Individual fertility assessment and counselling in women of reproductive age

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THE FOUR ORIGINAL PAPERS:

1. Birch Petersen K, Maltesen T, Forman JL, Sylvest R, Pinborg A, Larsen EC, Macklon KT, Nielsen HS, Hvidman HW, Andersen AN. Individual fertility assessment and counselling predicts time to pregnancy – a prospective two year follow up study of 519 women. Submitted.
2. Birch Petersen K, Hvidman HW, Forman JL, Pinborg A, Larsen EC, Macklon KT, Sylvest R, Andersen AN. Ovarian reserve assessment in users of oral contraception seeking fertility advice on their reproductive lifespan. Hum Reprod. 2015 Oct;30(10):2364-75
3. Birch Petersen K, Hvidman HW, Sylvest R, Pinborg A, Larsen EC, Macklon KT, Andersen AN, Schmidt L. Family intentions and personal considerations on postponing childbearing in childless cohabiting and single women aged 35-43 seeking fertility assessment and counselling. Hum Reprod. 2015 Nov;30(11):2563-74
4. Birch Petersen K, Sylvest R, Andersen AN, Pinborg A, Hvidman HW, Schmidt L. Attitudes toward family formation in cohabiting and single childless women in their mid- to late thirties. Hum Fertil (Camb). 2016 Mar 23:1-8

INTRODUCTION

The introduction of birth control in 1960 provided women and men the opportunity to plan their pregnancies. Family Planning clinics were established worldwide with the aim to enable individuals to determine freely the number and spacing of their children [2]. Although not initially intended, 'Family planning' successively has been used as a synonym for the use of contraception. Contraception and the women´s liberation movement changed the women´s participation on the labour market. In 1960, less than half of the Danish women were a part of the paid work force, and few had an education longer than the basic seven to nine years.

Today, the proportion of working women has increased to 71%. Additionally, today one-third of the women aged 25-34 years have a post-graduate educational length of more than three years (Statistics Denmark, 2008).

The women´s participation on the labour market and the increased educational length has influenced the reproductive patterns in recent decades. Women and men postpone parenthood. In Denmark, women´s age at first birth has increased from 22.7 years in the 1960´s to 29.7 years today (Statistics Denmark, 2016). Postponement of parenthood is associated with a higher rate of involuntary childlessness and infertility [4]. In 2014, 27,000 fertility treatments were performed in Denmark, and 8% of the Danish birth cohort is born by medical assisted reproduction (MAR) (Danish Fertility Society, 2015). In the female age group 35 – 40 years, 13.8% of all deliveries were conceived by assisted conception in 2011 [5].

New approaches to reverse this trend are highly warranted to improve reproductive health. So far, sexual health education has dominated the debate and pre-conceptional counselling has been lower prioritised [6]. Recently, a new tool "Reproductive Life Planning" (RPL) was introduced to encourage both women and men to reflect on their reproductive intentions and to find strategies for successful family planning [6, 7]. The tool consists of non-normative questions about considerations regarding child bringing and is, among other things, recommended to improve preconception health [8].
In line with this, the Fertility Assessment and Counselling Clinic (FAC clinic) was initiated in 2011 as an analogue to the 'family planning clinics' in the 1970s, but with a pro-fertility aim. The idea was to provide individual assessment of fertility risk factors, ovarian reserve and sperm concentration to help women and men to fulfil their reproductive life-plan [9]. "Fertility screening" on an individual level is a new concept and knowledge is needed to evaluate the scientific validity, reasons for seeking counselling, and usability for both the individual and fertility experts.

The aim of this thesis was to investigate, whether pre-conceptional and individual assessment of ovarian reserve, biological factors, medical conditions and lifestyle factors is able to predict future fertility, decrease the need for fertility treatments and provide information for women of reproductive age regarding their ability to conceive naturally.

BACKGROUND

The concept of the fertility assessment and counselling clinic (FAC Clinic)

The FAC Clinic was established in August 2011 and was from 2011 until 2014 funded by the European Union (EU), Interregional projects 'Reprosund' and 'ReproHigh'. The current funding of the FAC Clinic is provided by Rigshospitalet and the 'ReproUnion' collaboration.

The clinic is open to men and women living in the Capital Region of Denmark or southern part of Sweden. No referral is needed, the consultations are free of charge, and appointments are booked by phone on a weekly basis. The only restriction was regarding women/couples, who have already tried to conceive for more than a year in the present relationship. They were informed to seek medical assistance and infertility investigation instead. Baseline data is acquired by a web-based questionnaire on a Survey-Exact platform distributed by email before the consultation. The concept of the FAC Clinic is described in detail in the methods section.

All women were examined by a fertility specialist at the consultation, who performed a trans-vaginal ultrasound (AFC, ovarian volume, pathology), uptake of a full reproductive history, AMH measurement and a risk score assessment.

The risk assessment score sheet and risk factors

The women were informed of their potential risk factors and presumed ovarian reserve by a risk assessment score categorized as green (low), yellow (low), orange (medium) and red (high) for each risk factor (Figure 1). The risk assessment score sheet and definition of risk categories were based on the available literature in 2011 and rationale for the included risk factors will be explained in the following pages.

Name:		**Personal ID:**		
RISK FACTORS	**PARAMETER**	**LOW RISK**	**MEDIUM RISK**	**HIGH RISK**
FEMALE AGE				
Age	Age, years	Under 35	35-39	40 or above
OVARIAN RESERVE AND CYCLE LENGTH				
Cycle length	Days	23 – 35	More than 35	Less than 23
Antral follicle count (Sum of both ovaries)	N	11 – 30	5 – 10 or more than 30	Less than 5
Anti-Müllerian hormone	pmol/L	10-50	5-9 or higher than 50	Lower than 5
GYNAECOLOGICAL HISTORY AND GENERAL HEALTH				
Months of trying to conceive	Months	Less than 6	6 – 12	Longer than 12
Pelvic inflammatory disease	N	0	1-2	More than 3
Ectopic pregnancy	N	0	1	2 or more
Endometriosis	Yes / No	No	Yes	Endometriomas
Pelvic surgery	Yes / No	No	Intestinal surgery	Surgery in ovaries/tubes
Uterine fibroids (submucosal / intramural fibroids)	Major diameter	0	Less than 3 cm	More than 3cm
Intraperitoneal fluid/uterine malformation/hydrosalpinx	Yes / No	No		Yes
Previous chemotherapy	Yes / No	No		Yes
GENETIC DISPOSITIONS AND INTRAUTERINE EXPOSURE				
Maternal age at menopause	Age, years	Above 50	45 - 50	Less than 45
Mother smoked during pregnancy	Yes / No	No		Yes
LIFESTYLE FACTORS				
BMI	Kg/m^2	20 – 30	Lower than 20 or 30-35	More than 35
Waist/hip ratio		Lower than 0.80	Higher than 0.80	
Smoking	Number per day	0	1-10	More than 10
Alcohol	Drinks per week	0	1-6	More than 7
Caffeinated beverage	Cups per day	Less than 6	More than 6	
Physical activity		Mild/ moderate	Excessive	
WORK ENVIRONMENT FACTORS				
Stress		None/ moderate	Highly	

Figure I. Risk evaluation form used for structured risk evaluation of female clients attending the Fertility Assessment and Counselling Clinic (FAC Clinic) at Rigshospitalet, Copenhagen University Hospital, Denmark.

Female age in the score sheet

Age is one of the most important predictors of female fecundity. Fecundity refers to the capacity or ability to bear children [4]. Fecundability is defined as the probability of conceiving during a menstrual cycle among sexually active couples without the use of contraception [4]. Figure 2 illustrates the chance of conceiving and giving birth to a live born child and demonstrates the age-related decline of fecundity in women.

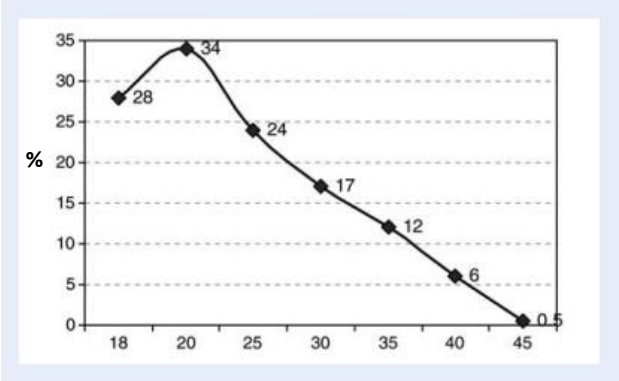

Figure 2: Graph based on calculations of the monthly hazard of live birth conception among Hutterite women based on Larsen and Yan (2000) [4].

The age-related decline in fecundity is indirectly associated to the follicular pool as the progressive reduction is accompanied by an associated decline in oocyte quality [10]. Furthermore, the poor monthly fecundity rate in women has been suggested to have a chromosomal basis - i.e., meiotically derived aneuploidy arises in 25% of conceptions and 50% or more of preimplantation embryos are chromosomally abnormal [11].
As illustrated in Figure 3, around seven million primordial follicles are present in the developing ovary during embryogenesis. The large majority of these follicles will be lost during foetal and post-natal life by atresia, and only 400–500 of them are ovulated before physiological menopause at the mean age of 51 years [12].

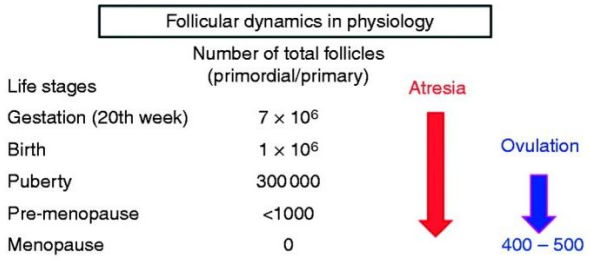

Figure 3: Follicular dynamics showing the number of total follicles in different life stages [12].

Studies have shown the peri-menopausal period from the onset of cycle irregularity until menopause to be approximately six years, regardless of the female age at menopause. Similarly, the onset of subfertility for each individual woman is believed to begin at a relatively fixed interval, presumably 10 to 13 years, prior to the menopause [13]. Ten percent of women below the age of 45, 1% of women below the age of 40 years and 0.1% of women below the age of 30 will enter menopause prematurely, either due to an accelerated depletion of the primordial follicle pool or a lower ovarian reserve at birth [13]. Hence, their subfertile period can begin in their early thirties or twenties (Figure 4).

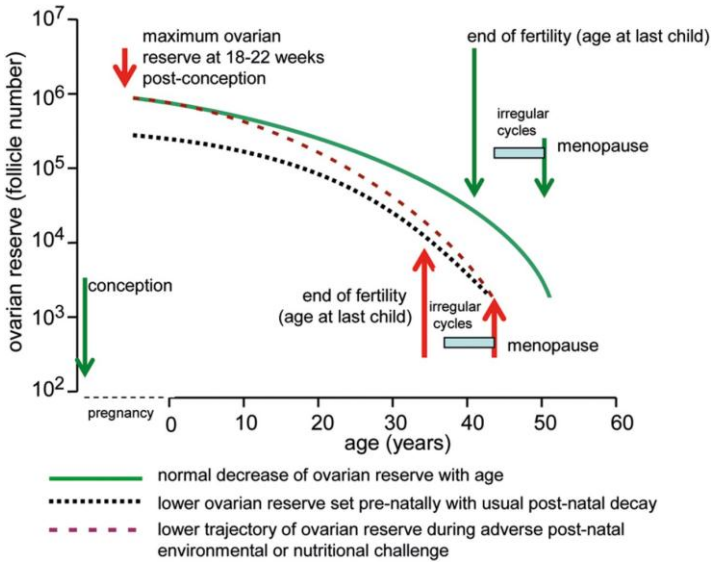

Figure 4: Decay of ovarian reserve with age [14]

Female age is associated to oocyte quality [15]. Studies on IVF oocytes have shown that the proportion of oocyte aneuploidy increases with age. In women aged 35 years or younger, the proportion is approximately 10%, but increases to 30% at the age of 40, to 40% at the age of 43, and to 100% in women age 45 or older [16]. A Danish prospective study of 1338 infertile couples demonstrated an increased chance of delivery (spontaneous/MAR), if the woman´s age was below 35 years compared to women aged 35 or older during MAR treatment [17]. Of the women below 35 years, 74.9% had delivered within five years compared with 52.2% of women aged 35 years or older. Therefore, age was included in the risk assessment score sheet. The risk colours were defined in accordance to the aforementioned knowledge of the age-related decline in fecundity and oocyte quality.

Ovarian reserve and menstrual cycle in the score sheet

Knowledge of women´s ovarian reserve provides essential information, when counselling women on their reproductive lifespan. The ovarian reserve is a term used to describe the functional potential of the ovary and reflects the number and quality of oocytes [18]. In this thesis, the ovarian reserve parameters were defined as; number of antral follicle count (AFC), level of Anti Müllerian Hormone (AMH) and ovarian volume. An ideal test of the ovarian reserve should predict the ability to conceive naturally, the current level of ovarian activity, and expected age at menopause [1, 19].

Antral Follicle Count (AFC)
The number of antral follicles can be measured by vaginal ultrasound and correlates with the ovarian reserve [20]. Low numbers of antral follicles may be a sign of ovarian ageing, and can be observed earlier than a rise in FSH serum level [21]. Furthermore,

AFC may be a better prognostic indicator of fertility outcomes than endocrine markers [22, 23]. Challenges with AFC may include variability among cycles, biological variation caused by age and OC [24, 25], and inter-observer differences [21]. Although, a recent study found insignificant intra-cycle variation of small antral follicles (≤ 6.0 mm) measured using 3D ultrasound [26].

