places for 5-HT synthesis in mouse embryos have been demonstrated at later fetal stages from the myenteric plexus at E15–E16 and the enterochromaffin cells in the digestive tract at E18 (Gross et al. 2016). In human fetoplacental vessels have been detected 5-HT at 7 weeks of gestation (Kliman et al. 2018). The presence of 5-HT in fetal vessels is probably due to the capacity of fetal platelets to uptake 5-HT mediated by SERT (Baković et al. 2021), similar to adult platelets. The presence of 5-HT in fetoplacental vessels in the first trimester of gestation could be a result of platelet uptake from maternal 5-HT provided by trophoblast and/or from a fetal source. Albeit the evidence still is scarce, it is proposed that the placenta supplies 5-HT to

the developing fetal brain until serotonergic neurons organize in the raphe nuclei and fetal synthesis begins, which is supported by the correlation between 5-HT levels in the human fetal brain and the placenta (Perić et al. 2022).

8.4 Critical Periods of Perinatal SSRI Exposition: Effects on the Placental Transfers and Brain Development

The hemochorial placenta constantly is challenged by potentially toxic and teratogenic agents (Myllynen et al. 2005). A teratogenic agent is a molecule with chemical potential that can disrupt fetal development and alters fetal morphology or a subsequent function. Includes infectious agents, physical conditions, a deficit of metabolic precursors, or fetal exposure to a broad spectrum of *noxas*. Teratogenicity depends upon the ability of the agent to cross the placenta. The critical period associated with the most susceptible influence of a teratogenic agent is during the high cell proliferation rates. Then, for particular organogenesis, each one had an inherent period of the most rapid cell division during embryo development (Prouillac and Lecoeur 2010).

The prevalence of mood-related disorders to major depression in pregnant women is 6–10%, and SSRIs are the most widely used drugs for treatment. The prescribed rate of 2–15% includes fluoxetine, paroxetine, escitalopram, sertraline, and fluvoxamine, among others (Fischer Fumeaux et al. 2019; Rosenfeld 2020). SSRIs act by binding to SERT, which leads acutely to elevated levels of 5-HT in the synaptic cleft, which increase the probability of activation of postsynaptic 5-HT receptors. This increased level of 5-HT in a brain region does not influence mood immediately, making the chronic administration of SSRIs a primary clinical approach for treating depression and anxiety disorders in the general population.

However, the effectiveness of these clinical approaches in pregnant women remains challenging due to the inherent use of drugs during pregnancy, environmental factors, and side effects (Reilly et al. 2020), especially related to undesired fetal exposure.

The placental transfer of SSRIs and metabolites has been determined in women taking citalopram, fluoxetine, paroxetine, or sertraline by umbilical cord blood samples (Hendricks et al. 2003). The mean ratios of cord to maternal serum concentrations of antidepressants and their metabolites ranged from 0.29 to 0.89. Citalopram produced the highest ratio, followed by fluoxetine, paroxetine, and sertraline, with the lowest ratio. These results suggest that sertraline may produce less fetal medication exposure than fluoxetine. Interestingly, whereas higher doses of fluoxetine and sertraline resulted in higher cord serum concentrations, the increase in the concentration of both drugs in the cord serum was not proportional to the increase in dose. This evidence means that an increase in maternal medication dose during pregnancy will not necessarily be traduced to a higher risk due to a significant increase in fetal exposure (Hendricks et al. 2003).

In vitro studies in HTR-8/SVneo cell lines, derived from chorionic villi explants of the human first-trimester placenta, treated with 30 μM of fluoxetine impairs glucose intake resulted in antiproliferative and cytotoxic effects (Correia-Branco et al. 2015, 2019). Similarly, fluoxetine and sertraline at 10 μM decreased cell proliferation by 94–100% in JEG-3, derived from placental human choriocarcinoma cells, and by 58.6% and 100%, respectively, in HIPEC, derived from human invasive proliferative extravillous cytotrophoblast cells (Clabault et al. 2018a).

Norfluoxetine, the major active metabolite of fluoxetine, at 0.03–3 μM increased the metalloproteinase activity by 2–44% in JEG-3 cells and decreased this activity in HIPEC cells by 64% at 3 μM dose. Sertraline 0.03 μM and venlafaxine 0.03–0.3 μM also had effects increasing the mRNA levels of TIMP metallopeptidase inhibitor 1 (TIMP-1) by 85–115%, a protein involved in the inhibition of metalloprotease activity, and ADAM-10 by 156–167%, a disintegrin and metalloprotease. Interestingly, the 5-HT2A receptor is expressed in JEG-3 and HIPEC cells

(Clabault et al. 2018a). Both cell lines are considered extravillous trophoblast models involved in cell migration and invasion during the first trimester of pregnancy. These processes are considered critical in a healthy placenta, and its alteration is associated with preeclampsia (Lyall et al. 2013). Fluoxetine 1 μM in H295R cell lines, derived from human adenocarcinoma, increases 17β-estradiol secretion (Lupu et al. 2017). In contrast, norfluoxetine has a disrupted effect on estrogen secretion by direct stimulation of 5-HT2A or by increasing extracellular 5-HT (Hudon Thibeault et al. 2017). In BEWO cells, derived from human placenta choriocarcinoma (Pattillo and Gey 1968), norfluoxetine decreased GJA1 and increased CGB gene expression, both biomarkers of syncytialization (Clabault et al. 2018b; Rosenfeld 2020). The JAR human choriocarcinoma cell line and the placenta express SERT, and the uptake of [^{3}H]-5-HT is inhibited by desipramine and fluoxetine in a concentration-dependent way (Martel and Keating 2003).

Likewise, in a concentration-dependent way, cell viability increases by DOI (5-HT2A receptor agonist) administration. Ketanserin, a selective 5-HT2A receptor antagonist, reverts this effect. 5-HT activation of the 5-HT2A receptor increases cell viability via the intracellular signalling pathways MEKeERK1/2 and JAK2eSTAT3, suggesting these subtypes of 5-HT receptor play a key regulator role in survival, differentiation, migration, and invasion controlling the placentation (Viau et al. 2009).

The main effect of 5-HT is the contraction of placental vascular smooth muscle, which is not under autonomic innervation regulation (Reviriego et al. 1990). The vascular effect is a consequence of 5-HT activation of 5-HT2A-5-HT1B subtype receptors, being 5-HT actions limited by its degradation by MAO (Huang et al. 1998). The vasoconstriction effect of 5-HT was blocked by ketanserin (Reviriego et al. 1990). In vivo placental vascular smooth muscle response to 5-HT2A receptor activation has been evidenced by ketanserin blockade in hypertensive and normotensive Wistar Kyoto rats, in which this blocker reduced systolic blood pressure and placental blood flow (Furuhashi et al. 1991).

In rodents, the effects of SSRI exposure have been reported using several experimental protocols such as acute or chronic exposure, varied routes of administration, different species or strains, and different timing for SSRI exposition. Therefore, we focus on three intervals for SSRI exposition in rodents: (1) during early intrauterine life from E1 to E5–E8 (2) during gestation from E5–E8 to E20, and (3) from newborns up to 3 weeks old during postnatal age; see Tables 8.1, 8.2, and 8.3.

Fetal and early postnatal age development involve developmental stages with high cell proliferation rates and differentiation, characterized by vulnerability (Erickson et al. 2019). Prenatal fluoxetine exposure from E7 to E20 can be correlated with increased maternal weight loss, neonatal mortality, decreased litter size at birth, low birth weight, and diminished weight gain during early postnatal development (Vorhees et al. 1994; Bairy et al. 2007). Also, Sprague-Dawley rats exposed from E8–E20 to oral gavage venlafaxine (3–100 mg/kg/day) altered the placental index (fetal body/placental weight ratio) and induced fetal cardiac anomalies (Laurent et al. 2016). Interestingly, studies applying citalopram 20 mg/kg (intraperitoneal) in mothers with gestation from E14 to E18 revealed that pregnancy modifies the pharmacokinetics of citalopram, being its maternal clearance and the fetal drug metabolic capacity increased at the latter fetal stages. Citalopram and its primary metabolite, desmethylcitalopram, can cross the placenta, reaching superior fetal levels compared to the mother 2 h after maternal administration. These results revealed that citalopram fetal levels depend on the maternal gestational stage and embryo development (Velasquez et al. 2016) (see Table 8.1).

The placenta can extrude a widespread range of xenobiotics based on phosphoglycoproteins (p-glycoprotein 1 or permeability glycoprotein, abbreviated as P-gp, also called multidrug resistance protein 1, MDR1, or ATP-binding cassette sub-family B member 1, ABCB1) in mice and human. The two murine isoforms, ABCB1a and ABCB1b, can be detected, among other types of tissues, in the placenta, where it is highly expressed in the placental syncytiotrophoblast

Table 8.1 Prenatal exposure SSRI during gestation from E7 to E20

References	Timing of exposition	Methods	Effects
Vorhees et al. (1993)	GD7-GD20	Fluoxetine, doses 0, 1, 5, and 12 mg/kg/day, gavage Sprague-Dawley CD rats	At fluoxetine 12 mg/kg/day ↑ maternal weight loss ↓ litter size at birth ↑ neonatal mortality All doses: No significant effects on locomotor activity, spontaneous alternation, passive avoidance, and water maize performance
Bairy et al. (2007)	6th to the 20th day of pregnancy	Fluoxetine, dose 8 and 12 mg/kg, orally by drinking water, Wistar strain	No change in the gestational length of pregnancy, no premature birth or miscarriage during pregnancy. At fluoxetine 12 mg/kg high dose of in utero: ↓ in birth weight of the offspring ↓ weight gain during the preweaning period No major congenital abnormalities ↑ delay in motor development and this poor motor activity was transient and not permanent ↑ favorable effect on learning and memory in water maze and passive avoidance tests
Velasquez et al. (2016)	GD 14–GD 18	Racemic citalopram hydrobromide, dose 20 mg/kg i.p., CD-1 mice	Maternal pharmacokinetics altered ↓ in peak concentration ↓ half-life time ↓ area under the concentration in a temporal course ↑ volume of distribution, and ↑ maternal drug clearance as gestation progresses
Laurent et al. (2016)	From E8 to E20	Venlafaxine hydrochloride, doses 3–100 mg/kg/day, gavage, Sprague-Dawley rats	↓ fetal placental weight, ↑ cardiac anomalies

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and prevents xenobiotics from entering the fetus. ABCB1 actively transports a comprehensive list of xenobiotics, including chemotherapeutic agents, antiretroviral HIV drugs, herbicides, pesticides, cardiac glycosides, morphine, epileptic drugs like phenytoin, and synthetic and endogenous steroids (Kalabis et al. 2005). In mice, the mRNAs of the two isoforms of ABCB1 can be detected in the invading trophoblast at E9.5, showing a peak at E12.5, and then a significant decay over the second half of gestation. Thus, the fetal susceptibility to xenobiotics and natural steroids from the maternal circulation increases toward the term of pregnancy.

It is worth noting that after 4 h of 1–10 mg/kg/day sertraline injected (intravenous) in pregnant FVB mice at E15.5, the ABCB1-mediated substrate efflux in the placenta increased, leading to reduction of drug transfer to the fetus. By contrast, sertraline decreased ABCB1-mediated substrate efflux at the fetal and maternal blood-brain barrier (Bhuiyan et al. 2012). This result means that sertraline treatment may improve the placenta barrier fetal protection. However, on the other hand, it may increase the susceptibility of the fetal brain to the influences of circulation drugs in critical periods of development (Fig. 8.2a).

Citalopram and its primary metabolite, desmethylcitalopram, cross the placenta into the fetal compartment. The fetal capacity for drug metabolism develops late in gestation, so elevated circulating and brain levels of desmethylcitalopram are observed toward the end of the

gestation (Velasquez et al. 2016). Fetal exposure to the SSRI citalopram is, therefore, influenced by both maternal gestational stage and embryonic development, suggesting potential time-dependent effects on fetal brain development in mice (Velasquez et al. 2016). Clinical evidence indicates that during the third trimester of pregnancy, some antidepressants show a prominent decrease in their dose-adjusted levels. In contrast, others, like citalopram and fluoxetine, show a smaller decrease or no changes in the dose-adjusted levels. By contrast, sertraline exhibited increased dose-adjusted concentrations (Schoretsanitis et al. 2020). These results show a broad variability in the direction and intensity of the changes, requiring adjustment of SSRI doses during pregnancy, as a consequence of changes in the metabolic capacity for SSRIs in the third trimester of pregnancy.

Perinatal SSRI exposure can affect the number of 5-HT neurons, behavioral tests, and the respiratory responses to hypercapnia in the offspring (see Table 8.2).

Recently, to investigate the impact of SSRI treatment on the maternal-fetal interface and offspring neurodevelopment, female mice were treated with fluoxetine 2 weeks prior to mating. Pregnant dams were induced for maternal immune activation at E11–E12, and the levels of serotonin, proinflammatory cytokines, and immune signalling were determined in the placenta and embryo brain 12 h after maternal immune activation. First, fluoxetine did not alter the 5-HT levels in the placenta but significantly

Table 8.2 Prenatal exposure SSRI during gestation from E5–E8 to E20 and early postnatal age to 3 weeks

References	Timing of exposition	Methods	Effects
Lisboa et al. (2007)	Fluoxetine exposure during pregnancy and lactation (weaning at P21)	Fluoxetine, 7.5 mg/kg, gavage, Swiss dams P30 and P70 (females) and on P40 and 70 (males)	In male pups, ↓ ambulation at P40 In female pups, ↑ immobility time in the forced swimming test at P30 and P70, which is interpreted as depressive-like behavior
Forcelli and Heinrichs (2008)	On gestational day 14 for 14 days	Fluoxetine, dose 10/mg/kg/day, osmotic minipump 21 days, female Wistar rats	P120 ↓ −9% nucleus accumbens cell count ↓ −35% serotonin transporter-like immunoreactivity in the raphe ↓ 21% overall activity in the elevated plus-maze. In the place conditioning trial, only the fluoxetine-treated group exhibited a significant place preference for the compartment paired previously with cocaine. ↑ (350%) in extinction response rate in a cocaine self-administration trial
McAllister et al. (2012)	E15–P12	Fluoxetine hydrochloride, dose 25 mg/kg/day administered via drinking water, C57BL/6 mice	Mice 20 weeks of age ↓ body weight ↓ in anxiety-like and ↓ depression-like behavior in early adulthood
Glover et al. (2015)	Throughout pregnancy and the postpartum lactation period until weaning on postpartum day 21	Paroxetine, dose 10 mg/kg/day, orally by drinking water, selectively bred low and high responder females, Sprague-Dawley rats	↑ susceptibility to behavioral abnormalities in selectively bred low responder offspring ↑ Forced Swim test immobility Selectively high responder offspring were resistant
Bravo et al. (2016)	From E5 to E20 and P0 to P8	Fluoxetine hydrochloride, dose 500 ng/mL osmotic minipump, CF-1 mice, chronic exposition	↓ Ventilatory responses to hypercapnia at P8-P40 ↓ hypercapnia-induced neuronal activation in RN and NTS ↓ respiratory frequency induced by acidosis in brainstem slices

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decreased the 5-HT induced by maternal immune activation and potentially increases the 5-HT levels in the embryo brain (see Fig. 8.2b). Also, fluoxetine alters the proinflammatory cytokine levels induced by maternal immune activation, increasing the placental levels of IL-6 (Fig. 8.2b). In the embryonic brain, fluoxetine potentiates the response to maternal immune activation, enhancing serotonin and cytokine signalling. The disruption of 5-HT tone in the placenta before E15 (see Fig. 8.1) could potentially have lasting consequences on offspring neurobiology (Zengeler et al. 2023). Still, the placenta is an understudied organ, and further research about the placental response to stress and SSRIs will be helpful to know details about neurodevelopmental biology.

8.5 Perinatal SSRI Exposure: Impact on Neural Networks and Their Functions

The daily delivery, through osmotic minipumps, of 7 mg/kg fluoxetine for 4 weeks starting from the gestational day E5–E8 reduced the ventilatory responses to hypercapnia at the postnatal day 8–40 (P8–P40), but not at P0–P5, and impaired the central respiratory chemoreception evaluated in brainstem slices (Bravo et al. 2016). By contrast, superfusion of control P8 brainstem slices with 500 ng/mL of fluoxetine, which is a concentration similar to that attained in plasma of fluoxetine-exposed dams, did not modify the basal fictive respiration at pH 7.4 and the respiratory responses to pH 7.2 (acidosis) and pH 7.6 (alkalosis). Such a result indicates that the effects of chronic fluoxetine exposure from gestation to early postnatal life upon the respiratory responses in frequency cannot be explained by a direct fluoxetine action on the respiratory network. Indeed they suggest changes in the long-term properties and circuitry of brainstem respiratory nuclei, likely on central respiratory chemoreception (Bravo et al. 2016).

To evaluate the effects of perinatal fluoxetine exposure upon offspring hedonic behavior, pregnant Wistar dams were exposed to fluoxetine by implantation on gestational day 14 of osmotic minipumps delivering 0 or 10 mg/kg/day fluoxetine for 14 days. Fluoxetine-exposed offspring exhibited a significant 9% decrease of cell count in nucleus accumbens and a 35% decrease in SERT-like immunoreactivity in the raphe nucleus at P120. This finding is consistent with a positive hedonic shift for conditioned reinforcing effects of cocaine evidenced by an increased extinction response rate by 350% in a cocaine self-administration trial, and a modification of the serotonergic neurotransmission, respectively (Forcelli and Heinrichs 2008).

Using a different protocol and route of administration, P40 male offspring born from Swiss dams treated daily with 7.5 mg/kg fluoxetine during pregnancy and lactation showed reduced ambulation. However, in female pups at P30 and P70, the perinatal fluoxetine exposure increased immobility time in the forced swimming test, which is interpreted as depressive-like behavior (Lisboa et al. 2007).

Glover et al. (2015) to explore the relation of vulnerabilities to SSRI exposure and emotional behavior associated to 5-HT circuitry, used selectively bred lines of Sprague-Dawley rats based on separate breeding of low (bLR) and high (bHR) responders to novelty. The bLR group showed higher levels of behavioral inhibition, spontaneous anxiety, and depression-like behavior than those observed in bHR group. Female rats were exposed daily to 10 mg/kg paroxetine through drinking water from 7 days before mating up to the third week postpartum. At P21, bLR offspring, more predisposed to an anxiety/depression-like phenotype, showed increased immobility in the forced swim test when exposed perinatally to paroxetine, whereas bHR offspring were resistant (Glover et al. 2015). On the other hand, 20-week-old C57BL/6 mice born from dams receiving 25 mg/kg/day fluoxetine via drinking water from E15 to P20 showed no difference compared to control mice in behavioral tests for assessing locomotor activity, motor coordination, startle response, prepulse inhibition, spatial memory, memory retention, working memory, or contextual fear

memory (McAllister et al. 2012). However, fluoxetine-exposed offspring mice showed a decrease in anxiety-like behavior by spending less time in the closed arms in the elevated plus maze and decreased depression-like behavior, taking a long time to become immobile in the forced swim test. The authors discussed that these effects are not explained by the direct pharmacological exposition to fluoxetine and its principal metabolite, norfluoxetine, because fluoxetine treatment ended 10 weeks before the beginning of the behavioral testing, being fluoxetine and norfluoxetine cleared from the brain in a range of a week (Ansorge et al. 2008; McAllister et al. 2012).

8.6 Brain Effects of Postnatal SSRI Exposure

Most postnatal studies evaluate the effects of SSRI exposure when applied in early postnatal age from P0–P5 to weaning age (approximately P21). The evaluation of the postnatal effects of SSRI exposure is illustrated in Table 8.3. The main SSIR effects are significant behavioral abnormalities concerning control mice only when the SERT protein is present. Genetic variants $SERT^{+/+}$ and $SERT^{+/-}$ suggest that the principal mechanism by which SSRIs affect behavior is through SERT blockade (Ansorge et al. 2004, 2008). Furthermore, several reports indicate that postnatal SSRI exposure in mice showed depression-like behavior, including significantly increased immobility, anhedonia, altered sleep, and diminished sexual behavior (Hilakivi and Hilakivi 1987; Maciag et al. 2006; Popa et al. 2008).

In P0–P6 Wistar rats, 10 mg/kg (subcutaneous) fluoxetine blunts thermal and tactile perceptions associated with a reduction in explorative activity. Morphological effects included the reduction in the number of branch tips of thalamocortical afferents into the somatosensory cortex; the reduction of the dendritic arborization, density, and field of the glutamatergic spiny stellate neurons in the somatosensory layer IV; and the increase in the spine length of mushroom-type and branched-type spines (Lee 2009).

Male, but not female, Long-Evans neonates receiving 10 mg/kg citalopram daily from P8 to P21 exhibited an exaggerated freezing response to a novel auditive tone presented at P25 and a reduction in the exploration of a novel object at P39, neophobic tendency in response to the presentation of visual or tactile stimuli, and uninterest in playing, showing a disrupted juvenile play behavior. They also exhibited abnormalities in raphe and callosal connections, sensory processing, and myelin sheath formation (Simpson et al. 2011).

Similarly, Wistar rats treated with citalopram (10–20 mg/kg i.p.) from P2 to P5 show suppression of the pattern of early thalamocortical activity, which contributes to the disruption of the barrel map development (Akhmetshina et al. 2016). These findings indicate that 5-HT is necessary for brain maturation in early postnatal life and has long-term effects.

The effects of postnatal exposition to SSRIs strictly during lactations are still unknown. In mice, brain concentrations of paroxetine in pups at P7 are sevenfold lower than those observed in brain tissue of adult drug-treated. This finding correlates with the assumption that only a fraction of the maternal dose is transferred via lactation. Despite its clinical importance, SSRI exposure in infants through lactation has yet to be largely reported (Glover et al. 2015).

8.7 Concluding Remarks

Serotonergic mechanisms control the contractility of vascular smooth muscle in the placenta, contributing to the regulation of placental blood flow and, in addition, contributing to the regulation of the blood placenta barrier that protects the fetus from the transfer of xenobiotics. Pathogenesis of pregnancy-associated disorders such as intrauterine growth restriction and preeclampsia are associated with 5-HT dysfunction (Ranzil et al. 2019).

5-HT is synthesized and degraded in the human and rodent placenta. Before and during placenta-

Table 8.3 Postnatal exposure SSRIs

References	Timing of exposition	Methods	Effects
Hilakivi et al. (1987)	Postnatal days 7–18	Zimeldine hydrochloride, dose 25 mg/kg i.p., male rats Wistar strain	Zimeldine-treated rats expressed ↑ lengthened immobility times in the water pail
Ansorge et al. (2004)	Postnatal days P4–P21	Fluoxetine, dose 10 mg/kg i.p., 5-HTT$^{-/-}$ and 5-HTT$^{+/-}$ mice	Abnormal emotional behaviors in adult mice
Ansorge et al. (2008)	P4–P21	Fluoxetine, 10 mg/kg, 5 ml/kg, i.p., desipramine 10 mg/kg, 5 ml/kg, i.p., citalopram 10 mg/kg, 5 ml/kg, i.p., and clomipramine, 20 mg/kg, 5 ml/kg, i.p., WT 129S6/SvEv mice, SERT$^{+/+}$ /SvEv mice, SERT$^{+/-}$/SvEv mice	Abnormal emotional behaviors in adult mice
Maciag et al. (2006)	Postnatal days 8–21	Citalopram, dose 5 mg/kg s.c, twice daily, male Long Evans rats	↓ in the rate-limiting serotonin synthetic enzyme TPH in DR and SERT expression in cortex that persist into adulthood. Changes in behavior in adult rats including ↑ locomotor activity and ↓ sexual behavior
Popa et al. (2008)	P5–P19	Escitalopram (10 mg/kg/day, s.c., CD1 mice)	No changes in body weight gain, litter size, and body weight of pups at birth ↑ exhibited signs of depression in the form of sleep anomalies, anhedonia ↑ helplessness in females offspring
Lee (2009)	P0–P6	Fluoxetine hydrochloride 10 mg/kg, s.c., Wistar rat	In P30 and P35, blunt thermal and tactile perceptions and less explorative activity Thalamocortical afferents to the somatosensory cortex have ↓ branches in layer IV and ↓ dendritic occupancy in spiny stellate neurons of layer IV, ↓complexity and spine density but ↑ elongated length in mushroom and branched spines
Simpson et al. (2011)	P8–P21	Citalopram, dose 5 mg/kg s.c., twice daily, female and male Long Evans rats	Including changes in raphe and callosal connections, sensory processing, and myelin sheath formation. Also, drug-exposed rat pups exhibit neophobia and disrupted juvenile play behavior
Akhmetshina et al. (2016)	P2–P5	Citalopram, dose 10–20 mg/kg i.p. Wistar rats	Suppression of the early thalamocortical activity patterns contributes to the disruption of the barrel map development

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tion, a maternal source of 5-HT may also be provided to the embryo (Côté et al. 2007; Bonnin and Levitt 2011). All the evidence indicates that maternal and placental 5-HT are required for a healthy placenta and morphogenesis at the early stages of embryonic development. In rodents, the supply of endogenous brain 5-HT begins at E14.5–E15. This period is interesting because maternal and placental sources of 5-HT cease as primary influences for brain development.

Failure in 5-HT homeostasis will cause short- and long-term effects on the offspring 5-HT homeostasis, which will impact overall fetal development, leading to postnatal dysfunctions. Therefore, using SSRIs during pregnancy demands a well-equilibrated balance between offspring risk and maternal benefits since SSRIs affect placental and fetal brain development, impacting brain structure and behavior in adulthood.

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9 Effects of Prenatal Cannabinoids Exposure upon Placenta and Development of Respiratory Neural Circuits

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Abstract

Cannabis use has risen dangerously during pregnancy in the face of incipient therapeutic use and a growing perception of safety. The main psychoactive compound of the *Cannabis sativa* plant is the phytocannabinoid delta-9-tetrahydrocannabinol (A-9 THC), and its status as a teratogen is controversial. THC and its endogenous analogues, anandamide (AEA) and 2-AG, exert their actions through specific receptors (eCBr) that activate intracellular signaling pathways. CB1r and CB2r, also called classic cannabinoid receptors, together with their endogenous ligands and the enzymes that synthesize and degrade them, constitute the endocannabinoid system. This system is distributed ubiquitously in various central and peripheral tissues. Although the endocannabinoid system's most studied role is controlling the release of neurotransmitters in the central nervous system, the study of long-term exposure to cannabinoids on fetal development is not well known and is vital for understanding environmental or pathological embryo-fetal or postnatal conditions. Prenatal exposure to cannabinoids in animal models has induced changes in placental and embryo-fetal organs. Particularly, cannabinoids could influence both neural and nonneural tissues and induce embryo-fetal pathological conditions in critical processes such as neural respiratory control. This review aims at the acute and chronic effects of prenatal exposure to cannabinoids on placental function and the embryo-fetal neurodevelopment of the respiratory pattern. The information provided here will serve as a theoretical framework to critically evaluate the teratogen effects of the consumption of cannabis during pregnancy.

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9.1 Introduction

There is a widespread tendency to promote cannabis use for medicinal or recreational use. However, studies about the consequences of consumption of cannabis during the gestational period are scarce and inconclusive concerning its potential teratogenic role.

Globally, some 192 million people regularly consume cannabis, according to the United Nations Office on Drugs and Crime in 2017 (SAMHSA 2017). In Chile, the first country in Latin America to be part of the OECD, cannabis use increased from 7.1% in 2012 to 11.4% in 2014, according to *Servicio Nacional para la Prevención y Rehabilitación del Consumo de Drogas y Alcohol* (SENDA 2015). In the United States, marijuana is one of the most widely used drugs. The increased social acceptance of consumption and a growing perception of safety in recreational use has intensified its consumption among pregnant women (SAMHSA 2017). In Canada, one of the countries with the highest index of quality of life and health status worldwide (UNODC 2016), the prevalence of cannabis consumption in pregnant women increased from 1.2% in 2012 to 1.8% in 2017 ($p < 0.001$), equivalent to a relative increase of about 50% (Corsi et al. 2019). A study in the United States with information from 2004 to 2018 showed that preconception and postpartum cannabis use increased significantly in states that had legalized recreational cannabis compared with those states that had not legalized it (Skelton et al. 2021). These backgrounds show that cannabis use during pregnancy increases regardless of the country's socioeconomic development level. In this review, we will collect evidence in animal models and human tissue related to prenatal exposure to cannabinoids and maternal-fetal physiology, mainly focused on neurodevelopment of the central respiratory network.

9.2 Cannabis Impact in Pregnancy Outcomes

THC (delta-9-tetrahydrocannabinol) is the main psychoactive ingredient in cannabis, and there is evidence that it has effects both in the placenta and directly on the embryo/fetus. In vitro studies on human slices of the placenta indicate that exposure to 20 mM of THC reduces the transplacental amino acid transport (Fisher et al. 1987), thus indicating an alteration in the feto-maternal solute transfer. In sheep, prenatal exposure to cannabis induces placental vasodilation and disrupts perfusion to the fetus (Clapp et al. 1988). THC crosses the placental barrier in mice and induces fetal death, resorption, size reduction, and neurodevelopmental changes (Harbison and Mantilla-Plata 1972). In Rhesus monkeys, THC rapidly crosses the placenta and enters the fetus (Bailey et al. 1987). In lambs, the timing of fetal respiratory movements is modified during maternal exposure to cannabis smoke (Szeto et al. 1992). Prenatal exposure to a CB1r agonist depresses the rhythmogenic network in the medulla in newborn mice (Tree et al. 2014).

Importantly, THC is detectable in fetal umbilical cord blood in humans due to the mother smoking cannabis during gestation (Blackard and Tennes 1984). A recent meta-analysis that included 32 studies with data from approximately 28.5 million women showed 52% increase in the occurrence of low birth weight, 47% increase in small for gestational age, and a 39% increase in preterm birth, associated with cannabis use during pregnancy. This study shows an independent effect of cannabis despite using other illicit drugs and tobacco during pregnancy (Baía and Domingues 2022). A previous study with a cohort of 12 million births showed that cannabis use during pregnancy (dependence or abuse) increased the risk for preterm prelabor rupture of membranes (46%), placenta previa (24%), chorioamnionitis (18%), intrauterine fetal demise

(50%), growth restriction (35%), and more extended hospital stay (17%) when compared with pregnant persons who did not use cannabis (Petrangelo et al. 2019). However, researchers are alert of the low quality of the evidence due to the methodological limitations of studies, high heterogeneity, and the possible risk of bias, mainly due to measuring errors of the cannabis exposure based on self-report. Importantly, a systematic review focused on cognitive performance of offspring exposed to cannabis in utero found that prenatal cannabis exposure was associated with few effects, negative or positive. The performance of children exposed to cannabis prenatally did not differ from nonexposed children on the majority of cognitive outcomes. The authors found evidence for scores being below the normal range in only 0.3% of the total sample, concluding that the evidence does not support an association between prenatal cannabis exposure and clinically relevant cognitive deficits (Torres et al. 2020). This conclusion controverts other articles that highlight several cognitive and behavioral domains affected by in utero exposure to cannabis (Nashed et al. 2021).

Despite the controversy and methodological limitations of studies, the clinical data alert about potential alterations of embryo/fetal and placental environment due to chronic or severe prenatal exposure to cannabinoids, especially in respiratory network development, considering the higher risk of preterm birth and fetal growth restriction mentioned above. A study by Scragg and colleagues (Scragg et al. 2001) showed that cannabis is a weak risk factor for sudden infant death syndrome (SIDS) (although the cause is unknown, SIDS might be associated with defects in the portion of an infant's brain that controls breathing and arousal from sleep). A recent study with a cohort of over 400,000 birth (211 SIDS cases) reported that a cannabis-related diagnosis in pregnancy conferred a 2.7-fold increased risk of SIDS, adjusted for maternal nicotine use. Given the increased cannabis use during pregnancy, this finding warrants additional consideration; however, the mechanisms underlying prenatal changes induced by THC and other cannabinoids over neural circuits remain unknown.

9.3 Phyto- and Endocannabinoids

The term "cannabinoids" was used by R. Mechoulam and Gaoni in the mid-1960s to refer to a set of 21 carbon chemical compounds with structural analogy and derivatives identified from the *Cannabis sativa* plant (Mechoulam and Gaoni 1967).

The two main cannabinoids initially identified from cannabis plant extracts, using chromatography, were tetrahydrocannabinol (THC) and cannabidiol (CBD). THC and CBD molecules are currently called phytocannabinoids to differentiate them from endogenous animal counterparts, the endocannabinoids, such as anandamide (AEA) and 2-araquidonil glycerol (2-AG) (Florian et al. 2009; Mechoulam et al. 1995, 2014). Currently, more than 90 phytocannabinoids have been isolated from cannabis plants, but the more abundant are delta-9-THC, cannabinol (CBN), and CBD (Ambach et al. 2014; Andre et al. 2016). From these, delta-9-THC has higher psychoactive effects than CBD and CBN, respectively (Pertwee 1997).

The endocannabinoid system is formed by molecules involved in cannabinoid-mediated signaling, i.e., cannabinoid receptors (eCBrs), their endogenous ligands, and the enzymes responsible for the synthesis and degradation of these ligands (Castillo et al. 2012; Mechoulam et al. 1994; Piomelli 2003). Initially, the entire study of the endocannabinoid system focused on the nervous system due to the effects of phytocannabinoids on psychological, cognitive, emotional, and motor functions (Mechoulam et al. 1994). The nervous system expresses a family of eCBrs, from which the most studied are the cannabinoid 1 receptor (CB1r) and cannabinoid 2 receptor (CB2r) (Deadwyler et al. 1995). CB1r is the first eCBrs discovered (Devane et al. 1988) and corresponds to a G protein-coupled membrane receptor (GPR) that is abundantly expressed in neurons and glia at the central nervous system (Moldrich and Wenger 2000).

