From Department of Oncology-Pathology

Karolinska Institutet, Stockholm, Sweden

# EFFECTS OF OVARIAN SURGERY ON OVARIAN RESERVE AND FERTILITY

Tekla Lind


Karolinska Institutet

Stockholm 2015

Published by Karolinska Institutet.

Printed by E-PRINT



ISBN 978-91 -7549 -813 -3

# Effect of ovarian surgery on ovarian reserve and fertility

## THESIS FOR DOCTORAL DEGREE (Ph.D.)

To be publicly defended at Aulan Södersjukhuset.

Friday 27th March 2015, 9.00 am

By


**Tekla Lind**


*Principal Supervisor:*

Kenny Rodriguez-Wallberg, MD PhD Associate Professor
Karolinska Institutet
Department of Oncology- Pathology
Karolinska University Hospital, Obstetrics & Gynecology and Reproductive Medicine

*Co-supervisor(s):*

Margareta Hammarström, MD PhD Professor
Karolinska Institutet
Department of Clinical Science and Education, Södersjukhuset,
Division of Obstetrics and Gynecology

Claudia Lampic, PhD Associate Professor
Karolinska Institutet
Department of Neurobiology, Care sciences and Society,
Division of Nursing

Jan I. Olofsson, MD PhD Associate Professor
Karolinska University Hospital
Reproductive Medicine
Department of Obstetrics and Gynecology

*Opponent:*

Torbjörn Hillensjö, MD PhD Associate Professor
Sahlgrenska Akademin
Department of Clinical Sciences
Division of Obstetrics and Gynecology

*Examination Board:*

Matts Olovsson, MD PhD Professor
Uppsala University
Department of Women and Children's Health
Division of Obstetrics and Gynecology

Britt-Marie Landgren, MD PhD Professor Emerita
Karolinska Institutet
Department of Clinical Science, Intervention and Technology CLINTEC
Division of Obstetrics and Gynecology

Britt Friberg, MD PhD Associate Professor
Lunds University
Department of Clinical Sciences, Malmö
Division of Reproductive Medicine

*To the women who participated in these studies*

# ABSTRACT

The objective of this thesis was to investigate short- and long-term effects of ovarian surgery on the ovarian reserve of women of reproductive age. This research also considered their reproductive outcomes with regard to conceiving spontaneously or using assisted reproductive techniques (ART). We also wished to explore how women perceived the information they received about fertility when planning for ovarian surgery.

In paper I, a cross-sectional study that included 106 women, we investigated patients' perceptions of the fertility-related information they received as well as fertility distress prior to ovarian cyst surgery. Although a high proportion of the women (72 %) desired to have children in the future, only half recalled receiving information about the potential impact of surgery on fertility. In a multivariable logistic regression, desire for children was the only association with recalled fertility-related information. Additionally, more than half of the women (54 %) reported fertility distress with VAS-scores ≥ 5 prior to surgery.

Paper II involves a prospective clinical cohort study that included 75 women planned for conservative ovarian cyst surgery (cystectomy, ovarian resection or unilateral oophorectomy). We investigated the effects of removed cysts' histopathology on women's ovarian reserve, estimated as serum concentrations of Anti-Mullerian Hormone, AMH. The AMH levels declined significantly from presurgery levels of 2.7 μg/L (0.2-16.9) to 1.6 μg/L (0.2-9.9) at three months, and the levels remained low at six months, 1.6 μg/L (0.2-8.3) ($p<0.001$, respectively). In patients who had a single cyst enucleation, a reduction was observed after surgery for both endometriomas and dermoid cysts. In a logistic regression, the only predictive factor found for the decline of AMH serum concentrations was a higher baseline AMH level prior to surgery.

In paper III, we prospectively investigated the changes in AMH after a long-term post-operative follow-up of two years in 66 of the women included in the clinical cohort study. The progressive post-operative decline of serum AMH concentrations continued over time. The AMH serum concentration significantly reduced from 2.7 μg/L at baseline to 2.0 μg/L at six months. We observed a further reduction to 1.0 μg/L at the two-year follow-up ($p$ = 0.001, respectively). Nonetheless, women who attempted pregnancy succeeded in 58 % of the cases. Women with normal or high AMH at baseline had a higher chance of conceiving, regardless of their AMH reduction over time.

In paper IV, we examined the clinical pregnancy rate after Assisted Reproductive Technology (ART) in a cohort of 76 women with a history of unilateral oophorectomy (UO), as compared to 12879 controls. Exposed women with UO and unexposed controls were included at three ART Swedish clinics. In this multicentre study, single embryo transfer was performed in 77 % of cases; analysis showed a significant reduction of 30 % in the clinical pregnancy rate of women with UO compared to controls, both crude and after adjustment for age.

**Conclusion:** The studies included in this thesis indicate that the ovarian reserve, estimated by its biochemical marker AMH, decreases significantly after conservative ovarian surgery for cysts in women of fertile age. The post-operative AMH decrease continues two years following the ovarian surgery, even though no macroscopic ovarian tissue was reported as being removed at the time of surgery. The reduction in AMH does not seem to affect fertility in women presenting with normal or high AMH serum concentrations prior to ovarian surgery. On the other hand, women of fertile age who had a previous surgical removal of one ovary had reduced chances of pregnancy, estimated as reduced clinical pregnancy rate after ART. Women require this important information in order to make decisions regarding their reproductive ability; however, our research evidenced that women were not properly informed of the potentially negative effects of ovarian surgery on reproduction.

# LIST OF SCIENTIFIC PAPERS

I. Lind, T, Lampic C, Hammarström M, Rodriguez-Wallberg K. "Young women's perceptions of fertility-related information and fertility distress before surgery for ovarian cysts." Acta Obstet Gynecol Scand. 2013: 92(11): 1290-1296.

II. Lind T, Hammarström M, Lampic C, Rodriguez-Wallberg K. "Anti-Müllerian hormone reduction after ovarian cyst surgery is dependent on the histological cyst type and preoperative anti-Müllerian hormone levels." Acta Obstet Gynecol Scand. 2015: 94; 183-190.

III. Lind T, Hammarström M, Lampic C, Olofsson JI, Rodriguez-Wallberg K. "AMH reduction following ovarian cyst surgery does not reduce fecundity in women with normal/high AMH."
Submitted.

IV. Lind T, Holte J, Olofsson JI, Hadziosmanovic J, Berglund L, Gudmundsson J, Rodriguez-Wallberg K. "Reduced pregnancy rates after IVF/ICSI in women with a history of unilateral oophorectomy. Results of a multi-centre cohort study."
Submitted.

# CONTENTS

# LIST OF ABBREVIATIONS

| | |
|---|---|
| AFC | Antral Follicle Counts |
| AMH | Anti-Müllerian Hormone |
| ART | Assisted Reproductive Technologies |
| Cd | Cycle day |
| CPR | Clinical Pregnancy Rate |
| CV | Coefficients of Variability |
| ET | Embryo Transfer |
| FPI | Fertility Problem Inventory |
| FSH | Follicle Stimulating Hormone |
| hCG | Human Chorionic Gonadotropin |
| hMG | Human Menopausal Gonadotropin |
| ICSI | Intracytoplasmic Sperm Injection |
| IMC | Integrated Morphology Cleavage Embryo Score |
| IVF | In Vitro Fertilization |
| LBR | Life Birth Rate |
| LH | Luteinising Hormone |
| MRI | Magnetic Resonance Imaging |
| OHSS | Ovarian Hyperstimulation Syndrome |
| OPU | Oocyte Pick-up |
| OR | Ovarian Reserve |
| ORT (s) | Ovarian Reserve Test |
| OSI | Ovarian Sensitivity Index |
| PR | Pregnancy Rate |
| VAS | Visual Analogue Scale |

# 1 INTRODUCTION

Fertility and childbearing are important aspects of most women's lives. Women today often delay childbearing to an age where the risk of infertility increases (1); therefore, it is critically important that healthcare providers give accurate and individualized information regarding each woman's fecundity. When a woman is considering ovarian surgery due to benign cysts, medical professionals should provide adequate information regarding the effect of surgery on ovarian reserve. Ovarian reserve is defined as the quantity and quality of the oocytes, and it greatly impacts a woman's ability to conceive. Over the last ten years, estimation of AMH serum concentrations has become an easily accessible hormone test evaluating the ovarian reserve, and it is regarded as one of the most reliable tests for this measure. With increasing age, both the quantity and quality of the ovarian follicle pool diminish, and AMH consequently decreases (2).

When this research project started in 2010, little was known about the effects of ovarian surgery on female fertility. Since then, the number of publications has substantially increased, indicating interest in this research area. The literature suggests that a reduction of fertility might occur after ovarian surgery for ovarian cysts. AMH measurements have spread and become easier, replacing earlier and less specific hormonal tests. Today, AMH is used for many purposes, including predicting and evaluating the chances of success in fertility treatments. Clearly, AMH testing has gained a place among reproductive medicine clinicians.

My interest in fertility and pregnancy emerged during my specialist training. I, like most gynaecologists, have performed minimally invasive surgery to remove ovarian cysts in young women. During these interventions, important questions emerged: what effects would this surgery have on the future fertility of patients and how could we provide adequate information when so little was known? During the development of this project, I recognized a special interest in women with a history of unilateral oophorectomy (UO), and I wished to investigate their chances of pregnancy. What information did these women need to make an informed decision? The studies designed for this project aimed to increase knowledge regarding the effects of ovarian surgery on ovarian reserve and fertility. If we could learn more about this topic, we could provide more accurate information to women in gynaecological care, better meeting their needs.

# 2 BACKGROUND

## 2.1 FERTILITY

Most people regard becoming a parent as highly important in their lives (3, 4). As social and economic development has increased during the last century, there has been a substantial decline in fertility rates (5). Meanwhile, age is the single most important factor affecting women's fertility. In western countries, women tend to postpone their childbearing period to a time when age-related infertility increases. This decision increases the probability of involuntary childlessness and/or smaller families. Over the last 30 years, the median age of women at their first birth in Sweden increased from 24 to 29 years (6). A similar trend has been noted all over Europe, where data show an increase in median age at first birth from 24.4 to 25.9 years of age from 1990 to 2003 (7).

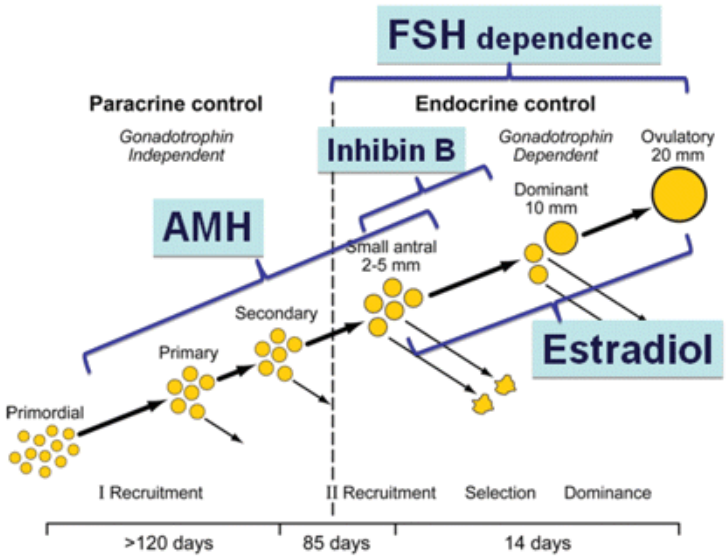
Female fertility is multifactorial, with quality and quantity of oocytes being the most important factor (1, 8). Other factors of importance are a normal uterine anatomy, a thick endometrium at time of implantation and permeable tubes. The infertility rate – defined as more than 12 months without clinical pregnancy – increased from 8 % among women aged 19-26 years up to 18 % among women aged 35-39 years old (9). Sexually-transmitted diseases, such as chlamydia, also negatively impact fertility by reducing the tubal permeability and by increasing the risk of extrauterine pregnancy (10). With increasing age, there is a higher risk of comorbidity of benign diseases, such as endometriosis or myomas, which affect women's fecundity. Endometriosis can cause infertility in 30-50 % of women through several proposed factors, such as adhesions, distorted pelvic anatomy and oocyte quality (11). Finally, the risk of cancer increases with age, and its treatment can cause infertility (12).

## 2.2 OOCYTE DEVELOPMENT

Oocytes are the reproductive female cells, colloquially known as eggs. The follicles include both the oocytes, contained in fluid, and the surrounding cell layers. The follicle fluid is rich in hormones and growth factors. A pre-granulosa cell layer surrounds the primordial follicle. In oocyte development, the primordial germ cells migrate from the yolk sac to the gonadal ridge, where the ovaries form. Gestational week 16 sees the formation of clusters of cells, known as primordial follicles. These follicles consist of the oogonia, developed from germ cells. After meiosis, the oogonia develop into primary oocytes during foetal life. When a female child is born, she has a follicle population consisting of 200000- 1milj follicles (13, 14). There is a gradual loss of follicles both before and after puberty. At puberty, the recruitment and maturation of oocytes begin. There is a transition from primordial follicles to

primary follicles, and thereafter, to secondary follicles and ovulation. The one follicle that escapes atresia will ovulate. Development from a primordial follicle to the ovulatory stage require several months (folliculogenesis) (15). The transition process from primordial follicles to primary follicles is not fully understood, but at least part of the process is controlled by growth factors. One group of growth factors is the transforming growth factor B (TGF-B) family. Secreted from the primary, secondary and small antral follicles, the Anti-Müllerian Hormone, AMH, is a glycoprotein that is part of this TGF-B family. A gonadotropin-independent process stimulates oocyte growth and differentiation to antral follicles, which takes about 70 days.

**Figure 1.** AMH and the folliculogenesis. AMH is produced by the primary, secondary and small antral follicles (16).




Reprinted with the permission of Human Reproduction. La Marca, A., F. J. Broekmans, A. Volpe, B. C. Fauser and N. S. Macklon (2009). "Anti-Mullerian hormone (AMH): what do we still need to know?" Human Reproduction **24**(9): 2264-2275.

### 2.2.1 Development of a mature oocyte to achieve ovulation

The oocyte must undergo developmental competence and maturation to be viable for fertilization. At the pre-antral follicle stage, the zona pellucida forms; thereafter, the process is gonadotropin-dependent. The process includes oocyte enlargement, vascularization and proliferation of granulosa cells and the theca layer. The granulosa cells can synthesize oestrogen when stimulated by follicle stimulating hormone (FSH). Both oestrogen and FSH stimulate the production of follicular fluid, antrum formation and formation of the cumulus oophorus, a granulosa cell layer that surrounds the oocyte. At the end of the luteal phase, just prior to menstruation, there is an increase of FSH, which stimulates the growth of several follicles. As follicles grow, more oestradiol is secreted from them, which has a negative effect

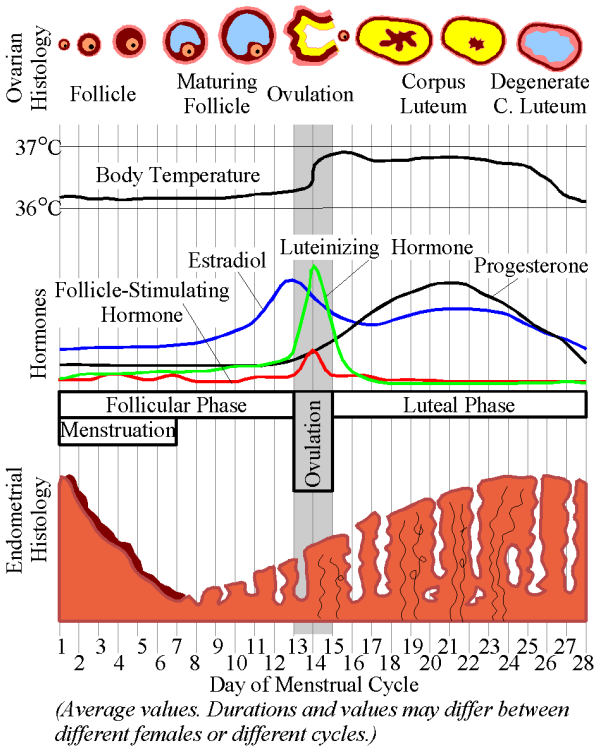
on the hypothalamus and the pituitary gland. This negative feedback inhibits the production of FSH, allowing only the follicle with the most receptors for FSH to dominate; hence, it grows and acquires the ability to ovulate during the luteinising hormone (LH) surge. Ability to convert androgens to oestrogens (aromatization) is crucial in the formation of a dominant follicle. With the influence of LH, the larger follicles are able to better develop and synthesize androgens in their theca cells. Under the influence of FSH, the androgens are aromatized to oestrogens in the proliferating granulosa cells. There is a higher sensitivity in the dominant follicle for FSH due to the increase of granulosa cells and higher FSH density. The negative feedback mechanism makes the smaller follicles even more clearly destined for atresia. The LH surge at ovulation is stimulated by the sustained production of oestrogen from the dominant follicle of about 15-20 mm. Oestrogen stimulates the pituitary gland to release LH. Ovulation can only happen in a mature follicle where the oocyte has undergone the final meiotic division and turned into a mature haploid oocyte with 23 chromosomes. Several hormones, prostaglandins and enzymes drive the process of ovulation. After ovulation, the ruptured dominant follicle forms the corpus luteum. In the process of corpus luteum formation, the theca cells luteinize into theca lutein cells and the granulosa cells into granulosa-lutein cells. Progesterone, synthesized from cholesterol, is secreted from the corpus luteum with the objective of maturing the endometrium, and it eventually sustains early pregnancy. If pregnancy occurs and human chorionic gonadotropin (HCG) is secreted, the corpus luteum will continue to produce progesterone. Otherwise, there will be a regression of the corpus luteum. The luteal phase lasts 14 (± 3) days (17).

### 2.2.2 Menstrual cycle

The menstrual cycle mirrors the ovarian cycle, as hormones produced from the ovaries stimulate and control the endometrium in the uterus. The endometrium is an important factor for implantation to occur. During the follicular phase, oestrogen, which is produced by the ovarian follicle, promotes the proliferation of the endometrium in the uterus. After ovulation, secretory glands develop in the endometrium under the influence of oestrogen and progesterone, which is secreted from the corpus luteum. At the time of implantation, around cycle day 21-22, the endometrium is edematous. In the absence of pregnancy, there is a fall in oestrogen and progesterone, leading to menstrual bleeding. When the menstrual cycle becomes ovulatory after puberty, the cycle tends to have a frequent interval of 28 days (18). The menstrual cycle length successively shortens with aging due to a progressive reduction in the pool of oocytes. The FSH serum concentration starts to increase, a trend that can already be detected in the late luteal phase; therefore, the subsequent follicular phase begins earlier in

the late luteal phase, resulting in a shortening in the cycle (19). In older pre-menopausal women, the menstrual cycle is irregular due to anovulation (20).

**Figure 2.** Schematic picture of the menstrual cycle showing both hormonal changes and development of the follicle and endometrium.

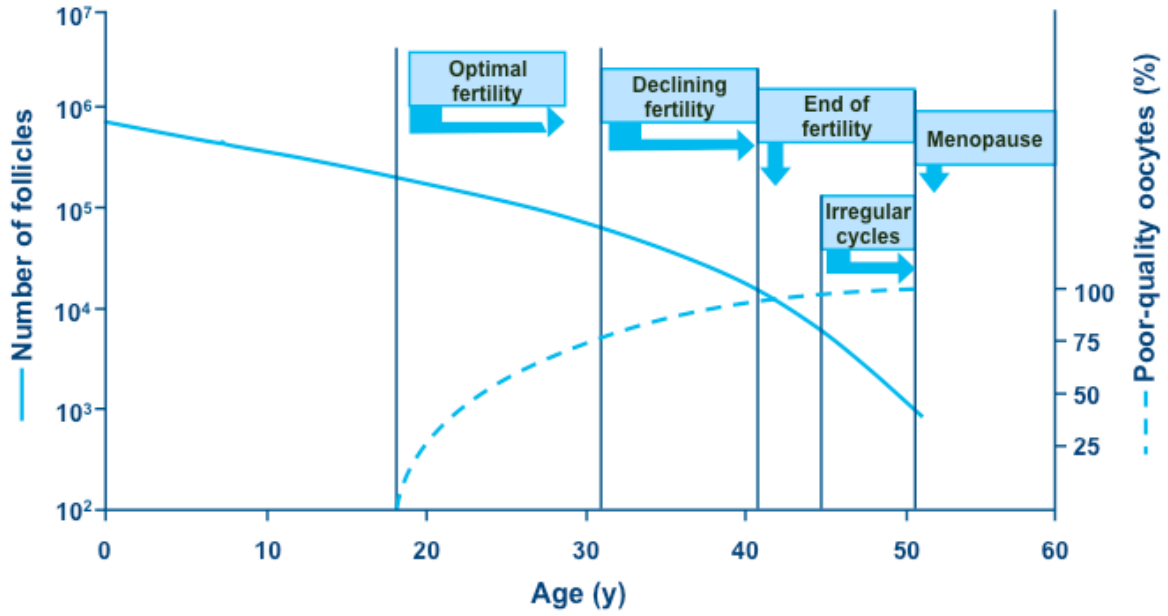



Attribution to Chris 73 / Wikimedia Commons [GFDL 1.3 (www.gnu.org/licenses/fdl-1.3.html) or CC-BY-SA-3.0 (http://creativecommons.org/licenses/by-sa/3.0)], via Wikimedia Commons.