Anti Müllerian Hormone (AMH)

AMH is a member of the transforming growth factor β-family. In women, AMH is solely produced by the granulosa cells of growing pre-antral and small antral ovarian follicles [21]. Measurement of serum AMH was first reported in the 1990s, and the test was initially developed to measure AMH as a marker for testicular function during childhood [1]. Serum AMH levels can be used as a marker of ovarian reserve representing the quantity of the ovarian follicle pool. The contribution of AMH by the pre-antral follicles is limited as the number of granulosa cells is much smaller[27]. A recent study showed that the antral follicles sized 5-8 mm contribute the most to the concentration of circulating AMH (~60% of serum AMH), 20-25% by 2.1-5 mm follicles and 15-20% by > 8 mm follicles [27]. FSH is an important factor for the pre-antral and early antral follicles that produces AMH. Yet, AMH reflects the number of growing follicles and is only a proxy for the number of primordial follicles [28] (Figure 5).

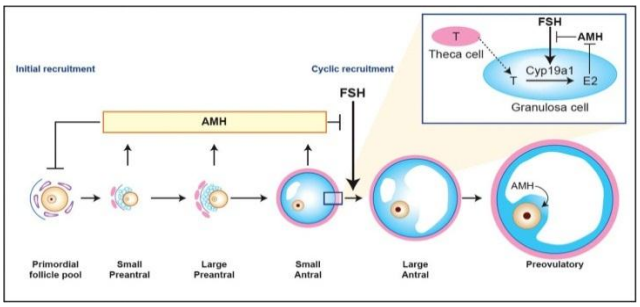

Figure 5: Schematic model of Anti Müllerian Hormone (AMH) actions in the ovary. AMH, produced by the granulosa cells of small growing follicles, inhibits initial follicle recruitment and FSH-dependent growth and selection of pre-antral and small antral follicles. In addition, AMH remains highly expressed in cumulus cells of mature follicles. The inset shows in more detail the inhibitory effect of AMH on FSH-induced CYP19a1 expression leading to reduced estradiol (E_2) levels, and the inhibitory effect of E2 itself on AMH expression. T, testosterone; Cyp19a1, aromatase; FSH, follicle stimulating hormone [1].

Several assays have been developed for measuring serum AMH [29]. So far, no international standard in order to maximize the clinical utility of AMH measurement has been established. Previous studies have shown that the inter-individual variability of AMH is high in similar age groups [30]. It has been suggested that AMH may be related to oral contraception [25], ethnicity [31], BMI [32] and smoking [33]. Although contradictory results have been reported for the latter two [34].

The inter-individual variability is primarily caused by the changeability in number of antral follicles, whereas the intra-individual variability in relation to measurements of AMH in the menstrual cycle appear to be random and minor, thus permitting AMH measurement independently of the cycle phase [1]. Furthermore, the fluctuations of AMH are randomly distributed during menstrual cycle which contradicts the necessity of a fixed cycle day (Figure 6)[1, 35].

AMH has proven to be a useful indicator of the time of menopause due to the age-related decline [19, 36, 37]. A study have suggested AMH to be an even more accurate predictor of individual time to menopause than mother's age at menopause [36].

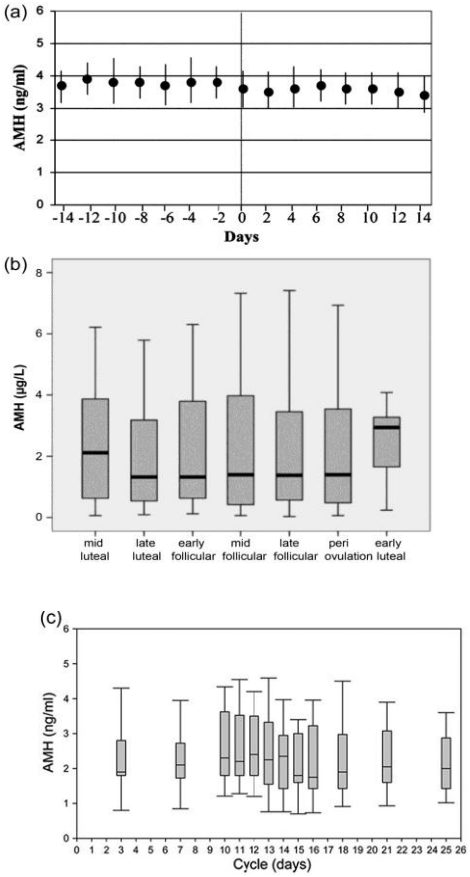

Figure 6: AMH variability throughout the menstrual cycle. Serum AMH appears to be stable. (Reproduced with permission from (a) La Marca *et al*., 2006, (b) Hehenkamp *et al.,* 2006 and (c) Tsepelidis *et al.,* 2007). Assays used for each data set were Beckman-Coulter for (a) and Diagnostic Systems Lab for (b) and (c) [1].

The literature on whether AMH is associated with time to pregnancy (TTP) is inconclusive. A recent prospective American study of 1202 women with 1-2 pregnancy losses did not find a correlation between AMH and TTP [38]. The authors of a Swiss observational study of 87 women with spontaneous pregnancies concluded that only age, and not AMH, as a continuous variable, was related to TTP [39]. A Danish study of 186 young women in their mid-20s found an association of prolonged TTP in women with a high AMH, but no impact if the AMH was low [40]. Contrarily, an American study of 98 women in their 30s concluded AMH to be a predictor of age-related reductions in fecundability [41].

Previous studies have shown a high correlation and one-to-one relationship among low numbers of AFC and AMH when using the Beckman Coulter Gen I assay in pmol/l [1, 24]. The rationale for the threshold value of 5 pmol/l, and hence an AFC of 5, as high risk answers in the risk assessment score sheet, was the 5th% percentile measured in a previous study of 1500 women in their mid-thirties, conducted by the Department of Clinical Biochemistry at Rigshospitalet, Denmark (unpublished).

Ovarian volume

Measurements of ovarian volume by ultrasound have been shown to be important predictors of ovarian ageing [42]. It is now well known that mean ovarian volume in premenopausal women is significantly greater than that in postmenopausal women. Furthermore, a statistically significant decrease in ovarian volume with each decade of life from age 30 to age 70 has been reported [43]. Kelsey *et al.* constructed a normative model of ovarian volume from conception to old age by searching the published literature for ovarian volume in healthy females, and using their own data from multiple sources (n= 59,994)(Figure 7)[44]. Ovarian volume was not included in the risk assessment score sheet, but was recorded for research purposes.

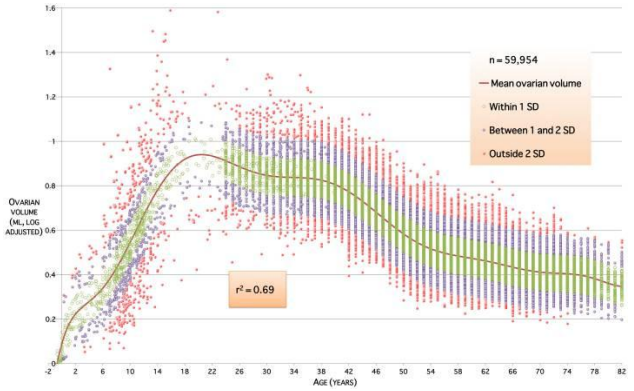

Figure 7: The validated model of log-adjusted ovarian volume throughout life.
The r^2 coefficient of determination indicates that 69% of the variation in human ovarian volumes is due to age alone. Colour bands indicate ranges within standard deviation from mean, within and standard deviations, and outside standard deviations [45].

Menstrual cycle length

A regular menstrual cycle depends on an integrative function of the hypothalamus, the pituitary gland and the ovaries causing a repetitive cyclic follicle recruitment, single dominant follicle recruitment, ovulation, and subsequently the formation of a corpus luteum [46].
The number of follicles in the human ovary declines with increasing age as explained in the section regarding Female Age. The peri-menopausal period is characterized by increasing irregularity in cycle length [47]. The rate of follicle loss more than doubles at approximately 37.5 years, when the numbers fall below the critical level of 25,000 [13]. It has been speculated that a threshold number of follicles is required to maintain a regular menstrual cycle [48].
When women reach the age of 35 the follicular growth begins to accelerate, causing an increased loss of the residual follicular stock in combination with a gradual increase in circulating levels of FSH [13]. The years prior to the menopause are usually marked by increasing variability in menstrual cycle length and frequency of ovulation, why menstrual cycle length was included in the risk assessment score sheet.

Menstrual cycle length is also associated to AMH and AFC levels. A previous study found increasing cycle length by one day, when serum AMH level increased by 14.0% (95% CI 10.2%–18.3%, P < 0.001). Similar association in cycle length were seen, when AFC increased by 7.4% (95% CI 5.0%–10.2%, P < 0.001)[24]. High AMH and AFC levels is related with polycystic ovarian syndrome (PCOS) and oligomenorrhea [49]. PCOS is associated with decreased fertility due to anovulation, why a long menstrual cycle was also included as a risk factor in the score sheet [50].

Gynaecological history and general health in the score sheet

Months of trying to conceive

Infertility is defined as a disease of the reproductive system with a failure to achieve a clinical pregnancy after 12 months or more of regular unprotected sexual intercourse [51]. Subfertility is defined as any form of reduced fertility with prolonged time of unwanted non-conception [52]. Most pregnancies (80%) occur in the first six cycles with regular intercourse in the fertile fase [52].

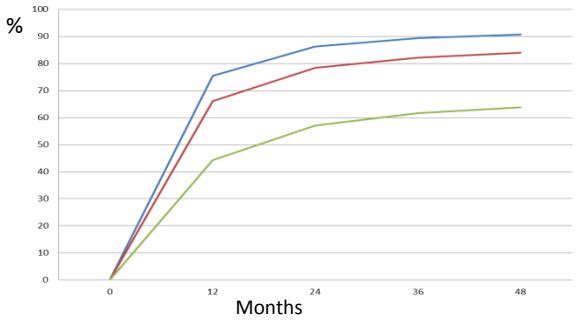

Figure 8: Probability for pregnancy according to the female age. Blue line: women aged 30 years. Red line: women aged 35 years. Green line: women aged 40 years. Adapted after [53]

As previously mentioned, and illustrated in Figure 8, the chance of monthly/yearly spontaneous conception is age-related. Leridon *et al.* constructed a model based on historical data between 1670 and 1819 including more than 106,000 children born and over 34,800 marriages during the same period [53]. The model demonstrated the following chance of conceiving spontaneously or after assisted reproductive technology (ART) stratified by age (Table 1):

Table 1: The chance of conceiving at age 30, 35 and 40 years; within 4 years without ART and within the next 2 years with ART. The table also displays the risk of remaining childless [53].

	Age of the woman when she starts to become pregnant		
	30 y	35 y	40 y
Women with children within 4 years without ART, %	91	82	57
Women with children within the next 2 years with ART, %	3	4	7
Women that will remain childless, %	6	14	36

In line with this, several studies have demonstrated that the duration of unprotected intercourse without conceiving is associated with a higher risk of infertility, and a decreased chance of spontaneous conceptions [54-56]. A Danish study explored the prevalence of infertility among 2,861 women. Among women

with current attempts of pregnancy the prevalence was 26% and 15.7% in the entire population [57]. The cut-off values in the risk assessment score were based on this knowledge as well as the definition of infertility (unprotected intercourse without conception > 12 months).

Pelvic inflammatory diseases incl. Chlamydia, ectopic pregnancies, previous pelvic surgery and hydrosalpinx

Tuboperitoneal factors have been estimated to be the main cause of subfertility in 11–30% of couples [11]. Tuboperitoneal factors are defined as post infectious tubal damage, tubal obstruction, hydrosalpinx, pelvic adhesions, and endometriosis [11].

In cohort studies, tuboperitoneal pathology is highly associated to a history of complicated appendicitis (OR 7.2, 95% CI 2.2–22.8), pelvic surgery (OR 3.6, 95% CI 1.4–9.0) and pelvic inflammatory disease (PID) (OR 3.2, 95% CI 1.6–6.6)[58]. Similar results were found in case–control studies, for a history of complicated appendicitis (OR 3.3, 95% CI 1.8–6.3), PID (OR 5.5, 95% CI2.7–11.0), ectopic pregnancy (OR 16.0, 95% CI 12.5–20.4), endometriosis (OR 5.9, 95% CI 3.2–10.8) and sexually transmitted disease (OR 11.9, 95% CI 4.3–33.3)[58].

A previous study stated that each episode of PID roughly doubles the risk of permanent tubal damage, irrespective of whether the infection is silent or overt [59]. The most common pelvic PID in Denmark and worldwide is *Chlamydia Trachomatis (CT)* with a prevalence of 30,000 new diagnosed cases per year nationally (National Danish Central Laboratory, 2015), and four to five million new cases worldwide [60]. *CT* infections of the lower female genital tract are frequently asymptomatic and remain undiagnosed or untreated. Thus, *CT* may ascend to the upper female genital tract and infect the fallopian tubes causing salpingitis. *CT* may lead to functional damage of the fallopian tubes and tubal factor infertility (TFI)[60].

A Swedish retrospective study of 1,844 women, all laparoscopically diagnosed with PID due to *CT*, found that 209/1,309 (16%) failed to conceive [61]. TFI was established in 141/1,309 (10.8%) patients with PID. The authors concluded that the rate of infertility was directly associated with the number and severity of PID infections. Every subsequent episode of PID approximately doubled the rate of TFI, i.e., 8% after just one *CT* infection, to 19.5% from two exposures resulting in infection, and an increase to 40% resulting from three or more exposures [60, 61].