The CB1r has been cloned in rats (Matsuda et al. 1990), mice (Chakrabarti et al. 1995), and humans (Gérard et al. 1991). It shows 97–99%

amino acid sequence identity between both species (Montero et al. 2005). This high degree of conservation strongly suggests a significant role in the physiology of the CNS. CB2r also corresponds to a GPCR, preferably expressed at the peripheral level and in immune cells (Munro et al. 1993). In addition, recent findings suggest nonspecific interactions of endocannabinoids with other GPR receptors (i.e., GPR55), nuclear receptors (i.e., PPARγ), and other ionotropic receptors such as the transient receptor potential vanilloid 1 (TRPV1), making the scenario increasingly complex (Oláh et al. 2017).

Endocannabinoid receptors are activated by endogenous hydrophobic ligands such as 2-AG and AEA, which are structural analogues of THC (Pertwee 1997). Lately, other endogenous cannabinoid receptor ligands have been described, such as hemopressin, pepcans, and other hydrophobic substances such as palmitoylethanolamine and oleoylethanolamine (Bauer et al. 2012; Pertwee 2015). AEA and 2-AG are biosynthesized and released from membrane lipids phosphatidylethanolamine and phosphatidylinositol, by the enzymes N-acyl phosphatidylethanolamine/phospholipase D (NAPE-PLD) and diacylglycerol lipase (DAGL), respectively. Then these can be coupled to their receptor or be internalized and degraded by different lipases and hydrolases, such as the monoacylglycerol lipase (MGL), fatty acid amide (FAAH), and the cyclooxygenase 2 (COX-2)(Di Marzo et al. 1999; Oláh et al. 2017). Generally, in the nervous system, endocannabinoid biosynthesis machinery is located at the postsynaptic membrane (Wilson and Nicoll 2002).

9.4 The Endocannabinoid System in the Brain

9.4.1 Cannabinoid 1 Receptor (CB1r)

In mice, the CB1 receptor is expressed from early E11.5 embryonic age mainly in the pallium, the layers of grey and white matter covering the cerebrum's upper surface in vertebrates. From day E13, CB1r is detected in diencephalic neuronal tissue and the hippocampus (Morozov et al. 2009). In the human brain, autoradiographic detection of receptors using synthetic agonists like [^{3}H]CP55.940 allowed us to establish that CBrs are expressed from the prenatal period up to the adult stage, mainly in the forebrain, midbrain, and hindbrain. CBRs express specifically in nuclei related to cognitive function, association/movement control, and sensory/motor/autonomic functions (allocortex, neocortex, thalamus, basal ganglia, cerebellum, and medulla) with the known effects of cannabinoids on higher cognitive and motor functions (Glass et al. 1997).

In rats, expression of CB1r mRNA has been observed in some neural tube cells since E11 and in different structures within the central nervous system in late embryonic stages E15 to E21 (Buckley et al. 1998). For example, discrete levels of expression of CB1r mRNA are detected in the cerebral cortex, hippocampus, caudate-putamen, midbrain, brainstem, and cerebellum from day E16 to adult (Berrendero et al. 1998; Padley et al. 2003).

In addition to neurons, glia in adult mammals can express CB1r. However, the existence of cannabinoid receptors on astrocytes during the early stages of development is controversial. Gliogenesis and astrocyte maturation occur belatedly during prenatal development. Thus, mature astrocyte markers such as GFAP, Glt1, and Kir4.1 have been detected since E18.5 in murine embryos (Sardar et al. 2020). Nevertheless, there is evidence that the expression of CB1r in astroglial precursor cells could be crucial during early postnatal development (Aguado et al. 2006).

A strong interaction between mature astrocytes and neurons has been demonstrated (Pasti et al. 1997; Perea et al. 2009). Astrocytes play an essential role in regulating the optimal conditions for neuronal communication (Stella 2010). It has been shown that astrocytes expressing CBrs can release gliotransmitters to control synapses. Since a single astrocyte can control a thousand synapses simultaneously, the effect of cannabinoids acting on astrocytes modifies the functioning of neural networks. For example, the endocannabinoid system controls synapses in the hippocampus in mice

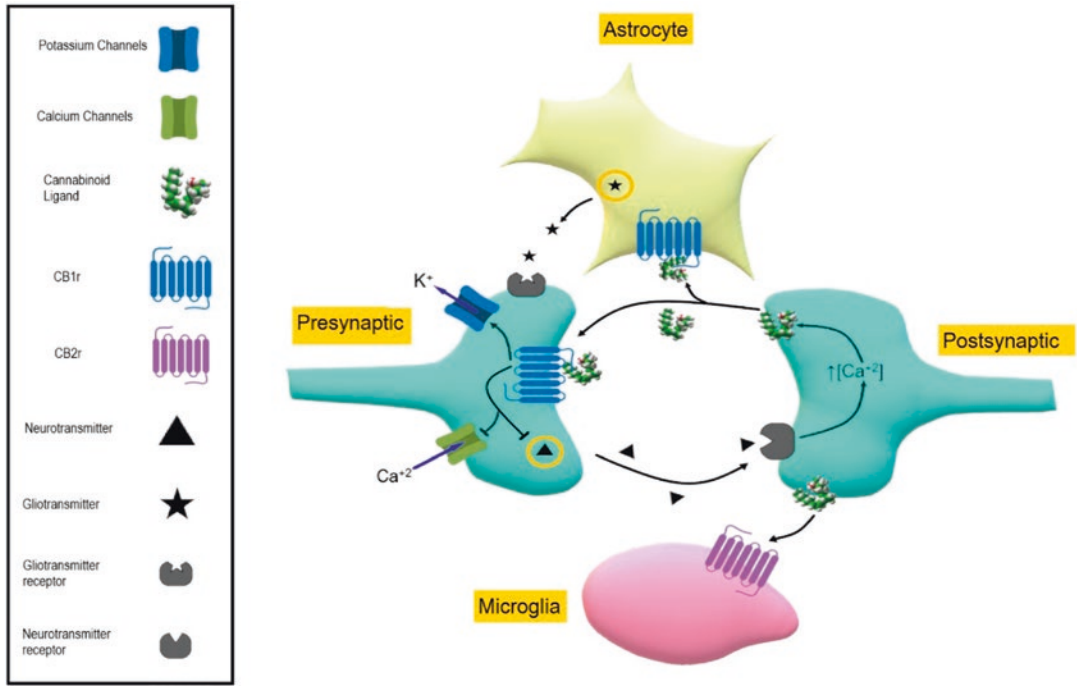

Fig. 9.1 Neuronal activity controls the endocannabinoid system. CBs are retrograde neuromodulators, synthesized in postsynaptic neurons. They inhibit neurotransmitter release and influence the membrane potential at the presynaptic neuron. The action of CBs originated at the postsynaptic neuron can also affect astrocytes and microglia, which makes this system more complex. A combination of CB1/CB2 receptors' independent mechanisms may contribute to cannabinoid neuroprotection

(Wilson and Nicoll 2002). Endocannabinoids (ECBs) released by pyramidal neurons act directly on the respective presynaptic terminal, reducing GABA transient neurotransmission (Kreitzer and Regehr 2001). However, the same ECBs released by pyramidal neurons can act on astrocytes through CBRs and elevate their intracellular Ca^{2+}, stimulating glutamate (GLU) release and enhancing the release of neurotransmitters in other distant synapses (Mulder et al. 2008; Navarrete and Araque 2010) (Fig. 9.1).

9.4.2 CB2r in the Brain

After the discovery of cannabinoid receptors, it was established that CB1r is preferably expressed in CNS neurons, whereas CB2r is expressed predominantly in basal cells and hematopoietic and other peripheral nonneural tissues (Galiègue et al. 1995; Griffin et al. 1999; Munro et al. 1993).

The existence of CB2r in the CNS tissue is controversial. Several authors report that the transcript and the protein CB2r are undetectable in the CNS (Buckley et al. 1998; Galiègue et al. 1995; Griffin et al. 1997). However, recent findings have questioned this affirmation. For example, CB2r quantitatively expresses in smaller amounts than CB1r in the CNS (Onaivi et al. 2006). Furthermore, CB2r can be found in the cerebral cortex, olfactory bulb, hippocampus, thalamus, cerebellum, and brainstem in neurons and glia (Atwood and Mackie 2010; Gong et al. 2006; Onaivi et al. 2006). Nevertheless, it is unclear whether brainstem expression is functionally significant (Van Sickle et al. 2005). On the other hand, CB2r is present in the microglia and CNS vascular elements (Ramirez et al. 2012; Stella 2010). Moreover, increased CB2r expression has been reported too in the neurons and glia in response to pathological environmental conditions (Stella 2010, 2012). Although the nature

and mechanism involved in this phenomenon are not yet clear, it is worth noting that, interestingly, CB2rs appear to be inducible, given the increase in its expression by disease, tissue injury, or inflammation (Carlisle et al. 2002; Maresz et al. 2005). For example, eCBs modulate cytokine liberation in microglia that express CB2r during neuroinflammation (Facchinetti et al. 2003; Maresz et al. 2005).

Other nonclassical eCBRs are expressed perinatally in the CNS. For example, GPR55, mRNA and protein, are already widely expressed in the murine frontal cortex at E14 (Cherif et al. 2015). In addition, GPR55 is expressed postnatally in the hippocampus, cerebellum (Kerr et al. 2013), striatum (Wu et al. 2013), brainstem, and spinal cord, mainly in neurons (Marichal-Cancino et al. 2017). In postnatal rats, PPARs, another type of cannabinoid receptor, are expressed in all tissues of the CNS (Moreno et al. 2004). In addition, TRPV1 mRNA is detected from E11.5 in adults in several brain regions (Thompson et al. 2014).

9.4.3 Enzymes Involved in the Biosynthesis and Degradation of Endocannabinoids

Components of the eCB system, including 2-AG and AEA ligands, their metabolizing enzymes diacylglycerol lipase (DAGL), N-acyl phosphatidylethanolamine/phospholipase D (NAPE-PLD), monoacylglycerol lipase (MAGL), fatty acid amide hydrolase (FAAH), and CB1r, are present early in mouse (E7.5) and chicken (HH stage 10) embryos. These data suggest that the endocannabinoid system is functionally active and could play a fundamental role in the early stages of neurogenesis (Psychoyos et al. 2012). However, experimental evidence obtained from work with murine *knockout* models for cannabinoid receptors, endocannabinoids, and their respective biosynthetic enzymes resulted in little apparent relevance of these components during the development of the central nervous system. These animals do not show significant deficiencies in organic neurodevelopment or the expected lethality known as "*knockout* paradox" (Harkany et al. 2007). Nevertheless, the absence of evident neurodevelopment alterations does not mean that there is no functional loss, since there is a lack of research in this regard. A relevant antecedent is the promiscuity of the endocannabinoid system in terms of other components that could compensate for the functional loss, though mechanisms have not yet been established. For further studies, the conditional genetic deletion of endocannabinoid components could be a tool to elucidate their specific contribution to neurodevelopmental processes.

9.5 Endocannabinoid System in the Placenta

The placenta is a specialized transitional organ (Gude et al. 2004) that provides oxygen, nutrition, and communication between mother and fetus (Fonseca et al. 2013). Its principal function is to support the fetus (Burton and Fowden 2015) through the provision of nutrients, hormones, and regulator factors, the exchange of gases, and waste elimination (Burton and Fowden 2015; Gude et al. 2004; Loke and King 1997), as well as to protect the fetus against any harmful stimulus, including maternal immune rejection (Fonseca et al. 2013). The coordination of these functions allows the placenta to be part of the proper development and growth of the fetus (Gude et al. 2004).

Among the cells forming the placenta, the trophoblast cells differentiate early and support the fetus' development during all gestation. These epithelial cells can originate from four subtypes of cells (Aplin 1991). The first subtype is the cytotrophoblasts, capable of proliferating and differentiating (James et al. 2006) into syncytiotrophoblast and extravillous trophoblast. Syncytiotrophoblasts are responsible for the endocrine function of the placenta and are located in direct contact with maternal blood, constituting a physical barrier between maternal and fetal blood (Kidima 2015). The extravillous trophoblast is an invasive component of the placenta that invades the decidua and the inner third of the myometrium (Huppertz et al. 2014). The remod-

eling of these tissues produces an increase in the diameter of the spiral artery, generating a decrease in resistance against blood flow and promoting maternal-fetal exchange. The fourth cellular subtype is the multinucleated giant trophoblast cells, formed by the aggregation and fusion of extravillous trophoblasts that restrict invasion of the trophoblast (Bischof and Irminger-Finger 2005; Costa 2016; Gude et al. 2004; Lunghi et al. 2007).

The expression of transcripts of CB1r and CB2r has been observed in rats during early gestational development (days 8–12) (Buckley et al. 1998), which suggests that the placenta during pregnancy is under cannabinoid regulation. In addition, the expression of these receptors was also detected in some types of placental cells, together with the TRPV1 receptor in all cell types between days E14 and E19 of gestation, with a gradual decline toward day E19 (Fonseca et al. 2012). In 1999, the first evidence of the presence of CB1r and CB2r receptors in the human placenta was published. The mRNA expression of both receptors was determined through northern blot analysis in human placenta and BeWo cells (Kenney et al. 1999), a model of human trophoblast (choriocarcinoma)(Al-Nasiry et al. 2006). In addition to expressing CBrs, BeWo cells respond to cannabinoid stimulation (Khare et al. 2006).

CB1r in the term placenta was immunodetected in all placental cell types, mainly in decidual, amniotic, and reticular epithelial cells, whereas in the chorionic cytotrophoblasts, there was moderate immunolabeling (Park et al. 2003). CB1r expression depends on the functional state of the placenta. It is differentially expressed in the term placenta, being higher in placenta from nonlaboring women than from laboring women (Acone et al. 2009). The cannabinoid receptors have also been detected since the early stages of gestation, particularly in the first-trimester placenta human. When measuring cannabinoid receptor transcript levels during weeks 7 and 12 of human gestation, CB1r expression appears highest at week 10, with a 91% decrease by week 12. By contrast, CB2r expression was constant throughout the period studied. CB1r was detected in all trophoblast cell types and endothelial cells from placental blood vessels but not in fetal or maternal infiltrating plasma cells. The cellular distribution of CB2r was similar to that observed for CB1r (Habayeb et al. 2008).

Additionally, it has been described in several tissues that cannabinoids can activate non-cannabinoid receptors, giving us new cannabinoid molecular targets, and the placenta is not an exception. Thus, Costa and colleagues (Costa et al. 2014a) demonstrated the expression of TRPV1 in human cytotrophoblasts and syncytiotrophoblasts in term placenta, using placental explants and the subsequent primary culture of cytotrophoblasts and syncytiotrophoblasts. In addition, mRNA and protein levels were consistent with the distribution of this cannabinoid target. Also, the expression of the GPR55 receptor in the placenta was analyzed. Interestingly, its expression was 5.8-fold increased in term placenta compared with the first-trimester placenta. Immunostaining and qPCR revealed that the expression of GPR55 in both placental stages is confined to the fetal endothelium. GPR55 was not detected in cytotrophoblast, syncytiotrophoblast, or villous stroma (Kremshofer et al. 2015). Regarding PPARγ receptors, their location in term placenta was confined to the syncytiotrophoblast (Capparuccia et al. 2002). However, their highest expression was detected in the placenta during the first trimester in the nuclei of the cytotrophoblasts, particularly in the nuclei of the extravillous cytotrophoblasts. Otherwise, PPARγ was not detected in the decidual cells (Tarrade et al. 2001). See details of cannabinoid receptors in the placenta in Table 9.1.

The placenta is also capable of expressing cannabinoid-metabolizing enzymes. During the middle and final stages of pregnancy in rats (days 14–19), there is higher expression and activity of NAPE-PLD (Fonseca et al. 2014) and DAGL, while COX-2 expression decayed. The levels of FAAH and MAGL remained unmodified (Fonseca et al. 2012). Subsequently, Vaswani and colleagues in 2015 partially confirmed these results in the rat placenta, reporting a differential expression of genes of different enzymes that are part of the endocannabinoid system, including upregulation of MAGL, NAPE-PLD, and COX-

2, while FAAH and DAGL remained constant (Vaswani et al. 2015).

In the human first-trimester placenta, FAAH mRNA levels peaked at week 11, and their levels subsequently declined. Its distribution involves extravillous trophoblast, cytotrophoblast, syncytiotrophoblast, and macrophages (Helliwell et al. 2004). In agreement, Habayeb and colleagues reported that FAAH expresses in all trophoblast cells. However, the syncytiotrophoblast exhibited higher levels of FAAH between weeks 10 and 11, and after, it became almost undetectable (Habayeb et al. 2008). In addition, FAAH is also expressed in the extravillous trophoblast that has not yet invaded the decidua (Chamley et al. 2008). In humans, FAAH in term placenta showed a higher expression in decidual and amniotic cells, whereas a deficient expression in the vascular layer and trophoblast, associated with immunolabelling in the syncytiotrophoblast (Park et al. 2003). In contrast, Acone and colleagues reported the lack of FAAH in term placenta obtained from laboring or nonlaboring women (Acone et al. 2009). Also, NAPE-PLD transcripts are present in the first-trimester and term placenta (Abán et al. 2013; Trabucco et al. 2009). Additionally, in term placenta, DAGL and MAGL enzymes have been identified in cytotrophoblast, syncytiotrophoblast, and chorionic villi and BeWo cells (Costa et al. 2014b, 2015a, b, c). In term placenta, COX-2 is expressed in syncytiotrophoblast, villous stroma, extravillous trophoblast, and capillary endothelium. In contrast, in the first-trimester placenta, it is expressed mainly in syncytiotrophoblast, villous stroma, and extravillous trophoblast (Meadows et al. 2004; Xu et al. 2005). Notably, the expression of COX-2 in the placenta is higher in pregnancies with preeclampsia, especially in syncytiotrophoblast (Cao et al. 2021; Hu et al. 2019), which indicates the pathophysiological association between placental COX-2 and placental disease, like preeclampsia.

Evidence shows that in the first-trimester and term placenta, components of the eCBS are expressed, including noncanonical components of this system that can be activated by eCBs and that could also be mediating their effects on the placental tissue (Table 9.1).

Table 9.1 Endocannabinoid system expression in placenta

	Location	References
CB1r	In early placenta, it is present in all trophoblast cells and endothelial cells of placental blood vessels	Habayeb et al. (2008)
	In the term placenta, it is found in all trophoblast cells, but mainly in the reticular, amniotic, and decidual epithelial cells	Acone et al. (2009), Kenney et al. (1999), and Park et al. (2003)
CB2r	In early placenta in all trophoblast cells, including placental endothelial cells	Habayeb et al. (2008)
	In term placental tissue and BeWo cells	Kenney et al. (1999)
TRPV1	In term placenta in cytotrophoblast and syncytiotrophoblast	Costa et al. (2014a)
GPR55	In fetal endothelium of term placenta, at a higher level of expression compared to first-trimester placenta	Kremshofer et al. (2015)
PPARγ	In extravillous trophoblast of early placenta	Tarrade et al. (2001)
	In syncytiotrophoblast of term placenta	Capparuccia et al. (2002)
FAAH	In the early placenta is present in all trophoblast cells and macrophages	Chamley et al. (2008), Habayeb et al. (2008), and Helliwell et al. (2004)
	In term placenta, higher expression in decidual and amniotic cells, with low expression in trophoblastic and vascular cells	Park et al. (2003)
NAPE-PLD	Present in early and term placenta	Abán et al. (2013) and Trabucco et al. (2009)
DAGL	In cytotrophoblast, syncytiotrophoblast, chorionic villi of term placenta, and BeWo cells	Costa et al. (2014b, 2015c)
MAGL	In cytotrophoblast, syncytiotrophoblast, chorionic villi of term placenta, and BeWo cells	Costa et al. (2014b, 2015c)

(continued)

Table 9.1 (continued)

	Location	References
COX-2	In syncytiotrophoblast, stroma, and villous trophoblast of early placenta	Meadows et al. (2004)
	In syncytiotrophoblast, villous stroma, extravillous trophoblast, and capillary endothelium of term placenta	Xu et al. (2005)

CB1r cannabinoid 1 receptor, *CB2r* cannabinoid 2 receptor, *TRPV1* transient receptor potential vanilloid 1, *GPR55* G protein-coupled receptor 55, *PPARγ* peroxisome proliferator-activated receptor gamma, *FAAH* fatty acid amide hydrolase, *NAPE-PLD* N-acyl phosphatidylethanolamine/phospholipase D, *DAGL* diacylglycerol lipase, *MAGL* monoacylglycerol lipase, *COX-2* cyclooxygenase 2

9.6 Endocannabinoid System Functions

9.6.1 Endocannabinoid System in Neural Process

The endocannabinoid system is conserved in all species, from invertebrates to vertebrates, suggesting critical biological functions (Onaivi et al. 2002). The first and most documented function of the endocannabinoid system is the modulation of synapses, specifically controlling the depolarization-induced suppression (DSI) or excitation (DSE) in neurons at the CNS (Wilson and Nicoll, 2002). The inhibition of the release of neurotransmitters can be achieved through three main mechanisms (Castillo et al. 2012): (1) Retrograde signaling: eCBs are mobilized from postsynaptic neurons and target presynaptic CB1rs to suppress neurotransmitter release. For example, studies in mouse striatum slice preparations show that CB1rs inhibit the presynaptic GLU release (Gerdeman et al. 2002). Similarly, CB1rs inhibit the release of GABA in hippocampal interneurons (Katona et al. 1999). (2) Non-retrograde signaling: eCBs produced in postsynaptic neurons activate postsynaptic CB1r or transient receptor potential vanilloid channels (TRPV1) in the nucleus accumbens (NAc) (Grueter et al. 2010), in the dentate gyrus (Chávez et al. 2010), and the bed nucleus of the stria terminalis (Puente et al. 2011). The activation of mGluR5, via PLC (Pan et al. 2008) and Ca^{2+} release from intracellular stores, promotes the synthesis of AEA that activates TRPV1 channels. (3) Neuron-astrocyte signaling: eCBs released from postsynaptic neurons stimulate astrocytic CB1r, thereby triggering gliotransmission. The gliotransmitter induces signaling cascades over the presynapse by inhibiting neurotransmitter release (Navarrete and Araque, 2008).

CB1 receptors are Gi/o protein-coupled receptors that mediate almost all the effects of exogenous and endogenous cannabinoids. 2-AG-mediated CB1r activation suppresses neurotransmitter release in two ways: First, the βγ subunits of the G protein inhibits voltage-gated Ca^{2+} channels (VGCCs), which reduce the influx of presynaptic Ca^{2+} and therefore prevent vesicular secretion. Second, the αi subunit inhibits adenylyl cyclase (AC) and cAMP/PKA, which is also involved in vesicular neurotransmitter transport (Castillo et al. 2012; Kano et al. 2009).

Brain CB2r alters neuronal activity and excitability. The bind of endocannabinoids (AEA or 2-AG) or exogenous ligands to CB2r activates a Gαi/o-mediated signaling cascade. This signaling leads to the inhibition of adenylate cyclase, the activation of intracellular kinases (including the PI3K-Akt pathway), and extracellular signal-regulated kinases (ERK). This mechanism culminates in the suppression of neuronal activity (Demuth and Molleman, 2006; Ibsen et al. 2017). For example, when JWH133 (highly selective CB2r agonist) is administered systemically or locally, there is significant inhibition of the dopaminergic neurons (DA) of the ventral tegmental area (VTA) both in vivo and ex vivo, in rats and mice. Thus, whole-cell patch clamped dopaminergic neurons of VTA, reduce dose-dependently their firing rate when exposed to CB2r agonists. This phenomenon is reversed by the CB2r antagonist AM630 and does not occur in neurons from CB2r-KO mice (Zhang et al. 2014, 2017). Compared to CB1r, CB2r has a higher affinity for Gαi than for Gαo (Glass and Northup, 1999; Ibsen et al. 2017). Like CB1r and other Gi-coupled receptors, CB2r activates G protein-

coupled potassium input rectifier channels (GIRKs) in cortical neurons (Stumpf et al. 2018). However, unlike other Gi protein-coupled receptors, JWH133 does not alter GIRK activation in VTA-DA neurons but instead activates M-type potassium currents, leading to hyperpolarization of the cell and inhibition of neuronal firing. Ma and colleagues (Ma et al. 2019) established that CB2R-mediated inhibition in VTA-DA neuron firing can be mimicked by M-current opener (10μM retigabine) and blocked by M-current blocker (30μM XE991). Moreover, the enhancement of neuronal cAMP by forskolin (10μM) reduced M-current and increased DA neuron firing rate. These results suggest that CB2Rs modulate VTA-DA neuron excitability mainly through an intrinsic mechanism, including a CB2R-mediated reduction of intracellular cAMP, and eventually enhancement of M-type K^+ currents.

CB2r inhibits the release of neurotransmitters in the nucleus accumbens and hippocampus. Both systemic and local administration of JWH133 in the nucleus accumbens (NAc) reduces extracellular dopamine (DA) levels in a dose-dependent manner, an effect that is blocked by co-administration of AM630 (potent inverse agonist CB2r) and that does not occur in CB2r-KO mice (Xi et al. 2011; Zhang et al. 2017). Through in vivo recordings of the dopaminergic terminals of the NAc, it was found that the activation of CB2r inhibits presynaptic DA release (Foster et al. 2016). In the hippocampus, local infusion of THC or JWH133 dose-dependently reduces the release of GLU or GABA by activating CB2r (Andó et al. 2012; Zheng et al. 2015).

9.6.2 Endocannabinoid System in Embryo Neurodevelopment

The endocannabinoid system components are expressed throughout prenatal development in the embryo and uterus, as well as in the embryonic annexes where cannabinoid signaling participates in crucial processes during neurodevelopment such as embryo implantation, the proliferation of neuronal and glial precursors, projection of the cone axonal, migration of neural precursors, neuronal morphogenesis, and neuronal differentiation (Harkany et al. 2007). Neurodevelopmental and embryotoxic effects of exogenous cannabinoids are indicated in Table 9.2.

Early murine embryos (blastocysts) express both CB1r and CB2r (Yang et al. 1996), and the uterus and the oviducts produce anandamide (Das et al. 1995; Paria et al. 1995). Basal levels of uterine AEA have been documented to decrease during the implantation process. Endogenous activation by AEA or exogenous activation by THC or WIN 55.212-2 of the CB1r and CB2r receptors negatively regulates uterine receptivity and blastocyst activation (Paria et al. 2001).

Cannabinoid agonists induce proliferation and subsequent astrogliogenesis in neural progenitor cells obtained from primary cultures of neonatal rats, a mechanism lacking in CB1r-KO rats (Aguado et al. 2006). Similarly, kainic acid-induced excitotoxicity produces the proliferation of neural precursors and neurogenesis of the hippocampus through a CB1r-dependent mechanism, which does not occur in CB1r-KO mice or in wild-type mice to which kainic acid and the CB1r antagonist, rimonabant, were co-administered (Aguado et al. 2007).

It has been established that the endocannabinoid system is actively involved in the growth of the axonal cone. Exposure of cultures of cortical neurons and hamster retinal explants to the specific CB1r agonist, arachidonyl-2′-chloroethyl amide (ACEA), induces the collapse of the axonal cone, which is reversed by the antagonist AM251 (Argaw et al. 2011). Similarly, adding the CB2r agonists JWH015 or JWH13 to the culture medium reduces the growth of the axonal cone in mice (Duff et al. 2013).

CB1r-mediated endocannabinoid signaling is also involved in migrating cholecystokinin-positive GABAergic interneurons from rats' hippocampus and cerebral cortex during late prenatal development. Furthermore, endocannabinoids suppress BDNF-dependent morphogenesis and differentiation of this neuronal type by inhibiting the branching and extension of neurites (Berghuis et al. 2005). Consistent with that, daily intraperi-

Table 9.2 Cannabinoids, neurodevelopment, and embryotoxicity

Experimental approach	Effect	References
Exposure in utero to cannabis extract in rats	Infertility, higher abortion rate, decreased litter size, and offspring survival rate. Fetal malformations such as microcephaly and phocomelia	Singh et al. (1981)
Rat embryonic primary cell culture of interneurons incubated with AEA y BDNF	Induction of chemotaxis, additive with BDNF-induced interneuron migration	Berghuis et al. (2005)
Exposure in utero to THC in mouse	Affects the directional growth of corticofugal axons (CFA) in the fetal brain	Tortoriello et al. (2014)
Exposure in utero to THC in mouse	Long-lasting functional alterations in the development of cortical neurons and increased susceptibility to seizures by their interference in CB1-dependent regulation of glutamatergic and GABAergic neuronal development	de Salas-Quiroga et al. (2015)
Exposure in utero to THC or win 55212-2 in mouse	Loss of hippocampal Cholecystokinin (CCK) interneurons	Vargish et al. (2017)
Exposure in utero to cannabis in humans	FGR and LBW	El Marroun et al. (2009)
Cannabis-related diagnoses in pregnant women	PTB and VPTB	Bandoli et al. (2021)
Cannabis exposure in pregnant women through routine urine toxicology screen	SGA	Kharbanda et al. (2020)
Cannabis-related diagnoses in pregnant women	SGA	Mravčík et al. (2020)
Cannabis dependence or abuse registered at birth	PTB, SGA	Petrangelo et al. (2019)
Cannabis dependence or abuse registered at birth	LBW, PTB, SGA	Shi et al. (2021)
Marijuana exposure of pregnant women based on urine drug screening during pregnancy	LBW, SGA	Straub et al. (2021)
Marijuana exposure based on urine drug screening at delivery, with nonexposed controls matched on multiple factors including other substance exposure	1 minute Apgar score significantly decreased	Bailey et al. (2020)
Self-reported cannabis exposure in pregnancy	5 minute Apgar score less than 4	Corsi et al. (2019)

FGR fetal growth restriction, *LBW* low birth weight, *PTB* preterm birth, *VPTB* very preterm birth, *SGA* small for gestational age, *win 55212-2* CB1r agonist

toneal injections of exogenous cannabinoids (WIN [0.75 mg/kg] or THC [5 mg/kg]) in pregnant mice from E10.5 until birth alters the morphology and reduce the density of hippocampal cholecystokinin interneurons (Vargish et al. 2017).

Endocannabinoid signaling is also involved in synaptic connectivity and responsiveness in the cortical circuitry. For example, maternal cannabis use during pregnancy disrupts the organiza-

tion of corticofugal axons by degradation of SCG10/stathmin-2 through CB1r signaling (Tortoriello et al. 2014) and Slit2/Robo1 pathways (Alpár et al. 2014). Thus, THC action involves the disruption of endocannabinoid signaling during development and postnatal effects on synaptic structure and plasticity (Tortoriello et al. 2014).

9.6.3 Endocannabinoid System and Placental Development

For normal placental development, there must be a balance between the proliferation, apoptosis, differentiation, migration, invasion, protein synthesis, and transport processes of the trophoblast. The endocannabinoid pathway is expressed and activated during placental development, which makes it possible for cannabinoids to act as regulators during placentation. Aberrant signaling at this stage can be associated with pregnancy disorders (which we will describe below).

Data suggest an interaction between apoptotic pathways and cannabinoids like AEA. In BeWo cells, activation of CB1rs and CB2rs stimulates the intrinsic apoptotic pathway, promoting the formation of reactive oxygen species (ROS) and nitric oxide (NO), producing an increase in intracellular calcium, a decrease in the mitochondrial membrane potential, and an increase in the activity of caspases 9 and 3/7, promoting apoptosis (Costa et al. 2014b, 2015b; Habayeb et al. 2008). In primary cytotrophoblast cultures, AEA can activate TRPV1, which modulates calcium signaling, increases ROS/NO, decreases the mitochondrial membrane potential, and increases the activity of the caspases mentioned above, leading finally to apoptosis (Costa et al. 2014a). Furthermore, AEA through cannabinoid receptors is capable of activating the extrinsic apoptotic pathway, increasing the activity of caspase 8 and the truncated Bid protein, and increasing the expression of the inhibitory protein of NF-κB (Costa et al. 2015b).

The syncytiotrophoblast plays an essential role in functions related to maternal-fetal exchange, such as nutrient transport, the supply of oxygen, and the protection of the fetus, in addition to being able to synthesize proteins, among which are some enzymes such as aromatase and placental alkaline phosphatase and hormones, such as human chorionic gonadotropin, estrogen, progesterone, and leptin. These hormones exert their functions in a paracrine and autocrine way, through which they can regulate different processes during embryonic development, such as placentation and angiogenesis (Costa, 2016). The differentiation from cytotrophoblast to syncytiotrophoblast is a coordinated and synchronized process carried out by several molecules and signaling pathways. Endocannabinoids can dysregulate the placenta's capacity for hormone synthesis (Costa et al. 2016). Studies suggest that CB1r, CB2r, and TRPV1 exert a regulatory role in the differentiation of cytotrophoblast to syncytiotrophoblast through 2-AG but not by AEA (Costa et al. 2014a, 2015b, c; Knerr et al. 2005).