## 2.3 OVARIAN RESERVE

The ovarian reserve represents the ability to conceive based on the quality and quantity of the ovarian follicle pool, which is composed of the small resting oocytes inside the primordial follicles. The ovarian reserve diminishes with age: of the initial 6 - 7 million primordial follicles presenting in the ovaries at the fifth month of foetal life, only 400,000 – 600,000 will remain at menarche. There is an important difference in the starting follicle cohort among women (21). Approximately 400 of the original follicles will ovulate, and the vast majority will undergo atresia by apoptosis (22). When women reach the age of 50 -51, about 1000 follicles remain, and the women reach menopause as a consequence. The median menopausal age in Sweden is 51 years (23). Several ovarian reserve tests have been used to evaluate ovarian reserve and fertility, but all of these tests and estimations remain proxies of the ovarian reserve. The most commonly-used tests include gonadotropins (FSH), AMH and the count of the antral follicles by ultrasound (AFC).

**Figure 3**. The decreasing follicle pool, increasing poor quality oocytes and its corresponding reproductive life events.




## 2.4 ASSESSMENTS OF OVARIAN RESERVE

Both clinical and biochemical markers are currently used to assess the female ovarian reserve.

### 2.4.1 Menstrual cycle length

The menstrual cycle changes over the course of the reproductive period. Research shows that the menstrual interval will shorten by two days between 20 and 40 years of age (18, 24). The length of the cycle has also been associated with fecundity. A cycle length of 30 - 31 days precedes the highest rate of fecundity (25). A study shows that live birth rates decrease for every menstrual length lower than 34 days in women undergoing IVF/ICSI treatment (26). Even though a shortening cycle length may indicate diminishing ovarian reserve, cycle length is not commonly used as an ovarian reserve test.

### 2.4.2 Follicle-Stimulating Hormone (FSH)

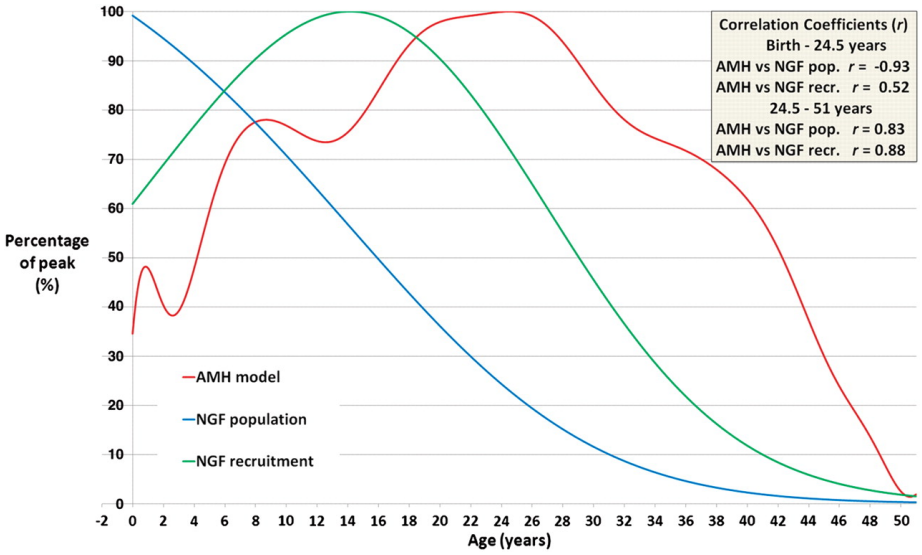
The pituitary gland secretes FSH, with the primary function of stimulating follicular growth. FSH levels are controlled by feedback mechanisms: the ovaries secrete oestrogen and regulatory peptides (Inhibin A and B) that control the amount of FSH secreted by the pituitary gland. When the oestrogen and inhibin levels are low, there is increased FSH secretion. In the menopausal transition, the FSH increases as fewer follicles are recruited every month, resulting in lower levels of oestrogen being secreted and a less efficient

negative feedback mechanism (27). The opposite occurs when there are high concentrations of oestrogen and inhibin (28). Serum FSH is measured early in the follicular phase to evaluate the ovarian reserve, usually on menstrual cycle day -3 when oestrogen levels are normally low. Elevated FSH is a late sign of decreased ovarian reserve and menopausal initiation.

### 2.4.3 Anti-Müllerian Hormone (AMH)

Research showing that the ovaries express this glycoprotein began 30 years ago (29), and there has been growing interest in the use of AMH over the last decade. The production of AMH takes place in the granulosa cells (30). Granulosa cells in secondary, preantral and small antral follicles up to 6 mm of size express the highest amount of AMH (31).

**Figure 4**. Comparison of AMH concentrations with non-growing follicles (NGF) population and with NGF recruitment. The red line illustrates log-unadjusted validated AMH, which peaks at 24.5 years. The blue line shows the decline of the NGF population, peaking at 18-22 weeks of gestation. The green line represents the number of NGFs recruited towards maturation, peaking at 14.2 years (14).




Reprinted with the permission of PLoS One. Kelsey, T. W., P. Wright, S. M. Nelson, R. A. Anderson and W. H. B. Wallace (2011). "A Validated Model of Serum Anti-Mullerian Hormone from Conception to Menopause." PLoS One 6(7).

Initially, researchers hoped that the AMH test would be able to predict the length of women's fertility period. Normograms of AMH levels at different ages for both infertile and fertile patients have been created (32, 33). Bentzen et al, has demonstrated a 5.6% yearly decline of AMH (34). In another study by La Marca et al, fertile women demonstrated a median yearly AMH decline of about 0.16 µg/L (35). There is a peak of AMH concentration in serum at

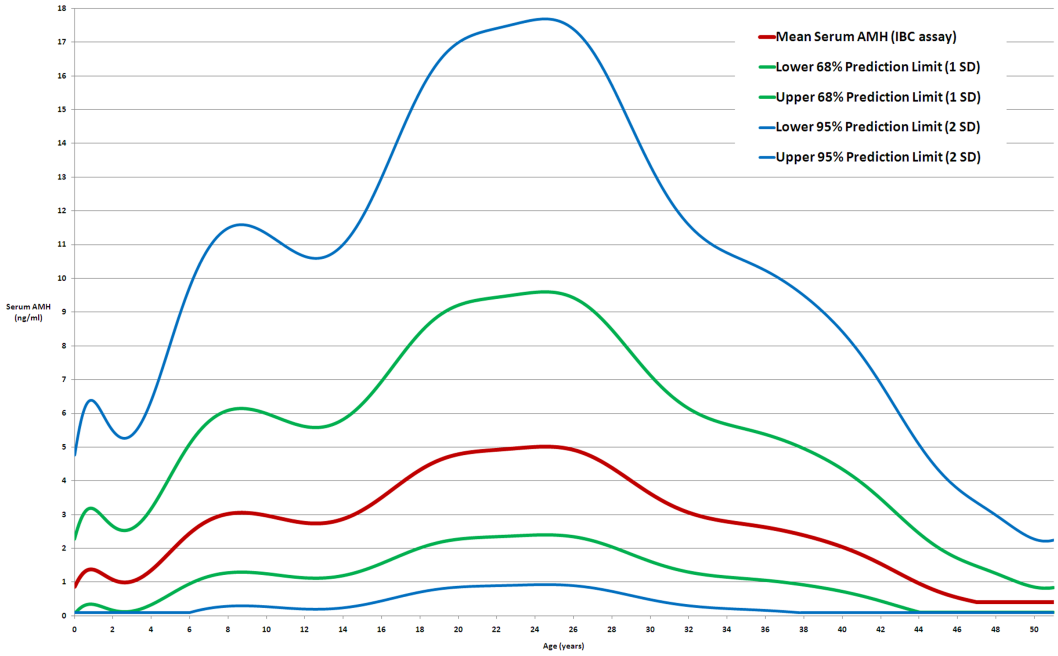
24.4 years of age (36). Age-related changes in AMH levels occur much earlier than in any other ovarian marker, such as FSH and Inhibin B (37). Several long-term follow-up studies show that serum AMH is an accurate predictor of age at menopause (38-40). The serum concentration of AMH can be low 5 to 10 years prior to the onset of menopause.

**Table 1.** Studies of AMH as a predictor of ovarian aging and menopause in cohorts of fertile women.

| | Study design | Number of women | Age | Follow-up time | Results |
|---|---|---|---|---|---|
| **Tehrani et al 2011 (41)** | Cohort study | 1265 | 20-49yr | 6 years with 3 years interval | Good correlation for prediction of menopause. 63 women reached menopause. |
| **Broer et al 2011 (42)** | Cohort study | 257 | 21-46yr | 11 years | Normogram, Model adequacy 90%. 48 women reached menopause. |
| **Freeman et al 2012 (43)** | Cohort study | 401 | 35-48yr | 14 years | If AMH < 0.2 µg/L, time to menopause was 6 yrs. 183 women reached menopause. |
| **Tehrani et al 2013 (39)** | Cohort study | 1015 | 20-50yr | 10 years | Model adequacy 92%. 277 women reached menopause. |

Clinical studies initially demonstrated that AMH was cycle-independent, displaying less intra-individual variation than any other current ovarian markers (44)**.** Recently, an inter-cyclic variation of AMH has been demonstrated (45). There are contradictory findings regarding the effect of exogenous sex-steroids or contraceptive treatment on AMH levels: some studies demonstrate no impact, while others show a tendency towards lower levels of AMH (46, 47). In the few studies published on the subject, there are discrepancies regarding AMH concentrations during pregnancy. One study demonstrated a decline of AMH (48), while another showed no significant difference in AMH levels during pregnancy (49). Pregnant women have an extra plasma volume of about 40 %, which might cause falsely low AMH concentrations.

**Figure 5.** Normal range of AMH in young adults and women based on a model presented by Kelsey et al (14).




Reprinted with the permission of PLoS ONE. Kelsey TW, Wright P, Nelson SM, Anderson RA, Wallace WHB (2011). A Validated Model of Serum Anti-Müllerian Hormone from Conception to Menopause.

AMH serum concentrations have shown clinical value in the context of IVF (50). In order to make ovarian stimulation well-tolerated and effective, it can be valuable to have previous knowledge of the woman's ovarian reserve and her possible response to ovarian stimulation (51, 52). Assisted reproduction techniques aim to avoid poor response/cycle cancellation or overstimulation of the ovaries (OHSS). No studies demonstrate the ability of AMH to predict live births and oocyte quality (53). In fact, live births occur even when very low AMH values have been detected (54). Studies present varying results for using AMH levels to predict spontaneous pregnancy. One study suggests that AMH could predict fecundity in women aged 30-42 years (55). However, the results could not be replicated in another study with a younger population of women (56).

### 2.4.4 Measurement of AMH

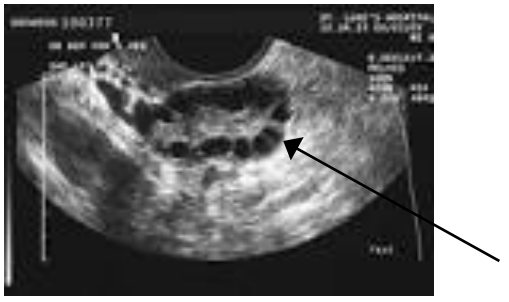
Since 2002, a number of assays have been used for measuring AMH in human studies, including the ELISA KIT by Immunotech (IOT) and the Diagnostic System Lab assay (DSL assay). In 2010, the Beckman Coulter microplate assay was introduced, which is an enzymatically applied two-site immune assay with a more stable antibody (57). The assay uses anti-AMH antibodies to bind to AMH. After incubation and washing, substances are added that give a colour change based on enzymatic reaction. The last step is to detect the intensity of light by spectrophotometry. In assay analyses, it is standard practice to report the

inter-assay and intra-assay coefficient of variability (CV). The inter-assay CV expresses the plate-to-plate consistency, and the intra-assay CV describes the variation within each data set of one experiment. Each laboratory produces its own inter- and intra-assay CV. Two studies have shown the inter- and intra-assay to be smaller (< 5 %) with Beckman Coulter's Gen II assay (58, 59). Beckman Coulter's Gen II assay has the advantage of greater sensitivity, with values as low as 0.08 µg/L compared to the IOT assay. There are also no cross-reactivates between inhibin, FSH or LH (59). Unfortunately, there is no comparability between the different assays (60), and poor assay reproducibility has been demonstrated (58). Gen II AMH concentrations were about 20 % lower compared to DSL (58). Pre-dilatation, storage at -20°C for five days or incubation at room temperature for seven days increased the AMH concentrations (58). In July 2013, Beckman Coulter released a field safety notice that AMH samples could be reported lower than expected. Differences of 70 % were observed for samples tested within 1-2 hours. Research has demonstrated that freshly-drawn or freshly-frozen samples have a higher risk of complement interference (61). In the same laboratory, the inter- and intra-assay variability has been small (58), but between laboratories, there has been a wide range of values (62).

### 2.4.5 Antral follicle counts (AFC)

It is not possible to estimate the number of primordial follicles in the ovaries, which constitutes the real reserve pool of eggs. However, the antral follicles growing at the beginning of the menstrual cycle can be imaged by ultrasound. Vaginal ultrasound can visualize small follicles of 1-2mm. Studies have shown that counting the antral follicles of 2-10 mm reflects the number of primordial follicles (63, 64). AFC can also predict oocyte yield (64), poor response to hormone stimulation (65) and live birth rates in ART treatments (66). Median AFC decreases by 0.4 for every year of increasing age (67). Research shows a correlation between AMH and AFC (68)

**Figure 6.** Vaginal ultrasonography illustrating an ovary with numerous antral follicles.

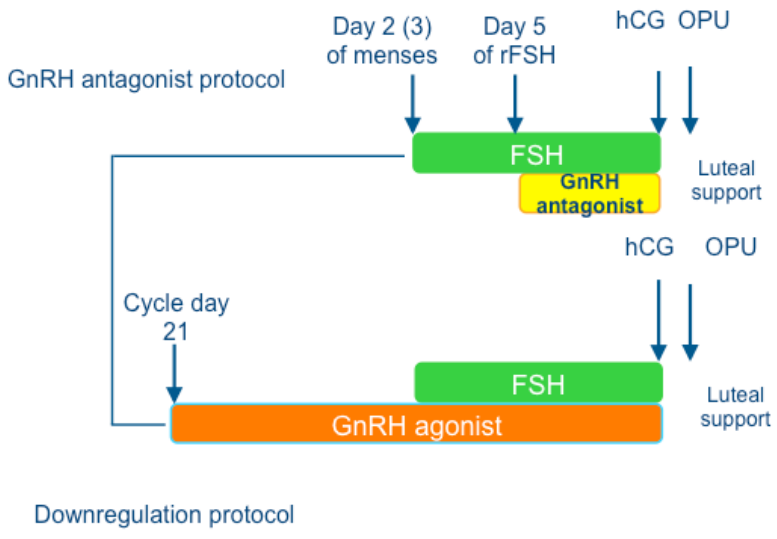


## 2.5 ASSISTED REPRODUCTIVE TECHNIQUE (ART)

A large proportion of the women who participated in the studies of this thesis have undergone fertility treatments by assisted reproductive technologies (ART). In Sweden, conventional In Vitro Fertilization Treatment (IVF) and Intracytoplasmic Sperm Injection (ICSI) are widely available within the tax-financed health care system. The aim of IVF/ICSI treatments is to obtain several oocytes to fertilize in the laboratory. The embryos obtained are cultured for several days and categorized according to their morphological aspects. Usually, one embryo is transferred into the womb during the treatment cycle, and supernumerary embryos can be frozen for later treatments. The recovery of several oocytes is achieved by the administration of supraphysiological doses of gonadotropins (FSH) to stimulate the development of several ovarian follicles during the same cycle. Gonadotropins can only recruit follicles that have reached the mature state of FSH dependency. Subcutaneous injections with individualized recombinant FSH (rFSH) or urinary-derived gonadotropin (hMG) are given for 10 ± 2 days. At day 5-7 of injections, the ovarian response is monitored by ultrasound, often in combination with serum oestradiol. The growing follicles produce a large amount of oestradiol, which can cause a premature LH surge through the negative feedback mechanism. To prevent potentially premature ovulation, there are two different treatment protocols: one uses down-regulation with GnRH agonists, and the other uses GnRH antagonists.

In the GnRH antagonist protocol, the FSH injections are started on cycle day 2-3, and a GnRH antagonist is added when the largest follicle reaches 10-12 mm or on a fixed day (day 5-6 of FSH stimulation) to be continued thereafter until ovulation.

**Figure 7.** Schematic illustration of the GnRH antagonist protocol and the long down-regulation protocol with GnRH agonist.



When using the long down-regulation of the pituitary gland, a GnRH agonist is usually started a week before expected menstruation, cycle day (cd) 21, and is continued until ovulation. The goal behind this process is exhaustion of the pituitary gland, which shuts off gonadotropin secretion. FSH injections are added after 2-3 weeks of GnRH agonist use. Ovulation is induced when three or more follicles reach the size of 17 mm, as measured by transvaginal ultrasound. The LH surge is stimulated by an injection of hCG. Oocyte pick-up (OPU) is undertaken by transvaginal ultrasound puncture 36-37 hours after the hCG injection. In the conventional IVF setting, the oocytes and spermatozoa are mixed, and a natural selection of fertilization occurs; in ICSI, a single sperm is injected into the oocyte. Embryo transfer is performed after 2-5 days of embryo culture. All women receive luteal phase support with progesterone.

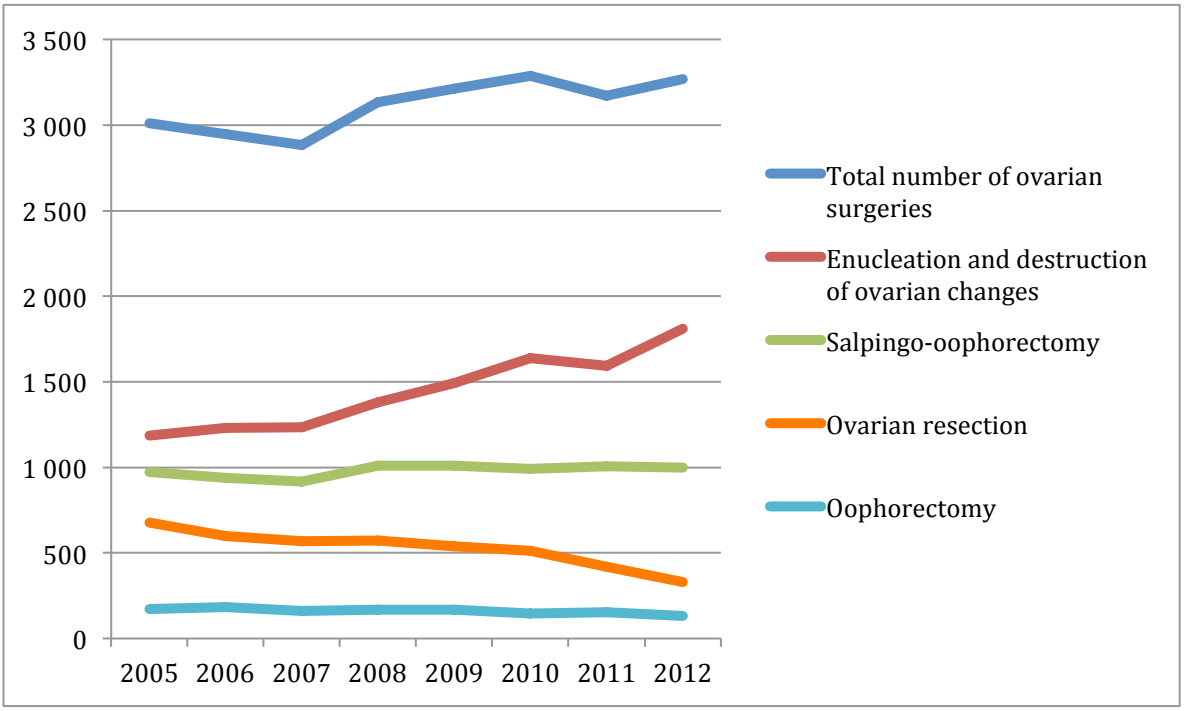
The clinical outcome of ART treatment can be reported as the clinical pregnancy rate (CPR) or the live birth rate (LBR) per started cycle or per OPU. This will give lower result rates compared to CPR or LBR/embryotransfer, as there are always some treatment cancellations. The clinical pregnancy rate or LBR per embryo transfer are usually reported as well. In the yearly ART surveillance from the United States, the cancellation rate was about 15 % in 2011, the LBR/started cycle was 31 % and the LBR/Embryo transfer was 37 % (69). The live birth rate in Sweden per ET for fresh cycles is between 25-27 % and about 20 % for frozen cycles (70).

## 2.6 OVARIAN CYSTS

Ovarian cysts are common among premenopausal women. A cyst is commonly defined as a fluid-containing structure of more than 30 mm. In young women, the prevalence of ovarian cysts is approximately 7 % (71, 72). The lifetime risk of ovarian cancer is one in 70 women, but this number increases with age and with a family history of ovarian cancer (73, 74). There are several different histopathologic types of benign ovarian cysts: follicular/functional cysts, corpus luteum cysts, endometriomas, cystadenomas (serous and mucinous) and teratomas. Some can be diagnosed easily through pattern recognition with ultrasound (75). Several guidelines from western countries attempt to describe when cyst surgery is necessary for women of pre-menopausal age (76-79). Most guidelines recommend diagnosis with ultrasound to discriminate between malignant and benign cysts. The International Ovarian Tumour Analyses (IOTA) group has developed simple ultrasound rules to help the clinicians differentiate potential malignant cysts form benign (80, 81). The guidelines recommend Magnetic Resonance Imaging (MRI) as a secondary step when the adnexal mass is unclear. Both the American College of Obstetricians and Gynecologists (ACOG) and the Royal

College of Obstetricians and Gynaecologists (RCOG) recommend expectant management of benign ovarian cysts if they are asymptomatic and smaller than seven centimetres. If the cysts are smaller than five centimetres, no follow up is needed (76, 79), and if surgery is needed, laparoscopy is the preferred choice. A recent review article by Legendre et al concludes that, with dermoid cysts, a "wait-and-see" attitude is probably acceptable if the cyst is asymptomatic and 4-6 cm (82). The same article also recommends expectant management for endometriomas.