Several studies have found TFI to be one of the major risk factor for ectopic pregnancies (aOR 2.23, 95% CI 1.93-2.58)[62, 63]. Apart from PIDs, TFI can also be caused by benign gynaecological disorders such as hydrosalpinx, which is associated with decreased cycle fecundity and impaired uterine receptivity (Figure 9) [64].

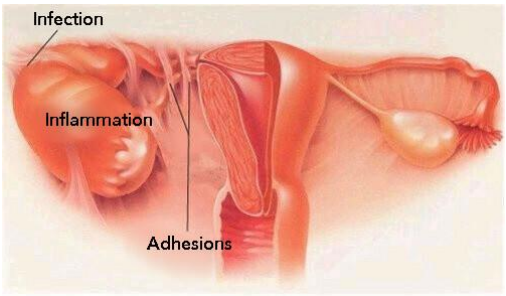

Figure 9: Previous PIDs and pelvic surgery can increase the risk of TFI and ectopic pregnancies by inflammation, adhesions and hydrosalpinx. PID: Pelvic Inflammatory Disease, TFI: Tubal Factor Infertility [64].

Based on the aforementioned and the risk for reduced fertility caused by TFI, previous PIDs including *CT*, ectopic pregnancies, hydrosalpinx and pelvic surgery were included in the score sheet.

Endometriosis

Endometriosis is associated with subfecundity and infertility, but a definite cause-effect relationship is still controversial [65, 66]. The prevalence has been estimated to affect up to 10% to 15% of reproductive-aged women [67]. The negative effects on fertility may result from reduced frequency of intercourse due to dyspareunia, from anatomical distortion and adhesions in more severe cases of endometriosis, or from more subtle alterations in the intra-ovarian and tubo-peritoneal environments [68]. Endometriosis impacts the ovarian microenvironment and endometrial receptivity by inflammatory markers such as TNF-α and IL-6, which are present in higher quantities within the granulosa cells as well as a higher rate of apoptosis (Figure 10) [67, 68].

Several data suggest that the monthly fecundity rate (MFR) is lower in women with mild to severe endometriosis than in those with minimal endometriosis [69]. Apparently, there seems to be a negative correlation between the MFR and the stage of endometriosis. This could be explained by the theory; that women with moderate-severe endometriosis have more adnexal adhesions and larger endometriotic ovarian cysts than those with minimal-mild disease. This may result in impaired fimbriae efficiency to pick up the ovulated egg from the ovarian surface and in impaired tubal transport of eggs, sperm, and embryos [69].

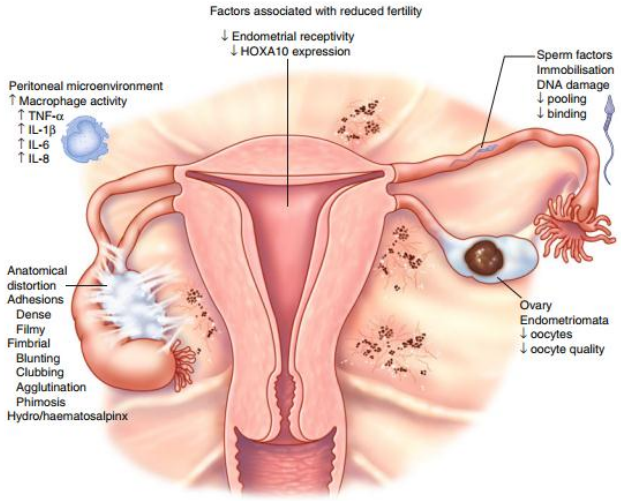

Figure 10: Factors associated with decreased fertility in endometriosis [68]

Uterine fibroids

Fibroids are the most common benign tumours of the upper female genital tract affecting 30– 70% of reproductive-aged women and are common in pregnancy (from 0.1 to 12.5% of all pregnancies) [70]. Fibroids are classed into subgroups according to their position and relationship to uterine layers; submucosal, intramural and subserosal [71]. Fibroids are associated with numerous clinical problems including a possible negative impact on

fertility [72]. The severity of the negative impact is linked to the size and position of the fibroids [73]. Anatomically, fibroids can distort the uterus and enlarge and even elongate the cavity, alter the contour and surface area of the cavity. Furthermore, fibroids can obstruct tubal ostia or the cervical canal, or displace the cervix in the vagina. These acquired abnormalities can inhibit migration of sperm, ovum, or embryo, and can impair implantation. Uterine function may also be affected, as fibroids may cause dysfunctional and altered uterine contractility, and thus hindering gamete transport and embryo implantation [73]. Studies have shown that fertility outcomes are decreased in women with submucosal fibroids with lower ongoing pregnancy rates (OR 0.5; 95%CI, 0.3-0.8), primarily through decreased implantation and removal seems beneficial [74].

Subserosal fibroids do not affect fertility outcomes, why removal is not advised due to the risk of serious complications. Intramural fibroids appear to decrease fertility, but the results of therapy are unclear [75]. There is inconclusive evidence regarding the size of the fibroids and impact on fertility. Due to the known association we chose to include fibroids as a risk factor, and the cut-off value of 3 cm was based on the available literature in 2011.

Uterine malformation

Subfertility can be related to uterine malformations such as a septate uterus, which is a congenital malformation. The septate is due to the longitudinal band separating the left and right Müllerian ducts, which form the uterus in the human female foetus, and has not been entirely resorbed. A uterine septum is present in 1% to 3.6% of women with otherwise unexplained subfertility [76]. Other anomalies can occur during this stage, where the two separate Müllerian ducts normally develop into the primitive right and left fallopian tubes, uterine horns, cervix, and upper vagina (Figure 11) [3]. The presence of uterine malformations may decrease the chance of spontaneous conceptions, why this was included in our risk assessment score sheet.

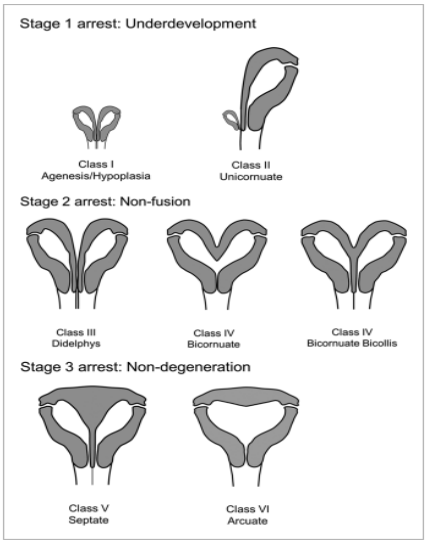

Figure 11: Three embryologic stages of normal uterine, cervical, and vaginal development. (a) Stage I: Two separate uterine, cervical, and vaginal segments develop. The upper 2/3 of the vagina develops with a transverse septum along the caudal aspect. This transverse septum will dissolve when the lower 1/3 of the vagina, which develops from the urogenital sinus, fuses with the upper 2/3 of the vagina. (b) Stage II: Midline fusion of the uterine, cervical, and vaginal segments. (c) Stage III: Degeneration of the midline fused segments in the uterus, cervix, and vagina [3].

Previous chemotherapy

Women suffering from a current of previous cancer that requires treatment with gonadotoxic drugs may experience cessation of reproductive function as a side effect due to obliteration of the ovarian pool of follicles [77]. Approximately, 40-80% of female cancer patients face possible infertility as a result of their cancer treatments (chemotherapy, radiation, and surgery) [78].

Genetic dispositions and intrauterine exposure in the score sheet

Maternal age at menopause

The mean age of female menopause is 51 years in Denmark [79]. A recent Danish study of 527 female healthcare workers aged 20–40 years old found a significant effect of the mother's menopausal age on both serum AMH levels and AFC in the daughters [80]. The analyses demonstrated a decline by 8.6% per year in median serum AMH concentration in the group with early maternal menopausal age (≤45 years), by 6.8% per year in the group with normal maternal menopausal age (46–54 years) and by 4.2% per year in the group with late maternal menopausal age (≥55 years). The study also found comparable declines in AFC. An earlier study of FSH in mothers and daughters, which is another marker of ovarian reserve, found similar associations between mother's and daughter's age of menopause [81]. Women whose mothers experienced earlier menopause had higher urinary FSH levels.

Intrauterine exposure to maternal smoking

Foetal exposure to tobacco smoke may decrease fecundability, which could be due to the accelerated ageing and follicle depletion [82-85]. Accelerated ageing and earlier menopause may be related to telomere length shortening. A recent study has demonstrated a positive association between shortened foetal telomere length and smoking during pregnancy [86]. Telomeres are complex nucleotide sequences that protect the end of chromosomes from deterioration and play a critical role in cellular division.

Over time, telomeres shorten and eventually reach a critical short length that leads to apoptosis. This shortening serves as a biomarker for cellular and biologic aging, longevity, and disease development. Shortened telomere lengths are associated with adverse health outcomes, such as cardiovascular disease, Alzheimer's disease, cancer, and early death [86]. Foetal exposure to maternal smoking was included as a risk factor, due to the well-established association with reduced fecundity in both genders [83].

Studies have demonstrated accelerated follicle depletion in human foetuses exposed to maternal smoking [87, 88]. A study of 24 human first-trimester foetuses, aged 37-68 days post-conception, obtained from women undergoing legal termination of pregnancy, found significantly reduced germ cells by 41% (95% CI 58-19%, P = 0.001) in embryonic gonads, irrespective of gender, in exposed versus non-exposed embryonic gonad [87].

Lifestyle factors in the score sheet

Body mass index (bmi) and waist/hip ratio

Obesity it is thought to be the sixth most important risk factor for mortality and morbidity worldwide [89]. Obese women are three times more likely to suffer infertility than women with a normal BMI [90]. Several studies have shown obesity to be risk factor for

subfertility due to anovulation [91]. Weight gain causes disturbances in the metabolic and reproductive system. The excess of free fatty acids causes liver lipid synthesis enhancement leading to insulin resistance and hyperlipidaemia. The increased glucose induces hypersecretion of insulin, which inhibits the hepatic SHBG synthesis (sex hormone binding globulin). This leads to increased testosterone and oestrogen, which can induce anovulation [92].

Furthermore, adipocytes synthesise and release chemical messenger peptides that participate in metabolic regulation, including the action of insulin (Figure 12). Leptin has been suggested to have the following effects in obesity: dysregulation of GNRH secretion, altered ovarian steroidogenesis, dysregulation of folliculogenesis, and dysregulation of perifollicular blood flow. Additionally, Leptin is observed in secretory endometrium, and may have a role in regulation of the embryo implantation and endometrial receptivity [93].

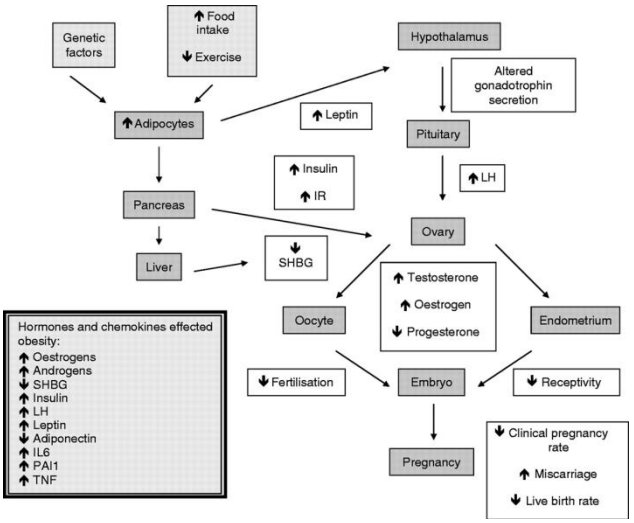

Figure 12: Putative effects of leptin in obesity [93].

Several studies have shown a detrimental effect of obesity upon oocyte quality and maturation. The mechanisms are not fully understood, but insulin resistance has been mentioned as a possible explanation. Another surrogate marker of oocyte quality is the fertilisation rate, which has been found to be significantly reduced in overweight and obese women [93].

An American study of 7,327 pregnant women found that the fecundity was reduced for the overweight (BMI>25 kg/m^2)(OR 0.92, 95% CI 0.84-1.01) and the obese (BMI>30 kg/m^2) (OR 0.82, 95% CI 0.72-0.95) women compared with optimal weight women [94]. This was even more evident for obese primiparous women (OR 0.66, 95% CI 0.49-0.89).

Fecundity remained reduced for overweight and obese women with normal menstrual cycles. Neither smoking habits nor age modified the association (Figure 13). Finally, the implantation rate has been found to be decreased in line with increased rate miscarriage and early pregnancy loss [95-97].

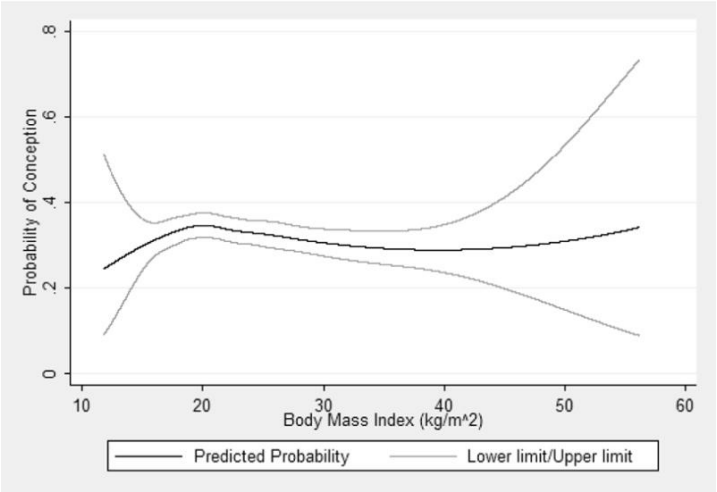

Figure 13: The predicted probability of conception with changing body mass index (kg/m^2), after adjusting for age, smoking, race, education, occupation, and study center is illustrated in this graph. The graph was constructed for 23-year old, non-smoking, white women with a high school diploma in white collar occupations enrolled at the Boston clinic. Pregnant women enrolled in the Collaborative Perinatal Project between 1959 and 1965. Adjusted fecundability odds ratios (ORs) were estimated using Cox proportional hazards modeling for discrete time data. Risk of infertility was: RR 2.7 with a BMI > 30 kg/m^2.Probability of pregnancy was reduced by 5% per unit of BMI exceeding 29 kg/m^2[94].

