REPRODUCTION

Mammalian foetal ovarian development: consequences for health and disease

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Abstract

The development of a normal ovary during foetal life is essential for the production and ovulation of a high-quality oocyte in adult life. Early in embryogenesis, the primordial germ cells (PGCs) migrate to and colonise the genital ridges. Once the PGCs reach the bipotential gonad, the absence of the sex-determining region on the Y chromosome (*SRY*) gene and the presence of female-specific genes ensure that the indifferent gonad takes the female pathway and an ovary forms. PGCs enter into meiosis, transform into oogonia and ultimately give rise to oocytes that are later surrounded by granulosa cells to form primordial follicles. Various genes and signals are implicated in germ and somatic cell development, leading to successful follicle formation and normal ovarian development. This review focuses on the differentiation events, cellular processes and molecular mechanisms essential for foetal ovarian development in the mice and humans. A better understanding of these early cellular and morphological events will facilitate further study into the regulation of oocyte development, manifestation of ovarian disease and basis of female infertility.

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Introduction

Normal ovarian development during embryogenesis is the key to fertility and reproductive success later in life. In mammals, reproductive capacity is limited by the size of the non-renewable pool of oocytes, which is established during foetal life. Follicles, the basic unit of the ovary, house the oocytes and are essential for their development and survival (Byskov 1986). In the early stages of development, follicles consist of an oocyte surrounded by somatic granulosa cells and the extra-cellular matrix (ECM; Fig. 1). As the follicle grows and differentiates, theca cells are recruited; these cells are the source of the oestrogen substrate, androstenedione (reviewed in Edson *et al.* (2009)). Each month throughout the course of a female's reproductive life, primordial follicles are activated to begin follicle development. This process culminates in a follicle that contains a mature fertilisable oocyte ready for release at ovulation. Disturbances in the initial steps or processes that facilitate foetal ovarian differentiation result in incomplete sexual development and may contribute to child-hood and adult diseases such as gonadal dysgenesis, infertility or ovarian cancer (de Boo & Harding 2006). This review discusses the highly regulated, unique processes and genetic mechanisms required for normal ovarian development. It is these events during foetal development that determine the fertility and reproductive capacity of the adult individual (Fig. 2).

Beginnings of the ovary

One of the earliest events in embryonic development is X chromosome inactivation, a process that occurs at the two-cell stage of the zygote and enables males and females to have equal transcript levels by genetic inactivation of one of the two X chromosomes in females. In mice, between the four- and eight-cell stage, inactivation of the paternally inherited X chromosome occurs in all female somatic cells (Huynh & Lee 2001). In the developing germ line, X inactivation is reactivated in primordial germ cells (PGCs) such that both X chromosomes are active in oogenesis (Sugimoto & Abe 2007). Epigenetic regulation of gene expression is an essential part of organogenesis and leads to heritable changes in gene function without inducing a change in the DNA sequence. DNA methylation or histone acetylation can affect such changes. The study of epigenetic events in the oocyte is an expanding field of research and involves studying the biology behind developmental processes such as genomic imprinting, X inactivation and transcriptional repression.

In the mouse embryo, oocytes develop from PGCs, which migrate into the extraembryonic mesoderm from about 6 days post coitus (dpc; Fig. 2; Tam & Zhou 1996). By 7.5 dpc, the germ cell pool is located in the allantois (yolk sac; Lawson & Hage 1994). Various genes and factors are involved in germ cell specification such as the pluripotency marker POU class 5 homeobox 1
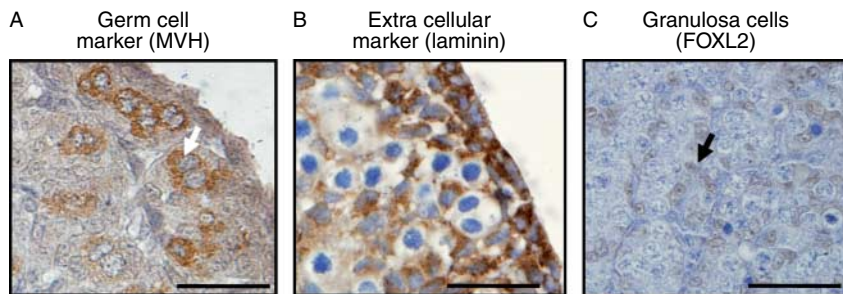
A Germ cell marker (MVH)   **B** Extra cellular marker (laminin)   **C** Granulosa cells (FOXL2)

Figure 1 Localisation of cell markers to ovarian structures. Mouse ovarian sections were subject to immunohistochemistry with antibodies for the following: (A) mouse vasa homologue (MVH), which stains the oocytes at 14.5 dpc (white arrow). (B) Laminin, which stains the extra-cellular matrix of the foetal ovary at 14.5 dpc (brown). (C) FOXL2, which stains the granulosa cells surrounding the oocytes at 15.5 dpc (black arrow). Scale bar = 0.5 μM.

(POU5F1/Oct4) and bone morphogenic proteins 4 and 8 (BMP4 and BMP8; Yoshimizu *et al.* 2001). Between 9 and 10.5 dpc in the mouse, the PGCs migrate from the yolk sac to colonise the gonadal (genital) ridge (Wylie 1999). The genital ridge is identical in both males and females and remains 'bipotential' until 11.5 dpc in the mouse (Figs 2 and 3A). In humans, PGCs proliferate and migrate into the mesoderm colonising the genital ridges until the 6th week of pregnancy (Baker 1963, Anderson *et al.* 1999). The gonads are indifferent but bipotential from 7 weeks of gestation until sex is determined (Fig. 4).