**Figure 8.** Trends in ovarian surgery in Sweden 2005-2012. From the National Board of Health and Welfare (83).




### 2.6.1 Impacts of ovarian cyst surgery on ovarian reserve

Several studies indicate that ovarian cyst surgery may induce a reduction of serum AMH concentrations (84) (85-87), thus suggesting a possible negative impact of cyst enucleation surgery. This effect might be due to unavoidable surgical damage to the normal ovarian tissue surrounding the cyst. Two systematic reviews have looked at heterogeneous studies published on the impact of ovarian surgery on ovarian reserve (88, 89). Both concluded that there is an association between ovarian surgery and AMH decline. Table 2 presents the available studies of AMH changes following ovarian cyst enucleation. Enucleation involves identifying the cyst wall and removing it from the ovarian cortex. In order to make results generalizable, it is important to define a proper and comparable study population. Studies presented in Table 2 only include women who have undergone cyst enucleation. No apparent removal of ovarian tissue was reported; however, they are heterogeneous, as they included women of different

age groups from a mean age of 28 years (87, 90, 91) to a mean age of 33 years (86, 92, 93). All of the studies used the same operation technique, the stripping method for cyst enucleation. Additionally, two of the studies included a control group that had a different operation; unilateral oophorectomy or three-step approach (94, 95). There were differences in the number of surgeons performing the operations. To reduce the risk of performance bias, two of the studies had two surgeons who performed all of the ovarian surgeries (96, 97). The studies also used different AMH assay kits, and as reported earlier, they are not comparable. Only a few of the studies reported low intra- and inter-assay variability estimated as coefficient of variability (CV) (86, 90, 91). Median AMH levels were reported by a few studies (85, 86), while the remaining studies only reported mean values that could be incorrect if the distribution was not normal or if they had extreme values that affected the results. In the available studies, the identified predictive factors of a decline in AMH levels included surgery for endometriomas, especially if bilateral (97). The prospective cohort study by Chang et al should be highlighted due to the high quality of its design. In this study, all AMH samples were analysed in the same assay after freezing the samples, and the intra- and inter-assay variability was low (86). A control group of non-endometriomas was also included. The most recent prospective studies by Kwon et al (97) and Uncu et al (96) tried to eliminate confounders using extensive exclusion criteria, and both studies had a control group with either women with other benign cysts or women without ovarian cysts. A very limited number of surgeons performed all ovarian surgeries, which minimized the risk of performance bias.

**Table 2.** Available studies on changes in ovarian reserve following ovarian surgery, estimated as changes in serum AMH concentrations. All but one of the studies (Tsolakidis et al, 2010) (94) were prospective cohort studies. The studies have used different AMH assays: Beckman Coulter's Gen II assay, the older ELISA KIT by Immunotech (IOT) and the Diagnostic System Lab assay (DSL assay). All of the studies used the stripping technique for removing the ovarian cysts.

| Study | Type of cyst investigated | No. of cases (unilateral/bilateral cysts) | Mean diameter (mm) | Presurgery AMH | Postsurgery AMH | Time of follow-up |
|---|---|---|---|---|---|---|
| **Change et al 2010 (87)** | Endometrioma (n=13)<br>Dermoid (n=6)<br>Other (1) | 19 (13/6) | NR | 2.23 (1.35-3.1) | 0.8 (0.7-1.6) | 3 months |
| **Biacchiardi et al 2010 (94)** | Endometrioma | 43 (33/10) | 37 ±11 | 3.0 ± 0.4 | 1.3 ± 0.4[a] | 9 months |
| **Tsolakidis et al 2010 (95)** | Endometrioma | 10 (7/3) | ≥30 | 3.9 ± 0.4 | 2.9 ± 0.2[a] | 6 months |
| **Ercan et al 2010 (91)** | Endometrioma | 47 (33/14) | ≥45 | 1.6 ± 1.1 | 1.4 ± 1.2 | 1 month |
| **Iwase et al 2010 (86)** | Endometrioma (n=29)<br>Dermoid (n=18) | 29 (16/13)<br>NR | NR | 3.0 (0.5-12.1)<br>3.9 (0.1-10.1) | 2.2 (0.1-7.2)[a]<br>3.3 (0.1-9.1)[a] | 1 month |
| **Lee et al 2011 (96)** | Endometrioma | 13 (13/0) | 40 ± 18 | 4.7 ± 2.5 | 3.3 ±2.1[a] | 3 months |
| **Hirokawa et al 2011 (93)** | Endometrioma | 38 (10/28) | 64 ±22 | 3.9 ± 2.5 | 2.1 ± 1.6[a] | 1 month |
| **Kitajima et al 2011 (85)** | Endometrioma (n=19)<br>Dermoid (n=13) | 19 (19/0)<br>NR | 67 ± 19<br>68 ± 21 | 4.3 ± 3.0<br>4.0 ± 2.2 | - 25% ± 29%[a]<br>-3% ± 34.4% | 3 months |
| **Ercan et al 2011 (99)** | Endometrioma | 36 (36/0) | 25 ± 23 | 2.0 ± 0.4 | 2.0 ± 0.6 | 3 months |
| **Hwu et al 2011 (100)** | Endometrioma | 31 (31/0) | ≥30 | 3.9 ± 0.4 | 2.0 ± 0.2 | 3 months |
| **Celik et al 2012 (88)** | Endometrioma | 65 (46/19) | 59 ± 21 | 1.8 ± 1.7 | 0.7 ± 0.8[a] | 6 months |
| **Urman et al (2013) (101)** | Endometrioma | 25 (25/0) | 51±15 | | 24% reduction from pre-operative levels | 6 months |
| **Uncu et al 2013 (97)** | Endometrioma | 30 (15/15) | 43 (36-52) | 2.8 ± 2.2 | 1.8 ± 1.3[a] | 6 months |
| **Alborzi et al 2013 (92)** | Endometrioma | 193 (121/72) | NR | 3.9 ± 3.6 | 1.8 ± 7.8[a] | 9 months |
| **Sugita et al 2013 (102)** | Endometrioma | 39 (22/17) | 80 ± 30 | 3.6 ± 2.1 | 2.1 ± 0.9[a] | 12 months |
| **Kwon et al 2014 (98)** | Endometrioma (n=68)<br>Dermoid (n=15)<br>Other benign cysts (n=17) | 68 (42/26)<br>32(24/8) | 63 ±25<br>60 ± 23 | 4.97 ± 2.83<br>5.03 ± 3.07 | 3.33 ± 2.08[a]<br>3.22 ± 2.09[a] | 3 months |

[a]p value < 0.05, NR: Non-Reported

### 2.6.2 Patients' fertility-related information prior to ovarian surgery

In many countries, women sign a consent form prior to surgery, but this is not the case in Nordic countries. International data suggests that even though patients sign a formal consent prior to surgery, they may not fully understand the content (102). Some people argue that information to patients will be sparse without a signed consent; in Sweden, however, a new patient law has just been implemented, regulating and extending the information duty towards patients and expanding the patients' participation in their own care. ACOG recommends patient-centred interviewing and caring communication skills, especially when informing of evidence-based specific risks and benefits of surgery (103). Only a few guidelines comment on the need for fertility information prior to surgery. The guidelines from the RCOG regarding complex gynaecological surgery state that "the outcomes of the proposed surgery should be discussed, including what loss of function is acceptable – in particular the possible loss of fertility" (104). The European Society of Human Reproduction and Embryology's (ESHRE) guidelines for endometriosis recommend that "clinicians should provide counselling to women with endometriomas regarding the risks of reduced ovarian function after surgery and the possible loss of the ovary"(105).

### 2.6.3 Effects of unilateral oophorectomy

A British cohort study of women born in 1946, who have been followed with yearly questionnaires from the age of 47 years, showed that women undergoing unilateral oophorectomy (UO) seem to reach perimenopause one year earlier than matched controls (106). Perimenopause was defined as irregular periods for 12 months or no period for three to 12 months. A recent study that used self-reported age at menopause has confirmed that women with one ovary reached menopause one year earlier than controls (107). As demonstrated in Table 3, the abrupt reduction of the number of ovarian follicles by unilateral oophorectomy may have a distinctive impact at different ages (108).

**Table 3.** Studies published on the effect of unilateral oophorectomy on age at menopause.

| | Study design | Total group (n) | UO (n) | Total group, Mean age at menopause | UO, Mean age at menopause | P-value | Data collection |
|---|---|---|---|---|---|---|---|
| **Cramer et al 1995 (109)** | Case control | 688 | 10 | | OR for earlier menopause<br>4.3(0.90-20.44) | NS | Questionnaire |
| **Hardy et al 1999 (106)** | Cohort | 1166 | 21 | 48 (perimenopause) | 47 (perimenopause) | 0.02 | Questionnaire |
| **Yasui et al 2012 (110)** | Cohort | 24152 | 827 | 52.1 (95% CI 52.0-52.2) | 50.9 (95 % CI 50.7-51.6) | <0.0001 | Questionnaire |
| **Thomas-Teinturer et al 2013 (111)** | Cohort | 706 | 40 | 49 years (95 % CI: 48–50) | 42 years (95 % CI: 40–46) | NR | Questionnaire of cancer survivors |
| **Bjelland et al 2014 (107)** | Cohort | 23580 | 1055 | 50.6 (95% CI 50.5-50.7) | 49.8 (95% CI 49.4-50.3) | <0.001 | Questionnaire |

UO: Unilateral Oophorectomy, OR: Odds Ratio, CI: Confidence Interval

Previous data on women with only one ovary who have undergone ART treatments (IVF) have reported similar pregnancy rates compared to women with two ovaries; however, the women with one ovary needed a higher FSH dose for stimulation, and fewer oocytes were retrieved at OPU (108, 112-116). Remarkably, in one previous study, an increased pregnancy rate was found after IVF/ICSI treatment in women with UO when compared to controls (117). Most of the previous studies were small in size, and multiple embryos were transferred (108, 112, 113, 116, 117).

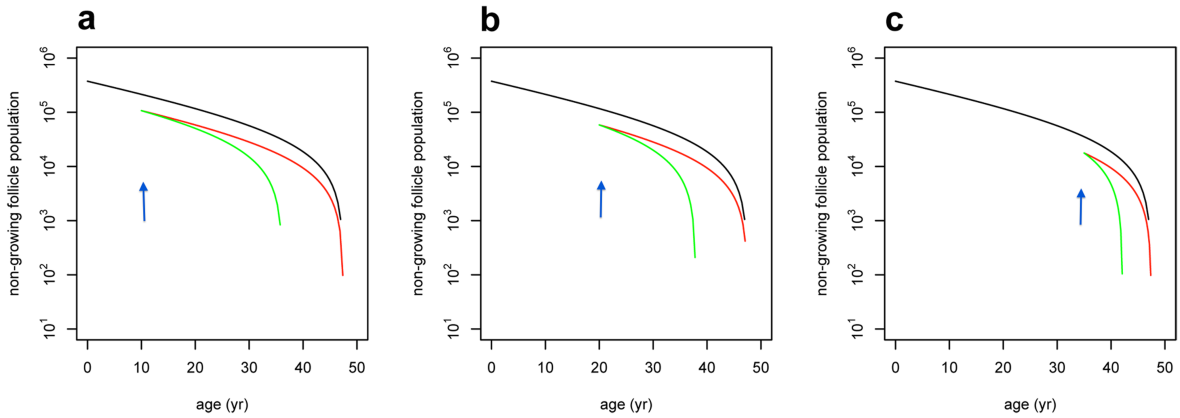
**Table 4.** Studies on women with a history of unilateral oophorectomy (UO) who underwent IVF/ICSI treatment (Exposed; Exp) compared to controls. In most of the studies, multiple embryos were transferred.

| Study | Women with UO (Cycles, n) | Controls (Cycles, n) | Mean Embryos transferred Exp vs controls | Main Outcome | Clinical Pregnancy rate (CPR), Live birth rate (LBR), Exp vs controls | Definition of pregnancy |
|---|---|---|---|---|---|---|
| **Alper et al 1985 (118)** | 14 | 14 | 2.2 vs 2.4 NS | FSH dose+ oocyte yield NS | PR: 14.3% vs 28.6 %. NS | NR |
| **Dodds et al 1987 (119)** | 16 (20) | 18 (34) | 1.7 vs 2.7 S | FSH dose+ oocyte yield NS | PR/ET 14 % vs 36 %. NS | NR |
| **Lam et al 1987 (120)** | 34 (60) | 335 (559) | 3 vs 3 | ↓ Oocytes in women with UO. S. | CPR/OPU 9.7 % vs 15.6 %. NS | Clinical pregnancy verified by ultrasound |
| **Boutteville et al 1987 (121)** | 86 (162) | 415 (788) | 2.3 vs 3 S. | ↓ Oocytes in women with UO. S. | CPR/ET 23.9 % vs 24.4 %. NS | Clinical pregnancy verified by ultrasound |
| **Dodson et al 1987 (122)** | (23) | (125) | 3.9 vs 4.5 NS | FSH dose+ oocyte yield NS | PR: 8.3 % vs 12.9 %. NS | NR |
| **Khalifa et al 1992 (123)** | (162) | (1110) | 2.4 vs 3.0 NS | ↓ Oocytes in women with UO. S. | CPR: 13 % vs 18 %. NS | Clinical pregnancy verified by ultrasound |
| **Penzias et al 1993 (124)** | 7 | 84 | 3.6 vs 4.0 NS | FSH dose+ oocyte yield NS | NR | NR |
| **Nargund et al 1995 (125)** | 17 (25) | 27 (33) | 1.9 vs 2.5 NS | ↓ Oocytes in women with UO. S. | CPR: Significantly lower in women with UO | Clinical pregnancy verified by ultrasound |
| **Lass et al 1997 (113)** | 58 (73) | 881 (988) | 2 vs 2 NS | ↑ FSH dos<br>↓ Oocytes in women with UO. S. | CPR/OPU 25.8 % vs 27.1 % NS<br>LBR NS | Clinical pregnancy verified by ultrasound |
| **Levitas et al 2000 (117)** | 10 (20) | 47 (60) | 3 vs 2.9 | FSH dose+ oocyte yield NS | CPR: 52.9% vs 20.8%. S | Clinical pregnancy verified by ultrasound |
| **Levi et al 2003 (112)** | 46 (46) | 123 (123) | 2.1 vs 3.3 | ↑ FSH dos<br>↓ Oocytes in women with UO. S. | CPR: 8.7 % vs 19.5 %. NS | Clinical pregnancy verified by ultrasound |
| **Al-Hasani et al 2003 (116)** | 24 (3) | 109 (191) | 2.6 vs 3 | ↑ FSH dos<br>↓ Oocytes in women with UO. S. | CPR: 22.2 % vs 17.2 %.NS | Clinical pregnancy verified by ultrasound |
| **Hendricks et al 2010 (115)** | 18 (22) | 44 | 2.6 vs 2.8 | ↑ FSH dos<br>↓ Oocytes in women with UO. S. | CPR: 31.8 % vs 43.2 %. NS | Clinical pregnancy verified by ultrasound |
| **Khan et al 2014 (114)** | 51 | 102 | NR | ↓ Oocytes in women with UO. S. | CPR: 33.3 % vs 52.9 %. S<br>LBR 31.1 % vs 46.8 % NS | Clinical pregnancy verified by ultrasound |

CPR= Clinical Pregnancy Rate, FSH= Follicle Stimulating Hormone, LBR= Live Birth Rate, NR= Not Reported, NS=Not Significant, OPU= Ovum pick-up, PR= Pregnancy Rate, S= Significant p <0.05, UO= Unilateral Oophorectomy

Researchers question whether reproductive aging involves a pre-determined decline of remaining ovarian follicles or an age-dependent process. In the pre-determined model, the unilateral oophorectomy induces a reduction of the follicle pool by half, but there would still be an equivalent decline in follicles. Therefore, a unilateral oophorectomy would have varying effects depending on age; a unilateral oophorectomy at an earlier age would lead to earlier menopause, as there is an equivalent decline the entire time in this model. In the alternative age-dependent model, a decline of half the rate of a non-operated woman would occur after unilateral oophorectomy; therefore, the age of the unilateral oophorectomy would have less impact on menopausal age (126). This alternative model aligns more with the previously-reported studies showing menopause only one year earlier following unilateral oophorectomy (106, 109-111).

**Figure 9.** An illustration of the two theories regarding the effects of a unilateral oophorectomy on the age of menopause onset. The green line represents the pre-determined model, and the red line shows the alternative age-dependent model for unilateral oophorectomy at ages 10, 20 or 35 (126).



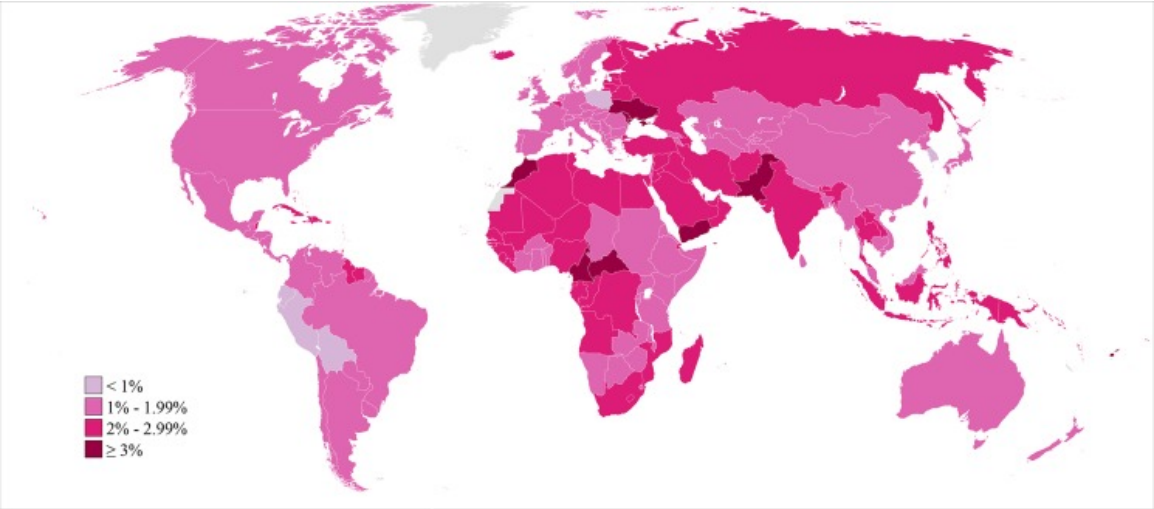

Reprinted with permission from PLoS One. Wilkosz, P., G. D. Greggains, T. G. Tanbo and P. Fedorcsak (2014). "Female reproductive decline is determined by remaining ovarian reserve and age." PLoS One **9**(10): e108343

## 2.7 ASPECTS OF INFERTILITY IN WOMEN

As a consequence of diminished ovarian reserve due to advanced age, ovarian surgery or cancer treatments, a woman might remain childless or have a smaller family than desired, a phenomenon known as primary or secondary infertility. Infertility is a global problem affecting women in developing countries more than women in developed nations because of

infectious diseases and lack of medical reproductive treatments (127). In 2010, there were approximately 48.5 million couples affected by infertility (127).

**Figure 10.** Worldwide prevalence of primary infertility among women seeking a child in 2010 (127).




Reprinted with permission from PLoS Med. Mascarenhas, M. N., S. R. Flaxman, T. Boerma, S. Vanderpoel and G. A. Stevens (2012). "National, regional, and global trends in infertility prevalence since 1990: a systematic analysis of 277 health surveys." PLoS Med **9**(12): e1001356.

Infertility can have cultural impacts for women in different societies. It can lead to both social and psychological consequences, such as divorce, social stigma and isolation (128, 129). One consequence of infertility is feeling a loss of control over one's own life (130). In social contexts, women have to deal with feelings of being left out and/or jealous of women who are pregnant (130). Women seem to use coping strategies more than men, including seeking social support, avoiding contact with people that remind her of her infertility (escape/avoidance) and accepting responsibility (believing that she is responsible for the infertility) (131). Infertile women have a higher risk of depression and diabetes (132). In one study, women reported that infertility and IVF treatment was one of the most stressful events of their lives (130). Information and communication are the most important variables in patient-centred care for infertile women (133). Guidelines recommend providing fertility-related information prior to medical treatments that can affect reproduction, such as ovarian surgery or cancer treatments (104, 105). Armuand et al report that less than half of women undergoing cancer treatment receive fertility-related information beforehand, and only 14 % are informed of fertility preservation options (134).

# 3 RATIONALE

In Sweden, nearly 3300 ovarian cyst surgeries are performed in women of reproductive age per year. More ovarian surgeries are performed on women over 30 years of age compared to younger women. The risk of having a benign cyst before attempting pregnancy has increased because women today are delaying childbearing. Therefore, these women have a greater risk of experiencing ovarian surgery, a potential threat to their fertility. Even though clinical guidelines recommend a conservative and more expectant attitude towards asymptomatic benign cysts < 7cm, there has been a slow increase in the annual rates of ovarian surgeries in Sweden. Prior to making a decision, patients require adequate information regarding the risks of ovarian surgery, its complications and potential effects on fertility.

Ovarian surgery for endometriomas has previously been shown to associate with a reduction of AMH up to one year after surgery. Further research is needed to study the long-term effects of ovarian surgery for endometriomas in the women's ovarian reserve, as well as for the various non-endometriotic cysts presenting in young women. Such research also needs to investigate the final effects of ovarian surgery on direct estimations of fertility, such as pregnancy rates. International guidelines state that reproduction should always be discussed before ovarian surgery, yet there are no published studies regarding the extent to which this information is provided. Also lacking from the literature are studies regarding women's informational needs prior to ovarian surgery.