Central obesity is defined by an elevated Waist-Hip Ratio and has a negative impact on fecundity. A Dutch study of 542 women found that an increase of 0.1 unit in WHR lead to a 30% decrease in probability of conception per cycle [98]. The authors concluded that increasing waist-hip ratio is negatively associated with the probability of conception per cycle, before and after adjustment for confounding factors. Body fat distribution in women of reproductive age seemed to have more impact on fertility than age or obesity [98].

As illustrated in Figure 13, underweight is also associated with decreased fecundity. A recent study of 1,950 women documented that being underweight at age 18 years (BMI less than 18.5) was associated with a longer current duration of pregnancy attempt compared with normal-weight women (time ratio 1.25, 95% CI 1.07-1.47) [99]. Another study of 33,159 North American Adventist women found underweight at age 20 was associated with approximately 13% increased risk of nulligravidity or nulliparity [100]. A British study of 2,112 women found a four-fold increased time to conception in women with a BMI < 19 (aRR 4.8, 95% CI 1.2–19.7)[101].

Smoking

In recent years, the detrimental effect of smoking in relation to fecundity has been well documented. The negative influence is caused by tobacco toxins, which can impair fertility by affecting the folliculogenesis, oogenesis, steroidogenesis, embryonic transport and implantation, endometrial angiogenesis, uterine blood flow and myometrial growth [102]. Additionally, the toxins may lower the age at natural menopause due to reduced level of circulating oestrogen caused by synthesis inhibition and endocrine disruption [102, 103].Two large studies have examined and documented a dose-dependent association between smoking and

time to pregnancy [104, 105]. A European multicentre study of 4,000 couples, divided in a non-pregnant population-based sample (aged 25-44) and a pregnancy-based sample, found a dose-response relationship between prolonged time to pregnancy and smoking habits in both groups (Table 2).

Table 2: Results of a parametric analysis of the distribution of waiting times to pregnancy in the European Study of Infertility and Subfecundity, according to couples' smoking habits, 1991-1993 [104].

	Median time to pregnancy	β ratio*	Standard error	95% confidence interval
Pregnancy sample				
Mothers' smoking (cigarettes/day)				
0	2.60	1.00		
1–10	3.20	1.26	0.09	1.08–1.44
≥11	3.94	1.61	0.12	1.38–1.84
Population sample				
First pregnancy				
Mothers' smoking (cigarettes/day)				
0	2.10	1.00		
1–10	2.52	1.20	0.09	1.03–1.36
≥11	3.23	1.54	0.11	1.32–1.75

An American study of 6,316 women found similar prolonged time to pregnancy according to the number of cigarettes smoked each day (Table 3).

Table 3: Odds ratios and 95% confidence intervals before and after adjustment for confounding factors of taking longer than 6 or 12 months to conceive, according to the number of cigarettes smoked each day by the mother [105].

No. of cigarettes smoked daily	Conception by 6 months		Conception by 12 months	
	Unadjusted OR	Adjusted OR	Unadjusted OR	Adjusted OR
Mother				
None	1.00 (reference)	1.00 (reference)	1.00 (reference)	1.00 (reference)
1–4	1.11 (0.85–1.44)	1.22 (0.92–1.62)	1.40 (1.01–1.94)	1.67 (1.18–2.38)
5–9	1.23 (0.97–1.57)	1.24 (0.93–11.64)	1.27 (0.92–1.73)	1.29 (0.88–1.90)
10–14	1.12 (0.90–1.38)	0.93 (0.71–1.22)	0.96 (0.71–1.30)	0.92 (0.63–1.36)
15–19	1.31 (1.07–1.59)	1.47 (1.15–1.87)	1.57 (1.22–2.01)	1.99 (1.48–2.69)
≥20	1.57 (1.32–1.86)	1.59 (1.28–1.99)	1.59 (1.27–2.00)	1.58 (1.18–2.12)
χ^2 value	31.65	27.15	29.87	33.39
P value	<.0001	.001	<.0001	<.0001

Women's Health Initiative Observational Study of 93,676 post-menopausal women aged 50–79 years examined the relationship between smoking and infertility, as well as smoking and age of menopause. The authors found an increased risk (OR) for infertility in active-ever smokers of 1.14 (95% CI 1.03 to 1.26) and an increased risk (OR) for earlier menopause than never-smoking women of 1.26 (95% CI 1.16 to 1.35). The active-ever smokers reached menopause 21.7 months earlier than the mean of 49.4 years for never-smokers not exposed to second hand smoking [103]. Surprisingly, second hand smoking increased the risk of infertility with OR 1.18 (95% CI 1.02 to 1.35). Likewise, there was also an increased risk of for earlier menopause OR 1.18 (95% CI 1.06 to 1.31). Women exposed to the highest level of second hand smoking reached menopause 13.0 months earlier than none-smoking women [103].

ALCOHOL

Women´s attitude toward alcohol, as well as the society´s, has changed within the recent 20 years. Today, the Danish Health Authority recommends total abstinence from alcohol when planning a baby [106]. The proportion of women drinking alcohol during pregnancy has dropped from 70% in 1998 to 17% in 2013 [107]. The literature is inconclusive regarding the impact of alcohol on fecundity. It has been hypothesized that the detrimental effect is caused by an alcohol-induced rise in oestrogen, which reduces FSH secretion, hereby suppressing folliculogenesis and ovulation. Furthermore, it may also have a direct effect on the maturation of the ovum, ovulation, blastocyst development and implantation [108, 109]. Previous studies have suggested a detrimental dose-response relationship between alcohol consumption and fertility [108, 110, 111]. Yet, current evidence is unclear regarding what dose of alcohol which may be safe to consume with regards to monthly fecundity [109]. Hence, the risk assessment score regarding alcohol was chosen in compliance with the recommendation from the Danish Health Authority [106].

CAFFEINATED BEVERAGES

Caffeine, a mild neurostimulant, is currently the most popular pharmacologically active substance worldwide [112]. Caffeine´s impact on fecundity has been examined in several studies due to a supposed harmful effect [111-114]. The mechanism is unclear, but alterations to hormone levels, and therefore impact on ovulation and the corpus luteum function, has been suggested [109]. Previous studies have found an association between prolonged TTP > 12 months and a possible dose-response effect. Consumption of more than three cups of coffee per day increased the risk of TTP > 12 months compared to no intake (OR 2.24, 95% CI 1.06-4.73) [115-117].

Other studies have shown opposite results [114]. Studies on caffeinated beverages are often based on retrospective data, which could induce recall bias [112, 113]. Due to inconclusive data the recommendation is presently to reduce the daily caffeine intake below 250 mg (2-3 small cups of coffee or 8 caffeinated soft drinks), when attempting to become pregnant [118]. Yet, a higher value was chosen in the risk assessment score due to previous inconclusive results.

Physical activity/exercise

Reproduction and metabolism are strongly connected and reciprocally regulated in women [119]. The physiological activity of the gonads ensures continuous regulation of energy metabolism, due to their cyclic production of sex hormones throughout the reproductive period of life [119].

Hypothalamic dysfunction associated with strenuous exercise can result in delayed menarche and disruption of menstrual cyclicity, due to the resulting disturbance of GnRH pulsatility [120]. The susceptibility of the reproductive axis to exercise and diet-related stresses appears to be highly individual [121]. Exercise-induced or athletic menstrual dysfunction (amenorrhoea, oligo-menorrhoea, anovulation, luteal phase deficiency, delayed menarche) is more common in active women. Menstrual dysfunction can result in suppressed oestrogen levels and affect bone health and fertility. Several factors, such as energy balance, exercise intensity and training practices, bodyweight and composition, disordered eating behaviours, and physical and emotional stress levels, may contribute to the development of athletic menstrual dysfunction (Figure 14)[121].

Similarly, strenuous exercise has been associated with an increased risk of infertility, whereas PCOS patients may benefit from it. Yet, the evidence is still inconclusive [112, 122]. Therefore, strenuous exercise was not defined in detail in the score sheet, but was based on an individual assessment in collaboration with the woman´s perception of her training intensity. However,

four hours of intense weekly training was used as an arbitrary cut-off.

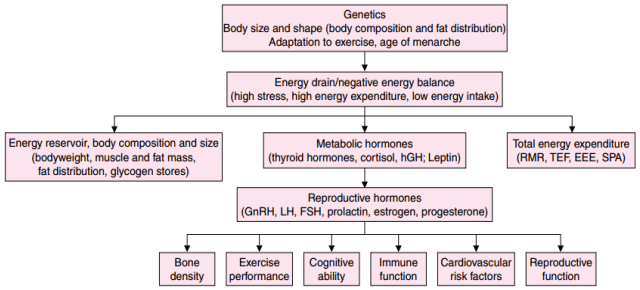

Figure 14: A model illustrating the influence of energy drain and high stress on the development of menstrual dysfunction in active women, and the potential health and performance outcomes due to low reproductive hormones and high cortisol levels; FSH = follicle-stimulating hormone; GnRH = gonadotropin-releasing hormone; hGH =human growth hormone; LH = luteinising hormone; RMR = resting metabolic rate; SPA = spontaneous physical activity; TEF =therapeutic effect of food.

Postponement of parenthood in general

The tendency of postponement of parenthood is well examined in OECD countries. Mills *et al.* displayed the differences in years of postponement from 1970 to 2008 in 24 countries. United States had the smallest difference of 1.5 years ranging to 5.2 in Iceland with a mean of 3.8 years for all countries [123]. The increasing age at first child has a direct influence on the total fertility rate (TFR). Firstly, due to shorter period the women are able to become pregnant. Secondly, the cumulative risk of age-related reproductive threats such as; PIDs, TFI, endometriosis and fibroids, which increases the risk of infertility [4].

TFR is a measure of reproductive performance and shows the average number of children each women would deliver in her lifetime, provided the age-related fertility rate observed in a period remains constant [4]. Under current mortality conditions, the average TFR needed to maintain population size, in the absence of migration, is slightly below 2.1 children per woman (accounting for childhood mortality)[124]. Europe is presently the continent with the lowest TFR, but as displayed in Figure 15, similar tendencies are seen in Japan, Russia and Taiwan [124].

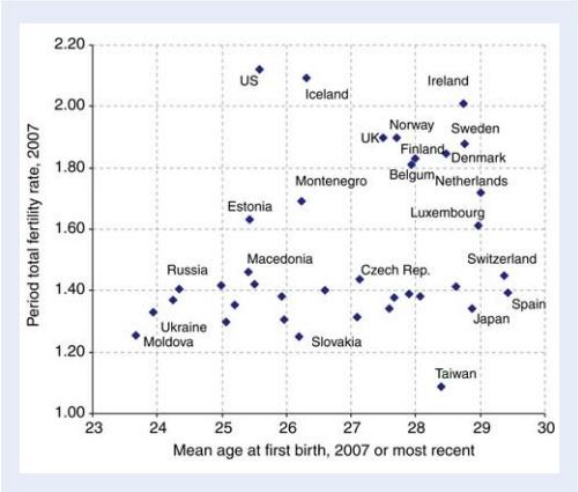

Figure 15: Total fertility rate and mean age at first birth in 37 developed countries of Europe, East Asia and the USA [4].

The Middle East countries and India report decreasing TFR due to increased education levels and use of contraception. In Iran the TFR has declined from 6.4 births per woman in 1984 to 1.9 in 2010 [125]. Although India has highest population growth rate of 1.6% per year, adding around 181 million people to the total during the decade, the TFR has dropped from 6.0 in 1966 to 2.6 in 2008 [126].

In brief, the following reasons for postponement of parenthood have been mentioned; 1) introduction of contraceptive technology, 2) increased educational levels and women´s labour force participation, 3) norm and value changes, 4) gender equity, 5) changing partnerships and increasing number of people living alone, and 6) housing and economic uncertainties [123].

The consequences of postponement of parenthood are a higher rate of involuntary childlessness and smaller families than desired [4, 127]. A recent Dutch study simulated a model based on previous publications and cohorts regarding the question; "Until what age can couples wait to start a family without compromising their chances of realizing the desired number of children?" [128]. The results were based on a 50%, 75% and 90% probability for achieving 1, 2 or 3 children, either by spontaneous conceptions or by ART (Table 4).

Table 4: Maximum female age (years) at which couples should start building a 1-, 2- or 3-child family, for a 50, 75 and 90% chance of realizing the desired family size, with and without IVF [128].

Chance of realization	1-child family	2-child family	3-child family
Without IVF			
50%	41	38	35
75%	37	34	31
90%	32	27	23
With IVF			
50%	42	39	36
75%	39	35	33
90%	35	31	28

Similarly, a previous study of the Swedish fertility patterns based on two birth cohorts from 1935-1939 and 1950-1954 found age at first child to be related to completed fertility rate (Figure 16) [129]. If a woman is aged 25, 35 or 40 at first birth, her TFR will be 2.3, 1.5 and 1.2, respectively.

Reasons for postponement of pregnancies were included as questions in the baseline questionnaire and further elaborated in the interviews (manuscript IV).