In the absence of the sex-determining region on the Y chromosome (*SRY*) gene or its downstream target *SRY (sex-determining region-Y)-box 9* (*SOX9*) (Sinclair *et al.* 1990, Koopman 1992), and in the presence of genes such as *wingless-type MMTV integration site family, member 4* (*WNT4*), *follistatin* (*FST*) and forkhead *box L2* (*FOXL2*), which have been independently implicated in female sex determination, the bipotential gonad forms an ovary and PGCs differentiate into oogonia (Fig. 5). No single regulatory gene 'switch' has been found to activate the ovarian differentiation pathway; however, various models of ovarian development have been put forward. The ovary-determining gene (*Od*) hypothesis ascribes to a gene located either on the X chromosome or on an autosomal chromosome that activates ovarian differentiation (Eicher & Washburn 1986). The 'Z model' proposes the existence of a factor that acts by suppressing pro-testis events in genetic males while promoting femaleness in the ovary (McElreavy *et al.* 1992, also reviewed in Ottolenghi *et al.* (2007)). Recent

advances in the field indicate that normal ovarian development requires both genes that lead to activation of the female pathway and genes that suppress the male pathway. The complex functional relationships between these genes and the pathways involved need to be clarified.

Differentiation events leading to a normal ovary

In mice, it is not until birth that the oocytes become enclosed in follicles. Before birth, foetal ovarian PGCs develop in cyst-like clusters following colonisation of the genital ridge (Byskov 1986). The mouse gonads are surrounded by the mesonephroi that, at the bipotential stage (11.5 dpc), harbour both the Müllerian and the Wolffian ducts (Fig. 3). The Müllerian duct gives rise to the oviduct, uterus, cervix and upper part of the vagina, while the Wolffian duct differentiates into the epididymis, vas deferens and seminal vesicle (Behringer 1994). After sex determination, the Müllerian duct is maintained in the ovary while the Wolffian duct regresses (Fig. 3).

In the mouse, between 10.5 and 13.5 dpc, the germ cells undergo mitosis and become closely associated in clusters called germ cell cysts (Figs 2 and 4). By 13.5 dpc, oogonia cease dividing and enter into meiosis to form oocytes. By 14.5 dpc, oocytes associate with gonadal somatic pre-granulosa cells to form 'ovigerous cords', loose foetal epithelial structures made up of clusters of pre-granulosa cells and meiotic germ cells (Odor & Blandau 1969, Pepling & Spradling 1998, Mazaud *et al.* 2005, Figs 1 and 4). The role of cyst and ovarian cord
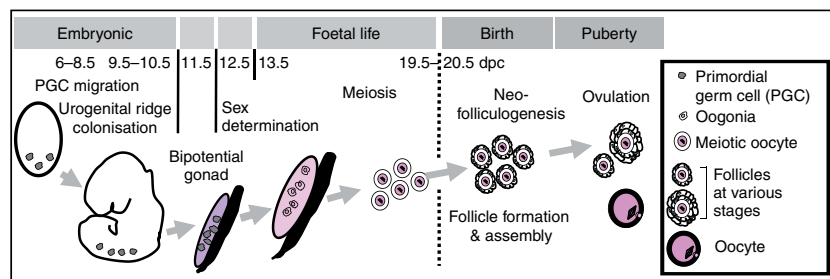

Figure 2 Healthy foetal gonadal development: the path to fertility. Normal oocyte formation is critically dependent on the stages occurring from the primordial germ cells (PGCs). Mouse PGCs migrate along the hindgut until they reach the genital ridges at 10.5 dpc. PGCs undergo cell division in the bipotential gonads (11.5 dpc) until sex determination (12.5 dpc). In females, PGCs enter meiosis at 13.5 dpc, become oocytes and then progress through prophase I and arrest until birth. After birth, primordial follicles form and folliculogenesis ensues; ovulation occurs once puberty is attained.
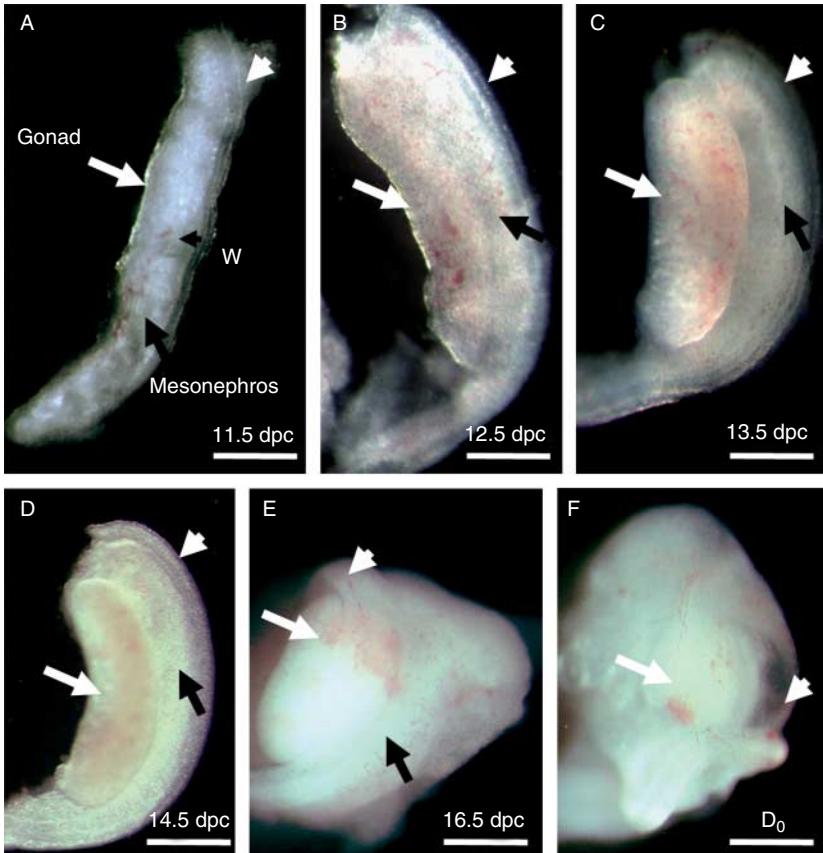
Figure 3 Dissected foetal mouse ovary and mesonephros complexes from the bipotential gonad up until the day of birth. (A) 11.5 dpc, (B) 12.5 dpc, (C) 13.5 dpc, (D) 14.5 dpc, (E) 16.5 dpc and (F) day of birth D_0. White arrow points to gonads, black arrow to mesonephros, white arrow head to Müllerian duct and black arrow head to Wolffian duct. Scale bar=0.5 mM.