This thesis aims to contribute to the knowledge regarding the effects of ovarian surgery on ovarian reserve and female fertility so that healthcare professionals might provide improved information and counselling adapted to the individual patient. After receiving such individualized information, women of today can make informed decisions with regard to their fertility issues and surgery.

# 4 AIMS

The overall aim of this thesis was to investigate short- and long-term effects of ovarian surgery on the ovarian reserve of women of reproductive age, their reproductive outcomes, and their perceptions of information received about fertility and their ovarian surgery.

*Specific aims*

Paper I To investigate patients' perceptions of fertility-related information received and fertility distress prior to ovarian cyst surgery.

Paper II To prospectively investigate the effects of ovarian cyst surgery for several cyst types on women's ovarian reserve, estimated as serum AMH concentrations, three and six months following ovarian surgery.

Paper III To prospectively examine the impact of changes in serum AMH following ovarian cyst surgery on reproductive outcomes in women attempting to achieve pregnancy within a two-year follow-up period.

Paper IV To investigate if women with a history of unilateral oophorectomy treated by IVF/ICSI would have a reduced likelihood of achieving clinical pregnancy compared to women with intact ovaries.

# 5 METHODS

## 5.1 STUDY DESIGN

This thesis includes two studies reported in four papers (Table 5). The studies have different methodological designs. Study I resulted in three papers: paper I had a cross-sectional design, while papers II-III involved a prospective cohort design with a follow-up cut-off time of six and 24 months, respectively. The study population in study I (papers I-III) was enrolled at Södersjukhuset prior to ovarian surgery for possible benign ovarian cyst. Study IV is a multicentre cohort study based on clinical registry data from three large reproductive medicine centres. The clinics that participated are Carl von Linné Clinic Uppsala, Reproduction Centre Uppsala University Hospital and Reproductive Medicine Karolinska University Hospital. The women included in the study underwent their IVF/ICSI treatments between January 10, 2003 and January 13, 2014.

**Table 5.** Overview of the general design of the project.

| Study | Design | Data collection | Participants | Paper | Analysis |
|---|---|---|---|---|---|
| 1 | Cross sectional | Study-specific measures from questionnaire, FPI | 106 women answered the questionnaire when they were planned for ovarian cyst surgery. | I | Logistic regression |
| 1 | Prospective cohort Inclusion from 2011 to 2012; follow-up completed Sept 2012. | Clinical data Ultrasonography of AFC Blood samples for serum AMH | Clinical prospective cohort of 75 women who underwent ovarian cyst surgery. 3- and 6-month follow-up. | II | Shapiro-Wilks, Wilcoxon signed rank test, $\chi^2$test, Kendall's r, Logistic regression |
| 1 | Prospective cohort Inclusion from 2011to 2012; follow-up completed March 2014. | Clinical data Ultrasonography of AFC Blood samples for serum AMH Questionnaire at 24 month follow-up | 2-year follow-up of the clinical cohort. 66 women attended 24-month follow-up visit. | III | Wilcoxon signed rank, Mann-Whitney U test Kendall's r, |
| 2 | Multicentre cohort study. Exposed cohort with unilateral oophorectomy and unexposed controls were treated with IVF/ICSI between 2003 and 2014. | Clinical registry | Data on IVF/ICSI treatments were collected at three large fertility clinics in Sweden. 76 exposed women with previous unilateral oophorectomy and 12879 unexposed control women. | IV | GEE model |

AFC: Antral Follicle Counts, AMH: Anti-Müllerian Hormone, FPI: Fertility Problem Inventory, GEE: Generalized Estimated Equation

## 5.2 STUDY SUBJECTS

### 5.2.1 Study I (Papers I- III)

The inclusion period of study I (papers I-III) started in March 2011, and the last patient was included in March 2012. Eligible were all women of reproductive age (18-44 years) planning to have surgery for ovarian cysts at the Department of Obstetrics and Gynecology in Södersjukhuset, Sweden. Indications for surgery included clinical symptoms, such as pain, pressure or fear of malignancy. Exclusion criteria were not being able to understand or write the Swedish language.

The women were invited to participate in the study at the pre-surgery consultation with a gynaecologist, usually the day before surgery. The women received both written and oral information about the study and the current lack of knowledge on the impact of ovarian cyst surgery on remaining ovarian function. A total of 167 women were scheduled for ovarian cyst surgery during the study period at the clinic. 20 women declined to participate, and 35 women were never asked to participate due to administrative failure.

#### *5.2.1.1 Paper I*

Six women did not complete the questionnaire in the cross-sectional design, which resulted in a response rate of 80 % (N = 106).

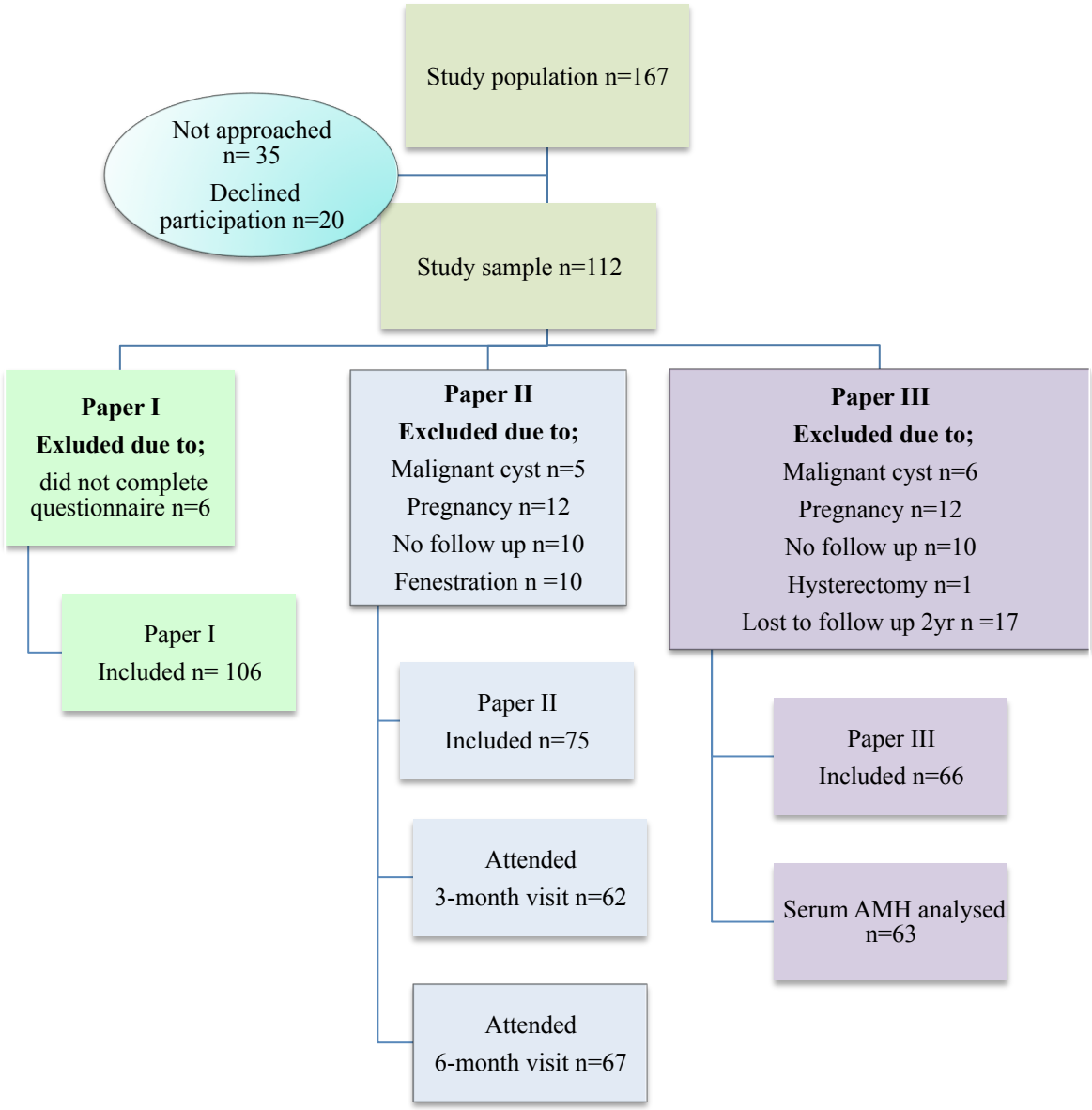
#### *5.2.1.2 Paper II*

Before surgery, participants in study I were offered the opportunity to participate in a prospective follow-up study to investigate the ovarian reserve through ultrasound and laboratory methods at three and six months. Of the 112 recruited women, 37 were excluded due to pregnancy, malignancy or because only fenestration was done at surgery and some were lost to follow up (Figure 11). Of the 75 women participating, 54 women attended both the 3- and 6-month follow-up visits. 62 women attended the three-month follow up, and 67 women attended the six-month follow up.

#### *5.2.1.3 Paper III*

In this prospective follow-up study, we invited all 75 participants from paper II plus ten women who underwent a cyst fenestration to participate in a two-year follow up. Two women were excluded, one due to malignancy and one due to hysterectomy. Of the 83 eligible women, 66 completed the two-year questionnaire, and 63 had a serum AMH test.

**Figure 11**. Flow chart of inclusion and participants in study I (papers I-III).




### 5.2.2 Study II (paper IV)

Data on women undergoing IVF/ICSI treatment were collected from the clinical registry at three large reproductive clinics in Sweden (Carl von Linné Clinic, Reproductive Medicine Karolinska University Hospital and Reproductive Centre Uppsala University Hospital). Women were excluded if they underwent IVF/ICSI treatment with donor gametes or if the treatment included preimplantation genetic diagnosis (PGD). The study period was defined as January 10, 2003 until January 13, 2014. The exposed cohort consisted of 76 women with a

previous unilateral oophorectomy (UO). The unexposed control group consisted of 12879 women with intact ovaries who underwent treatment with IVF/ICSI during the same study period at the three centres. Patients were included regardless of duration or cause of infertility. At two of the public clinics, there is an age limit of 40 years for women undergoing IVF/ICSI treatment: Reproduction Centre Uppsala University Hospital and Reproductive Medicine Karolinska University Hospital. Only sibling treatments with frozen embryos are allowed at an older age on a self-paying basis. Carl von Linné clinic is a private clinic that treats women up to 43 years of age. As a result, there was a difference in mean age between the centres.

**Table 6.** Participants' age by ART-centre and number of treatment included/centre in study II.

| | **Total** | **Carl von Linné Clinic** | **Reproduction Centre, Uppsala University Hospital** | **Reproductive Medicine, Karolinska University Hospital** |
|---|---|---|---|---|
| **IVF/ICSI treatments in exposed women with UO (n)** | 139 | 95 | 25 | 19 |
| **IVF/ICSI treatments in control women (n)** | 22477 | 11777 | 4269 | 6431 |
| **Mean age (SD)** | 34.1(4.4) | 35.1 (4.4) | 33.4 (3.9) | 32.6 (4.2) |

IVF: In Vitro Fertilization, ICSI: Intracytoplasmic Sperm Injection, SD: Standard Deviation, UO: Unilateral Oophorectomy

## 5.3 EXPOSURE

### 5.3.1 Ovarian cyst surgery (papers II and III)

All women who participated in study I were included when planning for ovarian surgery, and all participants in studies II-III had ovarian cyst surgery either by laparoscopy or laparotomy. All surgical procedures were performed under general anaesthesia by an experienced gynaecologist. Laparoscopic surgery was performed with a 5-mm trocar with endoscope through an umbilical incision. Additionally, two to three 5-mm trocars were inserted suprapubically. The cyst cleavage plane was identified and traction with atraumatic forceps was used when there were no signs of malignancy. Sharp dissection with scissors was used to remove the adherent part of the cyst from the ovarian parenchyma. Haemostasis with bipolar electro-coagulation was applied with care, aiming to avoid damage to the normal adjacent ovarian tissue. The cysts were removed using an endobag and a 10-mm trocar. Adhesions or

endometriosis in the peritoneum were always removed. Adhesion prevention products were used in most cases (Hyalobarrier®). Sometimes, a laparotomy was indicated by a large cyst size; then, a transverse Pfannenstiel incision was performed. Unilateral oophorectomies or ovarian resection were performed when macroscopical absence of any normal ovarian parenchyma without a cleavage plane was found during the surgery.

### 5.3.2 Unilateral oophorectomy study II (paper IV)

Exposed women in the study had a previous unilateral oophorectomy. The clinical registries provided no detailed information regarding the reasons for surgery.

## 5.4 EVALUATED PARAMETERS

### 5.4.1 Questionnaire study I (paper I)

All women participating in study I completed a questionnaire, usually the day before surgery. The questionnaire included socio-demographic and reproductive variables. The questions had been used in previous studies (134, 135). Study-specific questions focused on education, household composition, pregnancy, parity, previous or ongoing hormone medication and prior infertility problems. One study-specific item assessed reproductive desire, asking participants to report their desire for children or additional children (Yes, No). Present infertility problems were assessed with a Yes or No question (including not having tried to conceive and lack of male partner). Fertility treatments were assessed by a question regarding specific treatment (insemination, ovulation induction with clomiphene citrate, IVF or oocyte donation). Fertility distress was defined as worry about future fertility and assessed with a visual analogue scale (VAS, endpoints 0=not at all worried and 10=very worried).

Four different questions assessed perceptions of fertility-related information. The questions were adapted from a recent study (134) and included the following: 1) “Did you discuss the impact of the planned surgery on your fertility with the gynaecologist?” (Yes, No, Don’t remember), 2) “Briefly describe what you learned about your surgery and the potential impact of surgery on fertility” (open-response format), 3) “How satisfied are you with the received information regarding the potential impact of surgery on fertility?” (VAS scale, with endpoints 0=not at all satisfied and 10=very satisfied) and 4) “If you didn’t receive any information about the impact of surgery on fertility, what information would you have wished to receive?” (open response format).

The Fertility Problem Inventory (FPI) (136) was used to assess distress related to the importance of parenthood using two subscales: 1) Need for parenthood (10 items) assessing the degree to which parenthood is perceived as an essential goal in life and 2) Rejection of a

child-free lifestyle (8 items) assessing negative views of a child-free lifestyle or status quo and future happiness being dependent on having a child. A six-point Likert scale ranging from "strongly disagree" to "strongly agree" were used for the responses. The FPI was initially developed for infertile patients; therefore, an additional response option – not relevant – was added (134). Women who provided ratings for at least half of the sub-scale items had their missing values and "non-relevant" responses replaced with the means of their individual ratings for those sub-scores. The FPI has shown high validity and reliability (136, 137).

### 5.4.2 Questionnaire (paper III)

At the two-year visit, the women answered a questionnaire similar to the presurgery questionnaire, including questions about reproductive desire, present infertility problems and fertility distress. Specific questions about reproductive outcomes since surgery were also added.

### 5.4.3 AMH measurement (papers II-III)

In study I, blood samples were drawn at the presurgery consultation, usually on the day before surgery and at three months and six months ± 2 weeks after the ovarian surgery.
All samples in study I (papers II-III) were analysed at the Department of Clinical Chemistry's laboratory at Karolinska University Hospital. The commercial enzyme-linked immunosorbent assay kit (ACTIVE AMH gen II ELISA, Beckman-Coulter Inc. Webster, USA) was used. All blood samples were centrifuged within four hours and then sent to the laboratory. Serum samples were stored for up to 24 hours in a refrigerator and thereafter in a freezer at -70°C. All stored samples were analysed sequentially within one week. Median time for analyses was six days. The lowest detectable AMH value of 0.16 µg/L changed to <0.20 µg/L during 2011. All values of <0.16 µg/L obtained between the 14$^{th}$ of March 2011 and the 2$^{nd}$ of March 2012 (N=13) were changed and recorded as <0.20 µg/L for appropriate comparisons. The intra-assay and inter-assay coefficients of variation were 5.4% and 5.6%, respectively.
In paper III, the AMH samples at the two-year follow-up were treated in the same way, with centrifugation within four hours and storage at -70°C. These AMH samples were analysed in four batches.

### 5.4.4 Antral follicle count (AFC) (paper II)

AFC was calculated as the total number of follicles between 2 and 9 mm. The investigation was performed with vaginal ultrasound. Nineteen women had an AFC performed at the pre-surgery visit by the gynaecologist in charge of that visit and at the six-month follow-up visit, all ultrasound examinations were performed by the same examiner (TL).

### 5.4.5 ART treatment study II (paper IV)

The women in this study underwent IVF/ICSI treatment either with long down-regulation GnRH-agonist protocol or GnRH antagonist protocol. In the long down-regulation protocol, a GnRH agonist (nafarelin Synarela; Pfizer 400 μg twice daily or buserelin Suprecur; Sanofi Aventis; 0.3 mg nasally three times a day) was administrated for two to three weeks, after which ovarian stimulation was initiated with gonadotropin, either rFSH, such as follitropin alfa (Gonal F; Merck Serono), follitropin beta (Puregon; Organon) or hMG (Menopur, Ferring). In the antagonist protocol, gonadotropin was initiated on menstrual cycle day two to three, and a GnRH antagonist was added (cetrorelix Cetrotide; Merck Serono or Ganirelix Orgalutran, Organon). The gonadotropin dose was individualized according to women's age, ovarian reserve and antral follicle count. An hCG injection (Pregnyl) or recombinant hCG (Ovitrelle, Merck Serono) was given subcutaneously for final oocyte maturation, when at least three follicles were 17 mm in size or larger, and oocyte retrieval was performed 36-37 hours later. Oocyte retrieval was carried out by transvaginal ultrasound puncture. Luteal phase support was given with micronized progesterone (Progesteron MIC, APL) or vaginal gel (Crinone, Merck Serono) until the day of the pregnancy test. The pregnancy test was performed 14 days after embryo transfer, and if positive, an ultrasound examination was performed at pregnancy week seven to nine. All biochemical pregnancies (positive pregnancy test but no confirmed pregnancy on ultrasound) were classified as negative in this study. Only ultrasound-verified pregnancies at week seven to nine were considered positive in calculating clinical pregnancy rates for this study.

The secondary outcome variables were as follows: ovarian sensitivity index, oocytes retrieved, supernumerary embryos obtained per treatment cycle and embryo score. Embryo score was calculated using the integrated morphology cleavage embryo score (IMC) (138) at two out of three clinics (Reproduction Centre Uppsala University Hospital and Reproductive Medicine Karolinska University Hospital). Reproductive Centre Uppsala University Hospital uses ESHRE's guidelines for cleavage embryos (139).

IVF was performed in 54 % of the cases, and ICSI or combined IVF/ICSI was performed in the remaining 46 %. About half of the women received one treatment only, including both those exposed with a history of unilateral oophorectomy (n= 44, 57.9 %) and controls (n= 7103, 55.1 %). A few women with UO underwent up to six IVF/ICSI treatments, and among women with two intact ovaries, a few underwent up to 10 IVF/ICSI cycles.

### 5.4.6 Reproductive outcome

Primary outcomes were clinical pregnancy rates after oocyte pick up (OPU) and after embryo transfer (ET), both in fresh cycles, and cumulative clinical pregnancy rate, including both fresh and thawed cycles. The secondary outcome was live birth rate (LBR), both after fresh cycle and cumulative, measured by OPU and ET.

### 5.4.7 Ovarian sensitivity index

The OSI was used to evaluate ovarian responsiveness. The OSI is defined as the responsiveness to ovarian stimulation, as measured by a ratio of the total number of oocytes retrieved and total dose of FSH administrated (140-142). The OSI is particularly useful as women in IVF/ICSI cycles are often treated with different stimulation regimes. The women were categorized into three different groups (poor, normal and high responders) after a normogram of OSI response (141).

## 5.5 STATISTICS

### 5.5.1 Paper I

FPI sub-scores were calculated using a 6-point Likert scale and interpreted as indicating low, average, moderately high or very high levels of infertility-stress based on norm values for individuals seen for infertility treatment (136). Responses to questions with an open-response format were analysed according to content and categorized into groups. The VAS-scores for distress and satisfaction were dichotomized according to the median value, which was five ($<5$ and $\geq 5$). Univariate logistic regression analyses were performed to investigate the relationship between socio-demographic data, clinical data and reproductive desire/worry and the outcome variable (perceived information about potential impact of surgery on fertility). Independent statistically significant variables (desire for children and age dichotomized as $<35$ or $\geq 35$) and variables selected for their potential importance regarding information given to patients (parity and education level) were entered into a multivariate logistic regression model. All statistical analyses were performed using PASW Statistics 19 (SPSS Inc., Chicago, IL). Statistical significance was set to a p-value of 0.05.

### 5.5.2 Papers II and III

Baseline characteristics were presented as absolute and relative frequencies with either means and standard deviations (SD) or medians with ranges, as appropriate. The distribution of AMH levels were skewed when tested with the Shapiro-Wilks test. Wilcoxon's signed-rank test was used to test the significance of differences in AMH levels over time. The Mann-Whitney U test and Kruskal-Wallis H test were used to compare AMH and AMH

concentration changes over time. The percentage of change was calculated as (AMH baseline – AMH 6 months)/AMH baseline and (AMH baseline – AMH 2 years)/AMH baseline. Kendall's tau correlation coefficient was used to investigate correlations between AFC, AMH and age.

### 5.5.3 Paper II

All baseline AMH levels were categorized into three groups (≤1.1, 1.2–4 and >4 µg/L). The women's groups were defined as having low, normal and high AMH concentrations. This categorization was done to see if percentage changes over time differed according to the women's baseline level of AMH. A multivariate regression analysis used baseline AMH levels, age, cyst size, duration of surgery and assessed intra-operative bleeding as covariates to identify predictors for AMH decrease at three and six months.