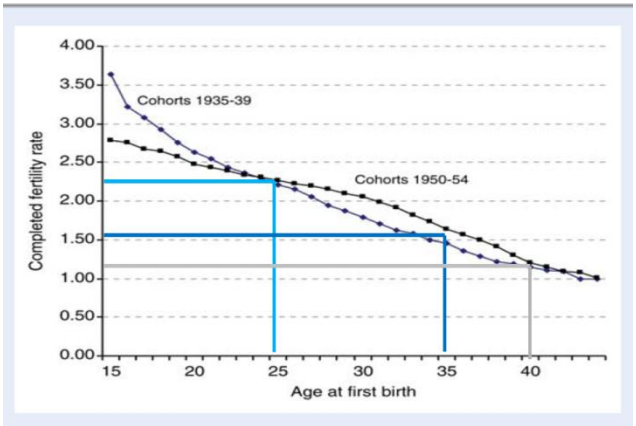

Figure 16: Age at first birth and completed fertility rate based on two cohorts of Swedish women born in 1935-1939 and 1950-1954. The fertility rates are highlighted for the ages 25, 35 and 40. Modified. [4, 129].

Work environment factors in the score sheet

Stress

Occupational stress, such as; night work, long hours, and physically demanding work was related to menstrual disturbances in a study of more than 6,000 nurses [130]. Similar tendencies were also found in an earlier study of rotating shifts among 71,077 nurses [131]. Self-reported psychosocial stress, anxiety, and depression were not associated with fecundity in a prospective American study of 339 women [132]. Contrarily, several studies have identified a relationship between higher stress levels and lower pregnancy chances and live birth rates in ART [112, 133, 134].

The pathophysiological rationale between the relation of stress and reproductive failure is a complex, immune, endocrine disequilibrium response to stress factors. There is evidence to suggest a stress-associated suppression of reproductive functions, such as the delay of menarche, hypothalamic amenorrhoea, ovarian dysfunction and early-onset menopause [112]. Still, further research into the effect of stress on fecundity is highly needed due to the discrepancy in the definition of stress in previous studies. Therefore, stress was included in the score sheet as self-reported perceptions, but without a defined stress score.

Rationale of the thesis

Fertility Assessment and Counselling is a new concept, which needs to be validated.

Firstly, several studies have examined the impact of solitary known or presumed risk factors on fecundity. Yet, only a few have combined the different risk factors and have been able to provide an estimate of female fecundity.

Secondly, although AMH is widely recognised as a valid estimate of the ovarian reserve, concerns have been raised in terms of interpreting values in users of combined oral contraceptives.

Thirdly, previous publications of attitudes toward family formation and fertility awareness have primarily been in general terms among students, infertile couples, and women and men of higher reproductive age. There is a sparse understanding of the considerations in relation to family intentions among older, childless women, who seek advice in relation to their reproductive lifespan. Similarly, there is limited information regarding the reasons for postponing parenthood in childless women, despite of advanced age and a wish for children.

DESIGNS AND MATERIAL

The following page and Figure 17 describe the design of each study, reasons for exclusion from the analyses and the distribution of the different cohorts.

MANUSCRIPT I:

A prospective cohort study including the first 570 women aged 20-43 years who consulted the FAC Clinic at Rigshospitalet, Copenhagen University Hospital from June 2011 to December 2013. The response rate of the follow up questionnaire was 91.1% (519/570).

MANUSCRIPT II:

A cross-sectional study of 971 women aged 19–46 attending the FAC Clinic from 2011 to 2014. In the analyses, 62 women were excluded due to: (i) pregnancy discovered at the consultation (n=9), (ii) present fertility treatment (n=1), (iii) no available baseline questionnaire (n=29), (iv) failed AMH analysis (n=3) or (v) no-show at the consultation (n=20). The women using progestin-only pills (n=21) and implants (n=1) were excluded in the analyses.

MANUSCRIPT III:

A cross-sectional cohort study of 397 women aged 35–43 examined at the FAC Clinic from August 1st 2011 to July 31st 2014. Eligible women were defined as heterosexual, childless and at least 35 years of age. Of the 397 women, we excluded 57 from the analyses due to: (i) lesbian relationship (n=3), (ii) unknown marital status (n=7) or (iii) women with children (n=46). In total, 340 women were included.

MANUSCRIPT IV:

The design was semi-structured qualitative interviews of 20 women aged 34-39 years attending the FAC Clinic from March to September 2014. Eligible women were defined as heterosexual, childless and aged 35 years and above. A total of 25 women were contacted of whom 22 wished to participate. Two were excluded due to pregnancy. To obtain the sample of 10 single women and 10 cohabiting women with equal distribution of postgraduate education length during the short inclusion period, two women aged 34 years were included.

Material

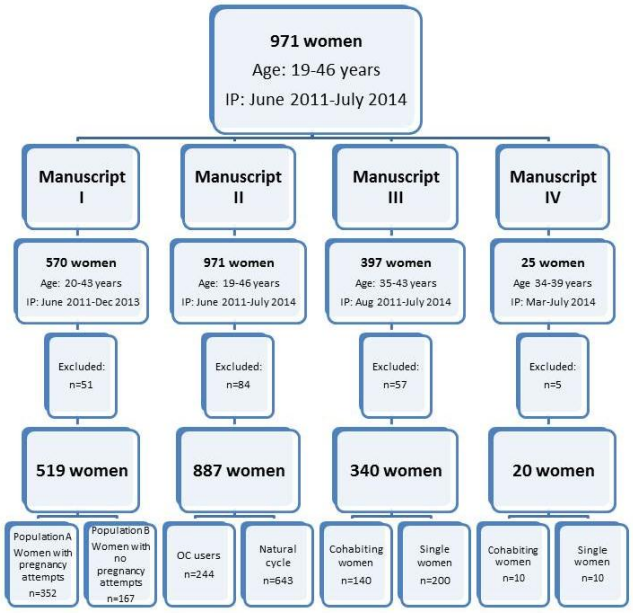

Figure 17: The distribution of the study populations in manuscript I-IV. IP=Inclusion Period, OC=Oral Contraception.

METHODS

FAC Clinic consultation in brief:

All women completed a web-based baseline questionnaire (Survey-Exact) before and an evaluation questionnaire immediately after the consultation. The baseline questionnaire was partly based on the validated Swedish Fertility Awareness Questionnaire by Lampic et al. [135] and a previous Danish study from our group [80]. The baseline questionnaire included items regarding socio-demographic background, reproductive and medical history, lifestyle and behavioural exposures, such as smoking, alcohol and exercise.

The evaluation questionnaire distributed after the consultation focused on the women´s reasons for attending the clinic, knowledge acquisition and whether they expected to plan a pregnancy within the next two years.

All women were examined by a fertility specialist, who performed a transvaginal ultrasound (AFC, ovarian volume, pathology), uptake of a full reproductive history, AMH measurement and a risk assessment. The women were informed of their potential risk factors by a risk assessment score categorized as; green (low), yellow (low), orange (medium) and red (high) for each risk factor (illustrated in Figure 1) and presumed ovarian reserve.

The ovarian reserve was assessed by AFC, ovarian volume and AMH. The number of antral follicles was counted and grouped into three predefined categories: 2-4 mm, 5-7 mm and 8-10 mm. The ovarian volume was measured by the formula for a prolate ellipsoid using the longest longitudinal (d1), anteroposterior (d2), and transversal diameters (d3): volume = d1 x d2 x d3 x π/6 [136]. Throughout the period the same team of five doctors examined the women.

The blood test for AMH was taken at the consultation. The serum AMH concentrations were measured at the Department of Clinical Biochemistry by an enzyme-linked immunosorbent assay (ELISA) (Immunotech, Beckman Coulter Generation I, Inc., Marseilles, France). The sensitivity was 0.7 pmol/l and the intra- and inter-assay coefficients of variation were 12.3% and 14.2% [25].

MANUSCRIPT I:

The follow up questionnaire was distributed by email two years after the consultation. The primary data in the follow up questionnaire were: changes in relationship status, change of contraceptive status, pregnancies, pregnancy loss, deliveries, time to pregnancy, attempts to conceive and whether the women had had fertility treatment, and if so, what types of fertility treatments. The questionnaire also addressed changes in health status and attitudes toward child bringing.

The population A was defined as women, who had attempted a pregnancy within the two years of follow-up after their visit to the FAC Clinic. In the questionnaire, the women reported the date(s) (day/month/year) within the two years at which the attempt(s) of pregnancy was initiated, and if relevant the date(s) at which pregnancy was achieved. Further, it was recorded whether the woman was still trying, or had given up at the end of follow-up. Had a woman been pregnant more than once during time of follow-up, the time to first pregnancy was used in the TTP analyses.

Thirty-two women had misunderstood the questionnaire and reported attempts for pregnancies prior to their visit to the FAC CLINIC. These were excluded from the TTP analysis. Pregnancies were categorized as spontaneous or after fertility treatment. Single women who achieved a pregnancy with insemination with donor semen (IUI-D) were pooled with spontaneous pregnancies in the analyses. The population B was defined as the remaining women without any attempts to conceive within the two years of follow up.

MANUSCRIPT II:

In the baseline questionnaire, the women were asked to report both the use of current and former contraceptive methods and the duration of each. The women were asked about the following contraceptives methods: (i) oral contraception with a combination of oestrogen and progestin, (ii) contraceptive patches, (iii) progestin implants, (iv) contraceptive vaginal ring, (v) progestin-only products (pills), (vi) intrauterine device (IUD) with copper or levonorgestrel, (vii) intramuscular depot of progestin, (viii) withdrawal, and (ix) “safe periods”. At the consultation, the women were additionally asked to report their current contraceptive method, if any.

These contraceptive methods were condensed into the following two groups for analytic purposes: a) OC-users) (n=244) (all ethinyl estradiol and progestin oral products or vaginal ring) and b) Non-users (n=643) (IUDs or no hormonal contraception).

MANUSCRIPT III:

The women were asked what they personally thought would be the most important prerequisites, expected benefits and consequences in relation to motherhood (personal considerations). To identify the most important prerequisites for childbearing the women were asked to answer 15 statements on a five-point scale

by i) very important, ii) important, iii) of some importance, iv) not very important or v) not important at all [135].

The statements primarily focused on relationship, job situation, and personal considerations. Similarly, 15 statements in random order answered by a four-point-scale described the expected benefits and consequences of motherhood: i) Agree, ii) mainly agree, iii) neither agree nor disagree or iv) mainly disagree.

All women were asked about their considerations toward fertility treatment (IVF/ICSI), adoption, and gamete donation (oocytes, sperm), if they were not able to achieve a spontaneously conceived pregnancy. The questions were answered by a five-point scale: by i) definitely yes, ii) most likely, iii) I don´t know, iv) probably not or v) definitely not. The same scale was used in relation to their attitudes regarding social egg freezing.
Knowledge acquisition and whether the women would bring forward the timing of pregnancy were likewise answered by a five-point scale.

MANUSCRIPT IV:
We developed a semi-structured interview-guide with open-ended questions focusing on family formation intentions. The interview topics were formed by knowledge and experiences from the researchers and by previous studies on family formation and fertility awareness [135, 137-139]. The interview took place one week before consultation at the FAC Clinic. The interviews were audio-taped and transcribed verbatim including non-verbal expressions like silence, laughter and tears. Transcripts were anonymised.

Transcripts were analysed according to qualitative content analysis [140]. The text was analysed with the concepts of meaning units, condensed meaning units, codes, subthemes and themes.

The analysis was performed in four steps: 1) scoping the interviews to obtain an idea of the content, 2) dividing the text into meaning units, which were defined as words, sentences or paragraphs in the text, where the content related to each other and to the aim of the study, 3) condensing the meaning units and labelling with codes, which were abstracted and compared for similarities and differences. The codes were distributed into categories and condensed into subthemes and, 4) comparing each subtheme, analysing and then unifying to a main theme. The consolidated criteria for reporting qualitative research were followed (COREQ) [141].

STATISTICAL ANALYSES

MANUSCRIPT I, II, III AND IV:
Baseline characteristics were summarised as; mean and standard deviation (SD) of continuous variables, or number and percentage of categorical variables. Continuous variables were analysed with two-sample t test and categorical variables with Pearson Chi-Square or Fisher´s Exact test. Descriptive statistics was made with the statistical software SPSS (version 22, Chicago, USA) and Microsoft Office Excel 2010.

MANUSCRIPT I:
Time to pregnancy analyses were carried out using a Cox regression type multi-state model in order to distinguish spontaneous pregnancies from pregnancies achieved by aid of fertility treatment.

States were defined as; 1) Attempting spontaneous pregnancy 2) Achieved spontaneous pregnancy, 3) Attempting pregnancy with fertility treatment, 4) Achieved pregnancy with fertility treatment, and 5) Given up. Women still trying to conceive at follow-up were censored. Potential predictors from the FAC Clinic questionnaire were assessed, but valid results could only be obtained for time to pregnancy and time to initiating fertility treatment. Predictors of time to pregnancy with fertility treatment and time to giving up could not be evaluated due to either too few cases (given up) or reduced sample size and time of follow-up (time to pregnancy with fertility treatment).

To enable more explicit statements about the chances of achieving spontaneous pregnancy additional logistic regression analyses were performed with spontaneous pregnancy within 3, 6, 9, and 12 months as outcome. Only women who had complete follow up of 3 (n=101), 6 (n=133), 9 (n=151), and 12 months (n=159), respectively were included in these analyses. Time to pregnancy analyses were performed with R (version 3.2.3, Vienna, Austria), using the timereg, survival, prodlim and rms package.

MANUSCRIPT II:
To determine the age-related decline in AMH, AFC and ovarian volume logarithmic transformation were applied due to skewed distributions. The transformation implied that the estimated levels of serum-AMH and AFC were expressed as medians, and estimated differences between groups were expressed as relative (i.e. %-wise) differences. In addition, the differences in ovarian reserve parameters between users and non-users of OC were estimated in multiple linear regression analysis which included potential confounders: hormonal contraception, smoking, BMI, preterm birth, prenatal exposure to maternal smoking, and maternal age at menopause.