Among the main regulators of invasion and migration of syncytiotrophoblasts, metalloproteinases (MMPs) and tissue inhibitor of metalloproteinases (TIMP) are found (Ji et al. 2013). In context, there is evidence that THC has anti-invasive effects on tumor cells by decreasing the expression of MMP 2 (MMP-2) or increasing the expression and activity of TIMP 1 (TIMP-1) through the activation of CB1r/CB2r (Blázquez et al. 2008; Ramer and Hinz 2008). On the other hand, Sun and colleagues demonstrated that the silencing of CNR1 dampens trophoblast invasion (Sun et al. 2010). Even in 2015, Kremshofer and colleagues (Kremshofer et al. 2015) observed that activating GPR55, through its agonist L-α-lysophosphatidylinositol (LPI), increases the migration of venous, but not arterial, placental endothelial cells, suggesting a role for this receptor in the function of the placental venous endothelium. The data suggest that eCBS participates in the invasion of trophoblastic cells and that since eCBS can activate GPR55, they could modulate endothelial cell migration.

On the other hand, endocannabinoids increase NOS activity, decrease intracellular levels of cAMP, decrease human chorionic gonadotropin and leptin, and decrease placental enzymes like

alkaline phosphatase, aromatase, and placental protein 13 (pp13), among others, which are related to regulatory signaling pathways of protein synthesis (Abán et al. 2013; Costa et al. 2015b, 2016). The incubation of normal placental villous with AEA increased the NO synthesis, to levels similar to those observed in preeclamptic samples. The NO synthesis in preeclampsia was decreased by incubation with CB1r antagonist AM251 (Abán et al. 2013). However, Accialini and colleagues (Accialini et al. 2021) showed that incubation with AEA enhanced NOS activity just in placenta from nonlaboring elective cesarean sections meanwhile decreased the enzyme activity in placental samples from patients with laboring vaginal deliveries. The authors propose that AEA plays an important role in labor, and its effects depend on the molecular context of the placenta.

TASK-1 channels are two-pore domain potassium channels expressed in humans, abundant in the brain, heart, placenta, and pancreas (Medhurst et al. 2001). TASK-1 play a crucial role in maintaining the resting membrane potential and contribute to the depolarization of the plasma membrane of excitable cells (Kim, 2005). These channels are inhibited by low concentrations of O_2 and acidic pH (Duprat et al. 1997; Lewis et al. 2001). However, in COS cells (fibroblast-like cell line derived from monkey kidney tissue), AEA has been determined to be a direct and selective blocker of TASK-1 channels (Maingret et al. 2001). Bai and colleagues (Bai et al. 2006) observed the presence of TASK-1 in cytotrophoblast cells and determined its functionality, establishing that the activity of this channel increased with pH (from 7.4 to 8.0). However, when applying 10μM of AEA, its activity decreased in both conditions, suggesting that TASK-1 channels are sensitive to extracellular pH and the presence of AEA in placental tissue. In parallel, Wareing observed the presence of TASK-1 mRNA in the placental vasculature and demonstrated that under hyperoxia (20% O_2), AEA increases the basal tone of the arteries and veins of the chorionic plaque, decreasing the activity of these channels (Wareing et al. 2006). Together, these results suggest that AEA could alter the plasma membrane potential in placental vascular tissue, at least through TASK-1 regulation.

It has also been observed that AEA may also affect folic acid transport in a contradictory way, since AEA acute administration reduces the transport, while its chronic administration increases it, suggesting that AEA may act as a modulator of placental transport (Araújo et al. 2009).

9.7 Placental Susceptibility to Cannabinoids

9.7.1 Effects of Prenatal Exposure to Endogenous and Exogenous Cannabinoids upon Placenta

In first-trimester placentas of women with spontaneous abortion, it was observed an increase in the expression of CB1r and NAPE-PLD and a decrease in FAAH levels, together with an altered expression of FAAH in peripheral lymphocytes, and high levels of AEA in plasma and in uterine natural killer cells (uNK) of the decidua. AEA activation affects uNK-dependent endometrial angiogenesis and decidualization (Fonseca et al. 2020; Maccarrone et al. 2000, 2002; Trabucco et al. 2009) (see Fig. 9.2).

Studies have registered alterations of the endocannabinoid system also in pathological conditions like preeclampsia. Preeclampsia is a disorder characterized by high blood pressure and proteinuria that manifests itself during the second half of pregnancy (Abalos et al. 2013), being one of the main causes of both maternal and perinatal morbidity and mortality (Brennan et al. 2014). Physiopathological findings of preeclampsia include exacerbation of vascular reactivity, imbalance between antiangiogenic and angiogenic factors, as well as an increase in oxidative stress and systemic inflammation, all contributing to insufficient trophoblastic invasion of the uterine spiral arteries, causing abnormal placentation and altered fluctuations in oxygen tension in the uteroplacental unit (Huppertz et al. 2014; Lim et al. 2015; Mellembakken et al. 2002;

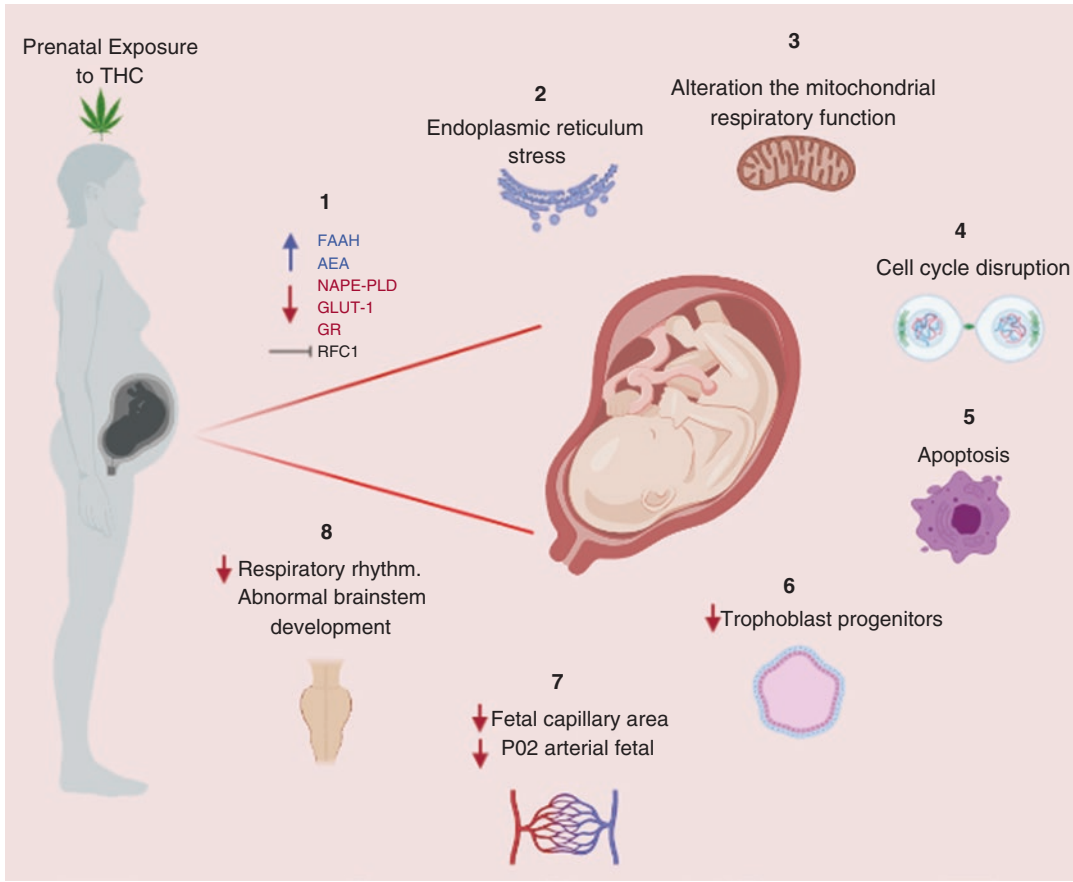

Fig. 9.2 Effects of prenatal THC exposure on placenta and brainstem. Prenatal exposure to THC causes changes at the molecular level such as enzymes and receptors, decreasing maternal-fetal transport, also causing stress of the endoplasmic reticulum and dysfunction of the mitochondrial respiratory chain, producing imbalances and interruption of the cell cycle, which leads to apoptosis of placental progenitor cells. It also decreases the fetal capillary area, which can lead to fetal hypoxia. At the fetal level, THC can influence the development of the nervous tissue through the effects on placenta, or directly affecting the properties of the circuit and synapses. Functional effects are diverse. As an example, the reduction of basal respiratory frequency is a consequence of the multiple effects of THC on the respiratory neural network as well as the abnormalities in brainstem development. As illustrated in Tables 9.3 and 9.4, cannabinoid receptors are found in respiratory-related circuits and their activation has impact on the respiratory neural network function

Powe et al. 2011; Redman and Sargent 2004; von Dadelszen et al. 1999; Walsh et al. 2009). Preeclamptic placentas show increased CB1r (Fügedi et al. 2014) expression and a decrease in AEA levels (Molvarec et al. 2015). Bienertova-Vasku and colleagues (Bienertova-Vasku et al. 2011) found a correlation between a single nucleotide polymorphism (rs806368) in the gene for CB1r and preeclampsia in the population of Central Europe. However, there are contradictory results on the levels of FAAH and NAPE-PLD, since the work of Abán (Abán et al. 2013) reports an increase of FAAH and a decrease in NAPE-PLD levels, while Fügedi (Fügedi et al. 2014) reported a decrease in FAAH and an increase in NAPE-PLD levels. In addition, an association between endoplasmic reticulum (ER) dysfunction and various reproductive pathologies has been proposed, including recurrent pregnancy loss and preeclampsia. It is worth mentioning that, recently, it has been reported that the endocannabinoid 2-AG is capable of inducing

Table 9.3 Cannabinoid receptors expression in neural respiratory-related tissue

	Location	References
CB1r in brainstem	Important CB1r expression in rat medulla since E16 that decays in adulthood	Berrendero et al. (1998)
	In the dorsal vagal complex, consisting of the area postrema, nucleus of the solitary tract, and the dorsal motor nucleus of the vagus in the brainstem of ferrets	Van Sickle et al. (2001)
CB2r in brainstem	Expression of CB2r mRNA and protein in brainstem neurons in rat, mouse, and ferret	Van Sickle et al. (2005)
CB1r in peripheral arterial chemoreceptors	High mRNA level in the carotid body superior cervical ganglia (SCG) and nodose-petrosal-jugular ganglia (NG-PG-JG) complex in newborn rats	McLemore et al. (2004)
TRPV1 in arterial peripheral chemoreceptors	In rat petrosal neurons innervating carotid body glomus cell clusters	Roy et al. (2012)

Table 9.4 Cannabinoids and respiratory effects

Experimental approach	Effect	References
Perinatal THC exposure in lambs	Increased fetal respiratory movements in lambs	Szeto et al. (1992)
Prenatal exposure to drug abuse and THC	Slow ventilatory response to hypercarbia in newborns	Ali et al. (2014)
KO CB1r	Mouse cardiorespiratory abnormalities	Silvani et al. (2014)
CB1r activation by win 55212-2 in the rostral ventrolateral medulla oblongata	Cardiorespiratory disturbances in anesthetized rats	Padley et al. (2003)
"en bloc" preparations[a] superfused with anandamide	Depresses the respiratory rhythm in neonatal mice	Tree et al. (2010)
win 55212-2 intracisternal application	Respiratory and cardiovascular dysregulation in anesthetized rats	Pfitzer et al. (2004)
Cannabis exposure based on urine drug screening at delivery, with nonexposed controls matched on multiple factors including other substance exposure.	1 minute Apgar score significantly decreased	Bailey et al. (2020)
Self-reported cannabis exposure in pregnancy	5 minute Apgar score less than 4	Corsi et al. (2019)

[a]"en bloc" preparation: isolated medulla-spinal cord preparation from newborn mice

ER stress and apoptosis, through a mechanism dependent on CB2r activation in placental cells (Almada et al. 2020b).

Recent evidence suggest that through cannabinoid receptors, phytocannabinoids have a negative impact on fertility, pregnancy outcomes such as preterm birth, and fetal health (El Marroun et al. 2009; Fried 1995; Grzeskowiak et al. 2020; Maia et al. 2019). THC presents a dual effect in primary cultures of human cytotrophoblast. At low THC doses, predominates an antioxidant effect, an improvement in the mitochondrial function of the syncytiotrophoblast, and a protective effect against cell death. By contrast, at high THC doses, predominates a cannabinoid alteration of the syncytialization process and a decrease in cell viability, in addition to the inhibition of trophoblast turnover. Although these results show a dual effect, they also are implying that THC alters the dynamic process of placental development (Costa et al. 2015a) (see Table 9.5). Maia and colleagues (Maia et al. 2019) reported that THC disrupts the placental endocannabinoid system by temporarily altering AEA levels and the expression of the metabolizing enzymes FAAH and NAPE-PLD, without observing alteration in the expression of cannabinoid receptors. Furthermore, long-term AEA levels are associated with a change in AEA biochemistry in an attempt to reestablish homeostasis in the endo-

Table 9.5 Effects of THC exposure on the placenta

Experimental approach	Effect	References
Exposure to THC in human placenta	Inhibition of amino acid uptake	Fisher et al. (1987)
	Delta-9-tetrahydrocannabinol transfer through the placental barrier	Hutchings et al. (1989)
	Low dose has antioxidant effect High doses decrease cell viability Inhibition of trophoblast turnover. Alteration of dynamic processes of placental development	Costa et al. (2015a)
	Short-term: increases NAPE-PLD levels and reduces FAAH levels without changes in AEA levels Long-term: decrease NAPE-PLD level, increases FAAH and AEA levels	Maia et al. (2019)
Exposure to THC in rat placenta	Symmetric FGR. Decrease in trophoblast progenitor cells Reduction of GLUT-1 and glucocorticoid receptors Decreased expression of placental CD31 Reduced fetal capillary area Higher levels of pericytes and collagen deposits	Natale et al. (2020)
BeWo cells exposed to THC	Chronic: decreases folic acid transport	Araújo et al. (2009)
	Induces endoplasmic reticulum stress Mitochondrial respiratory dysfunction	Lojpur et al. (2019)
	Cell cycle disruption and apoptosis	Almada et al. (2020a)
Primary culture of human cytotrophoblasts exposed to THC	Chronic incubation: decrease of folic acid transport and inhibition expression of the reduced folate carrier (RFC1)	Keating et al. (2009)
Cannabis use in the first trimester determined by a self-report questionnaire	Adaptations in fetal placental and cardiac blood flow, but not in cerebral blood flow	El Marroun et al. (2010)

cannabinoid system (Maia et al. 2019) (see Table 9.5). Moreover, a recent study has reported that exposure to THC induces endoplasmic reticulum stress and affects the mitochondrial respiratory function in BeWo cells (Lojpur et al. 2019) (see Table 9.5 and Fig. 9.2). On the other hand, as already mentioned, the silencing of CB1r produces premature differentiation and changes in genes related to the migration and invasion of trophoblast cells, leading to a defective invasion (Sun et al. 2010). However, this effect is not only produced by the silencing of CB1r but also the amplification of endocannabinoid signaling (Xie et al. 2012). Likewise, in PPARγ$^{-/-}$ mice, there was placental dysfunction, affecting the epithelial differentiation of trophoblast tissue and vascular processes (Barak et al. 1999; Kubota et al. 1999).

9.7.2 Maternal-Fetal Transfer and Cannabinoids

Maternal-fetal transfer is a key process through which, the fetuses obtain nutrients, passive immunization, hormones, growth factors, perform gas exchange (oxygen comsuption, carbon dioxide elimination) and waste products to support their development and maintenance. Hence, any impairment of the placental-fetal circulation indisputably affects this exchange and, therefore, affects the fetus. In 1987, Fisher reported that THC in human placenta inhibited the uptake of the amino acids α-aminoisobutyric acid and

valine, suggesting that THC is placento-toxic (Fisher et al. 1987) (see Table 9.5). Furthermore, it has been reported in BeWo cells that synthetic cannabinoids and THC can lead to an interruption of the cell cycle and subsequent apoptosis of placental cells, being able to affect fundamental cellular processes of gestational development (Almada et al. 2020a). In addition, it has been observed that THC interferes with the uptake of folic acid. After 48 h of exposure to THC in BeWo cells, the folic acid transport decreased. However, acute exposure produces an increase in folic acid transport (Araújo et al. 2009) (see Table 9.5 and Fig. 9.2). In agreement with these results, primary cultures of human cytotrophoblast chronically exposed to THC show inhibition of both the folic acid transport and the expression of the gene RFC1. This inhibition later leads to a reduction in folate carrier 1 that participates in the transfer of placental folate (Keating et al. 2009) (see Table 9.5 and Fig. 9.2).

A study by Clapp and colleagues (1988) tested whether maternal marijuana smoking impairs placental oxygen transfer in late ovine pregnancy by disrupting the perfusion balance between the maternal and fetal placental circulations. They observed that changes in fetal oxygen tension suggest that perfusion balance is disrupted at a microcirculatory level. In addition, cannabis consumption has been associated in humans with an increase in placental weight, a response that could be related to chronic hypoxia induced by exposure to the drug (Carter et al. 2016). Through the impairment of oxygen transfer, cannabinoids could significantly deteriorate the development and maintenance of the fetus. The impact of maternal exposure to THC on placental, fetal, and neonatal development is clear in rats. THC causes a symmetric fetal growth restriction, with size recovery on postnatal day 21. Besides, the placenta showed an increase in the labyrinth area and a decrease in trophoblast progenitor cells, glucose transporter 1 (GLUT1), and glucocorticoid receptor (GR), which are essential for fetal development. In addition, THC decreases placental expression of CD31 (endothelial marker), suggesting that it may alter angiogenesis. Even more, THC decreased the fetal capillary area and increased the recruitment of pericytes with greater collagen deposition, which may contribute to reducing fetal blood space. The results suggest that the decrease in fetal growth is due to placental insufficiency, where an increase in the maternal blood space and a decrease in the fetal blood space involve impaired nutrient transport (Natale et al. 2020) (see Table 9.5 and Fig. 9.2).

Both phytocannabinoids and endocannabinoids are mostly hydrophobic substances with the potential to cross biological membranes (Harbison and Mantilla-Plata 1972). Phytocannabinoids' hydrophobicity suggests these molecules have no problem passively spreading through cell membranes, similar to other terpenes. Various preclinical models demonstrate the vertical transmission of cannabinoids from mother to child, either through the transition of substances through the placental barrier or during lactation in mice (Freudenthal et al. 1972), Rhesus monkeys (Bailey et al. 1987), and humans (Blackard and Tennes 1984).

9.8 Embryonic Susceptibility to Prenatal Exposure to Cannabis

9.8.1 Prenatal Cannabis Exposure and Embryo-Fetal Survival

Exposure to cannabis during preimplantation period in preclinical models increases embryo lethality and teratogenesis (Harbison and Mantilla-Plata 1972; Singh et al. 1981; Wilson and Fraser 1977). Prenatal maternal smoke (1.84% THC) exposition in ovines decreased fetal pO_2 and remained significantly depressed at 2 h after smoke inhalation (Clapp et al. 1988; Szeto et al. 1992). Litters that survive prenatal exposure show a reduced number of pups. However, it is of interest that postnatal survival is also affected by prenatal exposure to THC. In rats, maternal subcutaneous prenatal injection of THC (50 mg/kg/day) reduced litter survival compared to control associated with breastfeeding problems (Borgen et al. 1971). In rabbits, pup survival during the first 3 h postnatally is also sig-

nificantly decreased by 30 mg/kg/day in mothers exposed to cannabis from the seventh day of gestation (Sofia et al. 1979). Similarly, prenatal exposure of rats to the cannabinoid agonist win55.212–2 (0.5 mg/kg) induces an increased neonatal death, and for over 1 mg/kg, the entire litter dies before the first postnatal day (Shabani et al. 2012).

9.8.2 Respiratory Effects of Prenatal Exposure to Cannabis

9.8.2.1 The Respiratory Pattern Generator

The neural network called respiratory pattern generator (RPG) generates the respiratory rhythm in mammals. The inspiratory and expiratory neurons conforming to the mammalian RPG are located at the brainstem along the ventral (VRC) and the dorsal (DRC) respiratory columns (Feldman et al. 2013; Ramirez and Baertsch 2018). These neurons are already active early in fetal life and cease their activity at death. Most of them are excitatory, i.e., glutamate is their primary neurotransmitter. RPG neurons project into cranial (V, VII, IX, X, XII) and spinal cord (C3–C6, T1–T10) nuclei to innervate respiratory motoneurons that drive the rhythmical contraction of the respiratory muscles to generate the breathing motor behavior. The RPG activity appears from the coupling of three circuits in the VRC: the retrotrapezoid/parafacial respiratory group (RTN/pFRG) (Feldman et al. 2013; Onimaru and Homma 2003), the post-inspiratory complex (Anderson et al. 2016), and the inspiratory pre-Bötzinger complex (preBötC) (Feldman et al. 2013; Smith et al. 1991). At the level of the phrenic nerve (the main respiratory output innervating the diaphragm), a three-phase respiratory cycle is recorded, defined by the activity of each circuit (Anderson and Ramirez 2017; Richter et al. 1992). The RPG is regulated by sensors, among which chemoreception is one of the most important, since it allows matching breathing to physiological demands. Peripheral and central chemoreceptors send information to the RPG about changes in PaO_2, $PaCO_2$, pH, and blood flow at the level of great arteries (Eyzaguirre and Zapata 1984) and the interstitial and the cerebrospinal fluid of the brain, respectively (Nattie and Li 2012).

9.8.2.2 Neuromodulation of the Respiratory Network

Several neuromodulators at the RPG contribute to controlling and shaping the respiratory rhythm. Some of them are excitatory (e.g., GLU, substance P, cholecystokinin, thyrotropin-releasing hormone (TRH), and histamine), others are inhibitory (e.g., opioids, GABA, glycine, and somatostatin), and the third group are both excitatory and inhibitory depending on the cellular target at the neural circuit (e.g., dopamine, serotonin, and norepinephrine) (Doi and Ramirez 2008). We will discuss the cannabinoid effects on the respiratory network, focusing on two examples of neuromodulators: dopamine and serotonin inputs to the respiratory network.

9.8.2.3 Dopaminergic and Serotonergic Systems

Dopaminergic neurons appear early in rat neurodevelopment, approximately between E12 to E14 (Specht et al. 1981) and close to E10.5 in mice (Riddle and Pollock 2003; Smidt et al. 2003). In addition, some specific groups of dopaminergic neurons only exist prenatally in the brain (Reisert et al. 1990). These neurons induce the maturation of other dopaminergic neurons through trophic signals such as TGF beta (Luo et al. 2016).

Dopamine is present in almost all regions of the brainstem (Fujii et al. 2004; Hsiao et al. 1989; McNamara and Lawson 1983; Milner et al. 1986). Nurr1 mutant mice (lacking dopaminergic neurons) have severe respiratory disturbances and decreased hypoxia responsiveness (Nsegbe et al. 2004). Nurr1 is expressed in various regions related to the nervous control of cardiorespiratory activity, including the n*ucleus tractus solitarius* (NTS), *nucleus ambiguus*, the dorsal motor nucleus of the vagus, and the carotid bodies. Nurr1 mutant mice fail to develop midbrain dopaminergic neurons and do not survive beyond 24 h after birth.

Serotonergic neurons that persist in postnatal life in rats are already detectable by immunohistochemistry from E13 in embryonic rhombencephalic cells (Lidov and Molliver 1982; Wallace and Lauder 1983). 5-HT has a pivotal role in morphogenesis during brain development and in adaptive brain plasticity throughout life. Serotonin participates in the modulation of neuronal proliferation, migration, and differentiation, neurite development, axonal guidance, synaptogenesis, and efficiency of transsynaptic signaling (Daubert and Condron 2010; Mazer et al. 1997).

Serotonin participates in the respiratory neural network of the brainstem as a neuromodulator (Hodges and Richerson 2008). Furthermore, abundant evidence indicates that serotonergic neurons are chemosensitive to changes in pH and CO_2 in cerebrospinal fluid and perivascular nervous tissue, contributing to the modulation of respiratory rhythm generation and chemoreception (Nattie and Li 2010; Richerson 2004; Teran et al. 2014). This contribution is demonstrated when optogenetically stimulating serotonergic neurons of the raphe nucleus in adult mice induce an increase in the respiratory rate (Depuy et al. 2011). In addition, stereotaxically applying acidic artificial cerebrospinal fluid into the raphe nucleus increases the amplitude of the respiratory signal obtained from the phrenic nerve in anesthetized rats and cats (Coates et al. 1993).

9.8.2.4 Cannabinoid Exposure and Respiratory Function

CB1r *knockout* mice show significant cardiorespiratory abnormalities such as irregular breathing and increased apneas during non-REM sleep (Silvani et al. 2014). Blockade of CB1r in postnatal days 0–2 (P0–P2) in mice, by subcutaneous injection of CB1r antagonist AM251, increased basal minute volume, secondary to increases in basal tidal volume, suggesting that endocannabinoids exert a tonic drive on ventilation. In addition, the basal number of apneas and their durations were increased by the blockade of CB1r, suggesting that endocannabinoids support rhythm regularity (Tree et al. 2014). Accordingly, activation of brainstem cannabinoid receptors by exogenous cannabinoids depresses the respiratory rhythm. Microinjections of win55.212-2 or HU-210 (CB1r agonists) into adult rats' rostral ventrolateral medulla oblongata (RVLM) reduce the respiratory phrenic nerve activity, which was reversed with AM281 (CB1r antagonist) (Padley et al. 2003). Interestingly, the application by superfusion of 30μM of AEA also induces a decrease in the respiratory rate, observed in the isolated medulla-spinal cord preparation ("en bloc" preparation) from newborn mice (Tree et al. 2010).

P0–P2 neonates exposed prenatally to cannabinoids showed hyperventilation during basal conditions, altered ventilatory chemoreflex response pattern to hypoxia, and longer apneas (Tree et al. 2014). The daily interscapular injection of 0.5 mg/Kg of win55.212-2 from the fifth day of gestation in murine dams increased the basal minute volume and the basal tidal volume in P0–P2 pups and the basal minute volume and basal respiratory rate in P10–P12 pups, compared to controls. In the "en bloc" preparations, these changes in basal values were less prominent. Indeed, the respiratory frequency (fR) and the central respiratory drive (fR multiplied by the amplitude of the integrated inspiratory burst) were lower in "en bloc" preparations obtained from exposed pups than in controls (Tree et al. 2014).

9.8.2.5 Dopamine, Serotonin, and Prenatal Cannabinoid Exposure

After prenatal exposure to cannabinoids, considerable changes in the levels of neuromodulators in the embryo/fetus are expected. Accordingly, rat fetuses chronically exposed to THC show dopamine (Bloom and Kiernan 1980; Bonnin et al. 1996; Chen et al. 1990; Maître et al. 1970) and serotonin (Sofia et al. 1971) levels significantly altered. Furthermore, acute THC exposure of rodent neuronal synaptosomes elicited a concentration-related decrease in the uptake of ^{14}C-dopamine into crude synaptosomal preparations derived from mouse striata (Howes and Osgood 1974). On the other hand, prenatal exposure to THC in rats induces a hyperdopaminergic

phenotype at the brain level (Frau et al. 2019). The dopaminergic activity of the midbrain is influenced by both phyto- and endocannabinoids, through the activation of CB1rs present in glutamatergic (Marinelli et al. 2007) and GABAergic (Lecca et al. 2012) terminals, suggesting that cannabinoids regulate the activity of dopaminergic neurons through their actions on excitatory and inhibitory presynaptic signaling.

Tetrahydrocannabinol impairs spatial memory and significantly increases the 5-HT content in the ventral hippocampus. A microdialysis study showed that THC (6 mg/kg, i.p.) decreased 5-HT release in the ventral hippocampus. Furthermore, the 5-HT precursor, 5-hydroxy-l-tryptophan (5-HTP; 50 mg/kg, i.p.), the 5-HT reuptake inhibitor, clomipramine (0.01–0.1 mg/kg, i.p.), the 5-HT receptor agonist, 5-methoxy-N,N-dimethyltryptamine (5-MeODMT; 0.01–0.03 mg/kg, i.p.), and the 5-HT2 receptor agonist, 1-(2,5-dimethoxy 4-iodophenyl)-2-amino propane (DOI; 10μg/kg, i.p.), significantly attenuated the THC-induced impairment of spatial memory. These results suggest that the 5-HT neuronal system may be involved in THC-induced spatial memory impairment.

In rat ventral hippocampus, administration of THC (6 mg/kg, i.p.) increased the 5-HT content and decreased the 5-HT release detected through microdialysis (Egashira et al. 2002). Similarly, in mouse brain cortex slices preincubated with [^{3}H]serotonin and superfused with medium containing tetrodotoxin (TTX), a serotonin-reuptake inhibitor and a nonselective 5-HT antagonist, the electrical- and calcium-evoked 5-HT release was inhibited via cannabinoid receptors activation (Nakazi et al. 2000). Conversely, the pharmacological blockade of CB1r increases basal extracellular levels of 5-HT in the medial prefrontal cortex (Aso et al. 2009; Darmani et al. 2003). The single administration of THC in adult mice increases the 5-HT concentration globally in mouse brain (Holtzman et al. 1969) and similarly in rats (Egashira et al. 2002; Molina-Holgado et al. 1993; Sofia et al. 1971). In adult mice, increases in serotonin levels occur throughout the brain with doses of THC over 5 mg/kg. At a dose of 10 mg/kg, the brain serotonin content increases 45 min after the administration of THC and returns to its normal values 3–6 h later (Holtzman et al. 1969).

9.8.2.6 Cannabinoids, Dopamine, Serotonin, and Embryonic CNS Respiratory Disruption

As discussed above, prenatal exposure to cannabinoids induces hypoxia in the embryo/fetus. Prenatal hypoxia alters the postnatal development of the chemo-afferent pathways that participate in the regulation of ventilation and the development of postnatal chemosensory mechanisms against a hypoxic challenge (Peyronnet et al. 2000). Hypoxia per se can modify pre- and postnatal catecholamine levels at the brainstem (Seidler and Slotkin 1990; Slotkin et al. 2011; Soulier et al. 1992) as well as the catecholaminergic levels in the carotid bodies (Peyronnet et al. 2000). Additionally, to hypoxia, cannabinoids could exert respiratory effects on the embryo/fetus due to transient changes in the brainstem dopamine and serotonin levels. As mentioned above, acute exposure to cannabinoids transiently increases dopamine release in different brain regions. Whether extracellular dopamine reaches high levels in the brainstem, there is a risk of central respiratory depression through desynchronization of the respiratory rhythm (Fujii et al. 2006). Since cannabinoids have been detected in urine up to 27 days after regular human consumption (Ellis et al. 1985), the effect of prenatal cannabinoids on fetal CNS dopamine levels could persist during prenatal and early postnatal neurodevelopment. On the other hand, maternal exposure to THC from the fifth day of gestation to the first postnatal day produces a brain area- and sex-dependent effect on indolamine levels, particularly in the midbrain raphe nuclei (Molina-Holgado et al. 1996). Although these studies demonstrated an alteration in the serotonergic system, the specific effects of cannabinoids upon the development of serotonergic neurons in central respiratory circuits have not been systematically addressed.

9.8.2.7 Cannabinoids Stimulate Peripheral Arterial Chemoreceptors

In mammals, the peripheral chemoreflexes are mediated by specialized oxygen-sensitive glomus cells located in the carotid and aortic bodies (Lahiri et al. 1981). When the carotid body is stimulated by hypoxia, the glomus cells release excitatory neurotransmitters that excite the carotid and aortic nerve terminals containing axons originating from cell bodies located at the petrosal and nodose ganglia, respectively (Eyzaguirre and Zapata 1984). The central projections of peripheral chemosensory neurons finally reach the CNS innervating the NTS, one of the main nuclei of the brainstem involved in cardiorespiratory integration and control (Accorsi-Mendonça et al. 2011; Accorsi-Mendonça and Machado 2013; Berger 1979; de Daly 1997; Longhurst 2008). Activation of peripheral chemoreceptors in conscious rats evokes hyperventilation (tachypnea and increased tidal volume) (Cardenas and Zapata 1983), bradycardia, and a significant increase in arterial blood pressure. These hemodynamic and respiratory responses are abolished when the carotid bodies are removed (Eugenin et al. 1990). According to the above, it has been shown that NTS neurons receive projections directly from the carotid bodies.

Acute intravenous injection of THC produces hypotension, bradycardia, and bradypnea in rats, dogs, and cats (Estrada et al. 1987; Graham and Li 1973; Vollmer et al. 1974). Similarly, intravenous injection of the endocannabinoid reuptake inhibitor AM-404 (10 mg/Kg) induces a transient bradypnea and reduction in O_2 saturation associated with an increase in thoracic cavity distention (Iring et al. 2017). This effect is absent in CB1r-KO mice, and therefore, it appears to be mediated by this type of receptor. Similarly, intracisternal injection of cannabinoid agonists has a depressant effect on the respiratory rate. However, stimulation by cannabinoids at supraphysiological concentrations directly on peripheral chemoreceptors induces the opposite effect. Thus, rabbit carotid body perfused with 3μM AEA increases carotid sinus nerve activity in a similar pattern to normal responses to hypoxia (Kobayashi and Yamamoto 2010). This phenomenon does not appear to be mediated by the classical cannabinoid receptors CB1r or CB2r due to the quantitatively low expression of these receptors in both the carotid and aortic bodies, even a high level of CB1r mRNA in the nodose-petrosal-jugular ganglia (NG-PG-JG) complex (McLemore et al. 2004). Interestingly, a significant expression of a nonclassical cannabinoid receptor TRPV1 has been detected in afferents carried by the carotid nerve (Roy et al. 2012).