formation in mammalian oocyte development and female fertility is still unclear. However, clues from *Drosophila* indicate that cyst formation occurs as a result of incomplete cytokinesis during mitosis, allowing daughter cells to connect to each other by intercellular bridges (McKearin & Ohlstein 1995). One of the essential functions of the cysts in *Drosophila* is that both oocytes and nurse cells that support the oocyte arise from them (Spradling 1993). Pepling *et al.* (1999) demonstrated that cysts were highly conserved across species such as *Xenopus, Drosophila* and the mouse. Suggested functions for cyst formation included: altering the cell
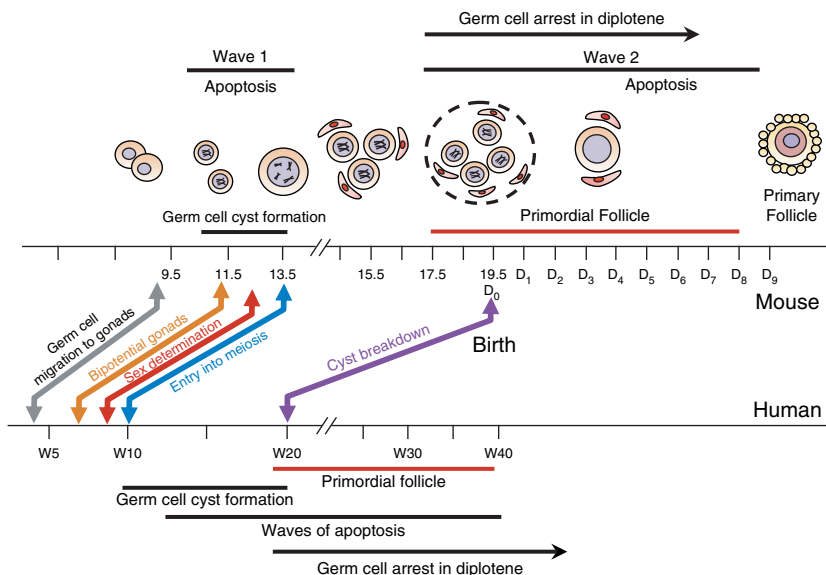

Figure 4 Mouse and human timelines for major processes taking place during foetal development (as reviewed in Pepling (2006) and Hartshorne *et al.* (2009)). This diagram is a schematic representation of the various processes occurring during mouse and human oogenesis starting from the migration of the PGC into the gonadal ridges until folliculogenesis commences. The mouse timeline is represented in dpc (days post coitus) while the human timeline is represented in W (weeks post coitus). D_0 is the day of birth in the mouse.
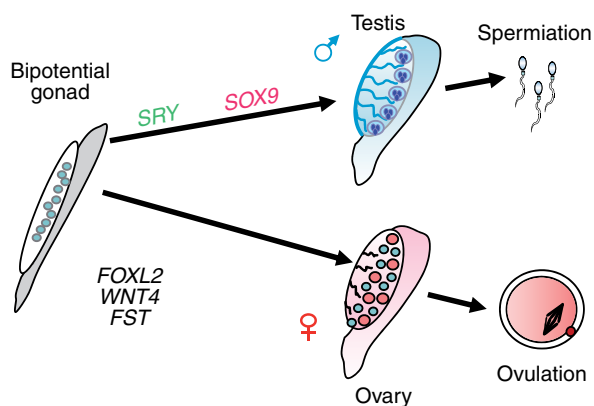
Figure 5 Ovary and testis differentiation from the bipotential gonad. The ovary and testis arise from the bipotential gonad. In the presence of the sex-determining region on the Y chromosome gene (*SRY*) and various male-specific genes such as *SOX9*, the gonad differentiates into a testis. In the absence of *Sry* and in the presence of female-specific genes such as *FOXL2, WNT4* and *FST*, the bipotential gonad differentiates into an ovary.

cycle and facilitating meiosis (Hawkins *et al.* 1996); determining germline sex (Pauli & Mahowald 1990) and enabling oocytes destined to form primordial follicles to acquire functional mitochondria (Jenuth *et al.* 1996). Recently, Nicholas *et al.* (2010) examined whether foetal ovarian cord structures are required for oocyte development. They observed that transplantation of disrupted and re-aggregated foetal ovarian cells from 13.5 to 15.5 dpc mouse embryos, under the kidney capsule of immunodeficient mouse recipients, resulted in a reduced number of oocytes (Nicholas *et al.* 2010). This study emphasised the importance of an intact ovarian structure during the window of development between 13.5 and 16.5 dpc for programming oocyte survival in mouse follicles after birth.