We imposed a non-inferiority assumption on the intercept of the model to compensate for the possible bias of non-randomly distributed missing data from the youngest participants with mothers experiencing normal to late onset of menopause as described in a previous Manuscript [80].

Non-linear regression models, previously described by Hansen *et al.* [142] and validated by Knowlton *et al.* [143], were applied to estimate the differences in median AMH, AFC and ovarian volume with adjustment for a potentially non-linear age-related decline. The overall fit of the nonlinear models was compared with the corresponding linear fits.

We used bootstrapping to ensure that p-values and 95% confidence intervals obtained from the nonlinear model were valid [144]. Multiple logistic regression with adjustment for age was applied to test whether the risk of having an AMH or AFC <3, 5 or 10 differed between users and non-users of OC. Duration of hormonal contraception was found to be highly collinear with age. Thus to assess a possible effect of duration on AMH, AFC and ovarian volume in OC-users, these were transformed to age-

adjusted Z-scores prior to analysis. We used the group of non-users as reference for computing the Z-scores.

RESULTS

MANUSCRIPT I: Individual fertility assessment and counselling predicts time to pregnancy – a prospective two year follow up study of 519 women

The predictive value of individual fertility assessment and counselling in terms of subsequent time to pregnancy was analysed in 519 women two years after the initial consultation.

The population A was defined as women, who had attempted a pregnancy within the two years of follow-up after their visit to the FAC Clinic. The population B was defined as the remaining women without any attempts to conceive within the two years of follow up. The majority (A: 67.8%, 352/519) had tried to conceive within two years after attending FAC Clinic. At follow up 73.6% (259/352) had achieved a pregnancy, 21% (74/352) were still trying and 5.4% (19/352) had given up. The remaining 167 women (population B) had no attempts to conceive within the two years following initial assessment.

The women in the population A and population B had the same mean age of 35.4 (±4.4) years (P=0.49) and the distribution among age groups was similar (P=0.30). Significantly more women in population A had an earlier or ongoing relationship with longer duration of unprotected intercourse without pregnancy (P<0.001), a moderate weekly alcohol intake than the controls (P=0.02) and a lower stress level (P<0.01). Otherwise, the two populations were similar with regards to; AMH, AFC, cycle length, previous pelvic inflammatory diseases including *CT* infections, endometriosis, previous pelvic surgery, myomas, abdominal fluid, previous chemotherapy, maternal age at menopause, prenatal exposure to maternal smoking, BMI, waist-hip ratio, smoking, coffee consumption and exercise.

Time to pregnancy and risk assessment score

Only three women (1.2%) had entirely green scores, why women with at least one yellow score were analysed as low risk. Two thirds of the women with only low risk scores (green/yellow) (33/51; 64.7%) conceived spontaneously within 12 months, while this figure was 101/194 (52.1%) among the women with a medium score (orange) and only 25/75 (32.5%) for women with at least one high risk score (red). The table below illustrates the reduced chance of achieving spontaneous pregnancy within 12 months with the presence of at least one orange or red score (Table 5).

Table 5: The reduced chance (OR) of achieving a spontaneous pregnancy within 12 months with the presence of at least one orange or red score.

	Univariate		
	OR	95% CI	P values
Risk assessment score			
Low - Green/yellow (categorical)	Reference		
Medium - Orange	0.59	0.31-1.11	0.109
High - Red	0.27	0.13-0.57	0.001*

The figure below displays the cumulated incidences of spontaneous pregnancies over 24 months of follow-up for women in population A grouped according to the estimated score after a consultation at the FAC Clinic.

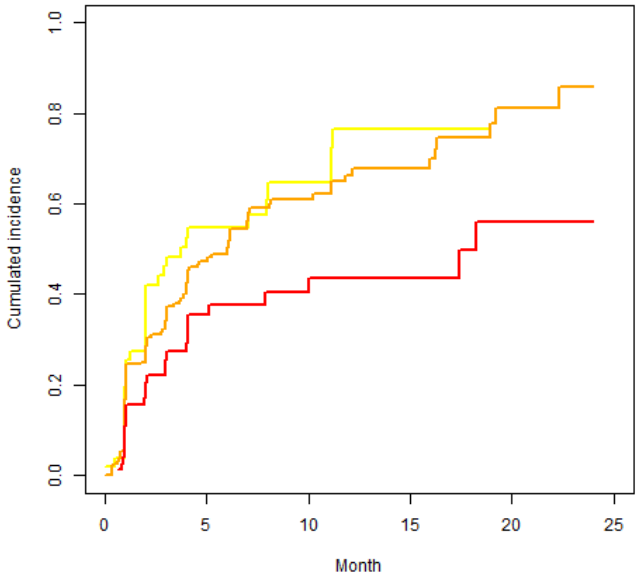

Figure 18: The cumulative incidence curve of spontaneous pregnancies over 24 months of follow-up for women in population A grouped according to the estimated risk assessment score (Yellow: Low risk score, Orange: Medium risk score, Red: High risk score).

Fertility treatment

Almost one third of the pregnancies (83/259; 32%) were achieved by fertility treatment. Intrauterine insemination with husband´s semen (IUI-H) was the most frequently used procedure among the 49 couples (20/49; 40.8%), and insemination with donor semen (IUI-D) among the 34 single women (19/34; 55.9%).

The following predictors displayed a significantly increased incidence of fertility treatment; age 35-39 years (HR 1.66, 95% CI 1.11-2.48, P=0.038) and cycle length < 23 days (HR 2.80, 95% CI 1.23-6.41, P=0.049) in univariate analyses. Also the incidence tended to be increased among women with a coffee intake ≥ six cups per day (HR 2.16, 95% CI 0.99-4.70, P=0.051). Contrarily, women with a weekly alcohol intake of 1-6 units had a decreased incidence of fertility treatment (HR 0.50, 95% CI 0.32-0.78, P=0.008).

MANUSCRIPT II: Ovarian reserve assessment in users of oral contraception seeking fertility advice on their reproductive lifespan

The impact of oral contraception (OC) on the ovarian reserve parameters, defined as AMH, AFC and ovarian volume, was analysed in 887 women seeking fertility assessment and counselling.

Of the 887 women, 244 (27.5%) used OC. The 244 users of OC were significantly younger than non-users with a mean age of 31.5 (SD 4.3) vs. 34.1 (SD 4.3) years (P < 0.001). Overall, and when stratifying by age groups, there was no difference between the two groups in relation to bodyweight, BMI, smoking habits, gestational age at birth, prenatal exposure to maternal smoking or maternal age at menopause. In linear regression analyses adjusted for age, ovarian volume was 50% lower (95% CI 45.1-53.7%), AMH was 19% lower (95% CI 9.1-29.3%), and AFC was 18% lower (95% CI 11.2-24.8%) in OC-users compared to non-users as illustrated in the figure below:

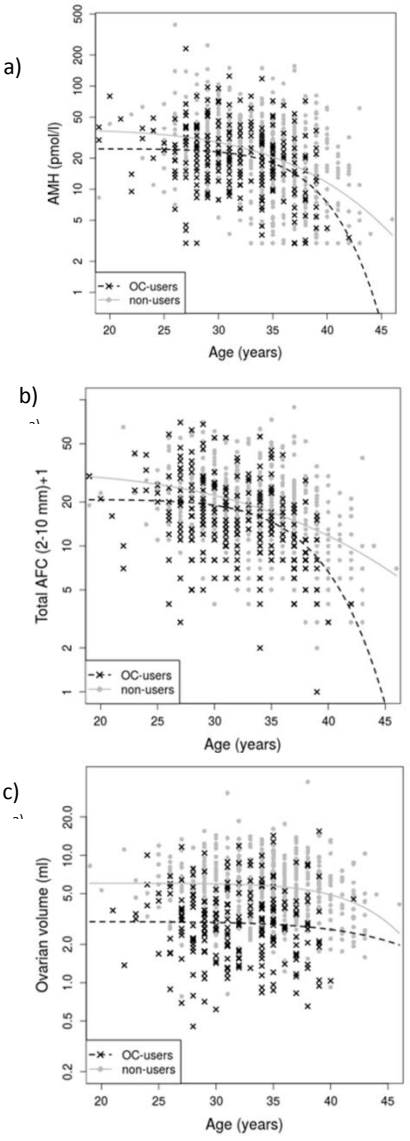

Figure 19: Relation between chronological age and ovarian reserve parameters among hormonal contraceptive users (n=244) compared with non-users (n=643).
(a) Hansen's power model and the non-linear association of age on AMH.
(b) Hansen's power model and the nonlinear association of age on AFC.
(c) Hansen's power model and the non-linear association of age on ovarian volume. Data are displayed in a logarithmic scale.

Anti Müllerian Hormone
We found significantly more women with an AMH < 5 pmol/l in the young age group from 19 to 29.9 years among OC-users than non-users (P=0.044). Yet, only for AMH < 10 pmol/l the negative influence of OC was significant (OR 1.6, 95% CI 1.1-2.4, P=0.03) based on a logistic regression adjusted for age. Similarly, the negative influence of OC on AFC was significant in all three groups: AFC ≤ 3 (OR 3.8, 95% CI 1.1-13.1, P=0.03), AFC < 5 (OR 4.4, 95% CI 1.8-10.5, P=0.001) and AFC < 10 (OR 2.4, 95% CI 1.6-3.6, P=0.0001) based on a logistic regression adjusted for age.

Antral follicle count
Overall, we found a decreasing proportion of the small AFC (2-4mm) with increasing age in both groups. Furthermore, we found a significant decrease in antral follicles sized 5-7 mm (P< 0.001) and antral follicles sized 8-10 mm (P<0.001) but an increase in antral follicles sized 2-4 mm (P=0.008) among OC-users compared to non-users.

Ovarian volume
Stratified by age groups, the significant reduction in the right ovarian volume ranged from 30% (40-46 years) to 50% (30-34.9 years) in OC-users. The reduction in left ovarian volume was likewise significant and ranged from 37% (40-46 years) to 53% (19-29.9 years).

MANUSCRIPT III: Family intentions and personal considerations on postponing childbearing in childless cohabiting and single women aged 35 to 43 seeking fertility assessment and counselling

The study analysed the characteristics of childless women aged 35 years and above seeking fertility assessment and counselling in relation to their reproduction, and whether there were significant differences between single and cohabiting women.

Characteristics, reproductive history, lifestyle and sexual behaviour
The majority of the 340 women (82%) were well educated and in employment. Their mean age was 37.4 years. Nonetheless, the main reasons for attending were to obtain knowledge regarding the possibility of postponing pregnancy (63%) and a concern about their fecundity (52%).
The two groups were comparable regarding BMI, smoking, alcohol consumption, use of antidepressants and a physically active life style.

One fourth of the women had a previous pregnancy (24.7%), but none resulted in a live birth. The majority only had one previous pregnancy, 60% of the cohabiting and 69% of the single women. Over 70% of the women had more than 10 previous sexual partners (cohabiting 71.2% vs. single 71.9%, P=0.142). Likewise, the groups were comparable in relation to previous chlamydia infections (cohabiting 29.9% vs. single 29.1%, P=0.877) and endometriosis (cohabiting 2.1% vs. single 3.5%, P=0.466).

Personal considerations in relation to childbearing
The primary reason for seeking fertility assessment and counselling among the single women was to gain knowledge on how long the women could postpone childbearing (70%). Among the cohabiting women the two main reasons were also to gain knowledge about the possibility of postponing pregnancy (54%) and a check because it was available (56%).

Overall, both groups listed "feeling mature" as the most important prerequisite for childbearing (89%). Significantly more

cohabiting women listed a “stable relationship” (cohabiting 93.7% vs. single 67.0%, P<0.001) and “to have a partner to share the responsibility” (cohabiting 83.5% vs. single 67.0%, P<0.001) as an important prerequisite to childbearing. Besides maturity the most important issues for the single women were “to have a job that can be combined with children” (78%) and access to day care (68%). In general, the women were aware of the declining female fecundity with age as 72% answered correctly when asked about the most fertile age.

Family intentions, desired number of children and ideal maternal age at first birth are illustrated in the table below (Table 6).

Table 6: Family intentions of the cohabiting (n=140) and single women (n=200).

Table V Family intentions of the cohabiting and the single women.

	Total	Cohabiting	Single	*P*-values[†]
Number	340	140	200	
Current desire to have a child				
Yes	173 (51.2)	83 (59.7)	90 (45.2)	0.024*,b
No	57 (16.9)	17 (12.2)	40 (20.1)	
Don't know	108 (32.0)	39 (28.1)	69 (34.7)	
Trying to get pregnant				
Yes	42 (14.7)	38 (30.4)	4 (2.5)	0.001*,b
No	243 (85.3)	87 (69.6)	156 (97.5)	
Ideal/desired number of children				
1	74 (22.2)	25 (18.1)	49 (25.1)	0.396[b]
2	176 (52.9)	81 (58.7)	95 (48.7)	
3	21 (6.3)	8 (5.8)	13 (6.7)	
4	1 (0.3)	0	1 (0.5)	
5	1 (0.3)	1 (0.7)	0	
Don't know	60 (18.0)	23 (16.7)	37 (19.0)	
Ideal maternal age at the birth of first child, mean, SD				
For you	33.2 (4.7)	33.1 (4.4)	33.3 (4.9)	0.750[a]
In general (personal opinion)	28.7 (2.8)	28.8 (2.6)	28.6 (3.0)	0.576[a]
Ideal maternal age at the birth of last child, mean, SD				
For you	39.4 (3.5)	39.3 (3.4)	39.5 (3.5)	0.386[a]
In general (personal opinion)	37.7 (3.8)	37.4 (4.0)	37.9 (3.7)	0.227[a]

Data are *N* (%) unless stated otherwise.
P-values [†]indicates the difference between the cohabiting (*n* = 140) and the single women (*n* = 200).
*Significant *P* < 0.05.
[a]Mann–Whitney *U* test.
[b]Pearson's chi-squared test.