All statistical analyses were performed using PASW Statistics 19 software (SPSS Inc., Chicago, IL, USA). Statistical significance was set at a two-tailed *p*-value of 0.05.

### 5.5.4 Paper III

To study differences in pregnancy rates according to AMH changes at six months, the women were categorized into three groups. Group A included women with low ovarian reserve with baseline AMH levels <0.7 µg/L which remained low after surgery. They were informed that low AMH levels are associated with reduced fertility. Group B included women with normal/high baseline levels that remained within the normal range. They were informed that their normal ovarian reserve, estimated by AMH levels, remained within normal range after surgery and that fertility should not be affected. Group C had similarly normal/high baseline levels that were reduced by 30 % or more at six months. The women were informed of a reduction in their AMH concentrations, but reassured that their levels were still within the normal range. A one-way Anova test, Kruskal-Wallis test and Pearson's chi-squared test were used to compare subgroups based on the desire for additional children at baseline, AMH subgroups and attempting pregnancy. The Mann–Whitney U test was used to compare AMH values and AMH concentration changes over time between groups in terms of histopathology (endometriomas *vs* non-endometriotic cysts), pregnancy or AMH group categories.

All statistical analyses were performed using PASW Statistics 21 software (SPSS Inc., Chicago, IL, USA). Statistical significance was set at a two-tailed *p*-value of 0.05.

### 5.5.5 Paper IV

Generalized estimating equations (GEE) models (143) were utilized, as two-thirds of the women had more than one treatment when including both fresh and thawed treatments. This

regression model makes it possible to take into account the dependence between repeated treatments for each woman. The same women can thus be included at different time points. The GEE model was used for continuous and dichotomous outcomes with and without adjusting for age, OSI and clinic. Effect measures were mean differences and odds ratios for continuous and dichotomous outcomes, respectively (with 95 % confidence intervals and p values with statistical significance set at $p < 0.05$ for two-sided tests). Effects were assessed in univariate and in multiple models while adjusting for women's age, ovarian sensitivity and clinic. An OSI normogram was used to categorize the women into three groups (141). Statistical analyses were undertaken with the SAS statistical program 9.4, SAS Institute.

# 6 ETHICAL CONSIDERATIONS

We followed the ethical standards of the Helsinki Declaration of 1975, which was revised in 2000. All participating women in the prospective cohort study were informed about the research and the current lack of knowledge about the potential impact of ovarian surgery on the ovarian reserve. They received both oral and written information about the study, and all participating women signed a formal consent. For the follow-up visits, they were contacted twice by telephone or by post. All women also received information regarding their individual AMH concentrations and any changes in these concentrations some weeks after each blood test. Knowledge of their individual AMH concentrations did not influence the type of ovarian surgery they underwent or their treatment in any way, as the AMH concentrations were not known at the time of surgery. There was only one woman in the study who preferred not to receive any information about her ovarian reserve.

In study IV, the clinical data was prospectively collected as part of the women's IVF/ICSI treatment protocols. The data was then anonymised and analysed at the group level.

All studies were approved by the Regional Ethical Review Board in Stockholm, Sweden: Study I (papers I-III) (D-nr 2011/107-31-4, 2012/314-32, 2013-532-34) and study IV (D-nr: 2011/1758-31-2, 2014/1360-32).

# 7 RESULTS

Study I included a cross-sectional design, and a prospective cohort followed for 3, 6 and 24 months, as reported in three papers. The participants' age and percentage of endometriomas were similar in the three different papers (papers I-III). Comparison showed no significant difference between study participants and women who were lost to follow up at 24 months in terms of their desire for children (paper III).

**Table 7.** Women's age and reproductive history among participants in study I.

| Paper | I | II | III |
|---|---|---|---|
| **Women (n)** | 106 | 75 | 66 |
| **Age, mean (SD)** | 32.3(6.4) | 32.1 (6.2) | 32.5 (6.4) |
| **Children, n (%)** | 42 (39.6) | 30 (40) | 24 (36.4) |
| **Infertility (%)** | 20 (18.9) | 15 (20) | 14 (21.2) |
| **Endometriomas (%)** | 33 (31.3) | 25 (33.3) | 21 (31.8) |

SD: Standard Deviation

### 7.1.1 Paper I

Only half of the women (n=51) reported that they had received information about the potential impact of ovarian surgery on their fertility. In a multivariate logistic regression model, desire for children was the only variable significantly associated with having received such information (p=0.02). Women who desired children were three times more likely to recall having received information about the impact of surgery on fertility compared to women with no such desire. Among the women who had received any fertility-related information about the surgery, 26 women (59 %) recalled receiving information that ovarian surgery could potentially have a negative impact on future fertility, and 20 (39 %) remembered receiving information about a lack of negative impact on fertility. Only 10 % of the women in our study population stated that fertility-related information was unnecessary for them.

About half of the participating women (N=57) reported moderate or high levels of worry about future fertility (VAS ≥5). Furthermore, among women with a desire for future children (n=76), high levels of distress were reported for the following FPI subscales: Need for parenthood (12 %) and Rejection of a child-free lifestyle (20 %).

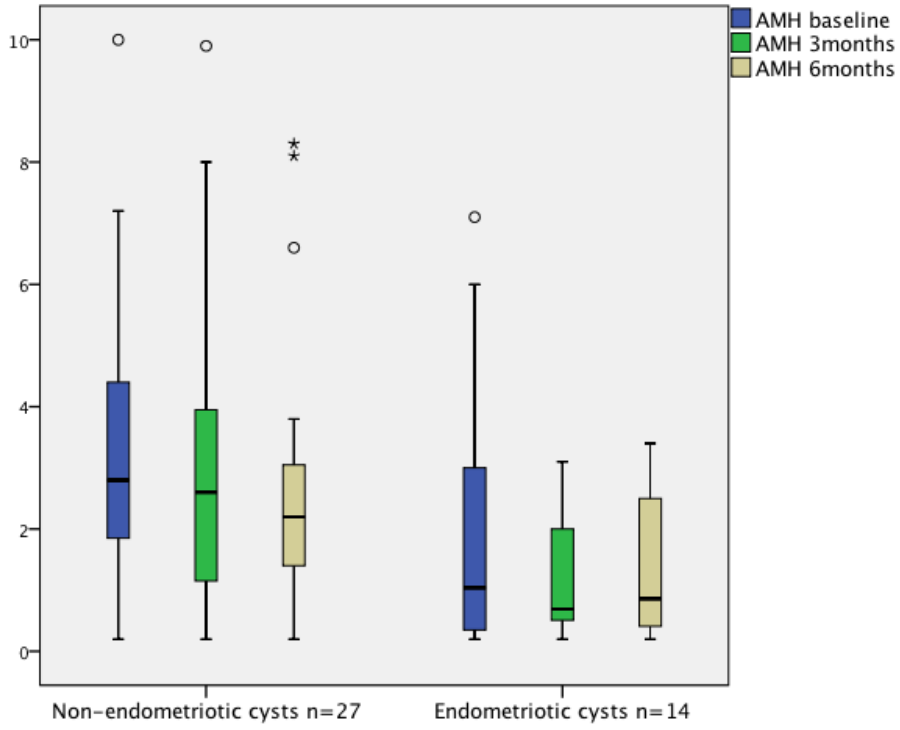
### 7.1.2 Paper II

In this paper, we described the changes of AMH observed after ovarian surgery within a six-month follow-up period. Based on baseline levels prior to surgery, there was a significant decline of AMH in the entire group of women. This statistically significant decrease was evident at three and six months. Median AMH levels decreased significantly from 2.7 µg/L (0.2-16.9) to 1.6 µg/L (0.2-9.9) at three months and were still low, 1.6 µg/L

(0.2-8.3), at six months ($p$<0.001, respectively). Women with normal baseline AMH concentrations (1.2–4 µg/L) or elevated AMH (>4 µg/L) showed a higher decrease of AMH levels at the six-month follow up compared to women with low AMH levels at baseline (≤1.1). We found a difference in the reduction of AMH concentrations according to the histopathology of the cysts. In the case of endometriomas, we observed the highest median reduction at six months (a reduction of 50 %). Women with dermoid cysts presented with a smaller reduction (25 %), and women with follicular cysts or cystadenomas showed a 34 % reduction of AMH levels. The study group was heterogeneous with regard to the surgical technique applied to removing the cysts: 65 women had a cyst enucleation, four had a resection of ovarian tissue, four had a unilateral oophorectomy, and two had surgery for bilateral cysts.

In order to follow changes in AMH concentrations, sub-analyses in a more homogenous group were performed. This group included only women with a clean cyst enucleation surgery without any obvious removal of ovarian tissue during the surgery.

**Figure 12. Comparison of women with endometriomas vs non-endometriotic cysts.** This figure illustrates AMH concentrations in 41 women who had a clean ovarian cyst enucleation for a single cyst and who had no previous ovarian surgery. There was one outlier: a woman in the non-endometriotic group who had a baseline AMH of 17 was removed to make the boxplot easier to comprehend. There was a significant decline in AMH at six months in the non-endometriotic cysts group ($p$=0.002), but this was not observed in the endometriotic group ($p$=0.060).



### 7.1.3 Paper III

This part of the study aimed to investigate pregnancy outcomes in relation to changes in AMH concentrations following ovarian surgery. During the study period, 26 women tried to conceive, and 15 achieved pregnancy (58%). The live birth rate was 46 %. A high percentage (82 %, n=9) of the women who tried to conceive without success had also reported fertility problems prior to surgery. Eleven women received some form of infertility treatment, which resulted in five live births. The treatments were as follows: one ovulation induction treatment with clomiphene citrate, one intrauterine insemination and one oocyte donation. Eight women received treatment with IVF/ICSI. Women with normal AMH concentrations at baseline who had a small or no reduction at six months showed the highest live birth rate of 62%. Among women who tried to conceive, there were no children born in the group of women with low ovarian reserve (AMH <0.7 µg/L) using their own oocytes compared to a live birth rate of about 50% among women with normal/high AMH levels (p=0.070).

Serum AMH decreased in the whole group from 2.7 µg/L (0.2-16.9) to 2.0 µg/L (0.2-11) at six months and continued to decrease to 1.0 µg/L (0.2-20.7) at the two-year follow up ($p$ = 0.001, respectively).

### 7.1.4 New subgroup analyses for this thesis

The new subgroup analyses included only women who attended all three follow-up visits at 3, 6 and 24 months (Figures 13-15). AMH levels decrease significantly after surgery for endometriomas and non-endometriotic cysts at 6 and 24 months postsurgery, respectively. The result was similar when including women (n=46) who had different types of ovarian surgery (cyst enucleation, fenestration or resection/unilateral oophorectomy) (Figure 13) and women (n= 32) who had a clean cyst enucleation (Figure 14). A significant reduction of AMH levels at three months was only demonstrated among women who had surgery for non-endometriotic cysts.

**Figure 13. Comparison of median AMH levels between women with endometriomas vs non-endometriomas who attended all four visits.** The figure illustrates the women (n=46) who attended presurgery and 3-, 6- and 24-month follow-ups. Four of the women had a fenestration of a cyst, two a resection/unilateral oophorectomy and 40 a cyst enucleation. P value between AMH levels presurgery and each of the follow-ups at 3, 6 and 24 months. * p<0.05

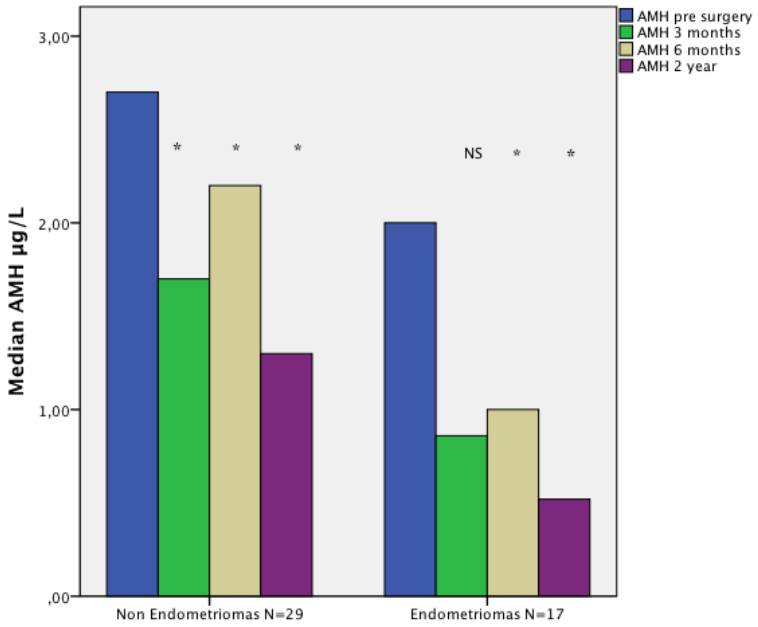



**Figure 14. Median AMH concentrations and changes over time in the 32 women who had a clean cystenucleation and completed all four AMH estimations (baseline, 3, 6 and 24 months).** Women who were breastfeeding, pregnant or had previous ovarian surgery were excluded (n=8). Wilcoxon's signed-rank test was used to test the significance of differences in AMH levels between presurgery and 3, 6 and 24 months. There was no significant difference in the percentage of AMH change between the women with endometriomas and those with non-endometriomas at 6 or 24 months. * p<0.05 and ** p<0.01.

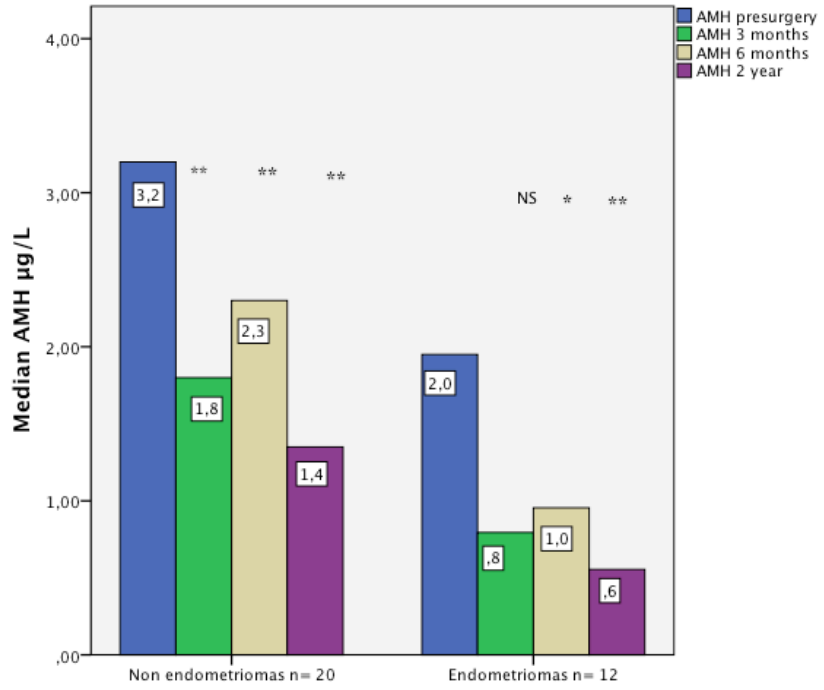
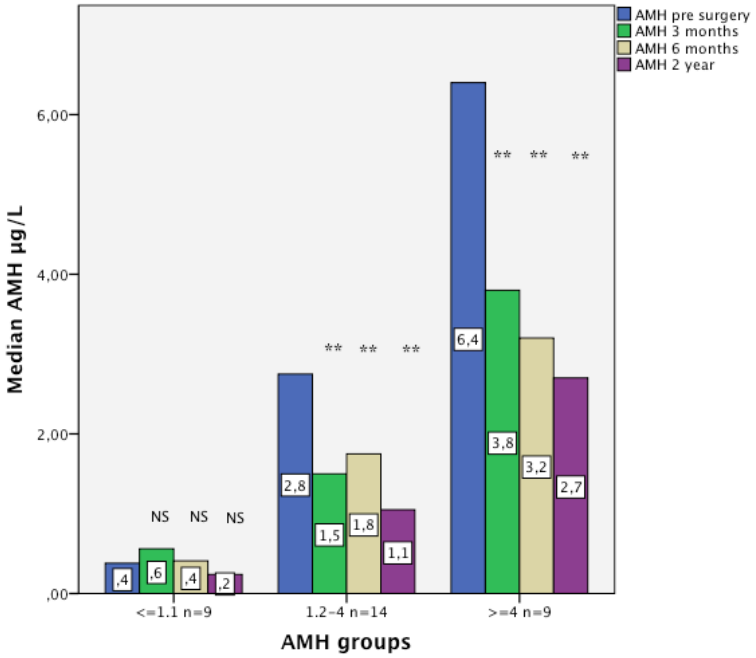


**Figure 15**. **Median AMH by category groups depending on baseline AMH concentrations and their changes after ovarian surgery in the 32 women who completed all four AMH follow-up estimations (baseline, 3, 6 and 24 months).** Women who were breastfeeding or pregnant at time of follow up were excluded (n=8). Wilcoxon's signed-rank test was used to test the significance of differences in AMH levels over time. * p<0.05 and ** p<0.01.




### 7.1.5 Paper IV

This study aimed to investigate if the clinical pregnancy rates differed in women with a history of unilateral oophorectomy (UO) compared to women with intact ovaries.

Our study showed that women with UO needed higher doses of gonadotropins for hormonal stimulation, and fewer oocytes were retrieved at OPU compared to women with intact ovaries. The OSI of women with UO was significantly lower than that of women with intact ovaries (p=<0.001). Nevertheless, there was no difference in the number of supernumerary embryos that could be frozen from the IVF/ICSI treatments or in embryo quality between the women with a history of UO and controls. The mean number of transferred embryos, including both fresh and thawed treatment cycles, was similar between the group of women with UO and controls (1.19 vs 1.16, p=0.198).

The clinical pregnancy rate/OPU in women with a history of UO was significantly lower compared to controls (24.5 % vs 32.4 %) both crude and adjusted for age (p=0.030 and p=0.021, respectively). After inclusion of treatments using both fresh and frozen-thawed embryos, the cumulative clinical pregnancy rate/OPU was still significantly lower in women with UO vs controls: 32.4% vs 42.9% (p=0.031). In a GEE model a 30% reduction in the chance of achieving clinical pregnancy was found for women with a history of UO

compared to women with intact ovaries, after adjusting for age (Table 8). Even though the odds ratio was reduced by 30%, the chance of achieving pregnancy was not significantly different after adjusting for age, OSI and clinic. Women with a history of UO also had a 30% lower chance of achieving live birth after IVF/ICSI treatment compared to women with intact ovaries of the same age, but this difference did not reach significance (Table 8).

**Table 8.** Clinical pregnancy rates and live birth rates (LBR) per OPU and ET in women with a history of unilateral oophorectomy; Odds ratios are age-adjusted.

| | **Odds ratio** | **95 % CI** | **P-value** |
|---|---|---|---|
| **Clinical Pregnancy/OPU (fresh)** | 0.66 | 0.44-0.99 | 0.043 |
| **Cumulative Clinical Pregnancy/OPU (fresh + thawed)** | 0.66 | 0.45-0.96 | 0.031 |
| **Clinical Pregnancy/ET (fresh)** | 0.68 | 0.45-1.01 | 0.058 |
| **Cumulative Clinical Pregnancy/ET (fresh +thawed)** | 0.64 | 0.42-0.96 | 0.031 |
| **LBR/OPU (fresh)** | 0.68 | 0.43-1.07 | 0.094 |
| **Cumulative LBR/OPU (fresh+ thawed)** | 0.71 | 0.47-1.05 | 0.090 |
| **LBR/ET (fresh)** | 0.70 | 0.44-1.11 | 0.129 |
| **Cumulative LBR/ET (fresh + thawed)** | 0.68 | 0.44-1.03 | 0.069 |

ET: Embryo Transfer, LBR: Live Birth Rate, OPU: Oocyte Pick-Up

# 8 DISCUSSION

In papers II-III, we were able to demonstrate that ovarian cyst surgery significantly reduces the ovarian reserve estimated as AMH serum concentration – even for conservative surgery, including only cyst enucleations with no macroscopic removal of ovarian tissue.
The AMH concentration consistently decreased over time at 3, 6 and 24 months post-ovarian surgery (paper II-III). For women who underwent conservative ovarian surgery in our clinical cohort, the total pregnancy rate was 58 % at the two-year follow-up, including spontaneous and ART-pregnancies, among those who tried to conceive (paper III); however, if a whole ovary was previously removed, the clinical pregnancy rate was reduced by 30 % after IVF/ICSI treatment when compared to age-adjusted women with intact ovaries (paper IV). Only about half of the women recalled receiving preoperative information about the impact of ovarian surgery on future fertility (paper I).

### 8.1.1 AMH changes following ovarian surgery

In our clinical cohort, the reduction of AMH is larger than that shown in normograms of women without ovarian surgery. The AMH decreased after all types of ovarian cyst surgery (2.7 µg/L to 1.0 µg/L) and also in the subgroup of women who had a clean unilateral cystectomy (2.8 µg/L to 0.9 µg/L) within a two-year period (paper III). Normograms of fertile women have reported a yearly reduction of AMH of 0.16 µg/L (35) or 5.6 % (34). This study is the first with a 24-month follow-up after ovarian surgery reporting not only the changes in women's ovarian reserve as measured by AMH but also fertility outcomes (clinical pregnancy rates). As a result, our data cannot be compared to that of previous reports. It is important to consider that age-related infertility usually precedes menopause by an average of 10 years (13). By using data from a previous study on AMH as a predictor of menopause, the ten women in our study with low AMH <0.7 µg/L and a mean age of 36.9 years would reach menopause at about 43 years (35-47 years) (41). According to another study, based on their AMH levels only (AMH <0.7 µg/L), these women would reach menopause in a median time of 12 years (95% CI 9.3-12.7) (43). Using the same predictor of menopause for the whole group of women who attended the two-year follow-up visit (n=63) (mean age 34.5 and median AMH 1.0 µg/L), menopause would be advanced by a couple of years: 48 years (40-54 years) (41) and 47.6 years (95% CI 47.3-47.8) (43). There are not yet any prospective studies published on the effect of ovarian surgery in terms of ovarian reserve, pregnancy rates and age at menopause.