Nevertheless, it is important to emphasize that despite cannabinoids applied locally in the carotid or aortic bodies stimulating the ventilatory chemoreflex, the systemic administration of cannabinoids depresses breathing. This dual effect is probably because the peripheral excitatory effect is counteracted by the inhibitory effect of cannabinoids on central respiratory nuclei or in other targets that have not yet been identified. Furthermore, the physiological plasma concentration of endocannabinoids such as AEA and 2AG varies according to various processes such as the circadian cycle, food intake, inflammation, and metabolism, among others, and is usually on the order of a few picomolar (Fanelli et al. 2012; Roy et al. 2012; Sparling et al. 2003). These data suggest that endocannabinoids could contribute to a basal tone of peripheral stimulation of the respiratory rhythm, which could change in the face of pharmacological stimulation of chemoreceptors during the perinatal period, modifying key processes such as the "reset" of the carotid bodies (Carroll and Kim 2013) or the sensitivity of the respiratory network to a hypoxic stimulus (Tree et al. 2014).

9.9 Concluding Remarks

With the information here exposed, we can say that there is abundant evidence that the endocannabinoid system plays a fundamental role in the development of the fetus, in the neural migration, in the growth of the axonal cone, in the control of the release of neuro- and gliotransmitters, and in synapse modulation. Therefore, the general over-

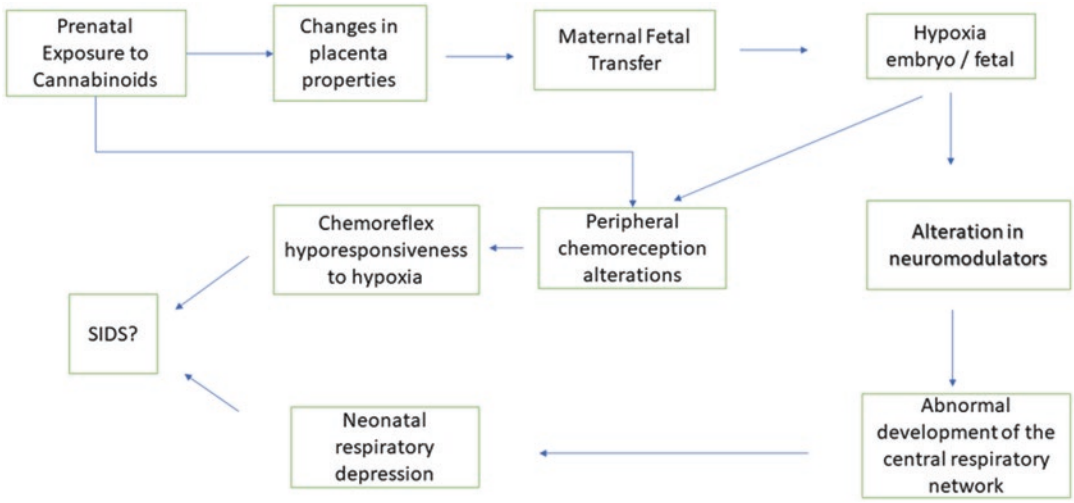

Fig. 9.3 Model of the effect of cannabinoids on the placenta and respiration Proposed model that relates perinatal exposure to cannabinoids with alteration in chemosensitivity to hypoxia, respiratory depression, and potential relationship with sudden infant death syndrome (SIDS)

view is that the consumption of cannabinoids during pregnancy is harmful to the development and function of the placenta and fetal health. Furthermore, this idea is supported by recent clinical studies that show an association between prenatal cannabis consumption and adverse obstetrics and perinatal outcomes.

The endocannabinoid system is essential for critical processes during placental and embryo/fetal development. An imbalance of this system is associated with alterations in maternal-fetal structures and directly on the embryo. Cannabis use induces alterations in the fetoplacental transport and vascular regulation, producing an increase in maternal blood flow and a decrease in fetal blood flow, leading to an embryo/fetal hypoxic condition. In parallel, cannabinoids could modify the incipient respiratory movements in the embryo/fetus/neonate in an indirect way through an alteration in the neurons responsible for brainstem dopaminergic and serotonergic signaling.

The use of cannabis in the gestational period is associated with a wrong perception of safety for both the mother and the fetus, but cannabis consumption could be related to early postnatal respiratory disorders and, eventually, to sudden infant death syndrome; therefore, studying these mechanisms is crucial for preventing fetal distress. We hope that the knowledge about the deleterious effects of cannabinoids on fetal development promotes self-regulation and the reduction in the prevalence of recreational cannabis use during pregnancy. It is important to note that there is still controversy about the neurodevelopmental effect of prenatal cannabis. Further studies are necessary to solve the questions about the dosage, frequency, and form of cannabis consumption and confounders, especially in socioeconomic disadvantaged populations. These studies are essential to design new public policies to regulate the recreational use of drugs and reduce the growing consumption of cannabis by pregnant women (Fig. 9.3).

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10 Impact of Prenatal Nicotine Exposure on Placental Function and Respiratory Neural Network Development

Sebastián Beltrán-Castillo, Karina Bravo, and Jaime Eugenín

Abstract

Smoking during pregnancy is associated with multiple undesirable outcomes in infants, such as low birth weight, increased neonatal morbidity and mortality, and catastrophic conditions like sudden infant death syndrome (SIDS). Nicotine, the most addictive and teratogenic substance in tobacco smoke, reaches and crosses the placenta and can be accumulated in the amniotic fluid and distributed by fetal circulation, altering the cholinergic transmission by acting on the nicotinic acetylcholine receptors (nAChRs) expressed from very early gestational stages in the placenta and fetal tissue. Because nAChRs influence the establishment of feto-maternal circulation and the emergence of neuronal networks, prenatal nicotine exposure can lead to multiple alterations in newborns. In this mini-review, we discuss the undeniable effects of nicotine in the placenta and the respiratory neural network as examples of how prenatal nicotine and smoking exposition can affect brain development because dysfunction in this network is involved in SIDS etiology.

Keywords

Nicotine · Acetylcholine · Cigarette · Placenta · Prenatal exposure · Prenatal nicotine

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10.1 Introduction

Despite decades of warning about its harmful effects on health, maternal cigarette smoking or exposure to environmental tobacco smoke during pregnancy remain a central public health problem. In a developing country like Chile, smoking prevalence in fertile Chilean women was around 37.5% (MINSAL 2010). Of the low-socioeconomic-status women, 27% have smoked before pregnancy, and 17% keep smoking during the first trimester of pregnancy (Mallol et al. 2007). If environmental tobacco smoke is considered, pregnancy exposure rises to 55%

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(Mallol et al. 2007). Smoke could be associated with around 11% of preterm birth and full-term low birth weight, 12% of deaths by preterm-related factors, and 35% of deaths diagnosed as sudden infant death syndrome (SIDS) (Cerda et al. 2017). Similar incidence rates have been reported in countries like the USA (Dietz et al. 2010). For instance, around 10% of women have smoked before pregnancy, and 7.1% keep smoking during the first trimester of pregnancy (Kondracki 2019). This health problem has been accentuated in the last decades because of the popularity, even during pregnancy, of using vaporizers, electronic cigarettes, or other electronic nicotine delivery systems (ENDS), sold with the marketing of a "safe way for smoking." However, ENDS delivers a similar amount of nicotine to traditional cigarettes (McCubbin et al. 2017; Whittington et al. 2018). Approximately 54% of women who smoke before pregnancy quit smoking before or during pregnancy (ACOG Committee 2020). Unfortunately, this is late because the teratogen effects on the respiratory neural network may occur before the first month of pregnancy.

Nicotine is a teratogenic substance contained in tobacco smoke (Joschko et al. 1991) acting as an agonist in the nicotinic acetylcholine receptors (nAChRs), a family of fast ionotropic cationic receptors with homo- or heteropentameric structure (Albuquerque et al. 2009). In mammals, nine α (1–7 and 9–10), four β (1–4), γ, δ, and ε nAChR subunits have been identified (Albuquerque et al. 2009). Many of these nAChR subunits are detected and are fully functional in the placenta (Hellstrom-Lindahl and Court 2000; Lips et al. 2005) and fetal brain (Atluri et al. 2001) at very early gestational stages. Because nAChRs are part of the feto-maternal circulation, prenatal exposure to nicotine can alter nAChR activity affecting placenta development and its function (Holloway et al. 2014). On the other hand, nicotine accumulates in amniotic fluid and the fetus's blood, reaching the fetal nAChRS (Wickstrom 2007), inducing an imbalance in the cholinergic activity that could promote neurodevelopmental abnormalities. Thus, fetuses exposed to cigarette smoke and nicotine could suffer developmental abnormalities due to a nicotine-associated placental dysfunction or by direct nicotine action on nAchRs expressed in the fetus. In brief, a smoking mother puts her child at considerable risk, with a higher incidence of spontaneous abortion, reduced weight at birth, premature ablatio placentae, or a high incidence of deformities and behavioral disturbances (Haustein 1999). A list of deleterious outcomes or pathologies in fetuses, newborns, and children associated with prenatal cigarette smoke exposition is illustrated in Table 10.1.

Table 10.1 Health problems in fetuses, newborns and children associated with maternal cigarette smoking exposure

Fetus
Alteration of fetal development by nicotine-induced hypoxia (Socol et al. 1982)
Increased rate of prematurity and mortality
Reduced intrauterine growth (Abraham et al. 2017)
Newborn
Respiratory dysfunctions (Campos et al. 2009)
Increased risk of SIDS
Low birth weight (Zheng et al. 2016)
Newborns with a greater need for handling and worse self-regulation (Stroud et al. 2009)
Childhood (meta-analysis)
ADHD (Huang et al. 2018).
Obesity and metabolic outcomes (Ino 2010).
Cardiac outcomes (Banderali et al. 2015)

SIDS sudden infant death syndrome
ADHD attention-deficit/hyperactivity disorder

Because smoking implicates the exposition of more than 3000 different substances potentially affecting directly or indirectly the fetuses or the placenta, a fine dissection of the specific nicotine effects using cigarette smoke as the stimulus is an impossible task. However, the animal models of prenatal nicotine exposure, as a single stimulus, are helping to isolate the nicotine-induced functional and structural effects on fetal development or placenta function. Using animals implanted subcutaneously with an osmotic minipump to deliver nicotine can mimic steady-state nicotine plasmatic levels like those described in moderate smokers (Benowitz and Jacob 1984). Unlike other animal models in which nicotine is delivered daily by injection, the use of an osmotic minipump facilitates the interpretation of the results because it reduces the daily stress associated with injections and avoids the plasma nicotine peaks

reached during smoking, which can lead to a reduction of uterine blood flow and fetoplacental intermittent hypoxia (Campos et al. 2009).

In this chapter, we discuss how nicotine can affect fetal development. First, we will discuss about the effects of nicotine on the development and function of the placenta. Next, we discuss the impact of nicotine exposure on the fetus, in particular, the induction of breathing dysfunction by alteration in the development of the respiratory neural network and how nicotine exposition can be part of the etiology and pathogenesis of SIDS.

10.2 Cholinergic Expression and Its Roles in the Placenta

In the placenta, an active non-neuronal cholinergic system emerges early, which includes the enzymes choline acetyltransferase (ChAT) and acetylcholinesterase (AChE) for synthesis and degradation of ACh, respectively, and the multiple muscarinic (mAChRs) and nicotinic (nAChRs) receptors (Chang and Gaddum 1933; Comline 1946; Fant et al. 1981). In trophoblast, specific anti-ChAT immunogold deposition is detected within the cell membrane, associated with placental microvilli and caveolae, in the cytosol, in the mitochondria, and within the nuclear membrane (Wessler et al. 2012). Whereas ACh agonist binding assays suggest a differential distribution of mAChR in syncytiotrophoblast, with M1 receptors expressed in the apical membrane and M2 receptors in the basal plasma membrane (Pavia et al. 1997), all the mammalian nAChRs subunits have been detected in term placenta or trophoblast cells in culture by RT-PCR, protein expression, or immunohistochemistry (Lips et al. 2005; Machaalani et al. 2014; Machaalani et al. 2018).

Unfortunately, the dynamic distribution patterns of cholinergic receptors in the placenta along gestation remain unclear. However, it is well known that the placental levels of ACh vary with gestational age, reaching a peak in the 20–22 gestational week (Koshakji et al. 1974). Additionally, the distribution and expression pattern of the different nAChR subunits in the human term placenta is compatible with the formation of many functional receptors, such as α7 homopentamers, α4β2 heteropentamer, as well as receptors containing α3 and α5 subunits in syncytiotrophoblast membrane, α4β2 and α4α6 heteropentamers intracellularly, and α9 homopentamers in the border of syncytiotrophoblast layer II (Lips et al. 2005). Furthermore, the basal membrane of syncytiotrophoblast is enriched with α9 subunits, whereas the apical membrane is enriched with α10 (Lips et al. 2005).

Despite the early high levels of ACh and cholinergic receptors in the placenta, their specific roles in this tissue still need to be better understood. However, ACh shows the ability to modulate, in an estrogen-dependent manner, the trophoblastic release of nitric oxide (NO). Such release of NO is associated with angiogenesis and the ability to alter cytotrophoblast function during modulation of the inflammatory microenvironment, allowing tissue remodeling and angiogenesis at the maternal-placental interface (Paparini et al. 2015), revealing that ACh participates in placental development (Babaei et al. 2003; Bhuiyan et al. 2006) (Fig. 10.1). Also, ACh in placenta has been associated with the uptake of amino acids (Welsch et al. 1981) and with the release of E2 and F2 alpha prostaglandins (King et al. 1991; Sastry 1997). On the other hand, ACh function as a regulator of vasoconstriction in the placental vessels is unclear. Whereas some studies show that ACh is a vasoconstrictor (Ciuchta and Gautieri 1964), others indicate that ACh is a weak vasodilator (Euler 1938) or even a molecule without any effect in the tone of the villous vascular smooth muscle (Boura et al. 1986). On the other hand, ACh, activating mAChRs, has essential functions during early mammalian embryogenesis. In mouse embryonic stem cells, inhibition of mAChRs suppressed the expression of Wnt3, which is essential for primitive streak formation and mesodermal progenitor differentiation (Arima et al. 2013). Whether mAChRs have similar actions on placenta development is an open question.

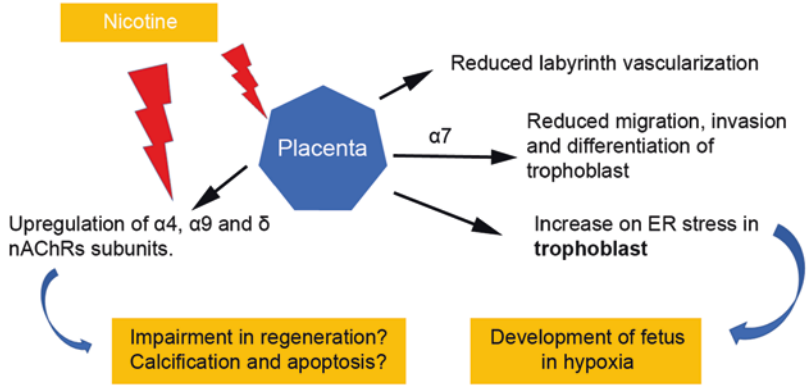

Fig. 10.1 Effects of nicotine on the placenta. The upregulation of nicotinic acetylcholine receptors (nAChRs) subunits could be associated with impairment of regeneration and calcification and apoptosis observed in the placenta of smoking mothers. The nicotine-induced alterations in labyrinth vascularization, trophoblast invasion, and endoplasmic reticulum (ER) stress significantly affect the development of the fetus in a hypoxia placenta environment

10.3 Gestational Cigarette Smoking, Nicotine Exposure, and Placenta

The main goal of the placenta is to facilitate and promote normal fetal growth and development, ensuring the exchange of nutrients, oxygen, carbon dioxide, and waste metabolites between the maternal and fetal circulatory systems (Gude et al. 2004). An adequate orchestration between differentiation and migration of trophoblast cells into the maternal decidua is essential to establish feto-maternal circulation (Strickland and Richards 1992; Cross et al. 1994). Because nicotine and cotinine, the main metabolite of nicotine, can activate nAChRs, gestational exposure to cigarettes can impact cholinergic transmission, altering the correct placenta development and resulting in impairment of its function and the development of the fetus.

The exposition of female Wistar rats to nicotine, from prior to mating until gestational day 15, resulted in increased placental hypoxia by reduction of labyrinth vascularization (Fig. 10.1), likely due to reduced levels of placental and circulating endocrine gland-derived vascular endothelial growth factor (EG-VEGF), and downregulation of the heart- and neural crest derivatives-expressed protein 1 (Hand1) and human glial cells missing-1 protein (GCM1) factors (Holloway et al. 2014), which impact placental angiogenesis, trophoblast differentiation (Scott et al. 2000), and the branching morphogenesis in the chorioallantoic placenta (Anson-Cartwright et al. 2000). The inhibitory effects of nicotine on trophoblast migration, invasion, and differentiation into interstitial trophoblast cells were confirmed using the rat trophoblast cell line RCHO-1 in culture (Holloway et al. 2014). In these cells, nicotine decreases matrix metalloproteinase 9 (MMP9) activity (Holloway et al. 2014). Moreover, the invasiveness of immortalized first-trimester extravillous trophoblast cell line (HTR-8/SVneo cells) is inhibited by the activation of nicotinic α7 receptor and by the reduction of C-X-C motif chemokine ligand 12 (CXCL12) expression, one of the most important regulators of trophoblast function and placental angiogenesis (Wang et al. 2015). Beyond the stress promoted by nicotine-mediated hypoxia, nicotine also increases the endoplasmic reticulum stress in trophoblast, contributing to placental insufficiency (Wong et al. 2015; Wong et al. 2016).

In part, these nicotine-induced placental dysfunctions can be a consequence of nAChR upregulation/downregulation or changes in their localization or activities (Holloway et al. 2014). For example, the levels of mRNA for α4 subunits are increased in rat trophoblast cell line RCHO-1 exposed in vitro for 24 h to nicotine (10^{-9} to 10^{-3} M) or in placenta obtained from rats injected daily with nicotine (1 mg/kg/day) from 2 weeks

before to 15 days after mating (Holloway et al. 2014). Other nAChRs, such as α9 and δ nicotinic receptor subunits, are upregulated in human placenta from cigarette-smoker mothers (Machaalani et al. 2014). The α9 activation has been related to the process of keratinocyte re-epithelization (Grando 2006). The nicotine-dependent upregulation of α9 could be involved in the impairment of the regeneration in the placenta, in addition to the direct effect of the nicotine exposition (Machaalani et al. 2014). On the other hand, the δ subunits are associated with increased Ca^{2+} permeability, which is associated with calcification and apoptosis (Machaalani et al. 2014). Because nicotine upregulates the levels of δ subunits, it is speculated that increased Ca^{2+} permeability increases calcification and apoptosis observed in the placenta of smoking mothers (Jauniaux and Burton 1992; Klesges et al. 1998; Genbacev et al. 2003; Wang et al. 2011; Machaalani et al. 2014).

Placenta and fetus development also can be affected by cotinine. Although cotinine acts as a weak agonist of the nAChRs, its levels are 12 times higher in the placenta of women exposed to smoking (from 2.3 to 27.2 ng/g) (Vyhlidal et al. 2013). Further, its half-life is ten times longer than nicotine, accumulating in the placenta, fetal serum, and amniotic fluid (Luck et al. 1985). Because cotinine contributes to the production of prostaglandin E2, which induces labor and initiates uterine contractions, its accumulation could contribute to the induction of preterm labor and spontaneous abortions (Rama Sastry et al. 1999).

10.4 Effects of Prenatal Nicotine Exposure on Fetus and Newborn

Prenatal exposition to tobacco has been associated with several dysfunctions and malformations in children's organs, including the lungs (McEvoy and Spindel 2017), kidneys (Kallen 1997; Taal et al. 2011), and heart (Malik et al. 2008). Moreover, many of these defects can also be observed in animals exposed to prenatal nicotine (Sekhon et al. 2001; Chen et al. 2015), strongly suggesting that nicotine is the principal teratogen agent associated with maternal cigarette smoking.

Although nicotine has a short half-life in humans (around 1–2 h) (Benowitz 2009), smoking addiction induced by nicotine and its ability to get through the placenta quickly (Wickstrom 2007) results in a prolonged exposition of the fetuses to high levels of nicotine. The nicotine in the fetal circulation and amniotic fluid can reach 15% and 88% higher levels than that in maternal plasma early in the first trimester (Luck et al. 1985; Pastrakuljic et al. 1998). Cotinine, resulting from nicotine degradation, has a longer half-life in the body than nicotine (Benowitz et al. 2009) and, thereby, is an excellent marker to trace fetal nicotine exposure (Eskenazi et al. 1995). In passive or active smokers, high cotinine levels have been detected in amniotic fluid at 7 weeks of gestation (Jauniaux et al. 1999). This evidence indicates that fetuses can be exposed to nicotine early in gestational age. Since nAChRs emerge early in the brain, within the first trimester of pregnancy (Dwyer et al. 2009), ACh can play a neurotrophic role in the brain (Drachman 1974). So, prenatal nicotine exposition can vastly alter the brain and neuronal network development. In the next section, we discuss the effects of prenatal nicotine exposure on the development of the respiratory neural network as an example of how harmful prenatal nicotine exposure is for nerve system development and how nicotine, affecting the respiratory neural network, can originate breathing defects and the increase in the risk for sudden infant death syndrome (SIDS) in newborn.

10.5 The Postnatal Breathing Dysfunction and Risk of SIDS: A Consequence of Prenatal Exposure to Smoke or Nicotine

The increased risk for sudden infant death syndrome (SIDS) is one of the most devasting post-birth outcomes associated with tobacco smoking and nicotine prenatal exposure (Anderson et al. 2005, 2019). According to the National Institute

of Health, SIDS is defined as the "sudden death of an infant or young child under the age of 12 months, which is unexpected by history, and in which a thorough postmortem examination fails to demonstrate an adequate cause of death" (Beckwith 1970). Its occurrence is associated with multiple risk factors, but intrauterine exposure to cigarette smoke or nicotine arises as the leading preventable risk factor for SIDS (Caleyachetty et al. 2014; Mitchell and Krous 2015). The risk of SIDS is also increased by a prone sleeping position (Mitchell et al. 1999), increased environmental temperature, or a history of maternal drug abuse, among others. Despite successful campaigns for reducing SIDS by promoting supine sleeping for infants, SIDS persists as the leading cause of death in infants under 1 year old in developed countries (CDCA 2018) and still is the leading cause of neonatal infant mortality in the USA with an overall rate of 0.39/1000 live births (CDCA 2018, Kinney and Haynes 2019). The major incidence rate is in males between 2 and 4 months of age (Kinney and Thach 2009). The "Triple-Risk model" (Filiano and Kinney 1994) postulates that the convergence of an underlying vulnerability in the infant, a critical developmental period, and an exogenous stressor as an asphyxia (for example, because the baby is sleeping in prone or face-down position), underlies the occurrence of SIDS. Thus, SIDS can arise from a failure in chemoreflex affecting the arousal and respiratory function (Campos et al. 2009) as the underlying vulnerability, occurring within a critical period during early postnatal life and triggered by a stressor as the asphyxia by the sleep position.

Evidence suggests that prenatal smoke exposure and nicotine are strongly related to infant breathing vulnerability during the critical period that would increase the risk of SIDS. On the one hand, infants deceased and classified as SIDS and infants born from smoker mothers show similar breathing dysfunctions. Many SIDS infants showed in their life span a background of breathing irregularities such as increased frequency of central apnea (Brady and McCann 1985; Schechtman et al. 1991, 1992; Kahn et al. 1992; Campos et al. 2009), sleep apneas (Shannon et al. 1977; Schechtman et al. 1991), and weakened chemoreflex such as impairment of spontaneous and hypercarbia- and hypoxia-evoked excitability (Campos et al. 2009). Infants born from smoker mothers showed an increase in the number of apneas, irregularity in the periodic breathing movements from fetal ages to early postnatal days (Gennser et al. 1975; Thaler et al. 1980), decrease in the tidal volume and increase in the respiratory frequency (Sovik et al. 1999) during sleep, increase in both the frequency and duration of obstructive apneas (Kahn et al. 1994), and decrease in the ventilatory responses to hypoxia (Ueda et al. 1999) and hypercapnia (Ali et al. 2014). Additionally, postmortem examination of brains belonging to infants exposed to maternal smoking or infants who died by SIDS revealed brainstem abnormalities in several neurotransmitters systems, in particular those contributing to the modulation of the respiratory neural network, such as the catecholaminergic system (Obonai et al. 1998) and the raphe serotoninergic (5-HT) system (Kinney et al. 2003, 2005, 2009; Paterson et al. 2006; Lavezzi et al. 2009; Kinney and Haynes 2019). Moreover, postmortem studies in infants who died from SIDS have detected different grades of hypoplasia in the arcuate zone (Lavezzi et al. 2009; Paradiso et al. 2018), a group of neurons located on both sides of the human medulla oblongata showing chemosensitivity to carbon dioxide and pH (Filiano et al. 1990; Zec et al. 1997).

Because many of these morphological abnormalities and ventilatory dysfunctions can be mimicked by prenatal nicotine exposure in animals (Campos et al. 2009), nicotine has been proposed as the component in cigarette smoke responsible for all these impairments. In animals, nicotine exposure during pregnancy induces hypoventilation and respiratory rhythm irregularity with an increased number of apnea during normoxia in mice and rats (St. John and Leiter 1999; Robinson et al. 2002; Huang et al. 2004; Eugenin et al. 2008). Reduction of ventilatory chemoreflex to hypoxia in awakening rats and sleeping lambs (St. John and Leiter 1999; Hafstrom et al. 2002; Huang et al. 2004;

Simakajornboon et al. 2004), reduction of auto resuscitation from primary apnea in rats (Fewell et al. 2001), and longer delays for the hypoxia-induced awakening response in lambs (Hafstrom et al. 2002). In mouse neonates, prenatal nicotine exposure reduced the ventilatory response to hypercapnia restricted to early postnatal life as a "critic period" (P0–P5) and reduced central chemosensitivity (Eugenin et al. 2008; Coddou et al. 2009). Defects induced by prenatal nicotine exposure have been associated with alterations in serotonin (5-HT) raphe system. For example, the reduced basal firing rate of raphe neurons in slices is likely due to an increase in 5-HT1A autoreceptor expression (Eugenin et al. 2008). Also, in isolated raphe neuron cultures, the exposure to nicotine resulted in a reduction in raphe neuron sensitivity to CO_2 (Avraam et al. 2020), but not in brainstem slices from prenatal nicotine-exposed neonates, where electrophysiological properties of raphe neurons, as well as their responses to hypercarbia, were similar to those in controls (Cerpa et al. 2015).

In the next section, we will discuss how nicotine can induce alterations in neuronal networks, as those observed in the respiratory neural network.

10.6 How Can Prenatal Nicotine Affect Neural Circuits, Functions, and Ontogenies?

The early emergence of nAChRs in fetal stages (Atluri et al. 2001), their potential to impact neural maturation and synaptic plasticity (Opanashuk et al. 2001, McKay et al. 2007; Dwyer et al. 2009; Placzek et al. 2009), and the imbalance in nAChR pattern expression induced by the nicotine exposition (Frank et al. 2001; Lv et al. 2008) can result in changes in neural circuits that could explain the outcomes observed in infants born from smoker mothers or in those who died from SIDS (Fig. 10.2).

Similar to what happens in the placenta, prenatal nicotine exposure changes the pattern of expression of nAChRs during intrauterine and postnatal life resulting in a transient increase of

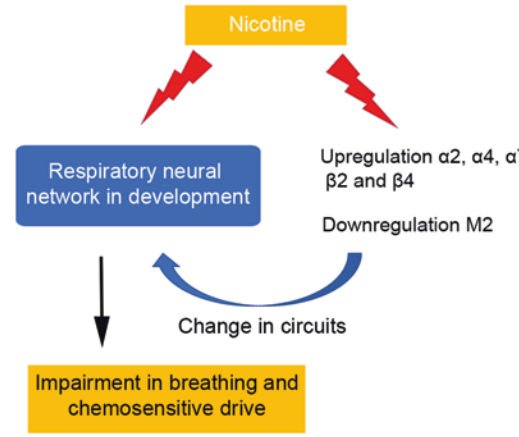

Fig. 10.2 Prenatal nicotine affects neural circuits. The alterations of nicotinic and muscarinic acetylcholine receptor subunits induce changes in the respiratory neural network during fetal development, inducing breathing dysfunction in newborns and children

α2, α4, α7, and β2 brain mRNA on fetuses (Frank et al. 2001; Lv et al. 2008) and early newborn rats (Shacka and Robinson 1998). Nicotine increases the binding of ^{3}H-Epibatidine in primary visual cortex of baboons exposed to nicotine from 86 until 161 gestational days, revealing the upregulation of heteromeric α2–4, β2, and β4 in the visual brain area (Duncan et al. 2015). Additionally, the binding of M2-muscarinic receptors is reduced in rat brainstem when nicotine is administered prenatally or during the first week of life (Slotkin et al. 1999), whereas M1 mRNA is increased in the forebrain (Frank et al. 2001). The nAChR upregulation and mAChR downregulation will impact the chemosensory drive of breathing. Through the activation of muscarinic receptors, the cholinergic system exerts an excitatory command upon the central respiratory chemoreception (Monteau et al. 1990; Eugenin and Nicholls 1997; Coddou et al. 2009). Prenatal nicotine exposure in mice switches the muscarinic to a nicotinic-mediated drive of central chemoreception (Eugenín et al. 2008; Monteau et al. 1990; Coddou et al. 2009). Moreover, the nAChRs can be desensitized by the prolongated exposition to their agonists (Wang and Sun 2005).

It is worth noting that ACh and nAChRs exert trophic, proliferative, differentiating (Aoyama

et al. 2016), cytotoxic (Roy et al. 1998) and plastic (Placzek et al. 2009) actions on other excitatory and inhibitory neurotransmitter systems. Such effects influence the development of circuits containing, among others, glutamatergic, GABAergic, catecholaminergic, and serotoninergic neurons (Wonnacott et al. 2006; Fregosi and Pilarski 2008).

Interestingly, through intersectional genetic strategy, combining ChatCre with Vglut2Flp, it was shown that some glutamatergic respiratory-related brainstem neurons adopt a cholinergic phenotype during critical periods of development. Such a transient population of cholinergic neurons provide synaptic input to developing glutamatergic neurons (Nasirova et al. 2020), and this latter may be under the influence of prenatal nicotine exposure (Nasirova et al. 2020). Such mechanism expands the vulnerable population of neurons to intrauterine exposure to nicotine.

Therefore, the nicotine-induced alterations in cholinergic receptors' expression and their activities during fetal development may influence how the respiratory neural network and modulatory-related circuits, like the serotoninergic input, are constituted, generating a profound impact on their functions, leading to a vulnerable respiratory neural network.

10.7 Summary

Prenatal nicotine exposure affects the normal development of fetuses, either by altering the placenta's functioning or by exerting a direct effect on the development of fetal organs and tissues. Due to the broad expression of multiple receptor isotypes, having different expression temporalities and localizations, the deleterious outcomes of nicotine exposition are fetal stage dependent. Even though society is more aware of the harmfulness of smoking, the popularity of ENDS, partly because of the false idea of safe smoking, continues to endanger the health of newborns and adults exposed to nicotine during intrauterine life.

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11

Language Impairment in Children of Mothers with Gestational Diabetes, Preeclampsia, and Preterm Delivery: Current Hypothesis and Potential Underlying Mechanisms

Language Impartment and Pregnancy Complications

Yesenia Torres, Cristian Celis, Jesenia Acurio, and Carlos Escudero

Abstract

Many conditions may impair or delay language development, including socioeconomic status, parent's education, or intrauterine environment. Accordingly, increasing evidence has described that pregnancy complications, including gestational diabetes mellitus (GDM), preeclampsia, and preterm delivery, are associated with the offspring's impaired neurodevelopment. Since language is one of the high brain functions, alterations in this function are another sign of neurodevelopment impairment. How these maternal conditions may generate language impairment has yet to be entirely understood. However, since language development requires adequate structural formation and function/connectivity of the brain, these processes must be affected by alterations in maternal conditions. However, the underlying mechanisms of these structural alterations are largely unknown. This manuscript critically analyzes the literature focused on the risk of developing language impairment in children of mothers with GDM, preeclampsia, and preterm delivery.

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Furthermore, we highlight potential underlying molecular mechanisms associated with these alterations, such as neuroinflammatory and metabolic and cerebrovascular alterations.

Keywords

Language impairment · Gestational diabetes · Preeclampsia · Preterm delivery · Brain · Offspring

11.1 Introduction

Speech and language are essential for human development. Speech includes articulation (how we make speech sounds using the mouth, lips, and tongue), voice (how we use vocal folds and breath to make sounds), and fluency (the rhythm of our speech). In contrast, language refers to the words we use to share ideas and get what we want (McIntyre et al. 2017).

Language is subdivided into components to facilitate clinical evaluation and research. In particular, oral language involves listening (or receptive) and speaking (or expressive) abilities used to communicate. Specifically, receptive language means understanding words, sentences, discourse, and conversation. In contrast, expressive language produces words, sentences, discourse, and conversation. Language is further subdivided into the domains of language form (syntax, morphology, and phonology), content (semantics), and use (pragmatics) (Fig. 11.1 and Table 11.1).