After birth, the first wave of folliculogenesis ensues, the granulosa cells become organised around the oocytes and the basement membrane undergoes remodelling (Pepling & Spradling 2001; Fig. 4). The ECM (Fig. 1B) is believed to play an essential role in maintaining cell-to-cell interactions and communication necessary for follicle formation and development within the ovary (reviewed in Woodruff & Shea (2007)). Further studies are required to clarify the mechanisms that regulate the differentiation of germ cells into normal oocytes, initiate cyst formation and elucidate the role of the ECM in ovarian development (Fig. 1). Transgenic and knockout mouse models, either conventional or conditional, continue to provide a wealth of information on early prenatal and post-natal ovarian development. In spite of the differences in temporal and spatial localisation of genes and proteins between the mice and humans (Fig. 4), common processes are involved in ovarian development, making knowledge from the mouse ovary informative for the humans (as will be further discussed).

Processes involved in mouse and human ovarian development

Organisation of follicular structures in the ovary

In humans, two processes are fulfilled during foetal ovarian development and before birth: the ovarian endowment of primordial follicles and the recruitment of resting primordial follicles into the growing follicle population (Fig. 4). In contrast, in the rodent, the majority of follicles form and are recruited to grow to primary/more advanced follicles after birth, although there is emerging evidence that follicles start to form at 17.5 dpc (Pepling *et al.* 2010). In both human and rodent species, follicle growth continues until the primordial follicle population is depleted (Peters *et al.* 1978, Gougeon 1996).

Entry into meiosis

Around the time of sex differentiation (12.5 dpc in the mouse (Fig. 3B) and 6–7 weeks of gestation in the human), germ cells enter a pre-meiotic state within the foetal ovarian germ cell nests (Motta *et al.* 1997). This time point marks the beginning of expression of the synaptonemal complex protein 3 (*Sycp3*) gene that encodes for the SYCP, a meiosis-specific protein that facilitates DNA synapses and meiotic recombination. Entry into meiosis occurs in a rostral to caudal (anterior to posterior) fashion in the mouse ovary reflecting the possibility that local factors and cues diffuse from the mesonephros at the anterior code of the ovary to promote meiosis (Bullejos & Koopman 2004). Once the germ cells enter meiosis, they initiate a relationship with the proximal somatic cells that will later differentiate into two subsets of granulosa cells: mural granulosa and cumulus cells (Matzuk & Lamb 2002). At 13.5 dpc in the mouse (Adams & McLaren 2002) and between 8 and 13 weeks in the human foetus (Baker 1963), meiosis is initiated, allowing oogonia to develop into oocytes (Figs 2 and 4). A large number of the oocytes undergo cell death while the remaining oocytes progress through prophase I of meiosis and arrest at diplotene (once primordial follicles are formed); here they remain until ovulation occurs (Borum 1967). A remarkable feature of primordial follicles is that many of them remain dormant for years or decades. Mutations or inactivation of genes known to affect the initial stages of meiosis such as meiotic control 1 (*DMC1*) and meiosis-specific sporulation protein (*SPO11*) result in the loss of oocytes and an inability to form follicles resulting in sterility (Villeneuve & Hillers 2001). Thus, the appropriate initiation of meiosis is essential for oocyte survival (Fig. 2).

Retinoic acid (RA), the active derivative of vitamin A, was identified as a key molecule promoting oogonia in the foetal mouse ovary to enter into meiosis and become oocytes (Bowles *et al.* 2006, Koubova *et al.* 2006; also

reviewed in Bowles & Koopman (2007)). RA was suggested to either directly or indirectly up-regulate the gene stimulated by retinoic acid gene 8 homolog (*Stra8*), which encodes a protein essential for pre-meiotic DNA replication. In contrast, in the foetal mouse testis, RA is degraded by the enzyme Cyp26b1 that prevents meiosis from occurring at 13.5 dpc.

More recently, a study investigated mice that were incapable of RA synthesis and found that *Stra8* expression in the foetal ovary did not require RA signalling (Kumar *et al.* 2011) and that Cyp26b1 prevented the onset of meiosis by a mechanism independent of RA. This finding will open new avenues for investigating the role of Cyp26b1 in meiosis.

Germ cells in the human foetal ovary do not enter meiosis in a rostral to caudal wave (Anderson *et al.* 2007) as they do in the mouse. Instead, the germ cells are arranged radially with undifferentiated PGCs located at the periphery of the ovary and the more differentiated germ cells confined to the centre, suggesting that local control of germ cell differentiation is important in the human foetal ovary. Recently, Childs *et al.* (2011) characterised the components of RA signalling in the human foetal ovary using cultured foetal gonads. They demonstrated that meiotic initiation may not be exclusively controlled by RA metabolism. Furthermore, they identified differences between humans and mice in regulating the initiation of meiosis in the ovary by RA. This leaves the field in a controversial state that requires further clarification.

Breakdown of oocyte cysts, oocyte loss and primordial follicle formation

Oocytes entering meiosis are clustered in cysts (as discussed previously) and lack close association with pre-granulosa cells (Gondos 1987, Pepling 2006; Fig. 4). Not all of the oocytes within these cysts survive to form follicles, some die leaving only one or two oocytes (Pepling & Spradling 2001). Regulation of oocyte numbers is an important process that occurs by cyst breakdown. Around the time of birth in the mouse and after 20 weeks of gestation in humans, the cysts breakdown so that primordial follicles could form (Fig. 4). The primordial follicles comprised oocytes surrounded by a single layer of squamous granulosa cells (Fig. 1).