The results presented in papers II-III add to the growing body of evidence that ovarian surgery affects ovarian reserve measured by AMH concentrations (30, 85, 87, 92-94, 96-98, 101).

### 8.1.2 AMH changes dependent on histopathology type of cysts

Reduction of AMH after cyst surgery for endometriomas has been described for 3-month (84, 95, 97), 6-month (87, 94, 96), 9-month (91, 93) and 12-month follow-ups (101). So far, there are only four published studies that have compared endometriomas with non-endometriotic cysts (84-86, 97). Two of these studies detected a significant reduction in AMH levels in the non-endometriotic cyst group as early as one month postsurgery (85, 86). However, these studies were relatively small: the first one included seven women with non-endometriotic cysts (six dermoid cysts and one cystadenoma) (86), and the second one included 21 women with non-endometriotic cysts (18 dermoid cysts, two cystadenomas and one struma ovarii) (85). The results of AMH decline three months postsurgery for non-endometriotic cysts are more inconsistent. A significant reduction of AMH three months postsurgery was demonstrated in only one study including 32 women with non-endometriotic cysts (15 women with dermoid cysts and 17 cystadenomas/functional cysts) (97). By contrast, Kitajima et al (84) showed no significant reduction of AMH in the non-endometriotic cyst group (8 women with dermoid cysts and 5 cystadenomas) three months postsurgery. Chang et al (86) also found no effect at three months despite observing a significant decline of AMH at one month. In paper II, we found significant median reductions in AMH concentrations six months following ovarian surgery following both surgery of endometriomas (n=20) and dermoid cysts (n=31); meanwhile, the median AMH reduction was non-significant in women with cystadenomas/functional cyst (n=15). The decline of AMH continued at the two-year follow-up, and it was similar for all women: there was no significant difference in median decline after surgery between women with endometriomas and non-endometriotic cysts (paper III). Researchers have argued that AMH decline after ovarian cyst surgery results from the removal of ovarian cortical tissue and ovarian follicles. Indeed, removal of healthy ovarian tissue occurs during surgery for ovarian cysts and differences have been demonstrated according to cyst histopathology. In the case of endometriomas, up to 54 % of healthy ovarian tissue has been removed; in dermoid cysts, the figure is up to 17 %, and in cystadenomas, it was 0 % (144, 145). Another study detected that normal ovarian cortex was accidently removed in 92 % of women who had surgery for endometriomas; in the non-endometriotic cyst group, there was diversity according to the histopathology type (146). With dermoid cysts, the ovarian cortex was removed in 82 % of cases, and for a corpus

luteum hemorrhagicum, it was removed 78 % of the times (146). In one of the largest studies published on the effect of ovarian surgery for non-endometriotic cysts, no significant difference was detected in follicles accidently removed between endometriomas and non-endometriotic cysts (97).

Until recently, there has been a belief that only surgery of endometriomas may affect the ovarian reserve, estimated as AMH concentrations. Earlier studies of non-endometriotic cysts only showed a decrease at one month (85, 86), which then improved at three months (86); another study showed no decrease at all (84). Our study both confirms the results of Kwon et al (97) and increases knowledge about the long-term effects on ovarian reserve following ovarian surgery. Surgery for non-endometriotic cysts also results in a post-operative decrease of AMH concentrations not only at 3 months but also at 6 and 24 months (papers II-III). As our study is the largest to date regarding AMH concentrations after surgery in women with non-endometriotic cysts (n=47), we were able to divide the group population and compare dermoid cysts and cystadenoma/functional cysts separately (paper II). In the two-year analysis, there were few women with cystadenoma/functional cysts; therefore, the cystadenomas/functional cysts and dermoid cysts were analysed as a group (paper III). In the group of women who had unilateral ovarian cystectomy, there was no difference in median change in percentage of AMH at 6 or 24 months between endometriomas (n=13), dermoid cysts (n=21) and cystadenomas/functional cysts (n=5), but the study sub-groups used for these comparisons were small.

### 8.1.3 Predictors for AMH changes following ovarian surgery

Already in 2012, a review article stated that no further research on the effect of ovarian cystectomy on endometriomas was needed, as a significant reduction of AMH had been demonstrated in several studies (88). Instead, further research on predictors for AMH decrease are necessary (88). Research associates bilateral endometriomas as a significant factor in AMH decline after ovarian surgery (92, 147). No previous studies, including ours, could demonstrate a difference in AMH decline between unilateral or bilateral surgery for non-endometriotic cysts (84-86, 147). This might result from the small number of women with bilateral non-endometriotic cysts available for comparison that were included in the studies, creating a lack of statistical power to detect a difference. In one previous study, preoperative AMH levels have been reported to positively correlate with AMH decline postsurgery (96). In our study, the only significant predictor for postoperative AMH reduction was the AMH concentration at baseline. Postsurgery, women with a higher baseline AMH level had an odds for reduction at three months of 1.9 (95 % CI 1.1-3.1), and at six

months of 2.5 (95 % CI 1.2-5.2) compared to women with low ovarian reserve <1.1 µg/L. These data are presented in paper II.

### 8.1.4 AMH changes following ovarian surgery and pregnancy rates

We could not detect a difference in pregnancy rates for women with normal AMH prior to ovarian surgery with regard to the magnitude of the AMH changes over time at the 24-month follow-up (paper III). In the women's group with AMH categorized as low prior to ovarian surgery (<0.7 µg/L), none of the women who tried to conceive achieved a pregnancy, but one undesired pregnancy occurred among these women. Our results confirm the study of Streuli et al (148), which indicated that there is no lower limit of AMH where a spontaneous pregnancy cannot occur. Results from previous studies are inconsistent regarding the predictive value of a low AMH level on fecundity. In a Danish study of younger women (< 35 years of age), there were no significant differences in time to pregnancy between women with low AMH <0.7 µg/L and women with normal AMH concentrations (56). A similar study that included women of an older age (30 – 44 years) showed a significant reduction of fecundability (fecundability ratio 0.38; 95 % CI 0.08-0.91) in women with AMH<0.7 µg/L (55). Both of the previous studies had a short follow-up period of six months. In our study with a longer follow-up period of 24 months, data were inconsistent regarding time to pregnancy, as this factor was not optimally assessed (paper III). We can conclude that there was a significant difference in pregnancy rates between the group of women with low AMH <0.7 µg/L and women with normal/elevated AMH concentrations despite changes in their AMH postsurgery (p=0.028) (paper III).

### 8.1.5 Pregnancy rates and histopathology of the cyst

Pregnancy rates did not differ between women with endometriomas and non-endometriotic cysts in our study (paper III). Only one previous study demonstrated a difference in pregnancy rates within 24 months following ovarian cystectomy for endometriomas (15% pregnancy rate) and non-endometriotic cysts (46% pregnancy rate) with a population size of 104 women (146). Otherwise, previous studies on pregnancy rates have primarily focused on women with endometriomas. A meta-analysis including 14 studies of pregnancy rates following surgery for ovarian endometriomas showed a weighted mean of 50% and a variation in pregnancy rates between 30-67% (149). The authors argue that the true pregnancy rate was probably lower due to selection bias, loss to follow up and confounding variables. A review article of surgery for borderline ovarian tumours (BOT) report a pooled spontaneous pregnancy rate of 54% (150). Unfortunately, results for cystectomy and unilateral oophorectomy were mixed, so the pregnancy rate per surgery type was not reported

(150). In that review article, the last discussed study reported a similar pregnancy rate between the unilateral oophorectomy group (89%) and cystectomy group (86%) in women with borderline ovarian tumour stadium 1 (151). No differences in pregnancy rates have been demonstrated between women who have undergone ovarian surgery or those following expectant management of ovarian endometriomas prior to ART (152). Such results have been interpreted differently. Some studies advise performance of surgery before ART and others point out the potential harm to the ovarian reserve. ESHRE's 2013 guidelines state that more studies are needed, but nonetheless recommend expectant management of painless endometriomas prior to ART. They also point out the importance of counselling women regarding the risk of losing an ovary and/or ovarian reserve with surgery (105).

### 8.1.6 Clinical pregnancy rate following ART in women with UO

By collecting data from three large Swedish ART centres, we were able to include 76 women with a history of UO and 12879 controls, and to demonstrate a 30% reduction of clinical pregnancy rates in women with a history of UO, compared to age-adjusted controls (paper IV). This is the first study to report a reduced pregnancy rate after IVF/ICSI treatment with mostly single embryo transfer in this population. There was also a 30% reduction in LBR in the UO group, even though the difference between UO and controls did not reach statistical significance. This finding might be explained by the relatively small sample size of exposed women, leading to insufficient power in investigating LBR differences. The effect of unilateral oophorectomy on female reproduction is still partly unknown and open for discussion. It has been shown that women with a history of UO tend to reach menopause one year earlier (107, 110). Previous studies of IVF/ICSI treatment in women with a history of UO showed no significant differences in pregnancy rates even though the women required higher gonadotropin doses, and fewer oocytes were retrieved at OPU (112, 113, 115, 116). One possible reason for our finding of a difference in pregnancy rates might be that our cohort of women, especially unexposed, was larger than that of previous studies. Embryo quality, according to ICM score, was similar between the two groups in our study (138). Miscarriage rates did not differ between the two groups. Most studies on women with UO have demonstrated a significantly lower amount of oocytes retrieved at OPU (112-116, 121, 123, 125), which was confirmed by our study. It has previously been concluded that there is a relationship between the number of oocytes retrieved, pregnancy and LBR (153). Therefore, in line with paper IV, it can be inferred that women with a history of UO might have significantly lower chances of pregnancy.

### 8.1.7 Fertility-related information prior to ovarian surgery

At the time of study I (paper I), a dawning knowledge of the negative effect of ovarian surgery on the ovarian reserve began to arise. In 2010, RCOG published guidelines highlighting the importance of discussing loss of function with the patient, especially the loss of fertility (104). In paper I, only half of the women included in the study recalled receiving preoperative information about the impact of ovarian surgery on future fertility. Desire for children was the only factor associated with fertility-related information. Several reasons might explain this finding. The gynaecologists might have provided fertility-related information, but the women failed to recollect it because so much information was provided during the visit. Patients might also experience presurgery anxiety affecting their perceptions and cognitive capabilities (154). Another explanation might be that the gynaecologists were unwilling to unnecessarily worry the patients about what they perceived to be low-risk outcomes. Only 10 % of the women included in our study regarded the fertility-related information as irrelevant to them. Reproductive knowledge seems to be sparse among childless women, both in terms of the consequences of delaying childbearing and the effectiveness of ART (4, 155). Several public campaigns have been launched in Great Britain and the United States in an attempt to improve fertility awareness. The effectiveness of such campaigns remains unclear. Online educational programs have sought to improve people's fertility awareness. Unfortunately, a recent study showed that six months after reading online educational information on fertility, participants' fertility beliefs and knowledge returned to pre-intervention levels (156). The lack of reproductive knowledge among women increases the risk of not receiving correct fertility-related information regarding the risks of ovarian surgery. All doctors must strive for shared decision-making with a well-informed patient prior to ovarian surgery (103), especially as reproduction and ability to become a parent is an important factor for many women (4). Prior to invasive treatment, information needs to be provided using structured communication tools that present risk to women in a way that they can understand. RCOG recommends that doctors inform patients of an event's probability in numbers, not in words (157).

### 8.1.8 Ovarian surgery in Sweden and ovarian reserve screening

In Sweden, there has been an increase in the number of ovarian surgeries performed for women aged 15-44 years over the last seven years, from 3000 to 3300 (Figure 8) (83). Reasons for this increase might be the improved diagnostic technique (transvaginal ultrasound) and the number of private clinics that perform laparoscopic ovarian surgery. This trend of increasing ovarian surgeries goes against all newly-published guidelines, which

recommend expectant management in benign asymptomatic cysts < 7cm (76-79). A change in ovarian surgery policy might occur as knowledge increases about ovarian physiology and the long-term effects of ovarian surgery.

Today, there is variety of ways to help women conceive. Still, if we harm women's ovarian reserve irreversibly through ovarian surgery, there is no guarantee that ART treatment will help. Live birth rates after ART treatment is about 37 % in the entire population who reached the embryo transfer stage, but younger women have the highest LBR (69).

Ovarian reserve screening has been advocated by measuring AMH concentrations. Such screening would give women a personalized risk profile of her reproductive capacity, and it might improve their motivation in reproductive life planning (158). ACOG's 2015 recommendation on ovarian reserve testing states that AMH should be measured for women over 35 years of age who have not conceived after six months of attempting pregnancy and in women who have had ovarian surgery for endometriomas (2). If AMH is diminished or decreased, the women should be counselled of their shorter reproductive window. It is hoped that such information will help women achieve pregnancy. This information aligns with our findings in paper III: we suggest that women with reduced ovarian reserve should be informed of their chances to conceive as well as the reduced reproductive window that can be estimated in some individual cases. In paper III, we also report that the information provided on AMH changes within two years following ovarian surgery did not affect women's reproductive behaviour, and that the information that was provided to the women in this study after their ovarian surgeries did not increase patients' worries about their fertility potential.

## 8.2 METHODOLOGICAL CONSIDERATIONS

### 8.2.1 Study design and validity

In paper I, we present results from a cross-sectional study of women who completed a questionnaire at the presurgery visit. The women answered the questionnaire directly after meeting with a gynaecologist to plan for ovarian cyst surgery. A strength of the study was that the assessment of the women's perceived information was done so quickly after consulting with the gynaecologist, decreasing the risk of recall bias. A disadvantage is that we could not study the extent to which doctors actually gave fertility-related information, as we did not perform a parallel survey asking the gynaecologists about their information disclosure routines. Information prior to surgery is standardised at the clinic, and all doctors were aware of the present study, which might lead them to provide more detailed information about the effects of ovarian surgery on fertility.

The response rate in the study was relatively high (80%). Only 20 women declined participation, and six women did not complete the questionnaire. Because not all women answered or filled out the questionnaire, there is a risk of selection bias. It might be that women with a desire for children were more prone to participate in the study. This might imply that the true proportion of women with a desire for children is lower than reported. Södersjukhuset, where the study was undertaken, is a tertiary care center that receives a variety of women from the population, not just infertile women. This makes the study more representative and increases its generalizability.

Because knowledge about the effect of ovarian surgery on ovarian reserve has increased during the last couple of years, it would have been interesting to study if there was a difference in the percentage of received fertility-related information prior to surgery over this time period. Unfortunately, the inclusion time of a year was too short to study this time dimension. Today, with more widespread knowledge, the results of our study might be different.

In papers II-III, we present results from a prospective cohort study. This design was useful as we could study the relationship between exposure (ovarian surgery for cysts) and outcome (AMH changes over time and pregnancy rates). It would have been unethical to randomize women to surgery or not, as all cysts were symptomatic. A possible confounder was age, as women who had a unilateral oophorectomy were older, and age affects AMH levels. To adjust for this possible confounder, we used multivariate logistic regression. Another strength of this study was a longer study period compared to previous studies; in addition, both ovarian reserve and pregnancy outcome was measured. The reliability of Beckman Coulter Gen II ELISA AMH kit was questioned during the study period. As described earlier, fresh AMH samples have been found to increase progressively in room temperature, but all AMH levels in our study were analysed from frozen samples with a median freezing time of six days; therefore, the risk of false values was minimized. Another strength is that all AMH samples were analysed in the same laboratory with low intra- and inter-assay coefficients of variability.

In prospective cohort studies, there is always a risk of selection bias due to loss to follow-up. In paper II, 72 % of the women attended both the 3- and 6-month follow up. The other 21 (28%) women attended either the 3- or the 6-month visit. No difference was demonstrated regarding age, baseline AMH or desire for children between the women who attended both the 3- and 6-month follow ups compared to the women who only attended one of these visits. Seventeen women (20 %) were lost to follow up in paper III. Analysis of these women showed that they had the same age, desire for children, baseline AMH concentrations and 6-

month AMH concentrations as the women who completed the two-year follow-up visit. Therefore, the risk of selection bias was probably low, and a representative group of the women attended all follow-up visits. Our results on ovarian reserve following ovarian surgery do not seem to depend on chance or so-called random errors, as a statistically significant reduction of AMH was demonstrated in all three follow-up analyses of AMH. Because these operations are generally performed in a similar way, external validity is high and applicable for the general population of women in Sweden who attend a university hospital where residents operate together with consultants in gynaecology.

Study IV is a population-based registry study. A retrospective registry study has inherent problems because the available information is restricted to the register's content on exposure, outcomes and confounders. When using retrospective data, the researcher also naturally depends on the quality of the data in the registry. A strength in our study is that it is multicentre, and that the same type of database was used by all three clinics. In this way, all variables regarding clinical features, embryology charts and IVF/ICSI treatment aspects were registered similarly, whit a minor risk for misclassification. A disadvantage was that no detailed information was available regarding the reason for ovarian surgery or pathology of the cysts. All presented data was age-adjusted, as age was considered a confounder. In study IV, the risk of selection bias was reduced because data on all exposed and unexposed women in the study cohort were collected from three ART centres. Further, the outcome variables, clinical pregnancy rate and live births rate, are reported to a national quality registry where validation is conducted on a regular basis. The quality register has 100 % coverage for patients and clinics. If data is incomplete or missing, the IVF clinic is informed, so the data can be completed. This strengthens our results, as the outcome variables have no missing values. External validity and generalizability are high as the treated women at the three different ART clinics were of different ages. In addition, they had different reasons for infertility and received similar treatment protocols for IVF/ICSI. Two of the clinics are funded through public health care, so the treatment is affordable for most patients, thus minimising socioeconomic differences.

# 9 CONCLUSION

Ovarian cyst surgery, even conservative, is associated to a reduction of the ovarian reserve and might affect pregnancy rates, at least for women receiving IVF/ICSI treatment after unilateral oophorectomy. Fertility issues must be discussed with women before they make the decision to have ovarian surgery.

## 9.1 CLINICAL IMPLICATIONS

Ovarian cyst surgery induces a significant reduction in ovarian reserve, which remains at least two years following surgery. However, the potential to conceive might be maintained in the sub-population of women who had normal or high serum AMH concentrations prior to their ovarian cyst surgery.

Fertility issues are important for women of reproductive age, and they must be addressed and taken into account when women are planning for ovarian cyst surgery. A shared decision between the gynaecologists and well-informed patients should be made before invasive treatments, such as ovarian cystectomy. Women's knowledge of eventual risks and intervention complications would increase awareness and satisfaction with their healthcare.

AMH should not routinely be assessed prior to ovarian cyst surgery in women of fertile age, as our research shows that surgery itself does not seem to impact women's fertility. However, women with decreased ovarian reserve prior to surgery, and older women with an expected low ovarian reserve, could benefit from awareness of a shorter fertile window.

Women with a history of unilateral oophorectomy should also be informed of the expected reduced number of eggs collected for IVF/ICSI treatment and the reduced likelihood to achieve clinical pregnancy by ART treatment.

## 9.2 FUTURE RESEARCH

A larger cross-sectional study regarding presurgery information would be beneficial in terms of what information women need and what information they perceive. This phenomenon could be studied using questionnaires/interviews with gynaecologists regarding what information they think is of importance to women. Similar questionnaires/interviews should be given to women with ovarian cysts to ascertain which information they find important. The presurgical visit could be recorded to actually interpret given and received information. The study could also include a follow-up interview with the women a few weeks following surgery about the given information and its adequacy. The level of anxiety should be measured to see if there is a possible association between anxiety and received information.

Studies of AMH ELISA assay methods could ensure their standardisation, including limits of normal AMH values. Such research would enable further studies to be more comparable.

Cohort studies of AMH concentrations and changes over time in a general population of women would assist in constructing normograms of AMH. These normograms should be accurate enough to predict women's future reproductive time span and menopause with a narrower confidence interval than earlier studies.

A prospective cohort multicentre study including women of reproductive age with different histopathology types of ovarian cysts would enable us to increase our knowledge regarding the effects of ovarian cysts treated conservatively or through ovarian surgery. Exposure should include different types of ovarian surgery, such as cystectomy, ovarian resection and unilateral oophorectomy. If possible, these women should be followed up with using yearly AMH samples, ultrasound and questionnaires regarding their reproductive behaviour for 10 years or until they reach menopause. Ideally, there would also be a control group of women without ovarian cysts included in the study.

Another research approach for learning more about the consequences of unilateral oophorectomy would be a register study including all women with a history of unilateral oophorectomy and aged-matched controls. The outcome variable would be parity.

# 10 SAMMANFATTNING PÅ SVENSKA

Vid födseln har kvinnan ungefär sex miljoner oocytanlag (ägganlag) i ovarierna (äggstockarna). Av dessa kommer de flesta självdö. Vid menstruationsdebut finns endast 400 000-600 000 oocyter (ägg) kvar. Minskningen av oocytanlag fortsätter sedan successivt. Under kvinnans fertila period kommer ungefär 450 av folliklarna (äggblåsorna) som oocyterna ligger i ha ägglossat och de andra, både folliklar och oocyter, kommer tillbakabildas. Ovarialreserven (antalet omogna ägg i äggstocken) speglar möjligheten för kvinnan att bli gravid. Förlusten av oocyter är åldersberoende och hastigheten ökar 10-12 år innan menopaus inträder. Vid 50-års ålder är det så lite kvar av ovarialreserven att klimakteriet inträder. Då återstår endast några hundra till tusen folliklar. I Sverige är medelåldern för menopaus 51år.