Receptive and expressive language development typically occurs in relative synchrony, with receptive skills more advanced than their expressive counterparts in the early phases of language development (Feldman 2005). Moreover, this complex process stages in continuous evolution naturally learned through interactions with the environment (Perez-Pedraza and Sameron-Lopez 2006), making language development a good indicator of cognitive skills and a good predictor of future cognitive performance (Bashir and Scavuzzo 1992; Schonhaut et al. 2008; Murphy et al. 2016). Therefore, most children master the language by five and speech by seven without specific instruction. Conversely, identifying lan-

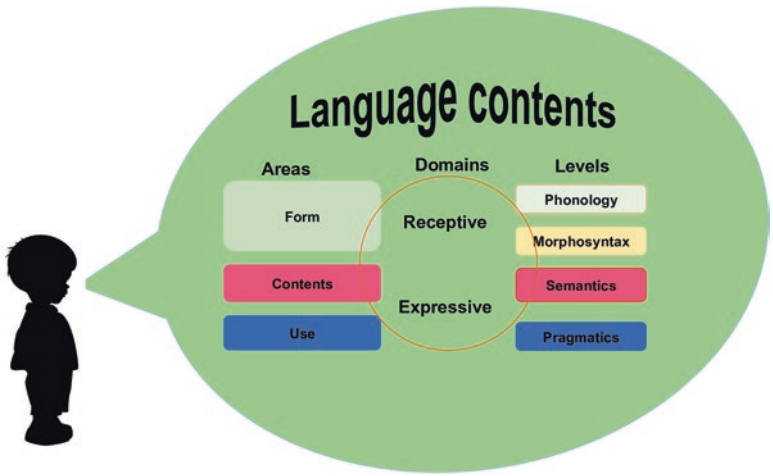

Fig. 11.1 Overview of the language content. Language can be divided in areas, domains, and levels. Areas involve form, contents, and use, while domains involve receptive and expressive abilities. The levels of the language include semantics, meaning of words; morphosinthax, structure and order of words or sentences; phonetics and phonology, production, structure, and sequence of sounds or phonemes; and pragmatics, use of language and its adaptation to conversational situations

Table 11.1 Receptive and expressive language development: average age of acquisition and age indicating significant delay or red flag and its main domains

Receptive language development: average age of acquisition and age indicating significant delay or red flag and its main domains			
Receptive language milestones	**Average age of acquisition**	**Significant delays and red flags**	**Main domains**
Alerts or quiets to sound	Birth to 1 mo	2 month	Pragmatics
Turns to the source of sound	Birth to 1 mo, then again 3–5 month	6 month	Pragmatics
Responds to own name	6–8 month	10 month	Semantic
Follows verbal routines/games (“Wave bye-bye”)	8–10 month	12 month	Pragmatics
Understands simple questions (“Where’s mommy?”)	9–11 month	15 mo	Semantics Morphosyntax
Stops when told “no”	9–10 month	15 month	Semantics Pragmatics
Understands at least 3 different words	10–13 month	15 month	Semantics
Points to 3 different body parts	12–16 month	18 month	Semantics
Follow simple commands (“Show me the ball” or “Get your shoes”)	12 month	18 month	Semantics Morphosyntax
Follows 2-part commands (“Get your shoes and give them to Dad”)	24 month	30 month	Semantics Morphosyntax
Answers simple questions (“Who is that?” or “What are you doing?”)	24–30 month	36 month	Semantics Morphosyntax
Expressive language development: average age of acquisition and age indicating significant delay or red flag			
Expressive language milestones	**Average age range**	**Significant delays and red flags**	
Cooing	2–3 month	6 month	Phonology
Babbling	6–8 month	10 month	Phonology
Nonverbal purposeful messages (requests with a reach, shows objects)	9–10 month	12 month	Pragmatics
Pointing	10–11 month	14 month	Pragmatics
Says 3 different spontaneous words	12–15 month	16 month	Semantics
Vocabulary at least 35–50 words	18–22 month	24 month	Semantics
Production of 2-word phrases (“Mommy sock”; “No water”)	18–22 month	24 month	Semantics Morphosintax Phonology
Simple sentences (“I want juice”; “Where’s my ball?”)	24–30 month	36 month	Semantics Morphosintax Phonology
Intelligibility to unfamiliar adult at >50%	30–36 month	42 month	Phonology
Able to tell about a past event with parent asking questions (personal narrative)	24–30 month	36 month	Semantics Morphosintax
Able to tell or retell a familiar story	36–48 month	54 month	Semantics Morphosintax
Fully intelligible to an unfamiliar adult (despite some immature sounds, such as consonant clusters or /r/ and /l/)	48–54 month	60 month	Semantics Morphosintax
Fully mature speech sounds	Up to 72 month	>72 month	Semantics Morphosintax Phonology

Feldman and Messick (2008)

guage development delays at the age of 2 years linked with speech or language disorders, adversely affecting domains of functioning, including learning, communication, social relationships, and response to therapy (Feldman 2005) (see Table 11.1).

Many conditions may impair or delay language development, including socioeconomic status, parent's education, or intrauterine environment (Carpita et al. 2018). Accordingly, babies who were born premature (<37 weeks of gestation) have a higher risk of presenting severe language impairment involving receptive and expressive language skills (Lamonica et al. 2016; Acurio et al. 2021). Additionally, other highly prevalent maternal conditions, including preeclampsia or gestational diabetes mellitus (GDM), have been associated with language delay and impairment in children. How these maternal conditions may generate language impairment has yet to be entirely understood. However, since language development requires adequate structural formation and function/connectivity of the brain (Gaudet et al. 2020), these processes must involve alterations of maternal conditions. For instance, structural alterations in the brain or the brain vascular anatomy are present in children born with preeclampsia (Ratsep et al. 2016a, b, c). At the same time, children born from women with GDM present structural brain alterations (Nelson et al. 2000; Vuong et al. 2017; John et al. 2018; Devarshi et al. 2019; Xuan et al. 2020; Lynch et al. 2021). However, whether those structural alterations are linked with language impairments is still unclear. Moreover, the underlying mechanisms of these structural alterations are also under investigation. Although common neuroinflammatory alterations found in the brains of offspring from intrauterine growth restriction, prematurity and GDM bring some light to these unknown processes (Boyle et al. 2017).

This manuscript critically analyzes the literature focused on the risk of developing language impairment in children of mothers with GDM, preeclampsia, and preterm delivery. Furthermore, we highlight the current hypothesis of potential underlying molecular mechanisms associated with these alterations.

11.2 Gestational Diabetes and Language Disorder

Gestational diabetes mellitus (GDM) is glucose intolerance with onset during pregnancy (Nomura et al. 2012). Pancreatic beta cells produce insulin to control glycemia, but in GDM, the response of these cells is inadequate leading to hyperglycemia (Devarshi et al. 2019). Diagnosis of diabetes in pregnancy considers the following types: preexisting type 1 diabetes (present before pregnancy), preexisting type 2 diabetes, and GDM. The prevalence of all these three types of diabetes is raised in pregnant women worldwide (Fraser et al. 2012). Thus, globally, 16% of live births had some form of hyperglycemia in pregnancy; 84% were GDM (Nomura et al. 2012; WHO 2017).

Although most infants of diabetic mothers are asymptomatic at birth, several acute perinatal clinical complications may occur, including macrosomia, hypoglycemia, respiratory distress syndrome, polycythemia, hyperbilirubinemia, cardiomyopathy, congenital abnormalities, and sudden infant death (Fraser and Lawlor 2014). Furthermore, since the onset of GDM coincides with a period of rapid growth of the fetal brain (Xiang et al. 2018), it may also impair the development of cognitive functions in children (Fraser and Lawlor 2014).

In particular, children born to women with GDM showed deficits in fine and gross motor function, lower verbal IQ, language disorder, more significant inattention, hyperactivity, and poorer general cognitive function than children born to normal pregnancies (Carpita et al. 2018). Accordingly, Clausen et al. (2013) showed that children of women with GDM had a significantly lower global cognitive score than the control population. The same pattern was observed in the subtests of cognitive function as vocabulary. Moreover, Taylor et al. (2018) showed that children born to GDM had the highest risk of late language emergence compared with children born to normal pregnancies. In the next section, we further analyze some available information regarding the association between GDM and language development in children.

11.2.1 Affected Language Skills Associated with GDM

Evidence describing the association between GDM and impairment in children's language development is sometimes contradictory (Table 11.2) (Veena et al. 2010) and comes from different populations, sample sizes, and study designs. This section discusses the most representative studies based on the sample size and directly analyzes language development.

Dionne et al. (2008) have performed one of the most detailed case-control longitudinal studies evaluating the association between language disorders in children born from women with GDM. This study included two birth cohorts from Canada, incorporating singleton and twin pregnancies. At least one language measure from 18 months to 7 years was available. Among other interesting findings, authors reported that children born to singleton diabetic pregnancies exhibited a significant reduction in expressive vocabulary at 18 months and 30 months compared to children born to nondiabetic pregnancies. Additionally, GDM children also showed a reduced score in oral communication tests at 72–84 months. These findings were partially confirmed in the twin cohort showing a reduction in expressive vocabulary at 30 months of age in GDM children. They reported multivariable analysis to adjust the findings to gender, socioeconomic status, and perinatal factors. Therefore, this study concluded that GDM hinders expressive language in offspring into middle childhood.

Another report (Torres-Espinola et al. 2015) performed a prospective case-control study of mother and child pairs from Granada (Spain) divided into four groups according to their pregestational body mass index and gestational diabetes status. These groups included overweight, obese, gestational diabetic, and healthy normal-weight controls. Results indicated no significant differences in language development in children born to GDM or normal pregnancy. However, an unexpected finding was that children born from mothers with pregestational obesity (a well-characterized risk factor for GDM) had significantly higher cognitive and language development scores at 6 months of age than children born from normal-weight pregnancies. Moreover, this difference remained even after adjusting for possible confounding factors. However, the beneficial effect of maternal obesity disappears at 18 months of age. Importantly, this article is a prospective study conducted in a Spaniard population instead of an English population, as much of the available information (Table 11.2). Also, the analysis was based on parent questionnaires and, notably, was performed in prelinguistic children in whom analysis of comprehensive and expressive skills is limited. Although this study did not find differences in language performance in children from GDM, we acknowledge that many other confounding factors not included in the analysis, such as parents' education, socioeconomic status, and socialization with friends or siblings, need to be taken into consideration.

Regarding socioeconomic status (SES), another study was performed on preschool and school-age children born to GDM, considering this confounding factor (Nomura et al. 2012). Thus, they evaluated four groups (Group 1, control group); Group 2, children exposed to mother's GDM but not low SES (GDM); Group 3, children exposed to low SES but not mother's GDM (Low SES); and Group 4, children exposed to both GDM and low SES (GDM+low SES). Results indicated that scores in language evaluation were lower in children in the low SES groups with or without GDM compared with control or GDM groups. Furthermore, the children in the GDM+low SES group exhibited the most negative effect in verbal IQ, general language, and functional communication composite scores (Nomura et al. 2012). Therefore, this study concludes that low SES negatively affects language development. Nevertheless, the harmful effect of low SES was further enhanced in the presence of GDM. Hence, despite the low sample size and the fact that this study does not support the harmful effects of GDM, this study emphasizes the necessity of including family socioeconomic status in the analysis of language development after pregnancy complications.

In contrast, Veena et al. (Veena et al. 2010) found that children born to GDM have a higher

Table 11.2 Language impairment in children of GDM

Authors	Ages	GDM	Control N	Language area	Test	Impairments	Conclusion
Ornoy et al. (1999)	Up to 9 year	23	24	Composite	WISC-R, (1974)	Verbal IQ	Negative affects
Bolaños et al. (2007)	7–8 year	5	5	Expressive Receptive	WISC-IV ENI	None	No differences
Dionne et al. (2008)	18 month 30 month 72 month 84 month	105 116 (twins)	1730 882 (twins)	Expressive Receptive	QLSCD QNTS NVIQ MCDI	Expressive language Communications skills	Negative affects
Nomura et al. (2012)	6 year	12/9	97/94	Composite Receptive expressive	ADHD RS–IV	Language Verbal IQ Language composite	Negative affects
Veena et al. (2010)	9–10 year	32	483	Composite Receptive expressive	KABC Atlantis WISC KBD	None	No differences Positive affects
Clausen et al. (2013)	8–27 year	153	118	Receptive	WAIS IQ	Vocabulary	No differences vocabulary
Krakowiak et al. (2012)	2–5 year	504	98	Composite Receptive expressive	MSEL receptive VABS Communication	Receptive Expressive Composite	Negative affects
Torres-Espinola et al. (2015)	6 month 18 month	58	81/44/31	Expressive Receptive composite	BSID-III Questionnaires	Expressive Composite language	Negative affects

WISC The Wechsler Intelligence Scale for Children, *ENI* Evaluación Neuropsicológica Infantil, *ADHD RS–IV* The ADHD Rating Scale–IV, *KABC* Kaufman Assessment Battery for Children-Second Edition KBD (Kohs block design), *QLSCD* The Quebec Longitudinal Study of Child Development, *QNTS* the Quebec Newborn Twin Study, *MCDI* McArthur Communicative Development Inventory-Short Form, *PPVT* Peabody Picture Vocabulary Test, *WAIS* Wechsler Adult Intelligence Scale, *MSEL* Mullen Scales of Early Learning, *VABS* Vineland Adaptive Behavior Scales, *BSID-III* The Bayley Scales of Infant Development, Third Edition WISC-R, 1974

score in both verbal fluency ability (in particular verbal ability-names) and in the score of attention and concentration than children born to control mothers. Interestingly, these differences remain significant after adjustment to multiple confounding variables such as child's sex, gestational age, SES, parent's education, rural/urban residence, maternal age, BMI and parity in pregnancy, and child's weight and head circumference at birth. The reasons why this study showed contrasting results from those analyzed above are unclear. However, the authors suggest that some population characteristics, including high education status in mothers with GDM, could be part of the explanation. However, differences with other studies are also related to the questionnaires used, particularly those used to language skills analyzed.

Besides, Krakowiak et al. (2012) have associated GDM with delay development (DD) and autism spectrum disorder (ASD). Thus, this study showed that metabolic disorders in mothers, including GDM, were associated with a twofold increase in the likelihood of DD relative to controls. Nevertheless, in the analysis of language skills, the receptive and expressive skill scores were lower in children of GDM than in the respective control group. Furthermore, when the analyses are on the ASD group, the scores in language skills were the worst in children with ASD born to GDM. So then, language disorder in ASD is significant; however, it seems enhanced in infants born from GDM pregnancies.

In agreement with those findings, a systematic review (Adane et al. 2016), including 14 case-control studies, describes that 8 out of 14 studies supported the negative association between GDM and offspring's cognitive and language development. The authors also concluded that the negative effect was substantial in the offspring's language development at a young age, particularly when analyzing verbal IQ. However, those studies were geographically limited and mainly included a small sample size. Moreover, it did not adjust for important confounders, including SES, prepregnancy BMI, SES, alcohol use, smoking during pregnancy, or offspring-related covariates.

In summary, children born to women with GDM have an increased risk of language disorders. However, the degree of affection, especially in language and communication skills, is nowadays undetermined in those children. Additionally, the available information is geographically limited, basically focused in English, including a mix of patients in the diabetic group, and the study design is usually retrospective. Then, we encourage future confirmatory population studies.

11.2.2 Potential Mechanisms

Differences in neurodevelopment between children born from GDM and normal pregnancy have been described in the literature. However, little is known about potential underlying mechanisms. The following sections will analyze the contribution of the three main factors: alterations in the brain structure and connectivity, brain hypoxia, and neuroinflammation as a potentially impaired process involved in the pathophysiology of language disorders in children born to GDM.

11.2.2.1 Structural Brain Alterations

Brain structure malformation generated by GDM may lead to impaired cognitive function, including the alterations in the language development described in the previous section. For example, the hippocampus, a crucial brain structure for learning and memory in language processes (Krishnan et al. 2016), has structural abnormalities in children of GDM (Lynch et al. 2021). Also, GDM offspring showed microstructural white matter (WM) abnormalities in selected brain areas, such as the splenium of the corpus callosum, posterior limb of the internal capsule (PLIC), and thalamus, compared with controls. Interestingly, those abnormalities were associated with worse neurocognitive performance in children born to GDM (Xuan et al. 2020). However, whether these findings are related to impaired language development in children born to GDM is unclear. Using association analysis, we could indicate that structural alterations in the corpus callosum have been described in children with speech sound disorders (that included phonetics and phonology problems) (Luders et al.

2017). Then, it is feasible that children born to GDM might also present similar alterations.

Another affected area in GDM offspring is the PLIC (Xuan et al. 2020). The internal capsule is an extensive fiber system that conveys information from motor areas, frontopontine peduncles, and thalamic peduncles to the brainstem and cerebellar regions. Additionally, it links the thalamus to the prefrontal cortex (Sullivan et al. 2010). PLIC is associated with language skill development and efficiency in cognitive processing (Deniz Can et al. 2013). Moreover, white-matter intensity in a cluster located in the left PLIC/cerebral peduncle at 7 months was positively associated with receptive language skills at 12 months (Deniz Can et al. 2013). Why PLIC may be particularly affected in GDM is unknown. However, it is well established that midgestation, when GDM happens, is a crucial stage in which white-matter myelination occurs.

The thalamus was the fourth brain region linked with language skills (Krishnan et al. 2016). The thalamus is a structure in the middle of the brain that projects to all areas of the neocortex, including those in the frontal, temporal, and parietal cortical regions commonly associated with language (Llano 2013). At the same time, alterations in the thalamus function were related to particular language skills, including anomia, perseveration, memory, and acceleratory effect (Hebb and Ojemann 2013). Also, a reduction in the activation of the thalamus, particularly in the gray matter, is related to poor reading skills independent of the native language (Jednorog et al. 2015). So then, any alteration in the thalamic region caused by GDM may alter language development.

11.2.2.2 Metabolic and Pro-inflammatory Underlying Mechanisms

The neurodevelopment of the fetal brain requires an adequate supply of nutrients, such as glucose, that favor the synapses and myelination process in neurons. Glucose crosses the placental barrier to satisfy these fetal energy needs, but this process is altered in GDM (Jansson et al. 2001). The fetal hyperglycemia and hyperinsulinemia observed in fetuses of GDM increased the metabolism by about 30% compared with normoglycemic pregnancies (Sobrevia et al. 2016). Therefore, the fetus requires more oxygen that is not adequately supplied by the placenta, resulting in a chronic fetal hypoxia condition. Furthermore, we remarked through this manuscript that the cerebral cortex and the hippocampus are more sensitive to hypoxic damage (DeBoer et al. 2005).

In addition to chronic hypoxia, in fetuses from GDM, it has been observed that low blood iron levels and high ketone levels (DeBoer et al. 2005) may also contribute to the alterations in the normal development of the brain, including alterations in myelination or cortical connectivity, and aberration in hippocampal neurons (Prado and Dewey 2014).

GDM is also associated with high levels of maternal (Nergiz 2014), placental (Vuong et al. 2017), and umbilical (Li et al. 2020) pro-inflammatory cytokines, including interleukin 6 (IL-6) (Amirian et al. 2020). Moreover, increased maternal levels of IL-6 can cross the placenta and are linked with disruption of normal fetal brain development in experimental studies (Masi et al. 2017). Additionally, IL-6 can cross the placenta and blood-brain barrier (BBB) (Capuron 2011). Once in the brain, IL-6 generates a pro-inflammatory environment that negatively affects neurotransmitter function, neuroendocrine activity, or neurogenesis leading to alterations in the brain circuitry, decreased neuronal viability, or enhanced microglia activation (Capuron 2011).

Gathering all the information above, it is feasible that placental hypoxia, iron deficiency, and inflammatory processes present in GDM result in alteration in different brain areas that predispose to language disorder.

11.3 Preeclampsia and Language Impairment

Preeclampsia (PE) is a human pregnancy syndrome characterized by arterial hypertension (systolic >140 mmHg and diastolic >90 mmHg), which onset after the 20th week of gestation, coexisting with proteinuria (>300 mg/day) or

dysfunction of maternal organs, including kidney and liver failure, neurological or hematological complications, or fetal compromise (2019). This syndrome affects ~10% of pregnancies worldwide (Duley 2009, 2011), and it is considered the leading cause of maternal, fetal, and neonatal morbidity-mortality, particularly in low- and middle-income countries (Duley 2009).

Several studies have attempted to investigate the association between preeclampsia and developmental alterations in the offspring that would, in turn, predispose them to a higher risk of metabolic, cardiovascular, and neurological disorders in adulthood (Alsnes et al. 2016; Davis et al. 2012; Escudero et al. 2014; Figueiro-Filho et al. 2017a; Jayet et al. 2010; Pinheiro et al. 2016; Ratsep et al. 2016a, b; Robinson et al. 2009; Tenhola et al. 2003; Tuovinen et al. 2012; Wu et al. 2008, 2009, 2011). In the context of brain-related disorders, children born to women with preeclampsia exhibit an increased risk of developing cerebral palsy, cerebral stroke, impaired neurological development, developmental delays at the age of 5 years, poor cognitive development, intellectual disability, anxiety, depressive symptoms, and attention-deficit/hyperactivity disorder in comparison with children born to normotensive women (Gumusoglu et al. 2020). Despite this evidence, the causality is not clear. However, the above studies present extensive multivariable statistical analyses tending to isolate the potential unique effect of preeclampsia. In agreement with this body of evidence, recent systematic reviews concluded that preeclampsia has independently been associated with neurocognitive and mood disorders in children (Figueiro-Filho et al. 2017a, b; Maher et al. 2018).

There is little evidence (Whitehouse et al. 2012; Acurio et al. 2021) describing the potential adverse effect of preeclampsia on the development of language skills in the offspring. In particular, those reports found that children born to preeclampsia may present language alterations in the narrative discourse, in the levels of semantics, such as morphology and syntax, and pragmatic language (see Table 11.3).

For instance, children of women with preeclampsia showed language alterations in semantic areas such as understanding narrative discourse. Specifically, Whitehouse et al. (Whitehouse et al. 2012) show that children (4–10 years old) born to mothers with preeclampsia and hypertension present reduced scores in the passive vocabulary compared to children born to normotensive mothers. Other alterations within the receptive language are observed at the morphosyntactic level, including difficulties in understanding complex sentences (Bishop 1997; Whitehouse et al. 2012). We also found specific alterations in the understanding of the macrostructure of narrative discourse in children (4–7 years old) of mothers with preeclampsia

Table 11.3 Language impairment in children born from women with preeclampsia

Authors	Age	PE	Control	Language area	Tests	Language impairment	Conclusion
Whitehouse et al. (2012)	10 year	34	1076	Receptive	PPVT-R RPM	Verbal IQ	Risk
Withagen et al. (2005)	4 year	193	1192 2189	Expressive	CBCL, Parents report	No difference	No relationship
Bishop (1997)	7 year	23 pairs	61 pairs	Receptive Expressive	Sub-test. CELF-R, EAT	Receptive vocabulary and grammar Phonetics	Affects
Acurio et al. (2021)	4–7 year	8	9	Receptive Expressive	PEFE TAR, EDNA	Narrative speech Receptive and expressive	Relationship

PPVT-R Peabody Picture Vocabulary Test Revised, *RPM* Raven's Progressive Matrices, *CBCL* Child Behavior Checklist, *CELF* Clinical Evaluation of Language Fundamentals, *EAT* Edinburgh Articulation Test, *TAR* Test de la Articulación en repetición (articulatory test by repetition), *PEFE* (Pauta de evaluación fonoaudiológica) Chilean language evaluation test, *EDNA* Evaluación del discurso narrativo (speech narrative skills) Chilean language evaluation test

compared to those born to normotensive mothers (Acurio et al. 2021).

In the analysis of expressive language and speech, the children of mothers with preeclampsia show alterations in the articulation compared with children born to normotensive mothers (Bishop 1997). Confirming this finding, our study (Acurio et al. 2021) showed that children born to preeclampsia exhibited a decreased capacity to express some particular aspects of the macrostructure of the discourse. In particular, those children have difficulties understanding and expressing characteristics of the information received as a tale. However, we did not analyze whether these alterations are related to other complications observed in children born to preeclampsia, including attention-deficit/hyperactivity disorder, or whether it may be generated/modulated by personal and family stories of language development. Also, further analysis is required to evaluate potential confounders, including paternal scholarship, other pregnancy-related complications, access to language therapists, and other factors that could play a key role in language development.

Therefore, despite the scarce evidence and with a limited number of participants (Table 11.3), it is suggested that both receptive and expressive language would be altered in children born to mothers with preeclampsia. Those alterations are characterized by a reduction in the understanding of vocabulary and speech articulation, which could trigger alterations in the macrostructure of narrative discourse. Further studies are needed to evaluate whether these language alterations persist later in life.

11.3.1 Evidence of Possible Pathophysiology

There is growing evidence in the literature testing biological plausibility and investigating potential underlying mechanisms that link preeclampsia with alterations in the cognitive development in the offspring (we recommend excellent reviews paper in this area Ratsep et al. 2016a, b; Lara et al. 2018; Gumusoglu et al. 2020). However, little is known about the possible underlying brain alterations associated with language development.

Alterations found in the human brain of offspring born to preeclampsia include magnetic resonance imaging (MRI) analysis. Thus, children born of mothers with preeclampsia showed structural changes in at least five regions, including the cerebellum, temporal lobe, brainstem, and right and left amygdala. Moreover, a more extensive volume was found compared to age-matched controls (Ratsep et al. 2016c). Also, Figueiro-Filho et al. (2017a) reported structural changes in brain connectivity. Thus, they found higher volumes of the tract for the superior longitudinal fascicle, the connective fasciculi that join posterior parietal cortical areas with different frontal cortical regions. They also observed less connectivity between the medial prefrontal cortex and the left occipital fusiform gyrus, as well as less connectivity between the left amygdala and bilateral frontal pole, the right amygdala and the left frontal pole, and the medial prefrontal cortex and the precuneus (Figueiro-Filho et al. 2017a). In turn, another report has shown reduced cerebral blood flow in the parietal and occipital lobes in children born to preeclampsia compared to their controls (Ratsep et al. 2016c). The authors proposed that these changes in the brain vasculature of the offspring might precede the structural alterations. Despite all these reports having tremendous relevance in the field due to a well-described matching between case and controls, we must indicate that they include a limited number of children and geographic limitations. Additionally, the authors belong to the same research group and studied the same group of children. Therefore, we encourage future research to confirm these findings in more extensive population studies. Also, we clarify that these results suggest an association but not causality.

Animal studies have confirmed the biological plausibility of these alterations reported in humans. For instance, structural alterations demonstrated by a reduced thickness of the brain cortex in pups born to preeclampsia-like syndrome have been reported (Liu et al. 2016). This finding was associated with a deficiency in neurogenesis

but not brain apoptosis in the offspring of the preeclampsia-like syndrome. Interestingly, these alterations observed early in brain development (postnatal day 0, P0) were associated with impaired spatial learning and memory in adulthood (P56). They also found disrupted adult hippocampal neurogenesis in the offspring of the preeclampsia-like syndrome, which may explain the cognitive deficiency. In agreement with these findings, structural alterations in the hippocampus of offspring born to preeclampsia-like syndrome were recently reported (Tachibana et al. 2019). This last study found high protein expression of the hypoxic inducible factor 2α in the hippocampus of fetuses exposed to the preeclampsia-like syndrome, associated with reduced expression of neuron development markers such as synaptophysin and myelin essential protein in the hippocampus of neonatal pups (P15).

Furthermore, based on brain vascular alterations observed in children (Ratsep et al. 2016c) and pups born to preeclampsia-like models, including the use of a high-fat diet (Whitaker et al. 2021) or hypoxia during gestation (Camm et al. 2021), we have suggested (Lara et al. 2018) that preeclampsia is characterized by an imbalance between pro and anti-angiogenic signals that impairs brain angiogenesis. Supporting this hypothesis, Whitaker et al. (2021), using a model of preeclampsia induced by a high-cholesterol diet, found that middle cerebral arteries exhibited less growth in the offspring at P30 in the preeclampsia group compared to the normotensive group. The lack of growth was associated with less maturation, evidenced by reduced stiffness or vasomotor response to increasing intraluminal pressure. Then, this study demonstrated structural and functional alterations in the cerebral vasculature of offspring from preeclampsia. Nevertheless, Trigiani et al. (2021), analyzing offspring born to dams chronically hypertensive, showed a more significant whisker-evoked cerebral blood flow response in the contralateral barrel cortex, an effect associated with longer-lasting impairments on an object recognition task as far as 12 months of age. However, whether these alterations may be present throughout life and may explain the increased risk of cognitive alterations in these individuals, for example, language impartment, is largely unknown.

11.4 Preterm Delivery and Language Impairment

Deliveries that occur before 37 weeks gestation are defined as preterm delivery. Within this classification, those deliveries that occur before 28 weeks are extremely premature (Mendoza Navas 2016). The prevalence rate of prematurity reaches 1% to 2% of all live births globally, affecting mainly low- and middle-income countries such as Latin American countries (Aylward 2002; Ramon-Casas et al. 2013; Charkaluk et al. 2019). For example, the reported rate in Brazil is 9.2%, while 9.0% in Bolivia, 8.8% in Colombia (Mendoza Navas 2016), and 7% in Chile (MINSAL 2015; Mendoza Navas 2016). Of them, about 3% were born before 34 weeks of gestation.

Premature babies are born in a fragile condition due to the lack of fetal maturation, which compromises their survival and well-being and predisposes them to possible deficits in neurocognitive development (Schirmer et al. 2006; Guarini et al. 2009; Lamonica et al. 2016; Dodson et al. 2018). Additionally, preterm infants are more susceptible to disease because they are more exposed to iatrogenic factors such as prolonged isolation in an incubator, side effects of medications or mechanical ventilation, and stress due to extended clinical care (Aram et al. 1991; Aylward 2002; Schirmer et al. 2006).

Additionally, children born prematurely present a higher rate of medical complications, such as a higher rate of obesity (Ou-Yang 2020), cognitive alterations (Lamonica et al. 2018; Duncan et al. 2019), or learning disorders that hinder their subsequent school performance (Chyi et al. 2008; Perez-Pereira et al. 2014). For instance, children born prematurely are more likely to suffer from neurological, cognitive, motor, and behavioral disturbances (Woodward et al. 2006; Hintz et al. 2011). Among these complications, premature babies exhibited a high risk of cognitive delay

(Sansavini et al. 2010), visuospatial difficulties, memory disorders (short-term and long-term and working memory) (Caravale et al. 2005), attention deficits, executive function problems, and language and reading impairment (Aarnoudse-Moens et al. 2009; Aylward 2014; Kovachy et al. 2015; Dodson et al. 2018). Thus, the alterations found in children born prematurely that affect neurodevelopment are multiple, including cognitive and motor impairment, anxiety disorders and depression, or even dyslexia and alterations in learning processes (Mendoza Navas 2016).

In language affections, several studies have shown that prematurity is one of the leading causes of delay or impaired language development (Barre et al. 2011; Aylward 2014; Dodson et al. 2018) (see Table 11.3). The following section will discuss the most recent evidence in this field, including systematic reviews.

11.4.1 Language Skills in Children of Preterm Delivery

Children born prematurely risk suffering from multiple language disorders (Barre et al. 2011; Aylward 2014; Dodson et al. 2018; Lamonica et al. 2018). Indeed, prematurity is often identified as a risk factor for future speech and language disorders. Moreover, several studies have shown that depriving the child of maternal sounds while in intensive care significantly altered the development of the auditory system (Suttora et al. 2020). Furthermore, this early deprivation of maternal sounds negatively impacts the brain's auditory system maturation and speech and language development (Lamonica et al. 2018). Therefore, in general, children born prematurely evaluated at 2 years of age exhibited a fourfold increase in the likelihood of having delayed motor coordination and language acquisition in the third year of life (Ruiz-Extremera et al. 2001) (see Table 11.4).

In addition, preterm infants scored significantly lower than term infants on reading assessments (Aarnoudse-Moens et al. 2009; Kovachy et al. 2015). Moreover, this difference is accentuated between 6 and 12 years, negatively impacting their school performance and precursor reading language skills (Olofsson and Niedersoe 1999; Dodson et al. 2018). Precisely about receptive language skills, several studies have reported that children born prematurely have a weakening in various language skills, including comprehensive vocabulary (Ramon-Casas et al. 2013; Charkaluk et al. 2019; Imafuku et al. 2019), narrative skills (Lamonica et al. 2018; Acurio et al. 2021), verbal memory, syntactic comprehension, speed of linguistic processing (Lee et al. 2011), morphological comprehension, and social reference (Lowe et al. 2013; Beaulieu-Poulin et al. 2016) in comparison with children born at term. In particular, a case-control study analyzed 40 children born premature (26–36 weeks) and controls at preschool age (48–71 months) (Lamonica et al. 2018). Among their results, children born prematurely showed fewer scores in the Peabody Picture Vocabulary Test (PPVT) than controls. However, although this study also included results from the observation of the communicative behavior test, they did not include any information regarding receptive skills such as communication function understanding specific or abstract situations. Additionally, they do not analyze potential confounding factors, including gestational age at delivery, newborn sex, or socioeconomic status.