Only one-third of the oocyte pool forms primordial follicles (Pepling *et al.* 2010), the remaining oocytes undergo apoptosis perinatally in mice or prenatally in humans (McGee *et al.* 1998). The fate of oocytes that are incorporated into primordial follicles is controlled by the oocyte; this is in contrast to the growing follicles that are controlled by somatic cell apoptosis (Fig. 4). Germ cell loss during oogenesis occurs through programmed cell death (PCD). Three types of PCD have been described in the ovary: type I, apoptosis characterised by nuclear condensation, fragmentation, cleavage of chromosomal DNA, shrinkage and packaging of the dying cell into apoptotic bodies without plasma membrane breakdown (Edinger & Thompson 2004); type II, autophagy or lysosomal degradation involving vacuole formation (Tilly 2001) and type III, non-lysosomal degradation involving necrosis characterised by plasma membrane breakdown, alteration in permeability of the cellular membrane, disruption of cytoplasmic structures and nuclear degeneration (Edinger & Thompson 2004).

In the mouse ovary, cell death by apoptosis is an essential component of ovarian development, which occurs at all stages of oogenesis with two main waves documented during foetal life (reviewed in Aitken *et al.* (2011)). The first wave of cell death coincides with the entry of oogonia into meiosis (13.5–15.5 dpc) and the second wave occurs between 17.5 dpc and the day of birth marking the breakdown of cysts and the beginning of primordial follicle assembly (Coucouvanis *et al.* 1993, Ratts *et al.* 1995; Fig. 4). In humans, depletion of the germ cells occurs in the foetal ovary during mid-gestation, with apoptosis highest between weeks 14 and 28 and decreasing towards birth (Vaskivuo *et al.* 2001; Fig. 4). At birth, about two million oocytes are present, 400 000 are left at puberty and 400 oocytes reach the ovulatory stage (Baker 1963). The non-ovulatory follicles undergo apoptosis and become atretic. Although the process of apoptosis is crucial for regulating oocyte number, its genetic control is just starting to become clear as a consequence of studies in animal models.

Two apoptotic pathways have been identified: the extrinsic and the intrinsic or mitochondria-associated pathways (reviewed in Jaaskelainen *et al.* (2010)). Some of the extrinsic pathway factors are soluble or membrane-bound ligands such as FAS ligand, tumour necrosis factor α (TNFA) and TNF-related apoptosis-inducing ligand (TRAIL/TNFSF10). The intrinsic pathway is operated by the B cell lymphoma-2 (Bcl2) family of cytoplasmic and mitochondrial proteins, which consists of anti-apoptotic (*Bcl2l1* (*Bcl-X$_L$*), *Bcl2* and *Mcl1*) and proapoptotic (*Bid, Bax, Bok* and *Bad*) genes. The degree of apoptosis depends on the balance of these competing family members. The analysis of transgenic mouse models has identified various genes as regulating oocyte survival, apoptosis or cyst breakdown.

These include *Bcl2* (Ratts *et al.* 1995), *Bax* (Perez *et al.* 1999, Greenfeld *et al.* 2007) and caspase 2 (Casp2; Bergeron *et al.* 1998). Boumela *et al.* (2011) extensively reviewed the expression patterns of the human BCL2 members and found them to be differentially expressed during oocyte differentiation and early embryogenesis. The proapoptotic factor BAX was constitutively expressed in the oocyte and early embryo, suggesting that BAX poses a continuous death threat unless other prosurvival factors such as BCL2 inhibit its proapoptotic activity. Understanding the balance between the driving

factors that cause an oocyte to undergo apoptosis or ensure its survival might give rise to potential biomarkers for oocyte quality and embryo developmental competence. A recent study using proteomic methodologies implicated proliferating cell nuclear antigen in promoting the apoptosis of oocytes in foetal and neonatal mouse ovaries (Xu *et al.* 2011).

Primordial follicles remain quiescent until selected follicles are activated to enter the first wave of follicle growth and commence the primordial-to-primary follicle transition (Hutt *et al.* 2006). These processes (reviewed in Uzumcu & Zachow (2007)) mark the earliest stages of folliculogenesis and affect the duration of the female reproductive lifespan (Skinner 2005). Normal follicular development also requires cell-to-cell communication between the oocyte and the granulosa cells, as well as between the granulosa and the theca cells (Eppig 2001, Skinner 2005). Earlier studies implicated Kit ligand and c-Kit as intraovarian factors acting via phosphatidylinositol-3-kinase to control oocyte growth and early follicular development (reviewed in Liu *et al.* (2006)).

The following section will provide an update on current knowledge pertaining to factors regulating mammalian foetal ovarian development.

Gene regulation and current mechanisms of ovarian development

Mammalian ovarian development and differentiation are complex processes that involve crosstalk between the germ cell and somatic cell types. Since the early stages of ovarian differentiation are independent of pituitary gonadotrophins, it is important to understand the intraovarian factors that regulate the germ and somatic cell populations of the ovary. Genetically modified mouse models and mutation analysis in patients with ovarian disease have supplied a wealth of information. The following sections discuss the genetic regulation of foetal ovarian development and the molecular involvement of a number of genes in germ cell and somatic cell regulation.

Germ cell regulation

DAZL enables the gonads to respond to ovarian cues

Deleted in azoospermia-like (DAZL) protein is an RNA-binding protein first detected in PGCs after they have migrated into the gential ridges; it persists in the cytoplasm of adult germ cells (Cooke *et al.* 1996). The ovaries of adult *Dazl* knockout mice lack oocytes as a consequence of germ cell loss (at the time of entry into meiosis) during foetal development (McNeilly *et al.* 2000, Lin & Page 2005). Expression pattern studies in the human foetal ovary indicated that immature germ cells express the pluripotency marker POU5F1, while the more mature

germ cells such as oogonia (in cysts) or oocytes express DAZL and the germ cell marker VASA (Anderson *et al.* 2007). Recent *in vivo* genetic analysis of C57BL/6 DAZL-deficient foetuses found that germ cell specification and migration processes were not affected while the response to ovary and testis cues that determine sex were disrupted (Gill *et al.* 2011). This led to the proposal that DAZL plays a role in enabling the gonads to respond to masculinisation or feminisation cues.