De mest pålitliga metoderna för att förutbestämma en kvinnas ovarialreserv och hennes möjliga svar på fertilitetsbehandling vid provrörsbefruktning är att mäta antalet folliklar (AFC) med ultraljud och att genom ett blodprov mäta Anti-mülleriskt hormon (AMH). AMH utsöndras endast från de små, < 6mm, folliklarna i ovariet och minskar med stigande ålder varför det anses vara en bra markör för ovarialreserven. AMH tycks även vara en god prediktor för menopaus. Fördelar med AMH är att det oftast inte är någon interindividuell skillnad så provet kan tas när som helst i menscykeln.

Godartade cystor på ovariet är vanligt förekommande. I Sverige sker årligen cirka 3300 operationer på grund av cystor i ovariet på kvinnor i fertil ålder. Operationerna har blivit mer fertilitesbevarande och idag görs det främst lapraskopiska cystektomier (borttagande av cysta) och inte borttagande av en del eller ett helt ovarium vilket tidigare var vanligare. Studier har visat att ovarialreserven mätt genom AMH sjunker efter en operation av cystor, särskilt vid operation av endometrios cystor. Det är dock oklart om ovarialreserven påverkas vid operation av andra sorters cystor, på längre sikt än några månader och ifall kvinnans möjlighet att bli gravid påverkas av cystoperation.

I artikel I, redovisas en tvärsnittsstudie, i vilken 106 kvinnor som skulle genomgå operation av ovariet på grund av godartad cysta inkluderades. Kvinnorna fick svara på en enkät med frågor av socioekonomisk karaktär, frågor om reproduktion och barnönskan samt frågor om vilken information de fått angående deras planerade operations eventuella påverkan på förmågan att få barn. I svaren framkom att bara hälften av kvinnorna sade sig ha fått information om cystoperationens eventuella påverkan på deras förmåga att få barn. En aktiv

barnönskan hos kvinnan var den enda faktorn som ökade chansen för att kvinnan skulle ha fått information om operationens eventuella påverkan på hennes fertilitet.

I artikel II, redovisas en prospektiv kohort studie som följde 75 kvinnor som genomgått cystoperation på ovariet. AMH togs före samt tre och sex månader efter operationen. En signifikant sänkning av AMH kunde påvisas från 2.7 µg/L (0.2-16.9) till 1.6 µg/L (0.2-9.9) vid 3 månader och till 1.6 µg/L (0.2-8.3) vid 6 månader (båda $p<0.001$).

I artikel III redovisas en prospektiv kohort studie med två års uppföljning av samma kvinnor som deltog i artikel II samt ytterligare 8 kvinnor som endast genomgått öppnande av en cysta (fenestration). Sammanlagt inkluderades 66 kvinnorna som svarade på en enkät med frågor om barnönskan, graviditeter och antal födda barn sedan operationen. Nytt AMH analyserades också. AMH sjönk från 2.7 µg/L före operationen till 2.0 µg/L vid sex månader och till 1.0 µg/L vid två år efter operationen ($p = 0.001$ för båda). Hos kvinnor som försökte bli gravida efter genomgången operation, både spontant och med hjälp av provrörsbefruktning, var graviditetsfrekvensen 58 %.

I artikel IV redovisas en multicenterstudie från tre IVF kliniker (IVF-provrörsbefruktning). Inkluderade var 76 kvinnor som genomgått borttagande av ett ovarium före sin IVF behandling. De 76 kvinnorna jämfördes med 12879 kontroller som också hade genomgått IVF/ICSI behandling under samma tidsperiod och på samma IVF kliniker. Möjligheten att bli graviditet var 30 % lägre hos kvinnor som tidigare genomgått borttagande av ett ovarium jämfört med kvinnor i samma ålder vid behandling med IVF/ICSI. De kvinnor som tidigare genomgått borttagande av ett ovarium behövde dessutom högre doser av hormonstimulering vid provrörsbefruktningen och färre ägg togs ut vid äggplock.

Slutsats: I denna avhandling framkommer att ovarialreserven mätt genom AMH minskar efter operation av olika sorters godartade cystor och inte bara efter operation av endometrios cystor. Ungefär hälften av kvinnorna som försökte bli gravida efter operationen lyckades. Borttagande av ett helt ovarium tycks påverka så att det vid provrörsbefruktning kan vara svårare att bli gravid. Mer kunskap behövs om effekten av cystoperation på kvinnors fertilitet så att kvinnor kan får noggrann och adekvat information om eventuella fertilitetsrelaterade risker med en operation.

# 11 ACKNOWLEDGEMENTS

*This work was made possible through good times with colleagues, friends and family. Having your help and support got me through this challenge.*

**Kenny Rodriguez-Wallberg,** my principal supervisor. I appreciate your great knowledge of reproduction and interest in research. Thank you for letting me be part of your world. I admire your kindness, optimism and ability to always see the possibilities.

**Margareta Hammarström**, my co-supervisor and former head of the Department of Gynecology and Obstetrics. Thank you for all your support and for giving me the opportunity to conduct this research.

**Claudia Lampic**, my co-supervisor. Thank you for your insightful help, encouragement and for always trying to get me to learn and improve my work with your thoughtful remarks.

**Jan Olofsson,** my co-supervisor. I appreciate your kindness and your accurate comments that have helped to improve our research.

**Marion Ek,** my mentor, colleague and dear friend. Without you, none of this would have been possible. You have both inspired me to undertake this research, and you made me continue all those times when I wanted to quit. Your support and ability to always go to the heart of the matter is a gift. You are always able to come up with solutions to private, professional and research issues. Our conversations are essential for my wellbeing.

**Mia Ahlberg,** my vivid and funny research school friend. Thank you for all your patience with my epidemiological questions.

**Mats Bergström,** former head of Gynecology at Södersjukhuset. Thank you for always sending me research updates on AMH and for being willing to engage in discussions about the clinical aspects of my research.

**Marie Söderberg Westergren,** head of Gynecology at Södersjukhuset. Thank you for inspiring me to begin the final writing of my thesis.

**Ingegerd Lantz,** head of the Department of Gynecology and Obstetrics. Thank you for letting me be a part of the exciting gynaecological outpatient clinic even though my research was not finished.

**Katarina Westman**, thank you for all the good times we had doing the schedule. Working with you is so fun and easy.

**Malin Brunes,** your ability to always think about the improvement/education of others is exceptional. I am so proud to be part of your ultrasound team.

To my friend **Petra Bygdevall**, spending time with you always puts me in a good mood. Our visualization of the worst dissertation scenario still, months later, makes me laugh.

**Eva Itzel,** colleague. Thank you for reading my thesis and giving me sound advice.

To my fantastic, clever and funny colleagues **Magnus Holmgren, Charlotta Ersmark, Charlotta Ericson, Caroline Kjellman Erlandson, Tove Wallström, Denise Golmann** and many more who took the time to help me to include all patients. You all make our clinic the best place to work.

To my friend **Johanna Cederholm**, for your expert knowledge of grammar, your analytical skills and ability to clarify things.

**Katarina Remaeus** and **Maria Bruzelius,** friends from research school. It would not have been the same without your humour and willingness to debate and question things.

To **Ola Olén** for giving us such a excellent education in Research school in epidemiology for clinicians.

**Gunilla Höjer** and **Annika Mauritzon-Golitats,** for assistance with patient recruitment and administrative help during the study.

**The wonderful team at our gynaecological out-patient clinic**, thank you for putting up with my sporadic energetic appearances.

**Jan Holte**, for your knowledge in research and for doing paper IV with us.

**Lina Benson**, statistician, for your wonderful help with statistics in papers I and II.

The **Berg**, **Ekelund** and **Carlblick** families**,** for helping out with the logistics of family life, reminding me of my core values and for giving me a good cup of coffee when I needed it the most.

My parents **Johan** and **Gunilla** for all your love, support, encouragement and help with both discussing/reading my data and taking care of my children. Among many other things, I'm so grateful that you were in Varkala with us and gave me time to finish this thesis.

My brother **Love** and my sister-in-law **Grace**, who are so good at enjoying the moment and always reminding me to take a break and socialize instead.

My sister **Klara** and my dear friend **Niklas**, for knowing and loving you. Thank you for all the years that have been and the ones that will come.

The happiness of my life, my lovely husband **Jonas,** with his patience for all my projects and our adorable, lovely children **Vita, Arvin** and **Iris**. You are my everything. All these long working hours during our travels are finally over. Now, I will start studying ants with you.

Supported by grants from the Local Research, Education and Development Council**,** Department of Obstetrics and Gynecology, Södersjukhuset, Stockholm.

# 12 REFERENCES


1. Broekmans FJ, Knauff EA, te Velde ER, Macklon NS, Fauser BC. Female reproductive ageing: current knowledge and future trends. Trends in endocrinology and metabolism: TEM. 2007;18(2):58-65.
2. Committee opinion no. 618: ovarian reserve testing. Obstet Gynecol. 2015;125(1):268-73.
3. Peterson BD, Pirritano M, Tucker L, Lampic C. Fertility awareness and parenting attitudes among American male and female undergraduate university students. Hum Reprod. 2012;27(5):1375-82.
4. Lampic C, Svanberg AS, Karlstrom P, Tyden T. Fertility awareness, intentions concerning childbearing, and attitudes towards parenthood among female and male academics. Hum Reprod. 2006;21(2):558-64.
5. Myrskyla M, Kohler HP, Billari FC. Advances in development reverse fertility declines. Nature. 2009;460(7256):741-3.
6. Sverige. Statistiska Centralbyrån. Population statistics 2009 (in Swedish). . Report no BE 0110TAB Orebro, Sweden, 2010.
7. Council of Europe. Recent demographic developments in Europe, 2004. Council of Europe Publishing, Council of Europe Publishing2005. p. 20-4.
8. Rothman KJ, Wise LA, Sorensen HT, Riis AH, Mikkelsen EM, Hatch EE. Volitional determinants and age-related decline in fecundability: a general population prospective cohort study in Denmark. Fertil Steril. 2013;99(7):1958-64.
9. Dunson DB, Baird DD, Colombo B. Increased infertility with age in men and women. Obstet Gynecol. 2004;103(1):51-6.
10. Paavonen J, Eggert-Kruse W. Chlamydia trachomatis: impact on human reproduction. Hum Reprod Update. 1999;5(5):433-47.
11. Practice Committee of the American Society for Reproductive M. Endometriosis and infertility: a committee opinion. Fertil Steril. 2012;98(3):591-8.
12. Rodriguez-Wallberg KA. Principles of cancer treatment: impact on reproduction. Adv Exp Med Biol. 2012;732:1-8.
13. te Velde ER, Pearson PL. The variability of female reproductive ageing. Hum Reprod Update. 2002;8(2):141-54.
14. Kelsey TW, Wright P, Nelson SM, Anderson RA, Wallace WHB. A Validated Model of Serum Anti-Mullerian Hormone from Conception to Menopause. Plos One. 2011;6(7).
15. Gougeon A. Dynamics of follicular growth in the human: a model from preliminary results. Hum Reprod. 1986;1(2):81-7.
16. La Marca A, Broekmans FJ, Volpe A, Fauser BC, Macklon NS. Anti-Mullerian hormone (AMH): what do we still need to know? Hum Reprod. 2009;24(9):2264-75.
17. Baerwald AR, Adams GP, Pierson RA. Ovarian antral folliculogenesis during the human menstrual cycle: a review. Hum Reprod Update. 2012;18(1):73-91.
18. Treloar AE, Boynton RE, Behn BG, Brown BW. Variation of the human menstrual cycle through reproductive life. International journal of fertility. 1967;12(1 Pt 2):77-126.

19. van Zonneveld P, Scheffer GJ, Broekmans FJ, Blankenstein MA, de Jong FH, Looman CW, et al. Do cycle disturbances explain the age-related decline of female fertility? Cycle characteristics of women aged over 40 years compared with a reference population of young women. Hum Reprod. 2003;18(3):495-501.

20. Johannes CB, Crawford SL. Menstrual bleeding, hormones, and the menopausal transition. Seminars in reproductive endocrinology. 1999;17(4):299-309.

21. Wallace WH, Kelsey TW. Human ovarian reserve from conception to the menopause. Plos One. 2010;5(1):e8772.

22. Vaskivuo TE, Tapanainen JS. Apoptosis in the human ovary. Reprod Biomed Online. 2003;6(1):24-35.

23. Rodstrom K, Bengtsson C, Milsom I, Lissner L, Sundh V, Bjourkelund C. Evidence for a secular trend in menopausal age: a population study of women in Gothenburg. Menopause. 2003;10(6):538-43.

24. Richardson SJ. The biological basis of the menopause. Bailliere's clinical endocrinology and metabolism. 1993;7(1):1-16.

25. Small CM, Manatunga AK, Klein M, Feigelson HS, Dominguez CE, McChesney R, et al. Menstrual cycle characteristics: associations with fertility and spontaneous abortion. Epidemiology. 2006;17(1):52-60.

26. Brodin T, Bergh T, Berglund L, Hadziosmanovic N, Holte J. Menstrual cycle length is an age-independent marker of female fertility: results from 6271 treatment cycles of in vitro fertilization. Fertil Steril. 2008;90(5):1656-61.

27. Burger HG, Hale GE, Robertson DM, Dennerstein L. A review of hormonal changes during the menopausal transition: focus on findings from the Melbourne Women's Midlife Health Project. Hum Reprod Update. 2007;13(6):559-65.

28. Fritz MA SL. Clinical Gynecologic Endocrinology and Infertility. 2011;8th ed. Philadelphia,: Lippincott Williams & Wilkins.

29. Hutson J, Ikawa H, Donahoe PK. The ontogeny of Mullerian inhibiting substance in the gonads of the chicken. J Pediatr Surg. 1981;16(6):822-7.

30. Lee MM, Donahoe PK, Hasegawa T, Silverman B, Crist GB, Best S, et al. Mullerian inhibiting substance in humans: normal levels from infancy to adulthood. J Clin Endocrinol Metab. 1996;81(2):571-6.

31. Weenen C, Laven JS, Von Bergh AR, Cranfield M, Groome NP, Visser JA, et al. Anti-Mullerian hormone expression pattern in the human ovary: potential implications for initial and cyclic follicle recruitment. Molecular human reproduction. 2004;10(2):77-83.

32. Nelson SM, Messow MC, Wallace AM, Fleming R, McConnachie A. Nomogram for the decline in serum antimullerian hormone: a population study of 9,601 infertility patients. Fertil Steril. 2011;95(2):736-41 e1-3.

33. Tehrani FR, Mansournia MA, Solaymani-Dodaran M, Azizi F. Age-specific serum anti-Mullerian hormone levels: estimates from a large population-based sample. Climacteric. 2014.

34. Bentzen JG, Forman JL, Johannsen TH, Pinborg A, Larsen EC, Andersen AN. Ovarian antral follicle subclasses and anti-mullerian hormone during normal reproductive aging. J Clin Endocrinol Metab. 2013;98(4):1602-11.

35. La Marca A, Spada E, Grisendi V, Argento C, Papaleo E, Milani S, et al. Normal serum anti-Mullerian hormone levels in the general female population and the relationship with reproductive history. Eur J Obstet Gynecol Reprod Biol. 2012.

36. Kelsey TW, Wright P, Nelson SM, Anderson RA, Wallace WH. A validated model of serum anti-mullerian hormone from conception to menopause. Plos One. 2011;6(7):e22024.

37. La Marca A, Volpe A. Anti-Mullerian hormone (AMH) in female reproduction: is measurement of circulating AMH a useful tool? Clin Endocrinol (Oxf). 2006;64(6):603-10.

38. La Marca A, Sighinolfi G, Papaleo E, Cagnacci A, Volpe A, Faddy MJ. Prediction of age at menopause from assessment of ovarian reserve may be improved by using body mass index and smoking status. Plos One. 2013;8(3):e57005.

39. Tehrani FR, Solaymani-Dodaran M, Tohidi M, Gohari MR, Azizi F. Modeling age at menopause using serum concentration of anti-mullerian hormone. J Clin Endocrinol Metab. 2013;98(2):729-35.

40. Dolleman M, Faddy MJ, van Disseldorp J, van der Schouw YT, Messow CM, Leader B, et al. The relationship between anti-Mullerian hormone in women receiving fertility assessments and age at menopause in subfertile women: evidence from large population studies. J Clin Endocrinol Metab. 2013;98(5):1946-53.

41. Tehrani FR, Shakeri N, Solaymani-Dodaran M, Azizi F. Predicting age at menopause from serum antimullerian hormone concentration. Menopause. 2011;18(7):766-70.

42. Broer SL, Eijkemans MJ, Scheffer GJ, van Rooij IA, de Vet A, Themmen AP, et al. Anti-mullerian hormone predicts menopause: a long-term follow-up study in normoovulatory women. J Clin Endocrinol Metab. 2011;96(8):2532-9.

43. Freeman EW, Sammel MD, Lin H, Gracia CR. Anti-mullerian hormone as a predictor of time to menopause in late reproductive age women. J Clin Endocrinol Metab. 2012;97(5):1673-80.

44. van Disseldorp J, Lambalk CB, Kwee J, Looman CW, Eijkemans MJ, Fauser BC, et al. Comparison of inter- and intra-cycle variability of anti-Mullerian hormone and antral follicle counts. Hum Reprod. 2010;25(1):221-7.

45. Hadlow N, Longhurst K, McClements A, Natalwala J, Brown SJ, Matson PL. Variation in antimullerian hormone concentration during the menstrual cycle may change the clinical classification of the ovarian response. Fertil Steril. 2013;99(6):1791-7.

46. Streuli I, Fraisse T, Pillet C, Ibecheole V, Bischof P, de Ziegler D. Serum antimullerian hormone levels remain stable throughout the menstrual cycle and after oral or vaginal administration of synthetic sex steroids. Fertil Steril. 2008;90(2):395-400.

47. Dolleman M, Verschuren WM, Eijkemans MJ, Dolle ME, Jansen EH, Broekmans FJ, et al. Reproductive and lifestyle determinants of anti-Mullerian hormone in a large population-based study. J Clin Endocrinol Metab. 2013;98(5):2106-15.

48. Koninger A, Kauth A, Schmidt B, Schmidt M, Yerlikaya G, Kasimir-Bauer S, et al. Anti-Mullerian-hormone levels during pregnancy and postpartum. Reprod Biol Endocrinol. 2013;11:60.

49. La Marca A, Giulini S, Orvieto R, De Leo V, Volpe A. Anti-Mullerian hormone concentrations in maternal serum during pregnancy. Hum Reprod. 2005;20(6):1569-72.

50. Toner JP, Seifer DB. Why we may abandon basal follicle-stimulating hormone testing: a sea change in determining ovarian reserve using antimullerian hormone. Fertil Steril. 2013;99(7):1825-30.

51. Broer SL, van Disseldorp J, Broeze KA, Dolleman M, Opmeer BC, Bossuyt P, et al. Added value of ovarian reserve testing on patient characteristics in the prediction of ovarian response and ongoing pregnancy: an individual patient data approach. Hum Reprod Update. 2013;19(1):26-36.

52. Broer SL, Dolleman M, van Disseldorp J, Broeze KA, Opmeer BC, Bossuyt PM, et al. Prediction of an excessive response in in vitro fertilization from patient characteristics and ovarian reserve tests and comparison in subgroups: an individual patient data meta-analysis. Fertil Steril. 2013;100(2):420-9 e7.

53. Iliodromiti S, Kelsey TW, Wu O, Anderson RA, Nelson SM. The predictive accuracy of anti-Mullerian hormone for live birth after assisted conception: a systematic review and meta-analysis of the literature. Hum Reprod Update. 2014;20(4):560-70.

54. Weghofer A, Dietrich W, Barad DH, Gleicher N. Live birth chances in women with extremely low-serum anti-Mullerian hormone levels. Hum Reprod. 2011;26(7):1905-9.

55. Steiner AZ, Herring AH, Kesner JS, Meadows JW, Stanczyk FZ, Hoberman S, et al. Antimullerian hormone as a predictor of natural fecundability in women aged 30-42 years. Obstet Gynecol. 2011;117(4):798-804.

56. Hagen CP, Vestergaard S, Juul A, Skakkebaek NE, Andersson AM, Main KM, et al. Low concentration of circulating antimullerian hormone is not predictive of reduced fecundability in young healthy women: a prospective cohort study. Fertil Steril. 2012;98(6):1602-8 e2.

57. Coulter B. AMH Gen II ELISA. 2014 Beckman Coulter, Inc. 2014;REF A73818.

58. Rustamov O, Smith A, Roberts SA, Yates AP, Fitzgerald C, Krishnan M, et al. Anti-Mullerian hormone: poor assay reproducibility in a large cohort of subjects suggests sample instability. Hum Reprod. 2012;27(10):3085-91.

59. Kumar A, Kalra B, Patel A, McDavid L, Roudebush WE. Development of a second generation anti-Mullerian hormone (AMH) ELISA. J Immunol Methods. 2010;362(1-2):51-9.

60. Li HW, Ng EH, Wong BP, Anderson RA, Ho PC, Yeung WS. Correlation between three assay systems for anti-Mullerian hormone (AMH) determination. J Assist Reprod Genet. 2012;29(12):1443-6.

61. Beckman Coulter. FOLLOW-UP URGENT FIELD SAFETY NOTICE – FSN 16241-4. 2013.

62. Zuvela E, Walls M, Matson P. Within-laboratory and between-laboratory variability in the measurement of anti-mullerian hormone determined within an external quality assurance scheme. Reprod Biol. 2013;13(3):255-7.

63. Scheffer GJ, Broekmans FJ, Dorland M, Habbema JD, Looman CW, te Velde ER. Antral follicle counts by transvaginal ultrasonography are related to age in women with proven natural fertility. Fertil Steril. 1999;72(5):845-51.