Expected benefits and consequences of motherhood
The most important benefits were “personal development” (89%) and “to give and receive love” (86%). Half of the women considered children “as the meaning of life” and one fourth anticipated that their “everyday life would be better” with children. The main concerns about childbearing were “less time to myself” (82%) and “less time to job and career” (76%).

Attitudes toward fertility treatment, gamete donation and social freezing
Of the single women, 70% would accept use of sperm donation compared to 25% of the cohabiting women (P<0.001). In general, 45% considered oocyte vitrification for social reasons, yet only 15% were positive toward oocyte donation.

MANUSCRIPT IV: Attitudes toward family formation in cohabiting and single childless women in their mid- to late thirties

The study explored attitudes toward family formation in 10 single and 10 cohabiting childless women of advanced age.

The data was comprised in four categories; ‘The biological clock’, ‘The difficult choice’, ‘The dream of the nuclear family’, and ‘Mother without a father’.
The categories were condensed into two subthemes; `Fear´ and `Expectations´ and gathered into one main theme ‘The conflict of choosing’, which reflected the women’s attitudes toward family formation prior to individual fertility counselling as illustrated in the figure (Figure 20).

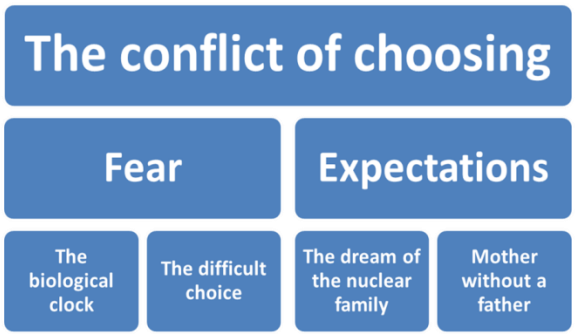

Figure 20: The conflict of choosing – model for analysis.

Fear
The women attended the FAC Clinic due to a concern about their fecundity and the fear of infertility. The women felt their biological clock was ticking. Their intention was to receive qualified advice on their remaining reproductive lifespan. Many expressed a wish to ‘buy more time’ independent of relationship status

Expectations
In general, the women had a dream of meeting “Mr. Right” and creating a nuclear family together.
Finding the right man is difficult and the next step – “is he ready to have children” was an issue. The women were frustrated because they had a feeling that the men were holding them back. Despite the women´s concerns of having children they were more resolved and expressed to be themselves more ‘ready’ than their male partners.

Mother without a father
The single and cohabiting women were ambivalent regarding solo motherhood, i.e., to become a single mother by choice. Several of the single women considered being a solo mother, although it was seen as not “natural” to have children on your own. None of the women considered solo motherhood as first choice but as a backup plan because time was running out.

The single women considered starting their own family without a man, mainly because of the wish to become a mother before it was too late. Being solution-oriented, the women contemplated their Plan B; to have the child before the man. All of the single women wished for a nuclear family in the future but accepted alternative family formations.

DISCUSSION
The FAC Clinic is the first of its kind worldwide. The concept was based on existing knowledge on fertility risk factors in 2011 and inspired by Ferti-STAT [145]. The main aims of this thesis were to; a) test the concept by evaluating the prognostic value of a risk assessment score (manuscript I), b) to quantify and discuss the impact of OC on ovarian reserve parameters in the FAC setting (manuscript II), and c) to explore and understand the concerns and considerations in relation to postponement of parenthood among childless women of advanced age (manuscript III+IV).

The concept of the FAC Clinic and individual fertility assessment is closely related to an estimate of the ovarian reserve. As discussed

in an opinion paper by Tremellen and Savulescu (2014), screening the ovarian reserve should be valued as a scientific and ethical analysis. A consultation at the FAC Clinic not only estimates the current ovarian reserve, but also provides an assessment of reproductive risk factors and important information for the individual in order to fulfil their reproductive life plan.

To evaluate the scientific validity and continuous justification of the FAC Clinic we chose to discuss to concept in accordance to WHO´s ten criteria for an acceptable screening procedure, as no previous studies exist regarding similar concepts. The criteria (I-X) for assessing the adequacy of a screening test were published in 1968 by Wilson and Jungner. These criteria have been discussed in the past 40 years and newer policy tools are now available as a supplement (Ib-Xb)[146].

Criteria I/Ib/IIb: The condition sought should be an important health problem/ The screening programme should respond to a recognized need/ The objectives of screening should be defined at the outset.

The onset of subfertility will begin 10 to 13 years prior to the menopause [13, 14]. Premature menopause will affect 10% of women below the age of 45, 1% of women below the age of 40 years and 0.1% of women below the age of 30 [13]. Accelerated follicle depletion or premature ovarian insufficiency (POI) is a "silent disease" and many women, although familiar with the age-related decline in fecundity, will not be aware of the imminent risk for infertility, when they cease their OC and commence child bringing in their mid to late thirties [147]. Infertility and involuntary childlessness have been associated with serious disorders such as; major depressions and eating disorders [148].

Additionally, 15-25% of women/couples will be infertile due to anovulation, TFI, endometriosis or reduced sperm quality [149, 150]. An individual risk assessment, as discussed in manuscript I and a previous publication by our group [9], would provide women and men useful knowledge of their current reproductive chances, in some cases a direct referral to MAR, and ultimately a chance to fulfil of their reproductive life plan.

Criteria II: There should be an accepted treatment for patients with recognized disease.
Participating in a screening programme will inevitably result in the diagnosis of pathology or low ovarian reserve in some women. Among the 352 women (population A) in manuscript I, 38 women (10.8%) were diagnosed with "major pathology/severe reproductive risk factors". Reproductive threats (hydrosalpinx, endometriosis, fibroids etc.) can be treated by surgical or medical intervention [67, 72]. Although, the effect of surgical intervention needs to be further assessed in RCTs.

The possibilities for women with a diagnosed low ovarian reserve are dependent on age. Young women without a wish to start at family until later in life may choose to cryopreserve their oocytes [151]. Women in their thirties have the opportunity to pursue a spontaneous pregnancy immediately. Should the attempts be unsuccessful, the results of ART in women under 35 years are promising [17, 152]. Single women in their late thirties may choose solo-motherhood by donor insemination, if they perceive their chances of finding a suitable partner in the near future as being sparse [153]. As discussed in manuscript IV, both single and cohabiting women of advanced age considered solo motherhood, but were ambivalent, as it was not perceived as "natural".

Criteria III: Facilities for diagnosis and treatment should be available.
Fertility experts in public clinics provide the FAC Clinic concept. The consultation is free of charge and subsequent referrals, if needed, are handled by the patient's general practitioner. The treatment of health related issues such as endometriosis, fibromas etc. are fully reimbursed by the Danish National Health Program. Childless women below the age of 40 years are entitled to three completed in vitro fertilization (IVF) or intracytoplasmic sperm injection (ICSI) transfer cycles, or oocyte recipient cycles with fresh embryos (only fertilisation with husband´s semen); an unrestricted number of thawed embryo transfer (FET) and three to six cycles of IUI-H or DI, likewise fully reimbursed by the Danish National Health Program [154]. Oocyte freezing is available in public clinics with a medical indication (cancer), whereas oocyte freezing for non-medical indications is available in private clinics.

An important reflection is whether attendance to the FAC Clinic induces premature actions toward cryopreservation or MAR. In our follow up study, one-third (32%) of the 259 reported pregnancies two years after the initial consultation were after fertility treatment (manuscript I). The majority were presumably unavoidable due to subfertility or solo status. Nevertheless, the FAC consultants must be aware of the responsibility and which impact their counselling could have. Hence, cautiousness regarding advices on the need for further actions is required.

Criteria IV: There should be a recognizable latent or early symptomatic stage.
Many gynaecological disorders can be asymptomatic (hydrosalpinx, TFI, endometriomas, uterine malformations/septae, low ovarian reserve). Diminished ovarian reserve can lead to irregular menstrual cycles. Oral contraceptives can "mask" bleeding irregularities and early signs of POI [155]. As 50–89% of women in Western countries use OC at some point in their lifetime and 32% of fertile women are current users in Denmark, many women are simply not aware of the disorder [156, 157]. The FAC screening by vaginal ultrasound (pathology, AFC), AMH, genetic dispositions and previous reproductive history can identify latent or "silent" disorders, otherwise unknown to the woman.

Criteria V/IVb: There should be a suitable test or examination/There should be scientific evidence of sceening programme effectiveness.
AMH and AFC are well-documented non-invasive markers and so far, the best available option to estimate the ovarian reserve in relation to biological age compared to chronological age [1, 158]. The new available AMH assays provide high precision results at increased speed and lower costs [159, 160].

Our study regarding the impact of OCs on ovarian reserve parameters (manuscript II) has shown consistent results with previous studies [25], why we would advocate for the continuous screening of OC-users with the knowledge of a possible decrease of approximately 20%, when estimating AMH and AFC. As far as the scientific evidence of screening programme effectiveness, the prediction of TTP in relation to risk assessment score provided by the follow up study (manuscript I), indicate a valid concept. Albeit, our population of 971 women was a selected group of highly

educated women living in the capital region of Denmark. The concept has to be tested on other populations and preferably other nationalities, before conclusions can be made.

Clinics based on same concept are already established in other university hospitals; Holbæk (DK), Hvidovre (DK), Herlev (DK), Malmö, and Southampton (UK). Initiatives to start up similar concepts are reported from Finland, Norway, Portugal and Spain. The FAC concept can be further improved by adjusting the score sheet to the acquired knowledge of these studies, updated literature on the field and a prolonged follow up.

Criteria VI/VIb/VIIb: The test should be acceptable to the population/There should be quality assurance, with mechanisms to minimize potential risks of screening/The programme should ensure informed choice, confidentiality and respect for autonomy.
There is an increasing interest for the possibility of ovarian screening on an individual level, from the public and health care professionals [155, 160-162]. An American study of 185 health care workers (75% female, 25% male) found that 47% of the females would have an ovarian test performed, if possible [161]. Furthermore, 52% of the males would encourage their female partner to take the test. The participants were asked, how they would respond to the answer of a low ovarian reserve. Almost half (48%) would bring forward the timing of pregnancy and only 14% would take no further action [161]. Another American study of 328 female students found similar results [162]. The far majority (79%) were interested in knowledge regarding their ovarian reserve and 80% would consider having children earlier, if they received unfavourable results [162].

These findings are in line with the results of manuscript I and manuscript III. The far majority of the cohort in manuscript I believed, that there was a need for more information on risk of age-related infertility (67.5%) and risk factors for infertility in general (81.2%). All of the women would recommend the FAC Clinic to others (99.3%). Similarly, more than half (53%) of the women included in manuscript III would bring forward the timing of pregnancy after the consultation at the FAC Clinic, regardless of ovarian reserve outcomes. Hence, we believe that the FAC concept is acceptable to the population.

To minimize the risk of screen failure in OC users, we offer a control vaginal ultrasound and AMH measurement in cases, where OC-users receive a low AFC and/or AMH answer (<10 pmol/l). We advise the women to stop the OC use for three months, before they attend the clinic again. As discussed in manuscript II, there is limited knowledge regarding the biological impact of OC in terms of; the variability of suppression among women (age, AMH, AFC, ovarian volume), the short term and long-term consequences of OC. As a further elaboration of our results in manuscript II, we have started a new PhD project, which will explore the physiological and biological changes of hormonal status including AMH, and alterations in ultrasound imaging immediately after pill termination with short intervals until six months after cessation.

Women and men that attend the FAC Clinic are informed of the concept as a research project. At the consultation it is underlined, that we do not provide a specific prognosis of their reproductive capacity, but a current and cautious estimate [9]. Additionally, the consultation is performed in an objective and educational manner, providing only facts and information with respect for the participants´ autonomy.

Criteria VII: The natural history of the condition, including development from latent to declared disease, should be adequately understood.
The age-related decrease in the female follicle pool and oocyte quality is well-established [10]. The decrease is associated to monthly fecundity as previously mentioned in the background section of ovarian ageing. It is important to emphasize the variance among women and the discrepancy between biological and chronological age regarding the ovarian reserve, which may be caused by genetic factors, intrauterine exposure or smoking [13, 14].

Criteria VIII/IIIb/VIIIb: There should be an agreed policy on whom to treat as patients/There should be a defined target population/The programme should promote equity and access to screening for the entire target population.
The target population is women of reproductive age with no current history of infertility, as the overall aim is counselling regarding reproductive status. Women who have tried to conceive for more than a year are in need of fertility treatment instead of counselling.

To promote equity and access for all, consultations are based on self-referral and free of charge.
Defining whom to treat as patients would necessarily be based on an individual assessment due to the complexity and difference in women´s wish for child bringing. As discussed in manuscript IV, women in similar age groups and even relationship status have diverse considerations and attitudes toward family formation.

A single woman in her late thirties with a reduced ovarian reserve are not necessarily prone to choose IUI-D as her first choice, although it may be her best chance to become a mother [137, 163]. Similarly, cohabiting women in their twenties facing POI do not necessarily wish to conceive with their current partner or due to educational or professional pursuits, and may choose to cryopreserve her oocytes which could diminish her chances for child bringing [162, 164]. Therefore, as previously mentioned fertility assessment and counselling should be cautiously performed by fertility experts with the aim of non-judgemental guidance based on the recognition of the weaknesses of the diagnostic tests and lack of knowledge about long-term prognoses.