Factor in germ line α and a role in primordial follicle formation

Factor in germ line α (*Figlα*) gene is a basic helix–loop–helix transcription factor required for ovarian follicle formation (Soyal *et al.* 2000). *Figlα* expression is detected in mouse oocytes from 13.0 dpc and persists into adulthood where it acts as an activator of zona pellucida genes (Liang *et al.* 1997). *Figlα* is essential for follicle formation as *Figlα* female knockout mice fail to develop primordial follicles perinatally and are consequently sterile (Soyal *et al.* 2000). Adult females have small ovaries that contain only cord-like structures formed by somatic cells. *In vitro* culture of normal foetal ovarian cells at various developmental ages found *Figlα* to be one of the determining factors for germ cell survival and normal primordial follicle formation (Lei *et al.* 2006).

Somatic cell regulation

R-spondin, WNT4 and β-catenin: roles in the foetal ovary

R-spondin 1 (*Rspo1*) has a conserved role in ovarian differentiation during embryogenesis across species such as the mouse, chicken and turtle (Smith *et al.* 2008). *Rspo1* was identified as an activator of the canonical WNT/β-catenin signalling pathway. Mouse *Rspo1* null XX ovaries display partially masculinised features including pseudohermaphroditism in genital ducts, depletion of foetal oocytes, male-specific coelomic vessel formation and ectopic testosterone production by the ovaries (Tomizuka *et al.* 2008). Loss-of-function mutations of the *RSPO1* gene in humans cause testicular differentiation in 46, XX females (Parma *et al.* 2006, Chassot *et al.* 2008). A recent study showed that human RSPO1 is up-regulated during pregnancy between 6 and 9 weeks after conception, a time critical for early human ovary development (Tomaselli *et al.* 2011; Fig. 4).

Another gene implicated in ovarian development is *Wnt4* that belongs to the wingless-type MMTV integration site family (WNT) of proteins. WNTs transduce their signals by binding to G-protein-coupled receptors of the Frizzled family to activate specific signalling pathways (Slusarski *et al.* 1997). *Wnt4* is expressed in embryonic mouse ovaries, and loss-of-function mutations cause masculinised XX gonads in the mice (Heikkila *et al.* 2005). Expression of *Wnt4* and its

downstream genes were down-regulated in *Rspo1*^{−/−} ovaries, suggesting that RSPO1 is positively regulating WNT4 signalling.

Both *Rspo1* and *Wnt4* exert their effects through β-catenin, a key transcriptional regulator and an intracellular mediator of the canonical WNT pathway. When β-catenin was ablated in the somatic cells of the foetal mouse ovary, female-to-male sex reversal was seen, with the loss of germ cells (Chassot *et al*. 2008). This phenotype is similar to that observed in the *Wnt4* knockout ovary, suggesting that WNT4 and β-catenin operate via a common pathway that is essential for female germ cell survival (Vainio *et al*. 1999).

Ablation of *Rspo1, Wnt4* and β-catenin genes induces development of seminiferous tubules in XX gonads (Chassot *et al*. 2008). It is thus postulated that these genes are required to suppress the male pathway in the XX gonad and that their signalling is essential for normal embryonic ovarian development and in opposing testis development.

The transforming growth factor β signalling pathway

The transforming growth factor β (TGFB) superfamily of growth factors has been shown to play multiple roles in the local regulation of ovarian function (Knight & Glister 2006). TGFB ligands act by binding to type II receptors, which recruit and phosphorylate type I receptors required for signal transduction. Binding of the ligands to the receptors is modulated by the non-signalling co-receptor betaglycan (TGFBR3; reviewed in Shi & Massague (2003)). Signal transduction is then mediated by the mothers against decapentaplegic homologues (SMAD; Kaivo-oja *et al*. 2006). Members of the TGF family include TGFB1, 2 and 3, activins, inhibins and bone morphogenetic proteins (BMPs), all of which are expressed in somatic cells or oogonia during foetal and neonatal ovary development (Weng *et al*. 2006).

Inhibins and activins

Inhibins (inhibin α and either βA or βB subunits) and activins βA and βB (homodimers of inhibin β subunits) have been characterised as gonadal-derived regulators of follicle-stimulating hormone (FSH; Risbridger *et al*. 2001). Inhibins and activins have been suggested to play autocrine and paracrine roles in steroidogenesis and folliculogenesis in the adult ovary (Findlay *et al*. 2000); their role in foetal ovarian development is becoming clearer. Inhibin βA or βB, but not inhibin α, subunit mRNA and protein are present in the human foetal ovary (Martins da Silva *et al*. 2004). *In vitro* culture of human foetal ovary with activin A resulted in an increase in germ cell proliferation. When human recombinant activin A was administered *in vivo* to neonatal mice, there was an increase in prepubertal primordial follicle numbers (Bristol-Gould *et al*. 2006). Activin and TGFB receptors are expressed on both germ cells and somatic cells of the ovary (Findlay *et al*. 2002, Kaivo-oja *et al*. 2006), whereas the active phosphorylated SMADS 2 and 3, which activin signals through, are present only in somatic cells. Thus, foetal somatic cells are targets of activin action (Coutts *et al*. 2008). Low levels of inhibin α and activin βA mRNA and protein were also detected in the stromal and granulosa cells of ovaries of foetal mice at 13.0 dpc (Weng *et al*. 2006). Yao *et al*. (2006) established a role for activin βB in the formation of the male-specific vasculature in XY gonads and identified it as a downstream target of *Wnt4* and *Fst* (Yao *et al*. 2006). Analysis of a somatic cell-specific, β-catenin conditional knockout mouse model provided evidence for the importance of somatic cells to germ cell survival by balancing positive (Wnt4/β-catenin) and negative (activin βB) germ cell regulators (Liu *et al*. 2010*b*), also reviewed in Liu *et al*. (2010*a*).