Several reports have shown reduced expressive language skills in children born prematurely. For instance, children born prematurely have a reduction in phonological awareness and decoding (Luu et al. 2011; Dodson et al. 2018), preverbal communication, development of expressive vocabulary (Ramon-Casas et al. 2013; Stolt et al. 2016; Brosch-Fohraheim et al. 2019; Charkaluk et al. 2019), morphosyntax (Sansavini et al. 2010; Lowe et al. 2013; Beaulieu-Poulin et al. 2016), and narrative skills (Ramon-Casas et al. 2013) compared to children born at term. In particular, a case-control study (Brosch-Fohraheim et al. 2019) used the expressive vocabulary test (AWST-R), which consists of verbalizing showed images. Image naming is a complex achievement since, to perform the requested task, the child must have stored phonological and syntactic knowledge since the name of the word is associ-

Table 11.4 Language impairment in children born preterm

Author	Age	Premature	Controls	Language area	Test	Language impairment	Conclusion
Vassar et al. (2020)	18–22 month	92	0	Receptive and expressive	BSID III	Receptive	Risk
Duncan et al. (2019)	1.5–2 year	397	0	Vocabulary verbal Comprehension	BITSEA BSID III	Verbal IQ	Cognitive and linguistic deficits
Dodson et al. (2018)	6 year	36	43	Receptive and expressive	CELF WAIS	Phonological awareness	Altered
Lamonica et al. (2018)	5–6 year	40	40	Vocabulary narratives skills	ABFW PPVT-R MacArthur	Narrative Expressive Vocabulary	Risk
Reidy et al. (2013)	7 year	198	70	Phonological awareness Receptive and expressive	CELF Magnetic resonance	Phonological awareness Expressive language	Altered

BSID-III The Bayley Scales of Infant Development, Third Edition WISC-R, 1974), *CELF* Clinical Evaluation of Language Fundamentals, *BITSEA* Brief Social Emotional Rating Scale for Infants and Children, *ABFW* Evaluación de Lenguaje ABFW – Parte A. Fonológica, *WAIS* Wechsler Adult Intelligence Scale (WAIS), *PPVT-R* Peabody Picture Vocabulary Test Revised, *MacArthur* Communication Development Inventory

ated with the existing knowledge of the meaning of words (semantic-conceptual knowledge). The test also contains nouns and verbs, which were presented explicitly. After analyzing the results, the researchers concluded that premature infants had significantly fewer correct answers than their controls, which was interpreted as impaired expressive language in those children. Lamonica et al. (Lamonica et al. 2018) confirmed these findings in another case-control study of children born premature (26–36 weeks) and controls invited to participate at the preschool age (48–71 months).

Accordingly, systematic reviews have reported that preterm children have difficulties with language function compared with their peers born at term (Barre et al. 2011; Zimmerman 2018). These systematic reviews examined language abilities in children born preterm, which mainly focused on expressive language, receptive language, semantics (expressive and receptive), and grammar (expressive and receptive) using a standardized test in early childhood children (2–12 years). In particular, Zimmerman et al. (Zimmerman 2018) analyzed the epidemiological association between preterm birth (<37 weeks of gestation) or low birth weight (<2500 g) and impairment in language development. This systematic review included 16 studies, with a total number of 2798 participants, from which 1515 were children born preterm and 1224 were children born at term. This meta-analysis indicates that preterm infants performed significantly lower in the following parameters: (1) overall language score (−13.2), (2) receptive language and expressive language (−6.2 points in each of them), (3) pragmatic (−8.3 points), (4) phonological awareness (−1.46 points), and grammar (−4.55 points) than their full-term peers at early school age. Then, this study concludes that children born preterm do not catch up with their full-term peers in their language abilities by early school age. Similarly, another non-systematic review included 13 studies showing an association between prematurity and language development in Brazilian children (n = 800, aged 1–71 months (Zerbeto et al. 2015). In this analysis, premature children exhibited delayed language development, particularly expressive language, and reduced vocabulary in all analyzed semantic categories.

Despite these reports, a causality of the language impairments in children born prematurely is challenging to demonstrate because of the complexity of the perinatal and demographic variables that may interfere with children's cognitive development including gestational age at birth (for instance, preterm versus severe preterm), birth weight, neonatal comorbidities including brain alterations, intraventricular hemorrhage, cerebral gray matter changes, pulmonary dysplasia, and extended hospitalizations, among others. Notwithstanding, the more likely mechanism for the differences in language outcomes in children born prematurely is originated due to general cognitive deficits linked to delay/impaired brain development (Zimmerman 2018).

11.4.2 Evidence of Possible Pathophysiology

Pathophysiology of the impact of preterm birth on cognitive development has been linked with alterations in brain development, but still, cause-effect analyses are lacking. However, magnetic resonance imaging (MRI) of premature infants has found abnormalities in brain areas involved in language skills and reading. For instance, a case-control study analyzed phonological awareness (a precursor of reading) and MRI of brain areas involved in languages, such as the arcuate fascicles of the left hemisphere, the left superior longitudinal fascicle, and the uncinate fascicle of the right hemisphere in children born prematurely (Dodson et al. 2018). They concluded that the main difference between children born prematurely and those born at term was the uncinate fascicle on the right hemisphere. Also, children born prematurely presented loss of volume of the periventricular white matter, as well as cystic abnormalities, ventricular dilation, and thinning of the corpus callosum. Nevertheless, they also found some similarities. For instance, the arcuate fasciculus dorsal tract of the left hemisphere was associated with phonological awareness in both

groups. These findings are complemented with evidence of a reduction in brain volume and particular brain areas, including the hippocampus, corpus callosum, reduction of gray matter in gyri of the temporal lobe, putamen, insula, left inferior frontal gyrus and precentral gyrus, sensory motor, premotor, mesotemporal, parietal, occipital, and subgenual (Peterson et al. 2000; Inder et al. 2003), or even in the cerebellar regions in premature infants (Kinney 2006; Back et al. 2007; Yung et al. 2007; Vassar et al. 2020).

It is unclear how these structural alterations lead to language impairment in premature children. However, the hippocampus is a critical structure of memory and language processing (Covington and Duff 2016). At the same time, the corpus callosum is related to the transfer and facilitation of associative information between the hemispheres required for language processing. Moreover, white matter lesions observed in children born prematurely are the neuronal substrate for their cognitive difficulties. In the linguistic setting, according to Foster-Cohen et al. (Foster-Cohen et al. 2010), alterations in these structures are predictors of alterations in the language in domains of semantics, grammar, phonological awareness, and speech of children born prematurely.

Accordingly, cerebrovascular lesions, mainly germinal matrix hemorrhage and ischemic injury to the periventricular white matter, are significantly common in preterm infants (Peterson et al. 2000; Maalouf et al. 2001; Aylward 2014). These vascular-dependent lesions increase with decreasing gestational age, predominantly affecting the white matter. These alterations are mainly associated with developmental immaturity in cerebral circulation. Because brain angiogenesis and vasoregulatory development continue after birth (Marin-Padilla 2012), immaturity in infants born preterm has been linked with the severity and pattern of preterm brain injury (Brew et al. 2014). However, these kinds of "severe" brain vascular alterations in preterm delivery drive cognitive impairment and, in the frame of this manuscript, likely language alterations. Still, no direct evidence has been reported regarding brain angiogenesis or function of the BBB in premature offspring associated with language impairment. However, indirect evidence suggests impaired vascular formation or function, such as lower levels of proangiogenic factors in the umbilical cords of preterm newborns than their term controls (Gródecka-Szwajkiewicz et al. 2020) (Hellgren et al. 2021). Furthermore, increased chemokines and pro-inflammatory cytokines, collagenolysis of the extracellular matrix, metalloproteinases, and activation of leukocytes in the brain of premature offspring have been found (Romero et al. 2008; Menon and Shorvon 2009). These alterations may be prone to modification in the brain structures involved in language development. However, currently, there is no causality analysis in this regard. Nevertheless, the ELGAN study gives us an integrative point of view, including pro-inflammatory, angiogenic, and growth factors, including vascular endothelial growth factor (VEGF) and brain-derived neurotrophic factor (BDNF) (Leviton et al. 2018). They analyzed the circulating levels of several proteins in premature children who had a cranial ultrasound scan during their stay in the intensive care nursery. These markers were quantified on 2 days at least a week apart during the first postnatal month. Based on complex statistical analysis, they defined four patterns of interrelationship among protein and brain ultrasound images. (1) Modulation of damage promotion pattern, in which elevated inflammatory cytokines were associated with elevated concentration of "protective proteins" (including IL-6R, RANTEs, and bFGF). This combination was associated with an increased risk of ventriculomegaly. (2) Damage promoter resistant to reduction pattern, in which elevated markers of inflammation, and growth factors (EPO, BDNF, IGF-1, and PlGF) did not appear to protect against the occurrence of ventriculomegaly or echolucent lesion. (3) Markers of brain damage patterns in which elevation of the markers are analyzed after brain damage, therefore, are a consequence rather than a cause. In this pattern, the elevation of circulating pro-inflammatory cytokines was associated with high levels of neurotrophic proteins. (4) Markers are the cause of brain damage. In this pattern, they speculate that the elevation of pro-inflammatory

and proangiogenic proteins can promote each other's leading to BBB dysfunction and brain damage. Although this study is mainly associative, we remark that it brings some light on the dynamic of the protein markers linked with neuroinflammation and brain recovery. It is intriguing to ask whether those dynamics might predict or be associated with significant brain damage such as ventriculomegaly and be associated with future prediction of impaired cognitive and language development.

11.5 Concluding Remarks

Consistent evidence has described that pregnancy complications, including GDM, preeclampsia, and preterm delivery, are associated with the offspring's impaired neurodevelopment. In particular, we have emphasized that those pregnancy complications increase the risk of language disorders, including language delay, evidenced by poor receptive and expressive language performance. For instance, premature children have difficulties in comprehensive and expressive vocabulary, verbal memory, syntactic comprehension, speed of linguistic processing, morphological comprehension, social reference, reduction in phonological awareness and decoding, preverbal communication, and narrative skills (comprehensive and expressive).

However, the degree of affection of neurological development, especially language and communications skills, is nowadays undetermined in those children. Additionally, the available information is geographically limited and focused in English, including a mix of patients in the pregnancy alteration groups. The study design is usually case-control studies or retrospective analysis. Then, we encourage future confirmatory population studies.

Whether some particularities are dependent on the pregnancy affection is unclear due to a lack of comparative analysis. Despite that, the information about language disorders in children born prematurely is abundant among the analyzed affections. This condition is well accepted as a risk factor for delay or impaired language development. However, evidence is less conclusive regarding the association between impaired language development in preeclampsia or GDM. Possibly, the lack of unification in standardized tests to evaluate the different language skills and the presence of multiple confounding variables that may interfere in the children's development, such as socioeconomic level, country, and the effect of language therapy, among others, make it challenging to analyze the real cause-effect contribution of those pregnancies complications.

In this last regard, the evidence analyzed in this manuscript shows that most of the research on language analysis in children born premature or from a GDM or women with preeclampsia is based on vocabulary. In comparison, few standardized tests measure other language skills, including receptive or expressive domains. Therefore, more instruments are required that cover all linguistic domains, preventing future alterations in these children.

Considering the plausibility of the association between pregnancy complications and an elevated risk for language disorder, further analysis of the potential underlying mechanisms must be conducted. Evidence present in this manuscript describes structural alterations in the brain of children born from GDM, preeclampsia, or preterm delivery. Those pieces of evidence emphasize microstructural WM abnormalities in areas of the brain associated with language development, such as PLIC, the thalamus, and the hippocampus. Some alterations in the connectivity of the brain areas associated with language development have also been described in children born to GDM or preeclampsia. It is unclear whether these areas are selectively affected depending on the pregnancy condition, but we think it would be improbable.

How pregnancy pathophysiology would generate brain structural changes is also still being determined. However, it is well described that the brain cortex and the hippocampus are highly affected by hypoxia. Importantly, hypoxia in the brain of children born from GDM, preeclampsia, or preterm delivery may have particular mechanisms. For instance, while in GDM, the elevated

glycemia may generate hypoxia due to high glucose metabolism in the brain, preterm delivery of preeclampsia may be associated with structural alterations in the brain vasculature. Other harmful mechanisms for the brain of children born from pregnancy complications include neuroinflammation, which may involve a BBB disruption.

Although the above underlying cellular mechanisms are not well understood, they may include common damaging mechanisms involving metabolic, pro-inflammatory, and vascular alterations in the brain of children born from pregnancy complications, including preterm delivery, preeclampsia, or GDM, as reviewed in this manuscript.

Relevance of studying language impairment after pregnancy complications include social interaction limitations that negatively affect behavior and future school performance and, eventually, cognitive alterations in life. These results reinforce the necessity of including pregnancy complications, such as GDM or preeclampsia, as a risk factor for language development alterations and also support the need to have periodic controls of infants born in this condition from biomedical interventions and the speech-language pathologist's area.

In conclusion, increasing evidence has described that pregnancy complications, including GDM, preeclampsia, and preterm delivery, are associated with the offspring's impaired neurodevelopment. Since language is one of the high brain functions, alterations in this function are another sign of neurodevelopment impairment. This manuscript emphasizes that pregnancy complications may increase the risk of language impairment, including language delay, evidenced by poor receptive and expressive language. However, many questions still need to be answered, including confirmation in population studies, or analyze the language as a whole complexity (and not only focusing on a few characteristics of the language), or using adjusted analysis including an increased number of relevant confounding variables. Despite that, evidence from imaging analysis (such as MRI or Doppler) reinforces the plausibility of the association between pregnancy complications and structural brain alterations in the offspring. How those alterations are linked with language development also needs further analysis. Finally, critical questions regarding underlying pathophysiological mechanisms are largely unknown. However, a typical harmful process, including neuroinflammation or reduced/impaired brain vascular function, has been described in the literature. Therefore, we encourage future studies addressed to discuss/analyze these points.

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12

COVID-19 on Pregnancy Outcomes, Mental Health and Placenta: Focus in Latin America

Marcelo González-Ortiz, Patricio Castro, Pablo Vergara-Barra, Patricia Huerta, and Carlos Escudero

Abstract

The COVID-19 pandemic has impacted many aspects of health and society worldwide. One vulnerable group that faced SARS-CoV-2 infection is pregnant women, who were considered to have potentiated risk factors. In physiological pregnancy, maternal systems have several changes and adaptations to support fetal development. These changes involve regulations of cardiovascular, respiratory, and immunologic systems, among others, which SARS-CoV-2 could severely alter. Furthermore, the systemic effects of viral infection could be associated with placental dysfunction and adverse pregnancy outcomes, which have been studied from the start of the pandemic to date. Additionally, pregnancy is a condition of more significant mental health vulnerability, especially when faced with highly stressful situations. In this chapter, we have collected information on the effect of COVID-19 on maternal mortality, the SARS-CoV-2 infection rate in pregnancy, and the impact on pregnancy outcomes, maternal mental health, and placental function, with a particular focus on studies that consider the Latin American population.

Keywords

COVID-19 · SARS-CoV-2 · Pregnancy · Perinatal mental health · Placenta · Latin America

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12.1 Introduction

During pregnancy, a series of maternal physiological adaptations allow the proper development of the fetus. These changes significantly involve the respiratory, cardiovascular, endocrine, and immune systems (Hegewald and Crapo 2011). For example, a significant increase in the respiratory rate, total volume of cardiac output, and circulating blood volume, associated with a substantial decrease in systemic vascular resistance, is required (Fu 2018) and leads to functional hypertrophy of the heart (Chung and Leinwand 2014).

These complex physiological adaptations during pregnancy alerted the medical health system about the potential higher prevalence of SARS-COV-2 infection and/or severe or critical illness derived from COVID-19 in pregnant women (Cheng et al. 2020). Indeed, reports of maternal deaths associated with COVID-19 in Brazil (Takemoto et al. 2020) and Mexico (Lumbreras-Marquez et al. 2020) increased wariness worldwide.

In this article, we will review the evidence published to date on the prevalence and effects of COVID-19 on pregnancy and maternal and fetal health, with emphasis in Latin American countries.

12.2 The Impact of COVID-19 on Maternal Mortality Rate in Latin America

Multinational cohort study reported that risk of maternal death increased by 22.2-fold in pregnant women with COVID-19 diagnosis compared with pregnant women without COVID-19 (Villar et al. 2021). Although some other reports showed that maternal mortality risk was similar to the nonpregnant population infected by SARS-CoV-2 (Espiritu et al. 2023; Pérez-López et al. 2022; Pettirosso et al. 2020). Apparent discrepancy could be explained by lack of analysis considering countries income, population-specific analysis of the COVID-19 prevalence, or complexity of the maternal care in countries with high SARS-CoV-2 infection rates, like Latino American countries.

The maternal mortality rate (MMR) is one of the sensitive indicators to characterize the performance of healthcare systems. In Mexico, the MMR increased by over 60% in 1 year during the pandemic, and COVID-19 was linked to 25.4% of maternal deaths (Mendez-Dominguez et al. 2021). In Chile, the MMR increased by 56% in 2020 compared with 2019 (prepandemic period) (González et al. 2023). The increase in MMR during 2020 represents a break in the historical trend of decline since 2011 in Chile. Similarly, in Peru, maternal deaths increased by 75% compared with a similar period in 2019 (83 deaths in 2019, up to 146 during 2020), and the MMR increased by 102%, which represents a significant disruption in this country from the last 5 years (Gianella et al. 2021). In Brazil, there was excess maternal mortality in 2020 by 40%, considering the trends of the past 5 years (Guimarães et al. 2023). Even considering excess mortality due to COVID-19 for the childbearing age female population, maternal mortality exceeded the expected number, and the risk of maternal death was 44% and 61% higher in black women and women living in a rural area, compared with the control group (Guimarães et al. 2023). A study with databases from eight countries in Latin America confirms an increased MMR during the pandemic, generating a setback in the region's achievements in the last decade. Thus, in 2020, the Latin American MMR was 88 deaths per 100,000 live births, up from 83 deaths per 100,000 live births in 2019 (Maza-Arnedo et al. 2022).

In the USA, a country significantly affected by COVID-19 and with a vast Latino-American community, maternal mortality increased by 33.3% during the pandemic. Specifically, the relative changes in MMR were higher for Hispanic (74.2%) and non-Hispanic Black (40.2%) than non-Hispanic White (17.2%) (Thoma and Declercq 2022). Underlying epidemiological or biological reasons for this increased MMR is largely unknown; however, it certainly will involve health conditions directly related to COVID-19 or exacerbated by this disease, including healthcare disruptors and perhaps higher vulnerability of Hispanic and non-Hispanic

Black pregnant women. So, the evidence demonstrates that COVID-19 significantly affected maternal mortality trends in Latin American countries and Latin American communities in the USA. The studies report that a percentage of maternal deaths can be directly associated with COVID-19, but there are other factors besides the biological effects of virus infections. The unequal access to healthcare and barriers to access to intensive care faced by pregnant women must be addressed by regional governments, along with the delay in providing greater protection to a vulnerable population such as pregnant women. The Director of Pan American Health Organization (PAHO), Dr. Carissa F. Etienne said, "Nearly all maternal deaths are preventable and even getting back to pre-pandemic levels of maternal mortality, which were already high, could take more than a decade." (PAHO 2021). These facts show severe deficiencies in the region's health systems to protect the health of pregnant women, especially in the period before vaccination.

12.3 The Prevalence of SARS-CoV-2 Infection in Pregnancy

At the beginning of the COVID-19 pandemic, some countries implemented universal screening for SARS-CoV-2 in pregnant women, making it possible to assess the incidence of SARS-CoV-2 according to the state of the spread of the pandemic. For instance, Australia informed zero or very low prevalence of COVID-19 in pregnant women (Rolnik et al. 2020), while in Italy, it was below 1% (0.6%) (Gagliardi et al. 2020). Countries with intermediate prevalence of COVID-19 in pregnant women include Sweden (5.8%) (Ahlberg et al. 2020), Chile (6.3%) (Díaz-Corvillón et al. 2020), United Kingdom (7%) (Khalil et al. 2020), and Turkey (7.8%) (Yassa et al. 2020), while a high prevalence was reported in the USA (37%) (Maru et al. 2020) and Mexico (29%) (Cardona-Pérez et al. 2021) (Table 12.1). In the case of the Australian and Italian studies, the universal screening was made in regions and periods with low rates of daily infections (0.58 cases per million inhabitants in Australia at the time of the study). In contrast, in the case of the USA (New York City), both Sutton et al. (2020) and Vintzileos et al. (2020) reported COVID-19 incidence of 503 and 600 cases per million inhabitants during the period of a widespread pandemic in that state, respectively.[1]

Whereas at the time of strict lockdown in New York City (NYC) (280 cases per million inhabitants), a prevalence of 3.9% was reported among 770 pregnant women (Campbell et al. 2020). Similarly, a second report in London 4 weeks after the peak of infections and after the lockdown showed a prevalence of SARS-CoV-2 of 3.9% in 180 pregnant women tested (Abeysuriya et al. 2020). In Portugal (Porto), two reports show 12% and 6% prevalence, with 64.8 and 50.8 cases per million inhabitants, respectively (Dória et al. 2020; Figueiredo et al. 2020). In Chile, Diaz-Corvillón et al. reported a prevalence of 6.3% positivity in 583 pregnant tested women in a period in which the rate of infections per million inhabitants was 26.3 (Díaz-Corvillón et al. 2020). In another Chilean study, with 73 women admitted to a hospital for pregnancy interruption, a prevalence of 9.5% was detected (Cornejo et al. 2020). It is possible to consider the study of Díaz-Corvillón and colleagues as representative of the public health situation in Chile during the first wave of pandemic, because the patients in the Cornejo study were admitted for pregnancy interruption, probably strictly controlled by medical staff.

In the study of Ahlberg et al. (2020) in Sweden (Stockholm), with 2682 patients, the prevalence was 5.8%. This study was conducted during higher contagion rates in the country (between March 25 and July 24, 2020), with a maximum of 107.8 infections per million inhabitants and an average of 58.9 infections per million throughout the study period.

As is shown in Table 12.1, the SARS-CoV-2 positivity prevalence in pregnancy ranged from 0 to 37%, and the asymptomatic prevalence ranged from 43% to 92%. Among the work with positive

[1]Cases per million inhabitants were obtained from www1.nyc.gov/site/doh/covid or www.ourworldindata.org/coronavirus

Table 12.1 Prevalence of infection in pregnancy from data obtained with universal test application for SARS-CoV-2

Country (city)	Sample size (*n*)	SARS-CoV-2-positive prevalence (%)	Asymptomatic (%)	References
USA (Queens)	124	37	72	Maru et al. (2020)
Mexico (Mexico City)	240	29	86	Cardona-Pérez et al. (2021)
USA (New York City)	161	20	66	Vintzileos et al. (2020)
USA (New York City)	215	15.4	88	Sutton et al. (2020)
Portugal (Porto)	103	12	92	Dória et al. (2020)
Chile (Santiago)	73	9.5	86	Cornejo et al. (2020)
Turkey (Istanbul)	296	7.8	52	Yassa et al. (2020)
UK (London)	129	7	89	Khalil et al. (2020)
Chile (Santiago)	583	6.3	43	Díaz-Corvillón et al. (2020)
Portugal (Porto)	184	6.0	82	Figueiredo et al. (2020)
Sweden (Stockholm)	2682	5.8	ND	Ahlberg et al. (2020)
UK (London)	180	3.9	86	Abeysuriya et al. (2020)
USA (New York City)	770	3.9	73	Campbell et al. (2020)
Italia (Several regions)	1566	3.1	55	Ferrazzi et al. (2020)
Spain (Madrid)	203	1.0	50	Herraiz et al. (2020)
Australia (Melbourne)	350	0	–	Rolnik et al. (2020)

ND Not determined

cases, the positivity mean was 10.5%, and the asymptomatic prevalence was 72.9%. A higher prevalence of pregnant women with COVID-19 symptoms was observed in Chile (57%) (Díaz-Corvillón et al. 2020), compared with the other studies in Table 12.1. Although it is complex to compare the situation between countries or regions due to the multiple factors that influence the registries, it is possible to observe that prevalence of COVID-19 in pregnancy correlates with the level of spread of the pandemic. For example, the infections per million inhabitants in NYC were 7.6-fold higher compared to London, and the prevalence of SARS-CoV-2 in pregnancy was 2.8-fold higher. Likewise, the establishment of strict lockdowns significantly impacts the prevalence of infection in pregnant women, highlighting that NYC decreased at a similar prevalence to London (3.9%) even when the spread of infections in the overall population was 7.3-fold higher. Therefore, we can consider the situation reported by Rolnik and colleagues (Rolnik et al. 2020) in Melbourne (Australia) as the best-case scenario, with a prevalence of zero in a context of

significant control of the pandemic, together with the experience in Madrid, which registered a prevalence of 1% in pregnant women after the peak of infections in Spain (Herraiz et al. 2020). The highest prevalence were reported in Mexico and NYC, where the Hispanic or Latin American communities were more affected, as described in the following paragraphs.

12.4 COVID-19 in Pregnancy and Inequality

From the World Inequality Report (2021), it is possible to obtain these data: In Latin America, the top 10% of wealthy citizens capture 55% of national income, compared with 36% in Europe. The bottom 50% earns 27-fold less than the top 10%; this difference is significantly lower in Europe (the bottom 50% earns ten-fold less than the top 10%). In detail, Mexico and Chile are two of the most unequal countries in the world, where the top 10% of the wealthiest earn more than 30 times more than the bottom 50%. The COVID-19 pandemic has exacerbated several forms of health, social, gender, and racial inequality within countries. The Latin American population is one or more affected, living in Latin America properly or as part of the immigrant community in wealthier countries.

In Mexico, in a period with one of the highest COVID-19 test positivity percentages worldwide, the prevalence of COVID-19 in pregnant women was 29% (Cardona-Pérez et al. 2021). This prevalence is higher than it reported worldwide (see Table 12.1). This prevalence correlates with 32.7% positivity rate in Mexico City at the time span of the study. In a report in NYC, among 126 pregnant women screened for SARS-CoV-2 between March 29 and April 22, 2020, 37% were positive (Maru et al. 2020). These studies are relevant because they exhibit universal screening results in pregnant women during peak periods of the pandemic and also because they consider a socioeconomically vulnerable population. Maru et al. showed significantly higher infection rates among Hispanics than non-Hispanics, consistent with data among all hospitalized patients in NYC. The positivity for SARS-CoV-2 was 46% in Hispanic pregnant women vs 22% in non-Hispanic women (Maru et al. 2020). The Hispanic ethnicity and Spanish as the primary language likely indicate that the patient was not born in the USA or is a first-degree descendant of Latin American immigrants. These communities have long faced barriers to healthcare access, challenges due to immigration status, and financial and labor instability. The COVID-19 pandemic has only aggravated issues in the vulnerable and often overlooked immigrant community (Behbahani et al. 2020).

Pregnant women's cultural and socioeconomic vulnerabilities are relevant since the spread of COVID-19 has been reported to be associated with multidimensional poverty. For example, in the nonpregnant Chilean population, COVID-19 rate was higher in populations with an upper score of multidimensional poverty (Telias and Figueroa 2020). Therefore, the highest percentage of pregnant women with comorbidities associated with high susceptibility to COVID-19 is concentrated in this population sector. This relationship could be corroborated by the study by Gurol-Urganzi et al. (Gurol-Urganci et al. 2021), which included 342,080 women with singleton pregnancies who gave birth in England (May 29, 2020, to January 31, 2021), of whom 3527 had laboratory-confirmed SARS-CoV-2 infection. In this period, SARS-CoV-2 infection was more likely in women residing in the most socioeconomically deprived areas. The socioeconomic score was evaluated by the "Indices of multiple deprivation (IMD)," which are widely used datasets within the UK to classify the relative deprivation (essentially a measure of poverty) of small areas. Using IMD score, 61.2% of COVID-19 pregnant women belong to more deprived areas, compared with 48.4% of nonconfirmed SARS-CoV-2 pregnant women (Gurol-Urganci et al. 2021). Globally, a meta-analysis involving 2,003,724 pregnant women from 57 countries showed that the overall rate of SARS-CoV-2 infection in pregnant women was 8%, with significant variations across geographical

regions and country income levels. The highest infection rates were reported in Latin America and the Caribbean countries (19%), associated with lower-middle income (13%). The rate of SARS-CoV-2 infection was 2.2-fold higher in lower-middle-income countries than in upper-middle-income countries (Sheikh et al. 2022).

In Chile, only one study has considered the socioeconomic status of pregnant women affected with COVID-19. Authors did not find a significant difference when analyzing the socioeconomic variables of patients with a positive (n = 68) or negative (n = 613) COVID-19 test (Vera von Bargen et al. 2022). However, this study considered patients from one public hospital in Santiago, representing a homogeneous population concerning the economic, social, and demographic levels. Therefore, further studies are required to understand the impact of the socioeconomic status of pregnant women on the prevalence of SARS-CoV-2 infection and obstetrics and perinatal outcomes in Chile and other Latin American countries, especially considering the social inequality within each country.

12.5 The Impact of COVID-19 on Pregnancy Outcomes

During pregnancy, a series of metabolic adaptations allow the correct development of the fetus. These adaptations involve all maternal systems relevant to the respiratory, cardiovascular, and immune systems. Due to these complex physiological adaptations, it was suggested that during pregnancy, the susceptibility to developing severe or critical COVID-19 could be increased (González et al. 2021). In a cohort of 91 pregnant patients diagnosed with COVID-19 in the gestational or postpartum period, 42 (46.2%) patients required hospitalization by COVID-19. Among women who gave birth at the time of the study (23 patients with an active infection at the time of delivery), prematurity was 34.8% (Barbero et al. 2020). A study in Sweden showed that positive pregnant women for SARS-CoV-2 presented a higher prevalence of preeclampsia (7.7%) compared to negative pregnant women (4.3%) without finding significant differences in prematurity (9% vs 7.5%) (Ahlberg et al. 2020). A report in the USA compared the clinical presentation of the disease between 8207 pregnant women and 83,205 nonpregnant women infected with SARS-COV-2. Hospitalization rates were 31.5% and 5.8% in pregnant and nonpregnant women, respectively (Ellington 2020). These hospitalization levels showed that women in gestation presented a higher prevalence of severe or critical COVID-19. However, pregnant women are in a system of greater medical surveillance, and it cannot be discarded that some hospitalizations have been due to pregnancy discomfort or extraordinary precautions due to minor symptoms of COVID-19. Although the first systematic review that collected 60 studies published until May 23, 2020 (considering 3830 pregnant patients in total), showed similar rates of severe or critical COVID-19 among pregnant and nonpregnant women (Pettirosso et al. 2020), the subsequent studies concluded that pregnant women had a higher risk of severe COVID-19 (Gurol-Urganci et al. 2021; Smith et al. 2023; Villar et al. 2021).

More recent reports indicate that pregnant women infected with SARS-CoV-2 manifested moderate symptoms (or asymptomatic) in 81–86%, severe illness in 9.3–14%, and critical illness requiring hospitalization in an intensive care unit (ICU) in 5% cases (Ayala-Ramírez et al. 2022). The multinational study of Villar et al. concluded that COVID-19 in pregnancy increases the relative risk of severe neonatal morbidity by fourfold and severe perinatal morbidity by 3.5-fold compared to negative SARS-CoV-2 pregnancies (Villar et al. 2021). Notably, the SARS-CoV-2 infection was associated with an increased risk of developing preeclampsia or eclampsia (presence of seizures in a hypertensive pregnancy) (Ferrara et al. 2022). This association between SARS-CoV-2 and preeclampsia or eclampsia has also been established in cases reported in Latin America (Ayala-Ramírez et al. 2022), which correlates with information that Latin America

and the Caribbean had the highest rates of stillbirth and neonatal intensive care unit admission in SARS-CoV-2-positive pregnancies (Sheikh et al. 2022).

12.6 The Impact of COVID-19 on Perinatal Mental Health

Concerning maternal mental health, the depressive symptoms in pregnant women increased almost three times from 15% (pre-pandemic) to 40.7% (pandemic), and anxiety (moderate or high) increased from 29% to 72% during the first months of the COVID-19 pandemic (Davenport et al. 2020). Subsequent studies consolidated the data with a prevalence of prenatal anxiety and depression during the pandemic of 59% and 37%, respectively, compared with incidences of less than 20% and 10% before COVID-19 (Lebel et al. 2020). A study with 3356 Spanish women in the perinatal period showed that the prevalence of anxiety and depression during the COVID-19 pandemic was 33% and 47%, respectively, which were more than double of what was reported in pre-pandemic studies (Motrico et al. 2022). Specifically, in the prenatal period, 40–60% of pregnant women reported symptoms of depression, anxiety, and post-traumatic stress disorder (Filippetti et al. 2022). A systematic review published in February 2023 including 13,401 pregnant women analyzed clinical symptoms of depression, anxiety, and stress employed standardized measures. Results indicated that depression rates ranged from 9.9% to 49% of participants, anxiety symptoms were experienced in 11% to 61% of participants, and stress symptoms were reported in 40% of women (Wall and Dempsey 2023). Importantly, from the 16 articles, 13 studies were conducted within Europe, with the remaining three conducted in North America, Asia, and Africa. In March 2023, Mesquita et al. published a cross-sectional study with 7645 participants from 12 countries (Albania, Brazil, Bulgaria, Chile, Cyprus, Greece, Israel, Malta, Portugal, Spain, Turkey, and the United Kingdom). The pregnant women or mothers in the postpartum period completed the Edinburgh Postnatal Depression Scale (EPDS) or the Generalised Anxiety Disorder Assessment (GAD-7). The overall rates for clinically significant symptoms of depression and anxiety in pregnant women were 26.8% and 20.1%, respectively. In postpartum women, the rates for depression and anxiety were 32.7% and 26.6%, respectively (Mesquita et al. 2023). The geographical analysis of the data indicates that in Brazil and Chile, the rates for depression in pregnant women were 37.7% and 34.9%, respectively, while in postpartum women were 47.2% and 33.8%, respectively. Additionally, anxiety was present in 34.3% and 36% of pregnant women in Brazil and Chile, respectively. These rates were even high in postpartum women, who presented anxiety in 41.9% and 41.5% in each country, respectively. Then, this report indicate that Latin American countries (Brazil and Chile) showed higher rate of depression in pregnant women (36.3% vs 25%) than European countries (Albania, Bulgaria, Cyprus, Greece, Malta, Portugal, Spain, Turkey, and UK) (Mesquita et al. 2023). Another study in Colombia reported a prevalence of prenatal depression in 30.4% among 345 pregnant women (Gaviria-Arbeláez et al. 2022). The rate of anxiety in Latin American pregnant women was 35.2% vs 20.6% in European women. In postpartum women, the rates of depression and anxiety were also higher in Latin America than in European countries (40.5% and 41.7%, respectively, vs 28.5% and 20.6%, respectively). Thus, Fig. 12.1 shows that the prevalence of depression and anxiety in pregnancy was 1.5-fold and 1.7-fold higher in Latin America vs Europe (Fig. 12.1a). In postpartum women (Fig. 12.1b), depression and anxiety were 1.4-fold and 1.9-fold higher in Latin America vs Europe, respectively. Therefore, the data support a higher impact of the COVID-19 pandemic on perinatal mental health in Latin American countries than in Europe.