64. Broekmans FJ, de Ziegler D, Howles CM, Gougeon A, Trew G, Olivennes F. The antral follicle count: practical recommendations for better standardization. Fertil Steril. 2010;94(3):1044-51.

65. Broer SL, Mol BW, Hendriks D, Broekmans FJ. The role of antimullerian hormone in prediction of outcome after IVF: comparison with the antral follicle count. Fertil Steril. 2009;91(3):705-14.

66. Holte J, Brodin T, Berglund L, Hadziosmanovic N, Olovsson M, Bergh T. Antral follicle counts are strongly associated with live-birth rates after assisted reproduction, with superior treatment outcome in women with polycystic ovaries. Fertil Steril. 2011;96(3):594-9.

67. La Marca A, Spada E, Sighinolfi G, Argento C, Tirelli A, Giulini S, et al. Age-specific nomogram for the decline in antral follicle count throughout the reproductive period. Fertil Steril. 2011;95(2):684-8.

68. Panchal S, Nagori C. Comparison of anti-mullerian hormone and antral follicle count for assessment of ovarian reserve. Journal of human reproductive sciences. 2012;5(3):274-8.

69. Sunderam S, Kissin DM, Crawford SB, Folger SG, Jamieson DJ, Barfield WD. Assisted reproductive technology surveillance - United States, 2011. Morbidity and mortality weekly report Surveillance summaries. 2014;63(10):1-28.

70. Q-IVF Nkfab. Resultat-trender-öppna jämförelser. Årsrapport skapad 2014 Gäller behandlingar utförda 2012. 2014;Version 2:1-28.

71. Knudsen UB, Tabor A, Mosgaard B, Andersen ES, Kjer JJ, Hahn-Pedersen S, et al. Management of ovarian cysts. Acta Obstet Gynecol Scand. 2004;83(11):1012-21.

72. Borgfeldt C, Andolf E. Transvaginal sonographic ovarian findings in a random sample of women 25-40 years old. Ultrasound in obstetrics & gynecology : the official journal of the International Society of Ultrasound in Obstetrics and Gynecology. 1999;13(5):345-50.

73. Force USPST. Screening for ovarian cancer: recommendation statement. Annals of family medicine. 2004;2(3):260-2.

74. Reis LA HD, Krapcho M, et al. SEER cancer statistics review. last accessed 30 September 2014.

75. Levine D, Asch E, Mehta TS, Broder J, O'Donnell C, Hecht JL. Assessment of factors that affect the quality of performance and interpretation of sonography of adnexal masses. J Ultrasound Med. 2008;27(5):721-8.

76. RCOG. Management of Suspected Ovarian Masses in Premenopausal Women. 2011;Green-top Guideline, No. 62 2011. RCOG, London.

77. Dodge JE, Covens AL, Lacchetti C, Elit LM, Le T, Devries-Aboud M, et al. Management of a suspicious adnexal mass: a clinical practice guideline. Curr Oncol. 2012;19(4):e244-57.

78. Brun JL, Fritel X, Aubard Y, Borghese B, Bourdel N, Chabbert-Buffet N, et al. Management of presumed benign ovarian tumors: updated French guidelines. Eur J Obstet Gynecol Reprod Biol. 2014;183C:52-8.

79. American College of O, Gynecologists. ACOG Practice Bulletin. Management of adnexal masses. Obstet Gynecol. 2007;110(1):201-14.

80. Timmerman D, Valentin L, Bourne TH, Collins WP, Verrelst H, Vergote I, et al. Terms, definitions and measurements to describe the sonographic features of adnexal tumors: a consensus opinion from the International Ovarian Tumor Analysis (IOTA) Group. Ultrasound Obstet Gynecol. 2000;16(5):500-5.

81. Timmerman D, Testa AC, Bourne T, Ferrazzi E, Ameye L, Konstantinovic ML, et al. Logistic regression model to distinguish between the benign and malignant adnexal mass before surgery: a multicenter study by the International Ovarian Tumor Analysis Group. J Clin Oncol. 2005;23(34):8794-801.

82. Legendre G, Catala L, Moriniere C, Lacoeuille C, Boussion F, Sentilhes L, et al. Relationship between ovarian cysts and infertility: what surgery and when? Fertil Steril. 2014;101(3):608-14.

83. Statistical database, Operationer i slutenvård. Operations in inpatient care. [Internet]. 2014. Available from: http://www.socialstyrelsen.se/statistik/statistikdatabas/operationerislutenvard, (2 Dec 2014, date last accessed).

84. Kitajima M, Khan KN, Hiraki K, Inoue T, Fujishita A, Masuzaki H. Changes in serum anti-Müllerian hormone levels may predict damage to residual normal ovarian tissue after laparoscopic surgery for women with ovarian endometrioma. Fertil Steril. 2011;95(8):2589-91.e1.

85. Iwase A, Hirokawa W, Goto M, Takikawa S, Nagatomo Y, Nakahara T, et al. Serum anti-Mullerian hormone level is a useful marker for evaluating the impact of laparoscopic cystectomy on ovarian reserve. Fertil Steril. 2010;94(7):2846-9.

86. Chang HJ, Han SH, Lee JR, Jee BC, Lee BI, Suh CS, et al. Impact of laparoscopic cystectomy on ovarian reserve: serial changes of serum anti-Mullerian hormone levels. Fertil Steril. 2010;94(1):343-9.

87. Celik HG, Dogan E, Okyay E, Ulukus C, Saatli B, Uysal S, et al. Effect of laparoscopic excision of endometriomas on ovarian reserve: serial changes in the serum antimullerian hormone levels. Fertil Steril. 2012;97(6):1472-8.

88. Somigliana E, Berlanda N, Benaglia L, Vigano P, Vercellini P, Fedele L. Surgical excision of endometriomas and ovarian reserve: a systematic review on serum antimullerian hormone level modifications. Fertil Steril. 2012;98(6):1531-8.

89. Raffi F, Metwally M, Amer S. The impact of excision of ovarian endometrioma on ovarian reserve: a systematic review and meta-analysis. J Clin Endocrinol Metab. 2012;97(9):3146-54.

90. Ercan CM, Sakinci M, Duru NK, Alanbay I, Karasahin KE, Baser I. Antimullerian hormone levels after laparoscopic endometrioma stripping surgery. Gynecol Endocrinol. 2010;26(6):468-72.

91. Alborzi S, Keramati P, Younesi M, Samsami A, Dadras N. The impact of laparoscopic cystectomy on ovarian reserve in patients with unilateral and bilateral endometriomas. Fertil Steril. 2014;101(2):427-34.

92. Hirokawa W, Iwase A, Goto M, Takikawa S, Nagatomo Y, Nakahara T, et al. The post-operative decline in serum anti-Mullerian hormone correlates with the bilaterality and severity of endometriosis. Hum Reprod. 2011;26(4):904-10.

93. Biacchiardi CP, Piane LD, Camanni M, Deltetto F, Delpiano EM, Marchino GL, et al. Laparoscopic stripping of endometriomas negatively affects ovarian follicular reserve even if performed by experienced surgeons. Reprod Biomed Online. 2011;23(6):740-6.

94. Tsolakidis D, Pados G, Vavilis D, Athanatos D, Tsalikis T, Giannakou A, et al. The impact on ovarian reserve after laparoscopic ovarian cystectomy versus three-stage management in patients with endometriomas: a prospective randomized study. Fertil Steril. 2010;94(1):71-7.

95. Lee DY, Young Kim N, Jae Kim M, Yoon BK, Choi D. Effects of laparoscopic surgery on serum anti-Mullerian hormone levels in reproductive-aged women with endometrioma. Gynecological endocrinology : the official journal of the International Society of Gynecological Endocrinology. 2011;27(10):733-6.

96. Uncu G, Kasapoglu I, Ozerkan K, Seyhan A, Oral Yilmaztepe A, Ata B. Prospective assessment of the impact of endometriomas and their removal on ovarian reserve and determinants of the rate of decline in ovarian reserve. Hum Reprod. 2013;28(8):2140-5.

97. Kwon SK, Kim SH, Yun SC, Kim DY, Chae HD, Kim CH, et al. Decline of serum antimullerian hormone levels after laparoscopic ovarian cystectomy in endometrioma and other benign cysts: a prospective cohort study. Fertil Steril. 2014;101(2):435-41.

98. Ercan CM, Duru NK, Karasahin KE, Coksuer H, Dede M, Baser I. Ultrasonographic evaluation and anti-mullerian hormone levels after laparoscopic stripping of unilateral endometriomas. Eur J Obstet Gynecol Reprod Biol. 2011;158(2):280-4.

99. Hwu YM, Wu FS, Li SH, Sun FJ, Lin MH, Lee RK. The impact of endometrioma and laparoscopic cystectomy on serum anti-Mullerian hormone levels. Reprod Biol Endocrinol. 2011;9:80.

100. Urman B, Alper E, Yakin K, Oktem O, Aksoy S, Alatas C, et al. Removal of unilateral endometriomas is associated with immediate and sustained reduction in ovarian reserve. Reprod Biomed Online. 2013;27(2):212-6.

101. Sugita A, Iwase A, Goto M, Nakahara T, Nakamura T, Kondo M, et al. One-year follow-up of serum antimullerian hormone levels in patients with cystectomy: are different sequential changes due to different mechanisms causing damage to the ovarian reserve? Fertil Steril. 2013;100(2):516-22 e3.

102. Akkad A, Jackson C, Kenyon S, Dixon-Woods M, Taub N, Habiba M. Informed consent for elective and emergency surgery: questionnaire study. Bjog. 2004;111(10):1133-8.

103. ACOG CommitteeOpinion No. 578: Elective surgery and patient choice. Obstet Gynecol. 2013;122(5):1134-8.

104. RCOG. (Obtaining Valid Consent for Complex Gynaecological Surgery (Clinical Governance Advice 6b). 2010.

105. Dunselman GA, Vermeulen N, Becker C, Calhaz-Jorge C, D'Hooghe T, De Bie B, et al. ESHRE guideline: management of women with endometriosis. Hum Reprod. 2014;29(3):400-12.

106. Hardy R, Kuh D. Reproductive characteristics and the age at inception of the perimenopause in a British National Cohort. Am J Epidemiol. 1999;149(7):612-20.

107. Bjelland EK, Wilkosz P, Tanbo TG, Eskild A. Is unilateral oophorectomy associated with age at menopause? A population study (the HUNT2 Survey). Hum Reprod. 2014;29(4):835-41.

108. Lass A. The fertility potential of women with a single ovary. Human reproduction update. 1999;5(5):546-50.

109. Cramer DW, Xu H, Harlow BL. Does "incessant" ovulation increase risk for early menopause? Am J Obstet Gynecol. 1995;172(2 Pt 1):568-73.

110. Yasui T, Hayashi K, Mizunuma H, Kubota T, Aso T, Matsumura Y, et al. Factors associated with premature ovarian failure, early menopause and earlier onset of menopause in Japanese women. Maturitas. 2012;72(3):249-55.

111. Thomas-Teinturier C, El Fayech C, Oberlin O, Pacquement H, Haddy N, Labbe M, et al. Age at menopause and its influencing factors in a cohort of survivors of childhood cancer: earlier but rarely premature. Hum Reprod. 2013;28(2):488-95.

112. Levi R, Ozcakir HT, Alatas E, Goker EN, Tavmergen E. The outcomes of assisted reproductive technology cycles in patients with one or two ovaries. J Obstet Gynaecol Res. 2003;29(5):321-5.

113. Lass A, Paul M, Margara R, Winston RM. Women with one ovary have decreased response to GnRHa/HMG ovulation protocol in IVF but the same pregnancy rate as women with two ovaries. Hum Reprod. 1997;12(2):298-300.

114. Khan Z, Gada RP, Tabbaa ZM, Laughlin-Tommaso SK, Jensen JR, Coddington CC, 3rd, et al. Unilateral oophorectomy results in compensatory follicular recruitment in the remaining ovary at time of ovarian stimulation for in vitro fertilization. Fertil Steril. 2014;101(3):722-7.

115. Hendricks MS, Chin H, Loh SF. Treatment outcome of women with a single ovary undergoing in vitro fertilisation cycles. Singapore medical journal. 2010;51(9):698-701.

116. Al-Hasani S, Asimakopoulos B, Nikolettos N, Diedrich K. Comparison of the response to ovarian stimulation between women with one ovary and those with two ovaries, in a program of ICSI/ET. Acta Obstet Gynecol Scand. 2003;82(9):845-9.

117. Levitas E, Furman B, Shoham-Vardi I, Lunenfeld E, Potashnik G. Treatment outcome in women with a single ovary versus patients with two ovaries undergoing in vitro fertilization and embryo transfer (IVF/ET). Eur J Obstet Gynecol Reprod Biol. 2000;88(2):197-200.

118. Alper MM, Seibel MM, Oskowitz SP, Smith BD, Ransil BJ, Taymor ML. Comparison of follicular response in patients with one or two ovaries in a program of in vitro fertilization. Fertil Steril. 1985;44(5):652-5.

119. Dodds WG, Chin N, Awadalla SG, Miller F, Friedman C, Kim M. In vitro fertilization and embryo transfer in patients with one ovary. Fertil Steril. 1987;48(2):249-53.

120. Lam SY, McKenna M, McBain JC, Baker HW, Johnston WI. Outcome of patients with one ovary in an in vitro fertilization program. Journal of in vitro fertilization and embryo transfer : IVF. 1987;4(6):319-23.

121. Boutteville C, Muasher SJ, Acosta AA, Jones HW, Jr., Rosenwaks Z. Results of in vitro fertilization attempts in patients with one or two ovaries. Fertil Steril. 1987;47(5):821-7.

122. Dodson MG, Young RL, Poindexter AN, Gibbons WE, Rossavik IK, Findley WE. Comparison of in vitro fertilization results in women with one or two ovaries. The Journal of reproductive medicine. 1987;32(5):359-62.

123. Khalifa E, Toner JP, Muasher SJ, Acosta AA. Significance of basal follicle-stimulating hormone levels in women with one ovary in a program of in vitro fertilization. Fertil Steril. 1992;57(4):835-9.

124. Penzias AS, Gutmann JN, Shamma FN, LaMorte AI, DeCherney AH. Ovulation induction with GnRH agonist and human menopausal gonadotropins: response in patients with one versus two ovaries. International journal of fertility and menopausal studies. 1993;38(5):270-3.

125. Nargund G, Bromhan D. Comparison of endocrinological and clinical profiles and outcome of IVF cycles in patients with one ovary and two ovaries. J Assist Reprod Genet. 1995;12(7):458-60.

126. Wilkosz P, Greggains GD, Tanbo TG, Fedorcsak P. Female reproductive decline is determined by remaining ovarian reserve and age. Plos One. 2014;9(10):e108343.

127. Mascarenhas MN, Flaxman SR, Boerma T, Vanderpoel S, Stevens GA. National, regional, and global trends in infertility prevalence since 1990: a systematic analysis of 277 health surveys. PLoS medicine. 2012;9(12):e1001356.

128. Dhont N, van de Wijgert J, Coene G, Gasarabwe A, Temmerman M. 'Mama and papa nothing': living with infertility among an urban population in Kigali, Rwanda. Hum Reprod. 2011;26(3):623-9.

129. Dhont N. The importance of being fertile. A call for a more balanced approach towards reproductive health. Facts, views & vision in ObGyn. 2013;5(3):243-6.

130. Cousineau TM, Domar AD. Psychological impact of infertility. Best Pract Res Clin Obstet Gynaecol. 2007;21(2):293-308.

131. Peterson BD, Newton CR, Rosen KH, Skaggs GE. Gender differences in how men and women who are referred for IVF cope with infertility stress. Hum Reprod. 2006;21(9):2443-9.

132. Herbert DL, Lucke JC, Dobson AJ. Depression: an emotional obstacle to seeking medical advice for infertility. Fertil Steril. 2010;94(5):1817-21.

133. den Breejen EM, Nelen WL, Schol SF, Kremer JA, Hermens RP. Development of guideline-based indicators for patient-centredness in fertility care: what patients add. Hum Reprod. 2013;28(4):987-96.

134. Armuand GM, Rodriguez-Wallberg KA, Wettergren L, Ahlgren J, Enblad G, Hoglund M, et al. Sex Differences in Fertility-Related Information Received by Young Adult Cancer Survivors. J Clin Oncol. 2012.

135. Armuand GM, Wettergren L, Rodriguez-Wallberg KA, Lampic C. Desire for children, difficulties achieving a pregnancy, and infertility distress 3 to 7 years after cancer diagnosis. Support Care Cancer. 2014;22(10):2805-12.

136. Newton CR, Sherrard W, Glavac I. The Fertility Problem Inventory: measuring perceived infertility-related stress. Fertil Steril. 1999;72(1):54-62.

137. Moura-Ramos M, Gameiro S, Canavarro MC, Soares I. Assessing infertility stress: re-examining the factor structure of the Fertility Problem Inventory. Hum Reprod. 2012;27(2):496-505.

138. Holte J, Berglund L, Milton K, Garello C, Gennarelli G, Revelli A, et al. Construction of an evidence-based integrated morphology cleavage embryo score for implantation

potential of embryos scored and transferred on day 2 after oocyte retrieval. Hum Reprod. 2007;22(2):548-57.

139. Alpha Scientists in Reproductive M, Embryology ESIGo. The Istanbul consensus workshop on embryo assessment: proceedings of an expert meeting. Hum Reprod. 2011;26(6):1270-83.

140. Li HW, Lee VC, Ho PC, Ng EH. Ovarian sensitivity index is a better measure of ovarian responsiveness to gonadotrophin stimulation than the number of oocytes during in-vitro fertilization treatment. J Assist Reprod Genet. 2014;31(2):199-203.

141. Huber M, Hadziosmanovic N, Berglund L, Holte J. Using the ovarian sensitivity index to define poor, normal, and high response after controlled ovarian hyperstimulation in the long gonadotropin-releasing hormone-agonist protocol: suggestions for a new principle to solve an old problem. Fertil Steril. 2013;100(5):1270-6.

142. Biasoni V, Patriarca A, Dalmasso P, Bertagna A, Manieri C, Benedetto C, et al. Ovarian sensitivity index is strongly related to circulating AMH and may be used to predict ovarian response to exogenous gonadotropins in IVF. Reprod Biol Endocrinol. 2011;9:112.

143. Zeger SL, Liang KY. Longitudinal data analysis for discrete and continuous outcomes. Biometrics. 1986;42(1):121-30.

144. Muzii L, Bianchi A, Croce C, Manci N, Panici PB. Laparoscopic excision of ovarian cysts: is the stripping technique a tissue-sparing procedure? Fertil Steril. 2002;77(3):609-14.

145. Alborzi S, Foroughinia L, Kumar PV, Asadi N. A comparison of histopathologic findings of ovarian tissue inadvertently excised with endometrioma and other kinds of benign ovarian cyst in patients undergoing laparoscopy versus laparotomy. Fertil Steril. 2009;92(6):2004-7.

146. Dogan E, Ulukus EC, Okyay E, Ertugrul C, Saygili U, Koyuncuoglu M. Retrospective analysis of follicle loss after laparoscopic excision of endometrioma compared with benign nonendometriotic ovarian cysts. International journal of gynaecology and obstetrics: the official organ of the International Federation of Gynaecology and Obstetrics. 2011;114(2):124-7.

147. Kwon SK, Kim SH, Yun SC, Kim DY, Chae HD, Kim CH, et al. Decline of serum antimullerian hormone levels after laparoscopic ovarian cystectomy in endometrioma and other benign cysts: a prospective cohort study. Fertil Steril. 2013.

148. Streuli I, de Mouzon J, Paccolat C, Chapron C, Petignat P, Irion OP, et al. AMH concentration is not related to effective time to pregnancy in women who conceive naturally. Reprod Biomed Online. 2014;28(2):216-24.

149. Vercellini P, Somigliana E, Vigano P, Abbiati A, Barbara G, Crosignani PG. Surgery for endometriosis-associated infertility: a pragmatic approach. Hum Reprod. 2009;24(2):254-69.

150. Darai E, Fauvet R, Uzan C, Gouy S, Duvillard P, Morice P. Fertility and borderline ovarian tumor: a systematic review of conservative management, risk of recurrence and alternative options. Hum Reprod Update. 2013;19(2):151-66.

151. Song T, Hun Choi C, Lee YY, Kim TJ, Lee JW, Bae DS, et al. Oncologic and reproductive outcomes of cystectomy compared with oophorectomy as a treatment for borderline ovarian tumours. Hum Reprod. 2011;26(8):2008-14.

152. Benschop L, Farquhar C, van der Poel N, Heineman MJ. Interventions for women with endometrioma prior to assisted reproductive technology. Cochrane Database Syst Rev. 2010(11):CD008571.

153. Ji J, Liu Y, Tong XH, Luo L, Ma J, Chen Z. The optimum number of oocytes in IVF treatment: an analysis of 2455 cycles in China. Hum Reprod. 2013;28(10):2728-34.

154. Carr E, Brockbank K, Allen S, Strike P. Patterns and frequency of anxiety in women undergoing gynaecological surgery. J Clin Nurs. 2006;15(3):341-52.

155. Daniluk JC, Koert E, Cheung A. Childless women's knowledge of fertility and assisted human reproduction: identifying the gaps. Fertil Steril. 2012;97(2):420-6.

156. Daniluk JC, Koert E. Fertility awareness online: the efficacy of a fertility education website in increasing knowledge and changing fertility beliefs. Hum Reprod. 2014.

157. RCOG. Presenting Information on risk (Clinical Governance Advice No. 7). 2008.

158. Tremellen K, Savulescu J. Ovarian reserve screening: a scientific and ethical analysis. Hum Reprod. 2014;29(12):2606-14.