Follistatin

Fst binds activin with high affinity and is a key regulatory factor in the foetal ovary (Hemmati-Brivanlou *et al*. 1994). Inhibin, activin and Fst are all produced by the adult ovary, and they have the capacity to act as endocrine feedback signals that modulate pituitary FSH secretion (de Kretser & Phillips 1998). *Fst* expression in the XX foetal mouse gonad first appears at 11.5 dpc and reaches its peak at 12.5 dpc (Yao *et al*. 2004). From knockout mouse analysis, it has been established that *Fst* acts downstream of Wnt4 to suppress the male pathway and inhibit formation of the XY-specific coelomic vessel in XX gonads. In addition, Fst facilitates meiotic germ cell survival in the ovary; however, the exact mechanism of its action is still unclear. *Fst*-null mice lacking both isoforms of *Fst* (315 and 288) die at birth due to respiratory difficulties (Matzuk *et al*. 1995). Transgenic studies where the *Fst* knockout mice were rescued using human *FST* showed that *Fst315* but not *Fst288* rescued most of the defects of the *Fst* knockout (Lin *et al*. 2008). Another transgenic knockin study showed that *Fst288* was able to rescue some of the defects and prevent prenatal death (Kimura *et al*. 2010). Further analysis of these *Fst* transgenic mouse models will identify the precise roles that *Fst* isoforms play in foetal ovarian development.

Anti-Müllerian hormone

Anti-Müllerian hormone (AMH) or Müllerian inhibitory substance is another TGFB superfamily member involved in the regulation of folliculogenesis (Baarends *et al*. 1995). A clear role for AMH has been identified during male foetal sex differentiation. AMH expressed by the testicular Sertoli cells causes the regression of the Müllerian derivatives that form the anlagen of the female reproductive tract; AMH prevents the development of the fallopian tube, uterus, cervix and upper vagina (Behringer 1994, Josso & di Clemente 1997). AMH is unlikely to play a direct role in foetal ovarian

development as no expression of AMH has been reported in the foetal mouse ovary; AMH is first detected in ovarian granulosa cells about 4 days after birth, at the onset of primary follicle formation (Durlinger *et al.* 2002). Recently, AMH was found to inhibit primordial follicle assembly in neonatal rat ovaries cultured *in vitro* (Nilsson *et al.* 2011). This same study found that AMH was expressed in the stromal tissue of the rat ovary during the postnatal stages of development, suggesting that stromal–epithelial interactions are required for primordial follicle assembly. In rodents, sheep and humans, AMH mRNA and protein were found to be expressed in granulosa cells of developmentally advanced follicles indicating a role in folliculogenesis (Baarends *et al.* 1995).

FOXL2: a key regulator of ovarian development

Another factor involved in ovarian differentiation is FOXL2, a member of the forkhead/HNF-3-related transcription factor family. *FOXL2* is highly conserved and plays roles in cellular differentiation and development. In the adult human ovary, *FOXL2* mutations result in premature ovarian failure (POF; Crisponi *et al.* 2001). FOXL2 has also been implicated in induction of female sex determination and follicle formation (Fig. 5). *Foxl2* expression is first detected in mouse ovarian somatic cells around 13.0 dpc, when the germ cells enter meiosis. In the ovaries of *Foxl2*-null mice, somatic cells fail to develop around growing oocytes preventing follicle formation (Uda *et al.* 2004, Ottolenghi *et al.* 2005). A recent microarray study compared ovarian transcriptomes from wild-type mice and mice lacking *Foxl2, Wnt4* or both genes to elucidate the role of these factors in gonadal development (Garcia-Ortiz *et al.* 2009). Analysis of the microarray data sets showed that loss of *Foxl2* caused expression changes in different pathways to those observed in the *Wnt4*-null ovaries. These findings suggest that FOXL2 acts independently of the WNT4 pathway, but both are essential for normal ovarian development. Further studies of this type will help to elucidate the genetic basis of female fertility and identify candidate genes and pathways involved in ovarian development.

The foetal ovary and the origins of disease hypothesis

Barker (1994) first espoused his 'foetal origins of disease' hypothesis after correlating low birth weight with the development of cardiovascular disease in adult life. In recent years, the hypothesis has been extended to include any insult that affects the foetal environment leading to maladaptive responses by the foetus, which engenders the propagation of disease in later life. Smoking, poor nutrition, pollution, pesticides, radiation, infection, drugs, etc. have been shown to be detrimental to foetal development (reviewed in de Boo & Harding

(2006), Edwards & Myers (2007), Swanson *et al.* (2009), Wadhwa *et al.* (2009) and Langley-Evans & McMullen (2010)). More recently, assisted reproductive technologies and obesity have been added to this list (Rinaudo & Lamb 2008, Freeman 2010). The programming mechanisms by which these changes are affected is not clear, although a number of hypotheses have been proposed, notably the thrifty phenotype, oxidative stress, catch-up growth and epigenetic changes that might incorporate DNA methylation or histone acetylation (Edwards & Myers 2007, Godfrey *et al.* 2010, Luo *et al.* 2010).