In Sweden, depression and anxiety were highly prevalent among pregnant women during the COVID-19 pandemic (Ho-Fung et al. 2022). This last study emphasizes that unemployment

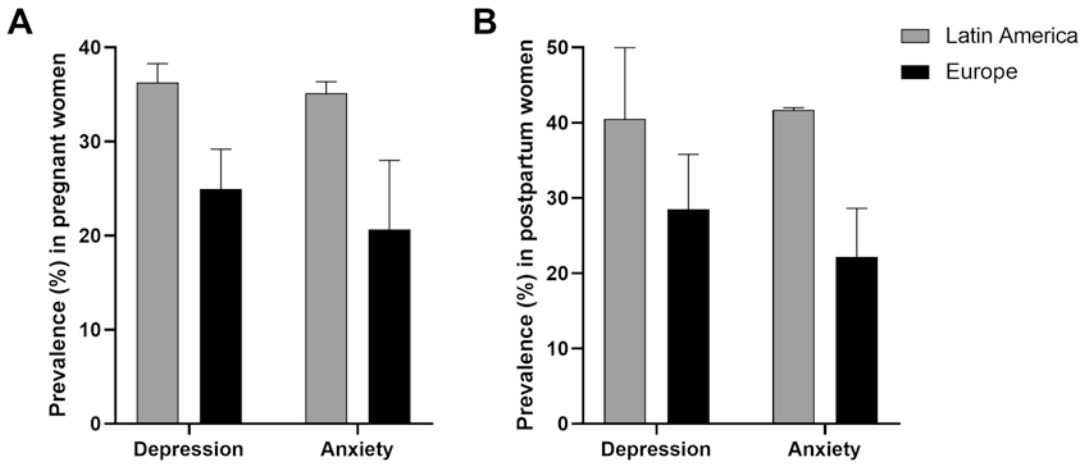

Fig. 12.1 Higher prevalence of prenatal and postpartum depression and anxiety in Latin America. The data from Mesquita and colleagues (Mesquita et al. 2023) were separated between Latin American (Brazil and Chile) and European (Albania, Bulgaria, Cyprus, Greece, Malta, Portugal, Spain, Turkey, and UK) countries to highlight the greater impact of COVID-19 pandemic on perinatal mental health disorders in Latin America

was an associated risk factor. Thus, younger age and higher educational level were protective, suggesting an essential role of socioeconomic factors on the impact of COVID-19 on perinatal mental health. In this line, an intersectional study between COVID-19 and socioeconomic mental health stressors in South African adolescent girls and young women concluded that various psychosocial risk factors disproportionally affect women's mental health, with the COVID-19 pandemic intersecting and overlapping with these preexisting social and environmental factors (Duby et al. 2022). These pieces of evidence are also important for countries like Chile, where there is a high correlation between the rate of violence against women and the percentage of households living in poverty (Onofri 2020). Gender violence and poverty are two of the most relevant risk factors for increased perinatal mental health disorders, which was potentiated by the COVID-19 pandemic.

Concerning gender violence, a systematic review involving 616,708 women from 51 countries reported the highest prevalence of perinatal depression in lower-middle-income countries (23 countries). In this group, the highest prevalence of perinatal depression (38.9%) was present in women who experienced intimate partner violence. The prevalence of depression was 37.0% among women who experienced physical intimate partner violence and 28.6% among women who experienced sexual intimate partner violence (Roddy Mitchell et al. 2023). During the lockdowns for the COVID-19 pandemic, emergency calls for violence against women increased 48% in Peru (Agüero 2021), 44% in Chile (Segovia 2021), 36% in Mexico, 32% in Argentina, 50% in Panama, 25% in Costa Rica, and 91% in Colombia (The World Bank 2022). The peak of phone calls to the police in Chile was between April and May of 2020, at the beginning of lockdowns, but maintained high during all periods of social restrictions. The helpline of the National Service for Women and Gender Equity (SernamEG) registered a 149% increase in phone calls because the women were forced to live 24 h a day, 7 days a week with their attacker, without being able to report it to the police (Segovia 2021).

Nevertheless, in the later stages of the pandemic, job losses and constrained financial sustainability for women were identified as potential drivers of increased abuse within the home (The World Bank 2022). Figure 12.2 shows the association between a significant increase in gender-based violence in Latin America and higher rates

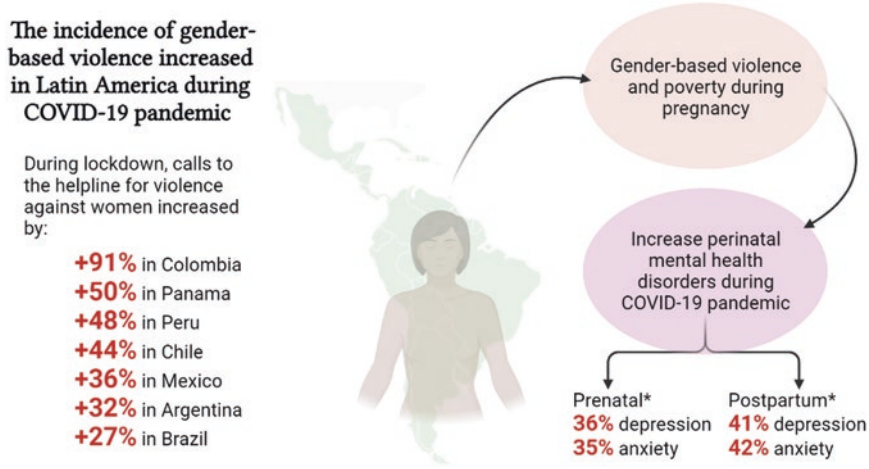

Fig. 12.2 Gender-based violence in Latin American pregnant women and association with perinatal mental health. During the COVID-19 pandemic, calls to the helpline for violence against women in Latin American countries were significantly increased, a relevant risk factor for perinatal mental health disorders. *Data from Mesquita and colleagues (Mesquita et al. 2023)

of perinatal mental health disorders. This association is supported by the data discussed previously and observational studies showing that domestic violence is associated with a higher prevalence of perinatal depression (Alvarez-Segura et al. 2014; Howard et al. 2013; Sørbø et al. 2014).

Regarding the conditions for giving birth during the pandemic, a study with pregnant women in England and Wales showed that a large proportion of individuals reported heightened levels of anxiety and distress due to poor or lack of communication from the hospital service, primarily related to whether their birthing partner would be able to attend the birth (Aydin et al. 2022). In Chile, considering the restrictions for partners to participate in the birth in the first year of the pandemic, it is highly probable that a large proportion of pregnant women lived similar experiences of anxiety and distress, especially with a positive diagnosis of COVID-19 (El Mostrador 2020; Leiva et al. 2021).

Significantly, prenatal stress during COVID-19 is associated with poorer infant socioemotional development (Duguay et al. 2022). Furthermore, higher levels of maternal postpartum depression were predicted or associated with lower levels of attachment/bonding, higher levels of stress related to parenting experiences, and a lower frequency of caretaking activities (Federica et al. 2023). Maternal depression during the COVID-19 pandemic could have impacted the development of affectionate and emotional connections to their infants. Recent evidence suggests that postpartum depression during the COVID-19 pandemic predicted poorer infant social-emotional development at 6 months of age (Harrison et al. 2022). This study is related to the findings that infants (6 months) born in the pandemic present neurodevelopmental alterations necessary to follow in the long term (Shuffrey et al. 2022). In this regard, a recent study showed that infection with SARS-CoV-2 during pregnancy increases the risk (odds ratio 1.86) of offspring neurodevelopmental disorder in 1-year infants (Edlow et al. 2022), which could be associated with placental and neurological inflammation in the fetus during SARS-CoV-2 infection at the third trimester of pregnancy (Shook et al. 2022). All these data strongly suggest that SARS-CoV-2 exposure during pregnancy may impair maternal mental health and children's neurodevelopment.

12.7 Placental Alterations in COVID-19

Vertical transmission (from a pregnant woman to a fetus in utero) is rare (Edlow et al. 2020). Still, the infection with SARS-CoV-2 and/or the development of COVID-19 have significant effects on the placenta. In infections during the third trimester of pregnancy, placental inflammation occurs, increasing the presence of Hofbauer cells (placental macrophages), activation of the maternal immune response in the placenta, thrombosis, placental infarcts, and poor fetoplacental perfusion (González et al. 2021). All these alterations can be associated with preeclampsia-like syndrome since there are similar placental alterations in pregnancies complicated with preeclampsia (Aouache et al. 2018; Harmon et al. 2016). It is important to note that placental pathology (inflammation, oxidative stress, poor perfusion) was observed in both severe and mild/asymptomatic cases of COVID-19 (Stenton et al. 2022; Sureshchandra et al. 2022; Ward et al. 2022). Moreover, a single-cell RNA sequencing study indicates mild/asymptomatic COVID-19 during pregnancy remodels the maternal-fetal interface's immunological landscape (Sureshchandra et al. 2022), showing the potential of long-term adverse outcomes for the offspring and mothers even in mild/asymptomatic cases.

Placenta from SARS-CoV-2-positive pregnant women shows poor uterine-placental perfusion, placental infarcts, evidence of atheroma in the decidua vessels, chorioangioma, and edema in placental villi (Shanes et al. 2020), also thrombosis in the chorionic plate and a significant decrease in capillaries of chorionic villi (Baergen and Heller 2020; Mulvey et al. 2020). Moreover, a study with 27 cases of asymptomatic or mildly symptomatic SARS-CoV-2-positive pregnant women showed the following features of fetal vascular malperfusion (FVM): chorioangiosis, intramural fibrin deposition, and vascular ectasia. Perivillous fibrin deposition was also significantly higher in placental histopathology (Jaiswal et al. 2021). The massive perivillous fibrin deposition and chronic histiocytic intervillositis were rarely observed in placentas before COVID-19 pandemic and are abnormalities that constitute the "placentitis" associated with SARS-CoV-2 infection (Schwartz et al. 2023).

Edlow et al. showed that maternal vascular malperfusion was presented in 16 of 44 (36%) SARS-CoV-2-exposed placentas and 8 of 44 (18%) unexposed placentas ($p = 0.06$). Furthermore, among SARS-CoV-2-positive pregnant women, the vascular lesions increased significantly with disease severity (odds ratio = 2.09; $p = 0.02$) (Edlow et al. 2020). Therefore, vascular lesions of the placentas can occur in pregnancies with SARS-CoV-2 infections, and the magnitude of the lesions is related to COVID-19 severity.

It should be noted that most studies failed to demonstrate whether placentas from SARS-CoV-2-positive mothers show higher levels of inflammatory markers. This aspect is relevant because there is a significant increase in inflammatory markers (including interleukin 1β or interleukin 6) in the placenta and plasma of women whose placenta tested positive for SARS-CoV-2 (Fenizia et al. 2020). Furthermore, SARS-CoV-2 infection induces a higher inflammatory response in the intervillous space (histiocytic intervillositis) and chorionic villi (villositis), with the presence of macrophages (CD68) and T lymphocytes (CD3) in the intervillous space (Hecht et al. 2020; Hosier et al. 2020; Hsu et al. 2020; Kirtsman et al. 2020; Patanè et al. 2020; Sisman et al. 2020; Smithgall et al. 2020; Vivanti et al. 2020). The placental inflammatory lesions are part of the pathophysiology of SARS-CoV-2 placentitis, which could be associated with preterm and stillbirth in COVID-19 (Schwartz et al. 2023).

Placental transcriptome analysis of COVID-19 cases showed increased expression of genes associated with immune responses, specifically a significant upregulation of the HSPA1A gene, which encodes the heat shock protein Hsp70 (Lu-Culligan et al. 2021). Furthermore, the study of Lu-Cullingan et al. also showed increased pro-

inflammatory genes and chemokines in both immune and nonimmune cell types in the placenta from COVID-19 cases. Additionally, the analysis of single-cell transcriptome reveals significant enrichment of genes encoding cytotoxic proteins in natural killer (NK) cells, upregulation of the activation marker CD69 in T cells, increased expression of interferon-induced protein ISG15, and the regulators of NFκB pathway NFKBIA/NFKBIZ in endothelial cells of COVID-19 cases (Lu-Culligan et al. 2021).

Remarkably, in patients with COVID-19 admitted to the intensive care unit, elevated levels of von Willebrand Factor (vWF) antigen and P-selectin were detected in plasma, as an indicative of endothelial dysfunction (Goshua et al. 2020). Also, the placenta of pregnant women with severe COVID-19 has higher expression of vWF in the endothelium from the decidua and chorionic villi and decreased expression of claudin-5 and VE-cadherin (Flores-Pliego et al. 2021), which correlates with findings that pregnant women with COVID-19 have a higher risk of hemostatic and thromboembolic complications compared with pregnant women without COVID-19 infection (Servante et al. 2021). These findings demonstrated that COVID-19 induces endothelial dysfunction and thrombosis in the placenta.

Whether placental vascular alterations detected in placentas positive for SARS-CoV-2 may impair fetal development is not entirely understood. For instance, Shanes et al. (2020) found 36% of small-for-gestational-age newborns (SGA) and 7% of prematurity in the infected group (15 cases), while Díaz-Corvillón et al. (2020) reported 5.4% of SGA with 10.8% premature newborns in the infected group (37 cases) vs 5.1% and 4.9% in the control group, respectively. On the contrary, another study, including a large number of cases (COVID-19 positive, $n = 155$) and controls ($n = 604$) (Ahlberg et al. 2020), did not find significant differences in the prevalence of SGA or premature between case and controls. On the other hand, an increase in stillbirth, low birth weight, and preterm birth have also been reported during the COVID-19 pandemic (Khalil et al. 2020; Schwartz et al. 2023; Villar et al. 2021), all adverse outcomes associated with placental dysfunction. The differences in these studies demonstrate the need to continue gathering information and elucidate the factors that affected the placental function and induced adverse perinatal outcomes in the COVID-19 pandemic, especially in Latin America.

12.8 Final Remarks

In summary, epidemiological and case-control studies showed that COVID-19 pandemic negatively impacts pregnancy outcomes, mothers' perinatal mental health, and eventually neurodevelopment in their children. Despite multifactorial mechanisms, we have highlighted socioeconomical, ethnical, and biological determinants of these association. We also emphasize the potential role of the dysfunctional placenta as one of the critical event that may constitute one biological mechanism.

As is shown in Fig. 12.3, multidimensional poverty, healthcare barriers, and financial instability particularly affect Latin American pregnant women. Also, the increased gender-based violence during the COVID-19 pandemic could significantly increase the deterioration of perinatal mental health in Latin American women (Fig. 12.2). These conditions increased the vulnerability to SARS-CoV-2 infection and the severity of COVID-19 disease. Furthermore, the pandemic disruptors and COVID-19 symptoms affected maternal mental health, associated with early development alterations. All the alterations induced by the COVID-19 pandemic have meant an increased maternal mortality ratio and severe neonatal and perinatal outcomes (see Fig. 12.3). Further studies are necessary to evaluate the long-term consequences of COVID-19 for mothers and children, to improve social and healthcare support and reduce the inequities in a vulnerable population.

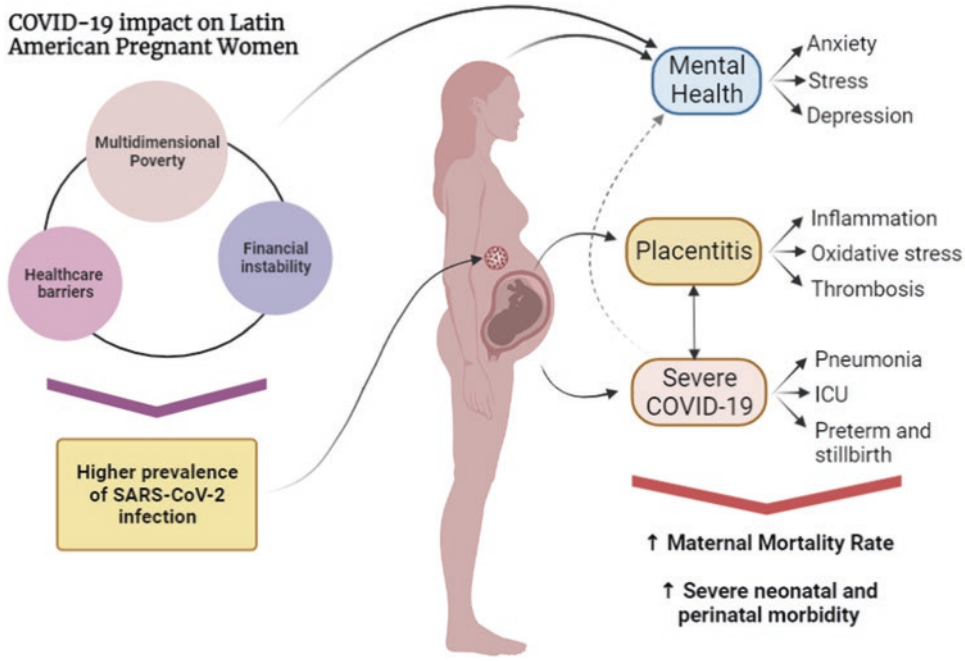

Fig. 12.3 Conditions of Latin American pregnant women to detrimental effects of COVID-19. The multidimensional poverty, healthcare barriers, and financial instability are burdens affecting Latin American countries and Latin American pregnant women living in wealthy countries. These conditions induce mental health alterations, potentiated by the COVID-19 pandemic and higher prevalence of SARS-CoV-2 infection. The maternal SARS-CoV-2 infection is associated with placentitis linked with severe COVID-19 during the third trimester of pregnancy. The maternal disease significantly increased maternal mortality and severe neonatal and perinatal morbidity

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13 The Placental Function Beyond Pregnancy: Insights from Latin America

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Abstract

Currently, more than 100,000 papers had been published studying the placenta in both physiological and pathological contexts. However, relevant health conditions affecting placental function, mostly found in low-income countries, should be evaluated deeper. This review will raise some – of what we think necessary – points of discussion regarding challenging topics not fully understood, including the paternal versus maternal contribution on placental genes imprinting, placenta-brain communication, and some environmental conditions affecting the placenta. The discussions are parts of an international effort to fulfil some gaps observed in this area, and Latin-American research groups currently evaluate that.

Keywords

Placenta · Placental genes imprinting · Placenta-brain communication · Pollution · Environmental · Phthalate

13.1 Introduction

The placenta is a transient organ that accomplishes key roles during pregnancy, constituting the mother and fetus interface (Burton et al.

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2016). For instance, the placenta must carry out functions, such as transport of nutrients, waste transfer and gas exchange, expression of hormones and protection of the fetus, oxygen, and carbon dioxide exchange, among others (Griffiths and Campbell 2015). To completely fulfil these functions, the placenta displays specialized structures, the placental villi.

Thousands of publications have described that dysfunctional placentas are related to pathological conditions, including preeclampsia, fetal growth restriction, gestational diabetes, or obesity. These pathological conditions can be perpetuated after birth, not only in the offspring but also in the mother. Then, there is no question about the relevance of studying the placenta to understand better the maternal complications associated with pregnancy diseases and programming of future cardiovascular and cerebrovascular disease in both mother and offspring. Also, the placenta can be used as a model to study several molecular mechanisms in human physiology.

Our international cooperation group (www.rivatrem.org) has set out to visualize the research carried out in Latin American countries in the area of vascular alterations during pregnancy complications (Giachini et al. 2017). Also, we want to encourage the worldwide research community to conduct studies on emerging topics focusing on the placenta. Very little is known but might have an enormous impact on mother and child health and disease. For example, we recently alert how little is known about placental damage due to neglected infections, including Chagas disease, Leishmaniasis, among other diseases (Ribeiro et al. 2020). This manuscript will provide essential points of discussion regarding emerging topics, including placental-brain communication, the role of gene imprinting, and how pollution and phthalate chemicals may impact placental function. Additionally, we would like to denounce how those problems are remarkably underestimated in Latin American countries.

13.2 Placenta-Brain Communication

A healthy pregnancy is characterized by significant physiological changes in practically all organ systems to maintain the mother's well-being and nurture the developing fetus (Ribeiro et al. 2020). Several adaptive changes are well recognized in the cardiovascular, endocrine, respiratory, digestive, and skeletal systems during pregnancy. However, significantly less scientific evidence is available about the impact of those changes or the pregnancy itself on the brain and maternal cognitive function (Brett and Baxendale 2001; John et al. 2018), although subjective cognitive impairment during pregnancy has been described for decades (Brett and Baxendale 2001). A meta-analysis (Davies et al. 2018) including 709 pregnant and 521 nonpreg-

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nant patients from 20 studies was focused on the measurement of cognitive function on these women. They reported that the overall cognitive functioning was poorer in pregnant women, particularly in the third trimester of gestation than in nonpregnant women. Also, the same meta-analysis described that it also diminished the overall memory performance in pregnant women. Still, no changes were found in executive functioning and attention in this group compared to nonpregnant women. Although one may argue that many confounding variables might be affecting these epidemiological observations, it is also intriguing to ask if these conclusions are correct and what would be the potential mechanism eliciting this cognitive deficit during pregnancy.

The brain is the most energetically expensive organ in vertebrates since it has been estimated to account for 20–25% of the resting metabolic rate in adult primates (Fonseca-Azevedo and Herculano-Houzel 2012). This demand is reflected in 50% of the total body glucose utilization to form adenosine triphosphate (ATP) (Fehm et al. 2006). This metabolic demand is affected in pregnancy, which indeed may constitute an energetic trade-off resulting in an energy-allocation dilemma for supplying the maternal brain and the developing fetus (Fehm et al. 2006). Considering this hypothesis, pregnancies affected by alterations in weight gain and fat deposition may generate a metabolic disequilibrium, impairing brain function. These conditions may include women with low access to nutrient (i.e., in low-income countries or starvation time), excessive access to nutrient (i.e., maternal obesity or glucose metabolism alterations including diabesity), in the condition in which the placenta can consume more (i.e., large placentas observed in gestational diabetes) or less glucose (i.e., small placentas observed in preeclampsia), among others.

In this regard, pathological conditions such as gestational diabetes (GDM) (John et al. 2018) or preeclampsia (Dayan et al. 2018; Elharram et al. 2018) have been associated with long-term maternal cognitive impairment. In particular, Keskin and colleagues report a cognitive decline in GDM patients (Keskin et al. 2015). Their study included a small group of women, with and without GDM, subjected to several cognitive function tests. GDM women scored less in the trial, analyzing whole cognitive function or spatial recall and visual memory test, while they had the more deficient speed of mental activity and attention than women with healthy pregnancies. The patients were also submitted to a test for assessing psychomotor speed, concentration, and integration (the symbol digit modalities, SDMT). Interestingly, the score obtained by GDM women in this test negatively correlates with plasma levels of glycosylated hemoglobin (HbA1c). Underlying mechanisms are unclear, but this association might suggest a metabolic origin of the cognitive impairment evidenced in GDM.

Similarly, preeclampsia is a well-characterized risk factor for acute or chronic cerebrovascular alterations (Martin et al. 2005; Newstead et al. 2007; Hammer and Cipolla 2015; Dang et al. 2016). For instance, a severe complication of preeclampsia is eclampsia or the new onset of generalized tonic-clonic seizures. Also, 30–70% of maternal deaths associated with preeclampsia are due to cerebral complications, mainly in low- and middle-income economies such as Latin American countries (Okanloma and Moodley 2000). Underlying brain alteration mechanisms are still under investigation but have been associated with brain endothelial cell dysfunction, manifested as cerebral vasogenic edema (Okanloma and Moodley 2000). This mechanism is reinforced with brain magnetic resonance imaging (MRI) since a majority of women with eclampsia show edema on the posterior cerebrum, a condition known as posterior reversible encephalopathy syndrome (PRES) (Legriel et al. 2012). Eclampsia and PRES have been thought to be reversible. Still, lately, it has been shown that women with previous preeclampsia are at higher risk of stroke, cerebral white matter lesions, vascular dementia, and cognitive failure (Brussé et al. 2008; Postma et al. 2013; Hammer and Cipolla 2015; Mielke et al. 2016; Andolf et al. 2017).

Eclampsia is commonly associated with hypertensive pregnancy disorders, although around 38% of seizures during pregnancy arose in women with mild hypertension or even without a hypertension diagnosis (Aagaard-Tillery

and Belfort 2005). Placental ischemia is associated with cerebral vasogenic edema in rats (Warrington et al. 2014). Also, placental ischemia was associated with a reduction in the threshold to seizure induced by pentylenetetrazole (PTZ) in a rat model of preeclampsia/eclampsia (Johnson et al. 2014). Therefore, placental ischemia may release "harmful factors" that generate brain vascular dysfunction, even without a mandatory increase in blood pressure.

Currently, little is known about which would be the harmful placental factors released from the ischemic placenta in preeclampsia. However, they are indeed circulating in the maternal bloodstream, as indicated in ex vivo studies, when preeclamptic plasma increased the brain-blood barrier (BBB) permeability compared to plasma from women with healthy pregnancy (Amburgey et al. 2010) (see Fig. 13.1).

Circulating harmful factors may include tumor necrosis factor alpha (TNF-α) (Warrington et al. 2015) and/or vascular endothelial growth factor (VEGF) (Amburgey et al. 2010), among others not tested yet. Accordingly, TNF-signaling inhibition, using soluble TNF-α receptor (etanercept), reverts the augmented brain vascular permeability elicited by the treatment with preeclamptic plasma. Similarly, the VEGF receptor (VEGFR) inhibition, using the inhibitor of tyrosine kinase activity (Calbiochem 676481), displayed a similar effect (Amburgey et al. 2010). Whether those molecules act synergically or independently is unknown. Also, underlying cellular mechanisms of TNF-α or VEGFR need to be further characterized. For instance, the underlying molecular target leading to increased BBB permeability in preeclampsia is unclear. The tight junction proteins or aquaporins could be part of this pathological mechanism.

Communication between the placenta and the brain, or at least with vascular brain endothelial cells, is plausible. Potential implications of this communication for acute or long-term complications in maternal brain function are currently speculative. But, considering epidemiological and preclinical studies in pathological pregnancies, such as preeclampsia, it is expected that significant acute consequences and long-lasting consequences for maternal cognitive function are expected. This evidence can be considerable in Latin American countries where a high incidence of pathological pregnancies, including preeclampsia, is well described (Giachini et al. 2017).

13.3 The Paternal Contribution to Placental Development

Maternal and paternal genes play a crucial role during placental development. However, some cell types, including those in the placenta, have a maternal or paternal monoallelic gene expression known as imprinting. The interplay between maternal and paternal gene expression is recognized as the "conflict theory hypothesis" or "kinship theory." This hypothesis states that the maternally expressed genes in the placenta are mostly engaged in restricting nutrients for the fetus, ensuring the possibility of future pregnancies. The paternally expressed genes stimulate fetal growth at the expense of the mother's well-being (Lim and Ferguson-Smith 2010). Besides low birth weight and intrauterine growth restriction, imprinting has also been associated with diabetes, obesity, cancer, and psychiatric disorders (Peters 2014). Then, placental imprinting is a relevant mechanism for neonatal well-being and, when altered, is accountable for the so-called developmental origins of health and disease (DOHaD).

Imprinting is recognized as an adaptive mechanism driven to nutrient availability as well as drug exposure. The deletion of paternal or maternal expressed genes results in intrauterine growth restriction and fetal overgrowth, respectively. For instance, in transgenic mice, the functional disruption of imprinted genes such as paternal-derived insulin-like growth factor 2 (*Igf*2) P0 isoform with exclusive expression in the placenta gives rise to both placental and fetal growth retardation (Peters 2014). Alternatively, the overexpression of the maternal *PHLDA*2 gene results in low birth weight (Apostolidou et al. 2007).

The most common studied mechanisms related to imprinting are DNA methylation and histone

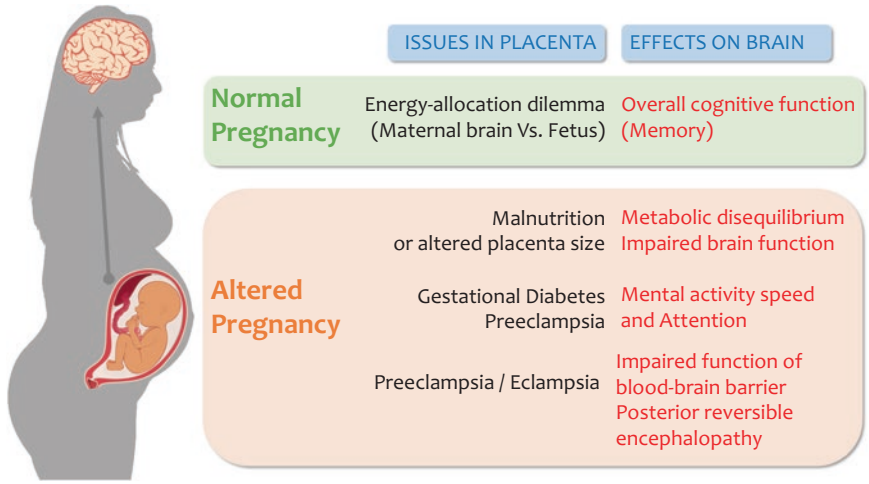

Fig. 13.1 Placenta and brain communication. During pregnancy, an interaction between the placenta and the brain is established in normal and altered pregnancies. In normal pregnancies, the placenta's energy requirements compete with the maternal brain requirement, an event known as an energy-allocation dilemma. Improper distribution of energy in a normal pregnancy could impact overall cognitive function. Pathological pregnancies, including malnutrition, abnormal placenta size, GDM, and PE, could affect impaired brain function, reduced mental activity, and increased vascular permeability in the brain and PRES syndrome. (The figure was made using vectorized images designed by Freepik.es)

modifications, which regulate gene expression nutrient the availability depending manner (Gheorghe et al. 2010). The methylation of DNA and the histone-binding proteins control transcription, replication, recombination, and DNA repair, which interact to trigger a cascade of transduction signals responsible for generating gene-silencing networks. Another regulatory mechanism is miR-127, a maternally expressed gene that works repressing *Rtl1*, a paternally expressed gene. The abolition of such a mechanism produces placental overgrowth (Ito et al. 2015). New mechanisms on placental imprinting have been recognized, such as (1) secondary imprints on differential methylated regions, (2) imprinted gene clusters, (3) methylation-independent noncanonical imprinting, and (4) endogenous retroviral (ERVs) insertions (Ito et al. 2015; Hanna 2020).

A microarray expression study showed that imprinting marks are acquired at early developmental stages in pregnancy, with some retained in adult tissues or erased (Babak et al. 2015). Initially, during gametogenesis, sperm and ovum display specific epigenetic marks. Imprinting marks are reprogrammed during fertilization and preimplantation processes, basically due to extensive demethylation of paternal pronucleus and the methylation of maternal pronucleus. Some genome sequences of the trophectoderm-derived placenta display a relatively higher global demethylation concerning inner cell mass-derived embryo (Chapman et al. 1984). After implantation, the embryo and extraembryonic tissues become highly and partially methylated, respectively (Decato et al. 2017).

The presence of both the paternal and maternal genomes is mandatory for adequate placenta/embryo development. This lesson is long known since it showed that the androgenetic mouse leads to a condition known as androgenetic hydatidiform moles. This mouse model contains two paternal DNA copies with no maternal DNA and developed only extraembryonic structures (Barton et al. 1984). Comparing androgenetic hydatidiform moles and healthy placental tissues has allowed the identification of paternally expressed genes, such as *DNMT1,* a critical regulator of methylation status (Monk 2015).

The evaluation of placentas spontaneously delivered in cases of extremely preterm infants

showed hypermethylation patterns in 216 genes, including those related to neuronal development. Indeed, the higher the placental methylation level, the higher the cognitive impairment level in children at 10 years of age (Tilley et al. 2018). Some imprinted genes, such as the paternally expressed *Peg3*, are expressed in the placenta and the adult brain. Then, besides its involvement in fetal growth, *Peg3* also influences maternal behavior regarding newborns' proper neonatal care (Li 1999). In a recent study, a new mutation generated in the last exon of *Peg3* reduced postnatal growth phenotype but no association with maternal care behavior (Denizot et al. 2016).