Criteria IX/Vb/Xb: The cost of case-finding (including diagnosis and treatment of patients diagnosed) should be economically balanced in relation to possible expenditure on medical care as a whole/The programme should integrate education, testing, clinical services and programme management/The overall benefits of screening should outweigh the harm.
It may be controversial, whether the FAC Clinic should be financed by public funds (government or health insurance) or by the individual in private clinics. Both options imply pros and cons. Public funding allows equal access for all, but raises the question of distributive justice in the allocation of limited medical resources [160]. Individual funding narrows the accessibility, but significantly reduces the resources for other competing medical needs. The counselling in public clinics may be perceived as more neutral, as there is no possibility for direct referral to MAR at present. Private clinics could be perceived as recruiting their own

patients. On the other hand, the absence of direct referrals can cause delays in fertility treatment and surgery.

When considering financial concerns and subsequent effect of postponement of parenthood, the consequences of infertility and involuntary childlessness on a personal level, for the closest relatives and society must be contemplated. As previously mentioned, infertility can cause anxiety with the risk of long-lasting psychological distress increasing the risk for depression and sick leave [134]. Additionally, similar psychological strains are associated with fertility treatment as well as the issue with time consuming treatments, possible conflicts with employers due to absence from work, and for some the difficulties in keeping the treatment a secret [165].

Other considerations regarding the consequences of postponement of parenthood are; i) the declining TFR, which is now below replacement level (Statistics Denmark), ii) the increased risk of adverse reproductive outcomes with higher maternal age, and iii) smaller labour forces and a greater burden on those in employment [4, 166].

The FAC Clinic concept has provided many opportunities for educational aspects. Ministries, Politicians, The Danish Family Planning Association, continuous media coverage, and a dedicated web page (fertilitetsraadgivning.dk) have made it possible to achieve attention from the public.

The introduction of a new screening procedure will inevitably induce the risk of false positive or false negative findings, as discussed in manuscript III and a previous publication from our group [9]. A presumed low ovarian reserve can cause concerns and influence the personal decision-making regarding actions toward motherhood. Contrarily, false 'negative' findings can induce a feeling of security and encourage women to postpone pregnancy even longer with the risk of future infertility and involuntary childlessness.

Criteria X/IX: Case-finding should be a continuing process and not a "once and for all" project/Programme evaluation should be planned from the outset.
We suggest that attendance to the FAC Clinic should be available for all men and women of reproductive age with the acceptance of women/couples with unsuccessful attempt to conceive for more than 12 months. Attendance should be voluntarily, based on the individual´s concern, and need for assessment. Limiting the access by age or oral contraceptive use can cause unnecessary anxiety, the risk of missing the young women with imminent or manifest POI, or denying fertility assessment and counselling to one-third of women of reproductive age.
The FAC Clinic concept needs to be continuously evaluated by long-term follow up studies in order to maintain a scientific valid screening option.

ALTERNATIVES
With the increasing female age at first birth, many commercial interests in ovarian screening and cryopreservation of oocytes have emerged in recent years. We are beyond the point of no return regarding the demand for ovarian reserve screening [160, 161, 167]. A large private clinic in Valencia has introduced free AMH analyses for all female inhabitants (personal information, Bosch E).

The internet provides numerous possibilities for "do-it-yourself-AMH-kit". Moreover, dedicated clinics for oocyte cryopreservation advertise: "retrieve, freeze, relax". New technologies have changed the possibilities for controlled family planning and delaying motherhood over a very short time frame. Oocyte preservation was considered as experimental in the UK and USA until 2012 [168]. Four years after it is an easy accessible procedure, even provided to their female employees by companies such as Apple and Facebook. However, the success of egg freezing is highly dependent on the woman´s age and ovarian reserve, and the cost is prohibitive for many individuals [169].

Another possibility is web-based self-assessments as provided by the Bunting and Boivin group: Ferti-STAT [145]. By answering questions regarding lifestyle, reproductive history and current attempt of pregnancy, a personalised risk score is calculated; blue (low), yellow (low to medium), orange (medium), red (high). The answer can enable women to gain
personalised guidance about reducing risks to their fertility and seeking timely fertility medical advice based on their own lifestyle and reproductive profile [145].

The major advantages of the Ferti-STAT are the low cost, easy accessibility and immediate response. A limitation is the lack of vaginal ultrasound and ovarian reserve parameters, which in some cases would be necessary to diagnose the "silent reproductive threats". Indeed, we found major pelvic pathology in a number of women (10.8%) and the presence of a specialist in reproductive medicine could grade the importance of such findings. Still there may be a benefit from easily accessible self-tests, but such test system should also be validated through long –term follow-up.

Other initiatives have been made to increase fertility awareness among women, as discussed in manuscript I [6, 170, 171]. Overall, the studies display that web-based interventions only have transient impact on knowledge and subsequent actions, whereas counselling on the individual level, such as the RLP tool and tailored education, showed significantly increased knowledge levels of reproduction [6, 170, 171].

LIMITATIONS OF OWN STUDIES
There are several potential limitations to our studies. Firstly, the non-randomised design and the lack of comparison with a control group make it difficult to evaluate the implications of the consultation at the FAC Clinic as an intervention. This could reduce the strength of our results, as the women may be more prone to bring forward the timing of pregnancy after the consultation. Furthermore, the women attended the FAC Clinic with a concern of their reproductive status, which could imply a selection bias.

Secondly, the homogeneity of the included women and relatively short period of follow up could weaken the prognostic values of the individual fertility risk factors in the risk assessment score sheet. This may explain why we found an association between the cumulative risk factors and TTP, whereas the individual continuous and categorical variables primarily indicated tendencies or no associations (manuscript I). Moreover, data regarding the partner´s sperm quality and considerations regarding parenthood, would have been valuable information in the TTP analyses (manuscript I) and provided further understanding of the women´s

considerations (manuscript III and IV), which may have strengthened our results.

Lastly, although the measurement of AMH has proven its value in terms of ovarian screening, uncertainties regarding other influential factors (OC use, BMI, smoking), and a possible intra-individual and circadian variability, must be considered when counselling women on their reproductive lifespan based on just one blood sample (manuscript I, II and III).

CONCLUSION

- The FAC Clinic concept seems usable and offers a tool for fertility experts, but need continuous validation and long term follow up, before conclusions regarding valid predictions of future fertility can be made.
- In terms of attendances, the FAC Clinic has been very successful and more than 2,000 individuals have now consulted the clinic, documenting the wish of men and women to protect fertility and be able to make the best possible reproductive choices.
- The FAC Clinic concept is in accordance with WHO´s original published, as well as the supplementary, criteria for an acceptable screening procedure.
- The FAC Clinic will never be able to outweigh the need for fertility treatment, but can work as a screening method for men and women of reproductive age with the aim of fulfilling their reproductive life plan.
- Physicians performing ovarian screening in oral contraceptive users should be aware of the hormonal impact on the ovarian reserve parameters, as well as the variability among women of similar biological age, and the risk of a latent or manifest POI concealed by OC.
- Childless women of advanced age consult the FAC Clinic to gain knowledge on how long they can postpone child bringing, despite awareness of the age-related decline in fertility.
- Our results indicated that the childless women aged 35-43, who consulted the FAC Clinic, overestimated their own reproductive ability and underestimated the risk of future infertility by postponing their pregnancies further.
- Women of advanced age are increasingly aware of single motherhood as a possible solution to family formation, but were ambivalent, as it was not regarded as “natural”.

FUTURE PERSPECTIVES

During the last five years, almost 1,600 women and 700 men have attended the FAC Clinic.
The reasons for attending the clinic differentiate between gender and relationship status [9, 172]. Within the next years, the risk assessment score sheet for men will be evaluated in relation to male fertility risk factors and sperm quality. Additionally, more knowledge is needed of the interrelation and dynamics between men and women in the FAC setting, and reasons for subsequent actions or counteractions toward parenthood. The FAC Clinic registration of couples provides the opportunity to link and analyse these circumstances.
The prognostic validity of the risk assessment score for both men and women will be further evaluated through a five-year follow up planned to be initiated in August 2016. As previously mentioned, a new PhD project began in February 2016 regarding OC´s impact on ovarian reserve parameters during use and after cessation.
Due to the initiation of similar clinics and ReproUnion, we will be able to compare the FAC Clinic concept both nationally and internationally within a few years. These collaborations imply promising possibilities for future projects on a larger scale.

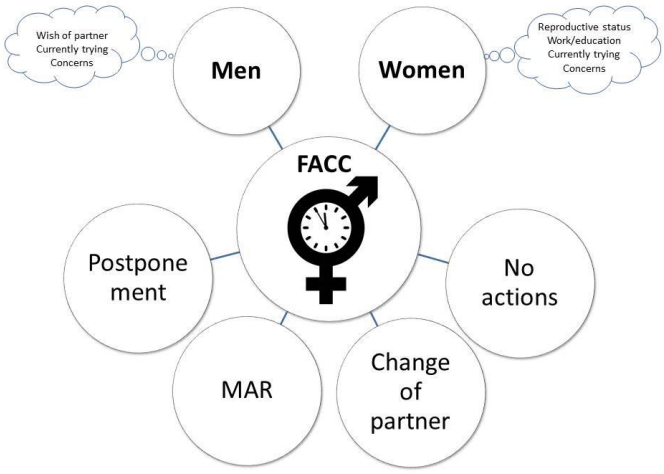

Figure 21: Men and women´s motivation for seeking counselling at the FAC Clinic and possible outcomes [9, 172]. FACC: FAC Clinic.

SUMMARY

The overall aim of this thesis was to validate the new concept of the Fertility Assessment and Counselling Clinic at Rigshospitalet. The intention was to; explore the prognostic value of fertility risk factors by a risk score and provide an estimate of female fecundity, to quantify the impact of oral contraception (OC) on ovarian reserve parameters defined as Anti Müllerian Hormone (AMH), Antral Follicle Count (AFC) and ovarian volume, and to gain knowledge of attitudes and considerations toward family formation in women of advanced age.

The thesis is based on the following four manuscripts:

Manuscript I describes the predictive value of individual fertility assessment and counselling in terms of subsequent time to pregnancy within two years after the initial consultation at the FAC Clinic. The follow up study comprised 519 women, of which 352 had tried to conceive.
At the time of follow up, 259/352 had achieved a pregnancy, 74/352 were still trying and 19/352 had given up. The remaining 167 women had no attempts to conceive.

The risk assessment provided a score based on the appearance of fertility risk factors: green (low), yellow (low), orange (medium) and red (high). Two-thirds of the women with only low risk scores conceived spontaneously within 12 months (65%), while this figure was only 32% for women with at least one high risk score (n=82). Accordingly, presence of at least one high risk score reduced the odds of achieving a pregnancy within 12 months by 73% (OR 0.27, 95%CI 0.13-0.57).

The FAC Clinic concept seems as a usable tool for fertility experts to guide women on how to fulfil their reproductive life-plan, but longer follow up studies are needed.

Manuscript II describes the impact of OC on ovarian reserve parameters in 887 women at the FAC Clinic. Of the 887 women, 244 (27.5%) used OC. The 244 users of OC were significantly younger than non-users with a mean age of 31.5 (SD 4.3) vs. 34.1 (SD 4.3) years ($P < 0.001$). Overall, there was no difference between the two groups in relation to bodyweight, BMI, smoking habits, gestational age at birth, prenatal exposure to maternal smoking or maternal age at menopause. In linear regression analyses adjusted for age, ovarian volume was 50% lower, AMH was 19% lower, and AFC was 18% lower in OC-users compared to non-users. Among the OC users there was a significant decrease in antral follicles sized 5-7 and 8-10 mm and an increase in the number of small follicles sized 2-4 mm. Physicians have to be aware of the impact of OC use on ovarian reserve parameters and possible concealment of premature ovarian insufficiency, when assessing the fertility status and estimating the reproductive lifespan in OC users.

Manuscript III describes the family intentions and personal considerations on postponing childbearing in 340 childless women of advanced age. The study comprised 140 cohabiting and 200 single women aged 35-43 seeking fertility assessment and counselling at the FAC Clinic. The majority (82%) was well-educated and in employment. Despite their mean age of 37.4 years, the main reasons for attending the FAC Clinic were to gain knowledge on the possibility of postponing pregnancy (63%) and due to a concern about their fecundity (52%). Both the cohabiting and single women expressed a wish for two or more children (60%).

The most important benefits were “personal development” (89%) and “to give and receive love” (86%). The main concerns about childbearing were “less time to myself” (82%) and “less time to job and career” (76%). The single women were more positive regarding the use of donor sperm (70%) compared to the cohabiting women (25%).

Our results indicated a general overestimation of the women´s own reproductive capacity and an underestimation of their risk of future infertility and childlessness with continuous postponement of pregnancies.

Manuscript IV describes attitudes toward family formation in 10 single and 10 cohabiting childless women of advanced age. The women were interviewed one week before their consultation at the FAC Clinic about their family formation intentions, considerations and concerns. The interviews were analysed and condensed into four categories: ; ‘The biological clock’, ‘The difficult choice’, ‘The dream of the nuclear family’, and ‘Mother without a father’. The categories were condensed into two subthemes; `Fear´ and `Expectations´ and gathered into one main theme ‘The conflict of choosing’, which reflected the women’s attitudes toward family formation prior to individual fertility counselling. The women attended the FAC Clinic due to a concern about their fecundity and a fear for infertility. Overall, the women expressed a dream of the nuclear family and finding “Mr. Right” and many with the wish of buying more time. Both groups would consider solo motherhood due to their advanced age, although it was considered to be Plan B, as it was not “natural”

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