The effect of the nutritional environment during the foetal period on reproductive health and fertility in later life has been investigated in a range of species. Studies by Engelbregt *et al.* (2002) investigated whether compromised nutrition during the foetal or postnatal period influenced ovarian development in the rat. The ovaries of postnatally undernourished rats stimulated with pregnant mare serum gonadotrophin contained more primordial follicles but fewer antral follicles and corpora lutea, suggesting that follicle maturation was impaired. In contrast, rats assigned to intrauterine growth restriction (IUGR) protocols experienced delayed puberty and their ovaries contained fewer follicles (of all types) compared with controls (Engelbregt *et al.* 2002). Studies analysing foetal human tissue post-mortem found no effect of IUGR on ovarian development (de Bruin *et al.* 2001). In 2009, a review undertaken by Gardner *et al.* (2009) to assess whether foetal under-nutrition correlated with fecundity and fertility in humans, primates, rodents and sheep concluded that there was insufficient evidence to support such a claim.

Polycystic ovarian syndrome (PCOS) is estimated to affect up to 18% of women of reproductive age (March *et al.* 2010) and is the most common cause of infertility. Heterogeneous in presentation, PCOS features include anovulation, polycystic ovaries, hyperandrogenism, elevated levels of LH, insulin resistance, dyslipidemia and obesity (review in Teede *et al.* (2010)). In addition, individuals with PCOS manifest an increased lifetime risk of type II diabetes, cardiovascular disease and endometrial cancer (Ehrmann *et al.* 2005). The origins of PCOS have been proposed to extend back to foetal life. Exposure to androgens during gestation led to the development of PCOS by female rhesus monkeys (Abbott *et al.* 2009), sheep (Veiga-Lopez *et al.* 2008) and rats (Demissie *et al.* 2008), with characteristics similar to those observed in humans. Interestingly, treatment of adult monkeys with androgens in various forms and regimens failed to induce PCOS (Billiar *et al.* 1985). An association between low birthweight and PCOS has been reported in human studies (Ibanez *et al.* 1998, Pandolfi *et al.* 2008), although more comprehensive analyses failed to support these findings (Sadrzadeh *et al.* 2003, Legro *et al.* 2010). Fibrillin 3, an ECM protein that regulates TGFB bioactivity, has been shown

to be expressed in the stroma of foetal ovaries during the period when follicles are forming (Hatzirodos *et al.* 2011). The authors postulate that expression of fibrillin 3 at this time may predispose the foetus to develop PCOS in later life. To prevent the propagation of PCOS or metabolic-related disorders from mothers to daughters, interventions to prevent increased weight gain/adiposity during adolescence have been proposed (Abbott *et al.* 2009).

Ovarian cancer is a heterogeneous disease, the subtypes of which are epithelial, germ cell and sex-cord stromal. Epithelial ovarian cancer (EOC) accounts for 90% of all ovarian cancers with serous, mucinous, endometrioid, clear-cell and transitional (Brenner) subtypes described on the basis of morphology. The origins of EOC remain unclear. Previously, the ovarian surface epithelium was thought to be the precursor for EOC, but in recent times, non-ovarian tissues such as the fallopian tube, peritoneum and endometrium have been touted as giving rise to specific subsets of EOC based on the tissue the cancer most resembles with (Kurman & Shih Ie 2010). If this is indeed the case, then the only primary ovarian cancers are gonadal stromal (granulosa cell tumours) and germ cell tumours. While the origins of ovarian cancer are not clear, a number of risk factors have been identified, these include early menarche, nulliparity and infertility (Beral *et al.* 1978, Whittemore *et al.* 1989, Barker *et al.* 1995). More recently, exposure of the foetus *in utero* to environmental toxins (chemicals, pesticides, etc.) has been espoused to increase cancer risk (Perera 2011), supporting the developmental origins of disease hypothesis. Barker *et al.* (2008) proposed that ovarian cancer is initiated by exposure of the foetal ovary to maternal sex hormones, notably gonadotrophins (Barker *et al.* 1995). Concentrations of these hormones may be higher in mothers who have had an early menarche. A higher rate of weight gain during infancy has also been linked to ovarian cancer.

The germ line stem cell hypothesis proposed by Johnson *et al.* (2004) called into question one of the basic tenets of female reproduction, that of the finite ovarian reserve. It is difficult to assimilate their hypothesis with the phenomena of the menopause, POF and infertility caused as a consequence of chemotherapy. If there were indeed stem cells in the ovary, one might expect them to be activated to produce more oocytes under these circumstances? Since 2004, there have been a number of studies investigating this claim and purporting to either support or refute the hypothesis (for a review, see Notarianni (2011)). In the context of the foetal origin hypothesis, one might suggest that any insult directly affecting oocyte numbers during foetal development would be remedied by activating germ line stem cells. To date, there have been no studies that address this supposition or investigate whether POF arises as a consequence of unfavourable conditions during the foetal period.

Conclusion

It is increasingly clear that the foetal environment and any protective measures the foetus invokes to maintain itself when exposed to detrimental stimuli will have repercussions in later life. Reproductive dysfunction may manifest in infertility, ovarian cancers or other ovarian disorders. There is still much to be learnt about the molecular mechanisms and genes involved in foetal ovarian development. For example, which gene regulation pathways are functionally active in the foetal ovary and at what points do these pathways intersect? There is also a need to identify cell-specific genes that could be used as markers for further study of ovarian developmental processes. The availability of novel transgenic mouse models and advanced technologies for detecting and sequencing genes will further our knowledge on foetal ovarian development and contribute new data to be assessed in light of the origins of adult disease hypothesis. Further knowledge is also required in the area of the somatic cell contribution to oocyte growth and loss. Questions such as 'how is cell death regulated?' and 'what determines the size of the primordial follicle pool in the ovary?' remain to be answered. Establishing the molecular basis of events that occur during foetal ovarian development will further our understanding of the factors that affect oocyte quality and quantity and lead to a better appreciation of the causes of infertility, ovarian diseases such as gonadal dysgenesis and reproductive disorders such as POF and PCOS.

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