Imprinted genes are also related to different processes associated with hypertensive pregnancy disorders (i.e., preeclampsia) with consequences for mother, baby, and development of chronic diseases later in life. Thus, a preeclampsia-like model was displayed in a pregnant mouse in the presence of a paternal copy of *p57Kip2* gene and a null allele. Meanwhile, the maternal copy and a null allele displayed no such phenotype (Kanayama et al. 2002). This fact reveals the importance of parental-specific alleles for the development of preeclampsia.

Additionally, at least 140 genes have been consistently found as deferentially expressed in preeclamptic, compared to normal placentas. Of those, 110 (78.6%) are considered as susceptibility genes associated with paternal/fetal fitness, and the remaining 30 (21.4%) as protective genes related to maternal fitness (Kobayashi 2015). Because the mother expresses genes in the placenta which are supposed to be involved in maternal protection, it seems reasonable to search for genes under-expressed in preeclamptic placentas. Thus, at least 50 downregulated genes were engaged in different pathophysiological mechanisms of preeclampsia. Among those 50 genes, 25 are located close to clusters controlling differentially methylated regions. Thus, the expressed genes have paternal or maternal expression, which supports the role of imprinting in preeclampsia development (Kobayashi 2016). This evidence was further confirmed by the same group (Christians et al. 2017), evaluating 61 imprinted versus 14.986 randomly non-imprinted genes that showed a significant higher dysregulation of the former in the preeclamptic placentas.

Nevertheless, preimplantation is an essential process in placental and fetal development controlled by *NLRP* imprinted genes (Zhang et al. 2008). Previous studies have revealed that mutations in the imprinted genes *NLRP*2 (Meyer et al. 2009) and *NLRP*5 (Docherty et al. 2015) are associated with impaired implantation and preeclampsia. Other evidence has only involved mutation in *NLRP7* in the pathogenesis of preeclampsia (Soellner et al. 2018).

Specific parental-expressed genes have been associated with preeclampsia. For instance, the maternally imprinted gene *DLX5* is overexpressed in the human trophoblast, and it has been associated with preeclampsia (Zadora et al. 2017). Also, imprinting control regions such as ICR1 and 2 regulate the expression of imprinted clustered genes such as *CDKN1C* responsible for the Beckwith-Wiedemann congenital overgrowth syndrome. In contrast, mutations on the maternally expressed gene *CDKN1C* are associated with preeclampsia (Romanelli et al. 2009). Thus, maternal and paternal evaluation should be carried out in pathologies, such as preeclampsia (Galaviz-Hernandez et al. 2019). In particular, some genetic studies showed that paternal markers might be protective for developing preeclampsia in the Mexican population (Galaviz-Hernandez et al. 2016).

Other maternal conditions related to imprinting derangements, including assisted reproductive techniques, have also been investigated. For example, after somatic cell nuclear transfer in mice, trophoblast stem cells showed loss of imprinting status for maternally expressed genes *Gab1, Slc38a4, and Sfmbt2*, which displayed a biallelic expression, resulting in aberrant placental development (Hirose et al. 2018). Conversely, one study conducted in humans, including 119 placentas, obtained from pregnancies induced by in vitro fertilization and intracytoplasmic sperm injection demonstrated no changes in placental weight and birth weight compared to controls, despite *H19* and *IGF2*, a couple of genes involved in placental and fetal growth, displaying biallelic expression (Sakian et al. 2015). These pieces of

evidence encourage future studies addressed to investigate the potential imprinting effect of associated reproductive techniques.

In summary (Fig. 13.2), the interplay between maternal and paternal genetic determinants is mandatory for placental and newborn well-being. Therefore, imprinting studies are needed to better comprehend maternal and paternal roles in placental-derived pathologies, such as preeclampsia. No studies evaluating the critical role of imprinting on preeclampsia development have been carried out in Latin America. It is required to start research lines considering the particular genetic diversity of the region.

13.4 Potentially Harmful Effects of Pollution in the Placental Function

Anthropogenic activity produces a variety of compounds that are released in terrestrial, aerial, and aquatic environments. Exposure to excessive levels and/or prolonged periods can result in harmful consequences for human health. The World Health Organization considers ten chemicals of public health concern, including air pollution and four specific heavy metals: arsenic, cadmium, lead, and mercury (Cámara de Diputados de Chile 2019).

13.4.1 Effects of Air Pollution on Placental Function

The damage caused by atmospheric pollutants is not limited to the lungs (Oyarzún and Valdivia 2012). Air pollutants can alter physiological parameters during pregnancy through several pathophysiological mechanisms, including placental inflammation, oxidative stress, and/or vascular dysfunction of this organ, among others (Salinas 2018). Increased levels of particulate matter (PM10 and PM2.5), sulfur dioxide (SO_2), and nitrogen dioxide (NO_2) are deemed dangerous for human health (WHO 2016).

In South America, Brazilian groups have led the research concerning air pollution and its potential effects on pregnancy. In Brazil, air pollution is a significant health problem, with cities like Rio de Janeiro or Belo Horizonte reaching PM2.5 levels of 36 μg/m^3 and 28 μg/m^3, respectively (WHO 2016). Sao Paulo is another large city with a long history of air pollution. Although patterns have improved, the problem is still considered relevant by the Brazilian health service

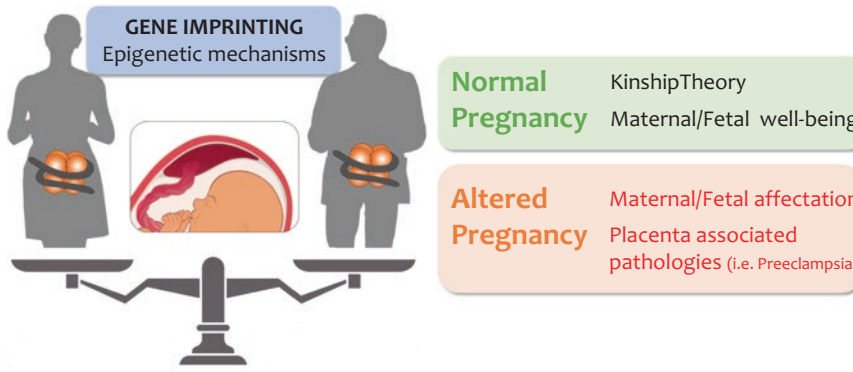

Fig. 13.2 Specific maternal and paternal gene expression is crucial for placental development and newborn well-being. Proper development of the placenta requires the expression of genes specifically inherited from the mother or the father. The phenomenon is known as imprinting and is governed by an epigenetic mechanism, including DNA methylation and microRNAs. For a normal pregnancy to get a proper balance between the expression of maternal or paternal alleles, which show the contrary effect on the placenta. The altered imprinting of genes (e.g., biallelic expression) has been associated mainly with preeclampsia, due to placenta-altered development. (The figure was made using vectorized images designed by Freepik.es)

due to repercussions on the population, especially during pregnancy.

For instance, Pereira and colleagues investigated the association between intrauterine mortality and pollutant concentrations (NO_2, SO_2, CO, ozone (O_3), and PM10) between 1991 and 1992 in Sao Paulo. They reported that the higher the NO_2 levels, the higher the intrauterine mortality (Pereira et al. 1998). In a study involving 299 low-risk pregnant women from Sao Paulo, levels of exposure to NO_2 and O_3 were determined with cellulose filters that women carried over 12 days (range 7–18 days), and vascular effects were evaluated using 3D power Doppler (Hettfleisch et al. 2017). Significantly, NO_2 exposition was associated with a negative influence of both the vascularization index (VI) and the vascularization flow index (VFI). The alterations of VI and VFI are associated with preeclampsia and fetal growth restriction. Thus, in pregnant women exposed to higher concentrations of NO_2, the placental VI and VFI were decreased in the first trimester, suggesting a negative influence on placentation, resulting in decreased placental vascularization (Hettfleisch et al. 2017).

In its Declaration on Air Pollution, the Chilean Academy of Medicine points out that the damage caused by atmospheric pollutants is not limited to the lungs (Oyarzún and Valdivia 2012). The concentration of populations and industrial activity around Santiago and Valparaiso regions (Chile) has created numerous environmental problems, especially air pollution (OECD Territorial Reviews: Chile 2009 2009). In the early 1990s, Santiago had air pollution levels similar to Sao Paulo and Mexico City, but with one-third of the population, compared to the other two cities (Pino et al. 2015). Among high-income American countries, Chile has the worst air conditions with national annual mean PM2.5 of 28 μg/m^3, compared with 12 μg/m^3 for the USA, 18 μg/m^3 for Uruguay, and 8 μg/m^3 for Canada (WHO 2014). In 2011, four Chilean cities were among the five American cities with the highest PM2.5 levels: Rancagua, 54 μg/m^3; Chillán, 53 μg/m^3; Temuco, 48 μg/m^3; and Talca, 44 μg/m^3 (WHO 2014).

Worldwide, other studies also have investigated the effect of air pollution on perinatal outcomes or even in the pregnancy itself. For instance, in one study, including more than 47 thousand singleton births registered in 28 hospitals from Japan, exposure to suspended PM and O_3 during early pregnancy (0–4 weeks) was associated with placenta previa (Michikawa et al. 2016). NO_2 and SO_2 were also associated, though to a reduced degree of significance. Researchers did not find these associations for exposures during 5–12 weeks of gestation. Thus, the authors concluded that early exposure, or during the pre-pregnancy period, likely affects implantation and trophoblast invasion mechanisms.

In another study involving 785 pregnant women in Spain, NO_2 exposure was associated with decreased birth weight. Small-for gestational-age newborns increased by 37% for each 10 μg/m^3 increase in NO_2 levels during the second trimester (Ballester et al. 2010). Even in regions with air pollution levels below WHO air quality guidelines, such as Sweden, there is a negative correlation between birth outcomes and air pollution, with an estimation of 9 g lower birth weight per 10 μg/m^3 increment NO_2 (Malmqvist et al. 2017).

Prenatal or early exposure to air pollutants also affects fetal thyroid gland function. Thus, exposure to PM was associated with higher total thyroxine levels in newborns, as observed in a cohort study of 2050 newborns in California, USA (Howe et al. 2018). Compatible with these results in humans, results from mice models suggest that gestational exposure to PM2.5 leads to spatial memory dysfunction and neurodevelopmental impairment (Zheng et al. 2019). Thus, early pregnancy exposure to pollutants may be harmful for placental function and the neuroendocrine axis and brain function in the fetus.

The underlying mechanisms of those alterations are unclear. But, some may include genetic, epigenetic, mitochondrial, and/or vascular modifications. For instance, in a study involving 336 mother-newborn pairs in Spain, exposure to NO_2 during the first trimester of pregnancy was inversely associated with length at 6 months in infants and placental mitochondrial DNA (mtDNA) (Clemente et al. 2017). There was a negative correlation between PM10 exposure and

placental mtDNA during the whole pregnancy, especially in the third trimester. Each 10 μg/m^3 increase in PM10 was associated with a lower placental mtDNA content of 10.1% in a Belgium cohort of pregnant women (Janssen et al. 2012). Changes in mtDNA can relate to mitochondrial dysfunction and decreased birth weight, with consequences in later life associated with alterations in fetal programming (Hanson and Gluckman 2014). Exposure to higher levels of PM2.5 during midpregnancy induces telomeres shortening, measured in cord blood and placental samples (Martens et al. 2017). Telomere length is a marker of biological aging that may be related to oxidative stress and inflammation in the placenta and the newborn, especially in the context of mitochondrial dysfunction. Moreover, epigenetic mechanisms in the placenta may also be altered by air pollutants. It is recognized that placental DNA and mtDNA methylation, dysregulation of microRNAs (miRNAs), transcriptional activity, among others, are targets by PM or indirectly by inflammatory mediators and/or reactive oxygen species (ROS) (Martens et al. 2017).

Besides, animal models have helped better understand pollution's harmful effect on placental function. For instance, female rats exposed to filtered air or concentrated fine PM, over 15 days, decreased placental size (Soto et al. 2017). The underlying mechanisms of disrupted fetoplacental vascularization were associated with impaired angiogenic mechanisms, driven by the vascular endothelial growth factor (VEGF) and angiotensin II (Soto et al. 2017). Together, these results show that air pollutants could affect the placenta's vascular function, increase vascular resistance, and decrease blood flow to the fetus.

Despite limited information on the effect of pollution on fetoplacental function and/or pregnancy outcomes in Latin America, it is essential to note that the impact of PM10 on health problems in children is documented. A study conducted in Santiago determined that PM10 is associated with clinic visits for lower respiratory symptoms in children fewer than 2 and 3–15 years old. For children under 2 years, 50 μg/m^3 elevation in PM10 was associated with a 4–12% increase in lower respiratory symptoms (Ostro et al. 1999). Considering the body of evidence presented here, it is feasible that children from Latin American cities, including Santiago and Rio de Janeiro, and smaller towns such as those in the south of Chile, may carry out alterations during intrauterine life. With this regard, they might be more sensitive to environmental stress and pathophysiological mechanisms that increase oxidative stress and inflammation.

13.4.2 Specific Effects of Heavy Metals on Placental Function

In cells, metal and nonmetal elements are crucial for the activity of several enzymes, for example, selenium, magnesium, and copper. However, metals can harm cells by several chemical mechanisms. Adventitious binding may induce steric rearrangement and impair function, such as the inhibition of heme synthesis by lead (Lee et al. 2012). Mimicry, or competition for metal-binding sites in proteins, can occur. For example, molybdate mimics sulfate competing for sulfonation reactions and sulfate carriers (Zhou et al. 2013). Oxidative damage may produce ROS as well as oxidative responses. Direct binding to DNA can occur, by producing adducts or inducing DNA-protein cross-linking, such as trivalent chromium (Zhitkovich 2005). Finally, heavy metals can also modify gene expression, exemplified by the arsenite-regulated NRF2 transcription factor activity (Kawai et al. 2011).

Several heavy metals may disrupt placental function, among them: cadmium, manganese, chromium, lead, mercury (Amaya et al. 2013), methyl mercury, selenium, zinc, copper (Sakamoto et al. 2013), titanium, nickel, antimony, tin, vanadium, arsenic (Cerrillos et al. 2019), boron, barium, calcium, iron, potassium, lithium, magnesium, manganese, molybdenum, sodium, strontium, vanadium, and zinc (Cerrillos et al. 2019) (see Table 13.1). Different protein systems are implicated in the transport of metal and metalloids through the placenta, including transporters from the ABC family (Liu et al. 2016), transferrin (Liu et al. 2016), and divalent metal transporter 1 (DMT1) (Somsuan et al.

2019). On the other hand, metals can also be stored in organs by metallothioneins, proteins of low molecular weight (3.5–14 kDa) with domains rich in cysteine residues that can trap metal irons (reviewed in Babula et al. 2012). Although ubiquitously expressed, they are suggested as important protective factors for cadmium's placental trafficking to the fetus (Espart et al. 2018).

Exposure to high levels of heavy metals has been associated with placental pathologies, such as exposure to cadmium and preeclampsia (Laine et al. 2015). Placental alterations have been described, including lower weight and decreased placental efficiency (newborn weight vs placental weight) related to chromium's placental concentrations (Punshon et al. 2019). Cadmium is implicated in several cellular alterations, including reduced migration capacity (Brooks and Fry 2017); the proliferation of trophoblastic cells (Zhou et al. 2016); and microscopic alterations, characterized by a more significant presence of syncytial nodes, deposits of fibrinoid material, chorioamnionitis and decidual inflammation (Phuapittayalert et al. 2014).

Although studies evaluate heavy metals' impact on newborns, most of them have been conducted in Europe (Ballester et al. 2010; Malmqvist et al. 2017; Sabra et al. 2017). Again, there is an urgent necessity for this kind of analysis in Latin America. Additionally, there is no available data about a histological examination of the placenta concerning heavy metal exposure in the region, which is critical for correctly understanding the impact of those contaminants on the placental function. We believe that in front of the vital increment of environmental contamination in Latin America, the study of its effects on maternal health, placenta, and newborn physiology is determinant for future generations' public health (Fig. 13.3).

13.5 Exposition to Plastic-Derived Endocrine Disruptors

Phthalates are a family of human-made chemicals (CDC 2017). They are classified as endocrine-disrupting chemicals (EDCs), particularly concerning early life exposures (Benjamin et al. 2017). Plastic materials for food packaging, flooring, and medical devices use high-molecular-weight phthalates like butyl benzyl phthalate (BBzP), di-2-ethylhexyl phthalate (DEHP), and mixtures of di-n-octyl phthalates (DnOP), most well-known as polyvinyl chloride (PVC) (Atwood and Paisley-Jones 2012; Zota et al. 2014). Recently, dinonyl phthalate (DiNP), and di-decyl phthalate (DiDP) have replaced DEHP in these applications (Zota et al. 2014). On the other hand, in cosmetics and personal care products, low-molecular-weight phthalates as dimethyl phthalate (DMP), diethyl phthalate (DEP), and dibutyl phthalate (DBP) are primarily used as solvents, fixatives, and adhesives (Zhao et al. 2016).

Phthalate chemicals and their parent materials have noncovalent bonds, then can be significant leaching and volatilization leading to environmental contamination and thus, ubiquitous exposures in the general population (Zhao et al. 2016). Metabolite biomarkers of eight major phthalates have been detected in 89–98% of the United States population (Zota et al. 2014), but unfortunately, similar information in the Latin American context is not available. Phthalates have been linked to adverse health effects, as they are classified as endocrine-disrupting chemicals, particularly with early life exposures (Benjamin et al. 2017).

Increasing evidence suggests that persistent organic pollutants can influence both male and female health and fecundability, which is the natural ability to conceive a pregnancy (Skakkebæk et al. 2001; Buck Louis et al. 2011). Prenatal exposures are also related to the risk of pregnancy loss, alterations in labor timing, either longer gestation or preterm birth, infant hormone levels, changes to infant birth weights, and infant and child neurobehavioral outcomes (Swan 2008; Wolff et al. 2008; Engel et al. 2009; Ferguson et al. 2014; Adibi et al. 2017) (Fig. 13.4). Phthalates cross the blood-placenta barrier and are associated with deficits in cognitive functions and behavior problems in offspring, as analyzed in the Columbia Center for Children's Environmental Health birth cohort (CCCEH) in

Table 13.1 Detrimental effects of heavy metal exposure in pregnancy and fetal development

References	Espart et al. (2018), Laine et al. (2015), Punshon et al. (2019), Brooks and Fry (2017), Zhou et al. (2016), Phuapittayalert et al. (2014), Ballester et al. (2010), Malmqvist et al. (2017), and Sabra et al. (2017)								
Effects on fetal development		Reduced Zn transport to the fetus	Reduced birth weight/ IUGR	Reduced IQ	Alteration in gonadic steroid genesis in male offspring	Reduced neonatal length	Reduced APGAR score	Cognitive disabilities	
Effects on placenta	Interference with the synthesis of endocrine hormones	Injury to vascular endothelium	Diminished placental permeability to Zn, Fe	Impaired trophoblast cell migration and proliferation	Early decidualization of human endometrial stromal cells	Increase presence of syncytial nodes	Chorioamnionitis and decidual inflammation	Abnormal glucocorticoid balance	Abnormal regulation of insulin-like growth factor-related proteins
Heavy metal exposure	Cadmium (Cd)								

References	Punshon et al. (2019), Sabra et al. (2017), and CDC (2017)							
Effects on fetal development	Reduced birth weight	Birth defects	Learning disabilities	Autism	Impairment in hearing	Impairment of psychomotor development	Reduced IQ	Neurodevelopmental disorders and subclinical brain dysfunction
Effects on placenta		Deregulation in placental hormonal secretion	Impaired placental amino acid transport	Impaired placental oxygen consumption	Alteration in membrane fluidity of trophoblast cells	Miscarriage/ stillbirth		
Heavy metal exposure	Mercury (Hg)							

(continued)

Table 13.1 (continued)

References	Sabra et al. (2017) and CDC (2017)									
Effects on fetal development	Reduced birth weight	Congenital malformations	Learning disabilities	Reduced IQ	Impairment in hearing	Impairment in motor development	Attention deficit disorders	Hematological alterations	Mental restriction	Neurotoxic effects and neurodevelopmental disorders
Effects on placenta					Interference with calcium-mediated cellular processes in synctiotrophoblast	Reduction of cytochrome oxidase activity				
Heavy metal exposure	Lead (Pb)									

References	Brooks and Fry (2017) and Sabra et al. (2017)							Zhitkovich (2005)
Effects on fetal development	Reduced birth weight	Increased chronic disease risk (e.g., cardio metabolic disease)	Deficits in learning and memory	Increased metabolic and metastatic disorders risks	Neurotoxicity	Behavioral deficits	Neurocognitive deficits	Reduced birth weight
Effects on placenta	Abnormal placentation?							Decreased placental efficiency
Heavy metal exposure	Arsenic (As)							Chromium (Cr)

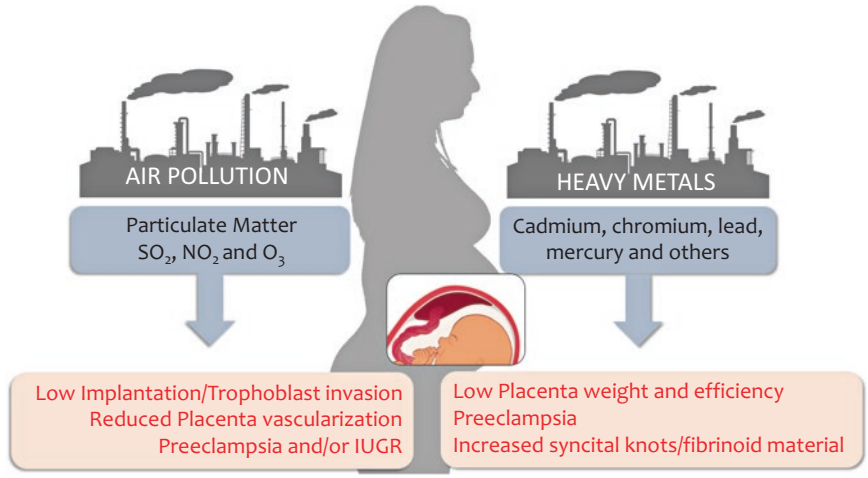

Fig. 13.3 Potential underlying mechanisms of air pollutant-mediated placental alterations. Not only particulate matter or nocive gasses affect the placenta but also heavy metals. Prolonged exposure to air pollution is strongly associated with early placental development alterations as reduced implantation or trophoblast invasion. In concomitance with reduced placenta vascularization, preeclampsia is one of the most observed pregnancy diseases in contaminated areas. Heavy metals can impact the placenta's size and weight with characteristic marks of cellular damage (i.e., increased syncytial knots) associated with preeclampsia. (The figure was made using vectorized images designed by Freepik.es)

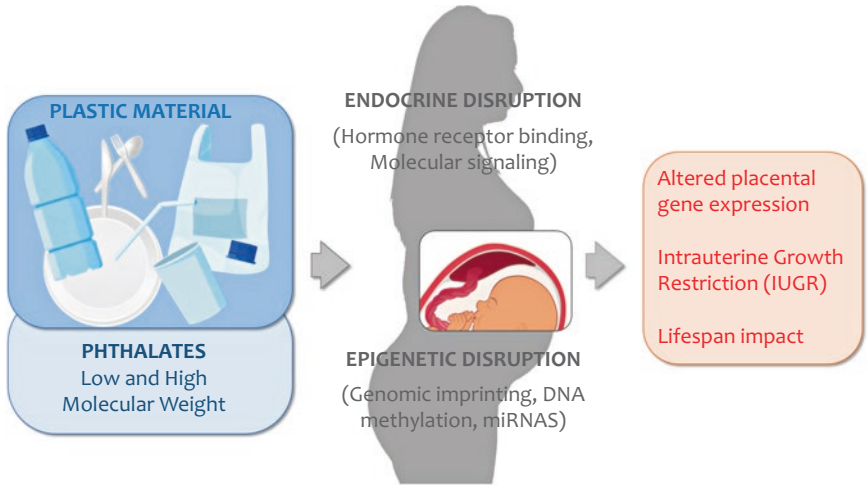

Fig. 13.4 Plastic-derived endocrine disruptor-mediated placental and fetal affectations. Many low- and high-molecular-weight phthalates are released from the plastic material. Practically all of them have the ability not only to affect the placenta but also the fetus. Mechanisms can be divided into endocrine disruption (i.e., the alteration of cellular signaling at extracellular and intracellular levels) and epigenetic disruption (or the alteration of crucial epigenetic mechanism for the placenta and fetus development). Both of them are tightly associated, influencing each other. As a result, plastic-derived endocrine disruptors alter placental gene expression associated with intrauterine growth restriction and neurological alterations in middle childhood. (The figure was made using vectorized images designed by Freepik.es)

which 209 mother-child pairs were included (Daniel et al. 2020). They concluded that phthalate exposure during pregnancy was associated with decreased motor functions among 11-year-old girls.

DNA methylation is a potential mechanism through which bisphenols and phthalates may be related to female reproductive disorders (Menezo et al. 2016). Bisphenol A (BPA) has been shown to target reproductive tissues and affects reproductive outcomes in numerous animal studies (Shelby et al. 2004). In a now-classic experiment, mice exposed to BPA during pregnancy were more likely to give birth to offspring with yellow coats due to decreased methylation upstream of the Agouti gene. The effect was negated when BPA-exposed dams were supplemented with folic acid, a methyl donor (Dolinoy et al. 2007). Folate depletion has been associated with global hypomethylation but also with targeted hypermethylation (Crider et al. 2012). A recent study among women undergoing infertility treatment reported that high urinary BPA concentrations were associated with lower probabilities of implantation, clinical pregnancy, and live birth, but only among women who consumed less than 400 mg/day of dietary folate (Mínguez-Alarcón et al. 2016). For instance, women from polluted areas had a higher placental expression of miR-146a, which may impact biological functions, including signal transduction, cell differentiation, and enzymatic activity through miR-146a target genes (De Felice et al. 2015).

Several studies have shown associations between phthalate or BPA exposure and altered placental gene expression patterns (Adibi et al. 2010, 2017; Xu et al. 2015; Li et al. 2016). Therefore, it is feasible to hypothesize that the effects of environmental chemicals on the placental epigenome can negatively impact placental and fetal growth (Strakovsky and Schantz 2018). As indicated previously, early placentation is an event that occurs under strict epigenetic control (Nelissen et al. 2011). Therefore, maternal environmental exposures may result in a sensitive process for disruption. Furthermore, as the pregnancy progresses, toxicant-induced epigenetic modifications to genes involved in placental function have the potential to alter placental development and efficiency. In a recent review, ten studies with evidence that the placenta is an epigenetic target (e.g., genomic imprinting, global DNA methylation, and miR expression) of these chemicals were analyzed (Strakovsky and Schantz 2018). They conclude that despite the relevance of the potential association between BPA or phthalate exposures and epigenetic changes in the placenta and future well-being of the offspring, additional research is needed to understand the molecular mechanisms behind those effects. These mechanisms may include the interaction between chemicals with hormone receptors, disrupting downstream molecular signaling, altering the chromatin state of placental cells, among others (CDC 2017). Alternatively, they might create a microenvironment within the placenta that is more conducive to epigenetic disruption (Atwood and Paisley-Jones 2012), such as increasing ROS or inflammation (Strakovsky and Schantz 2018).

Di(2-ethylhexyl) phthalate (DEHP) is a plasticizer with widespread exposure in pregnant women (Casas et al. 2011; Arbuckle et al. 2014) that is used in food packaging, toys, medical devices, and other PVC-containing products, and human exposure occurs primarily from consuming processed and packaged foods (Schettler et al. 2006). To note, a well-studied and vital gene cluster linked to placental and fetal development is placental IGF2/H19. In women with fetal growth restriction assessed phthalates in the third trimester observed that higher exposition to DEHP was associated with position-specific decreased methylation of placental IGF2 at delivery in biopsies pooled from the maternal side (Zhao et al. 2016). In another study, the increased mono (2-ethyl-5-hydroxyhexyl) phthalate (MEHHP) and SumDEHP (molar sum of MEHP, MEHHP, and MEOHP) metabolites were associated with decreased placental *LINE-1* methylation in women with fetal growth restriction (Zhao et al. 2015). These results once again support the association of maternal DEHP exposure with altered placental DNA methylation.

While specific dietary components (e.g., folate) indirectly contribute to DNA methylation by par-

ticipating in 1-carbon (methyl group) metabolism (Su et al. 2016) and the metabolic status of a cell drives the modifications on histone tails (Zhang and Kraus 2010; Mandaviya et al. 2014; Salminen et al. 2016), little insight is available regarding the precise epigenetic actions of EDCs. This might be because exposure assessment across pregnancy is challenging, and studying relationships between chemical exposures and placental epigenetic disruption is complicated by the temporal shifts in epigenetic marks across gestation (Luo et al. 2009), its relative genomic hypomethylation (Novakovic et al. 2010; Logan et al. 2013; Jensen et al. 2015), differential genomic imprinting (Court et al. 2014), or its unique intragenic methylation patterns (Schroeder et al. 2015) when compared to other tissues (Januar et al. 2015).

Unfortunately, no studies in Latin American pregnant women have been published. In a recent review of 59 scientific articles from 11 Latin American countries, published between the years 1999 and 2018 (Peña-Guzmán et al. 2019), the pollutants with a higher frequency of sampling were hormones and bisphenol-A.

13.6 Concluding Remarks

In this manuscript, we have highlighted challenging questions regarding the placenta study (Fig. 13.5). We have included information about potential communication between the placenta and the brain as an underlying mechanism of maternal complications such as eclampsia, PRES, or future cognitive function as a fascinating research area that needs mechanistic studies to elucidate molecular targets.

We further have emphasized that physiological mechanisms such as imprinting genes for eliciting the maternal and paternal roles in placental and fetal development are increasing research lines that need to expand in Latin American countries. Not only because limited information is available but also because of the high incidence of pregnancy diseases, such as preeclampsia. Despite this, Latin American people have a particular genetic diversity not fully included in the currently available literature.

Finally, societies' development has generated environmental damage that affects wildlife and

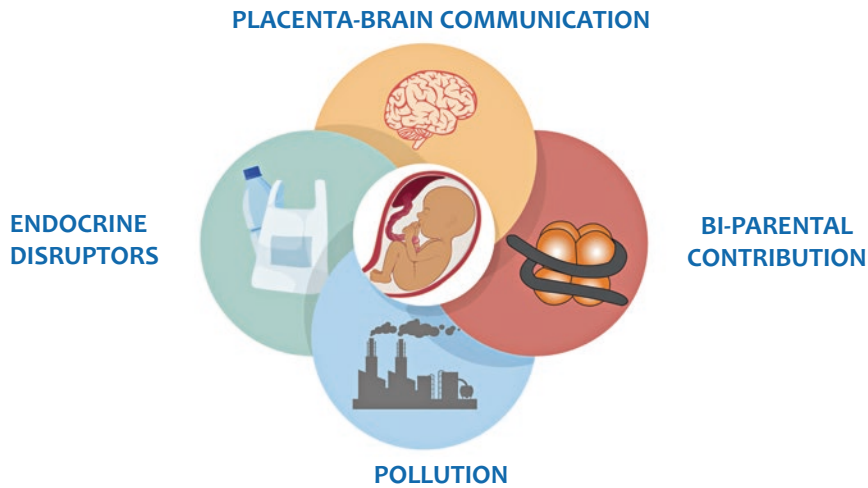

Fig. 13.5 An integrative model of challenging topics affecting the placenta. State of the art in the study of the placenta are challenged by new findings, generating new questions. Thus, both the induction of neurological dysfunction in the brain induced by normal or altered placental development and the involvement of epigenetic mechanisms in the activation or inactivation of parental specific alleles is an open field with big unanswered questions. On the other hand, societies' evolution has generated environmental damage that affects wildlife and human beings, including intrauterine life. Then, human activity directly impacts placental development and function and fetus well-being, by generating tons of contaminant molecules, including articulate matter, nocive gasses, heavy metals, and plastic-derived phthalates. Many of the new issues disturb placental function and, therefore, normal pregnancy development. However, underlying mechanisms are not yet completely understood

human beings, including intrauterine life. We have remarked on the potential detrimental effect produced by industry, such as agronomy or extractives industry, on the reproductive system and particularly in the placental function. In particular, pregnant women may be exposed to high EDC levels, harmful gasses, and heavy metals. There is a lack of knowledge about the specific mechanisms involved. Due to the impact of fetal life on the health of the population, it is necessary to further research in these areas to evaluate the effects of environmental saturation in each country on obstetric and perinatal outcomes and to propose public policies that guide an integral economic development that ensures well-being from the first stages of life. Also, the use of pollution or EDCs in the study of human pregnancy and development in experimental animal and cell models is unquestionably challenging. Such models could help establish mechanistic hypotheses that can be later tested in humans.

We applaud the international effort to elucidate the association between all these conditions/issues in both normal and pathological pregnancy and – in some cases – its impact in the generation of developmental alterations. Still, we want to emphasize the increased vulnerability in Latin American countries of the mother and children exposed to those conditions/issues, who unfortunately do not receive adequate attention in governmental policies or as a target for research.

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Conflict of Interest All authors declare no conflict of interest.

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