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# ASSISTED REPRODUCTIVE TECHNOLOGIES FOR FERTILITY PRESERVATION AND FERTILITY TREATMENT IN YOUNG WOMEN WITH CANCER

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# Assisted reproductive technologies for fertility preservation and fertility treatments in young women with cancer

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*“It is difficult to say what is impossible, for the dream of yesterday is the hope of today and the reality of tomorrow.”*

- Robert H Goddard

To all the women and girls
that these studies were about and for

# ABSTRACT

Loss of fertility as a potential side effect of anti-neoplastic treatments may have a significant negative impact on the survivors' quality of life. Assisted reproductive technologies (ART) have been adapted and further developed for fertility preservation (FP) and fertility treatments in young women with cancer. The last three decades have seen impressive achievements in this field, with the rapid introduction and development of methods in clinical practice. Still, the data on outcomes and safety of FP are scarce, and numerous questions await answering.

The four studies included in this thesis aimed to investigate: if a history of cancer may increase the risk of adverse perinatal outcomes in pregnancies achieved with donor oocytes (Study I), if return rates and utilization rates differ in patients with oncologic and non-oncologic indications for FP, and if the trends in decisions regarding preferred FP-methods have changed over time (Study II), if current approaches to controlled ovarian stimulation aimed at FP in women with breast cancer are efficacious and safe (Study III) and if long-term reproductive outcomes after breast cancer differ in women with and without FP (Study IV).

**Study I** was a single-center cohort study, including 25 live births in 20 women with a history of cancer and 244 live births in 212 women without a history of cancer, all after treatments with donor oocytes. Higher rates of pre-eclampsia (adjusted odds ratio (aOR) 2.79, 95% CI 1.07-7.34) and preterm birth (aOR 5.54, 95% CI 2.01-15.31) were observed in women with a history of cancer.

**Study II** included data on 1254 women that underwent FP counseling due to oncologic and non-oncologic indications at Karolinska University Hospital during a 20-year long period (1998-2018). Oncologic indication for FP was associated with a slightly lower likelihood of returning for counseling (OR 0.72, 95% CI 0.51-1.0) and a significantly lower likelihood of returning for pregnancy attempt (OR 0.41, 95%CI 0.27-0.62), when compared to non-oncologic FP indication. Among different FP methods, cryopreservation of unfertilized oocytes has become the preferred one in recent years, both by adult women (with or without partners) and by post-menarchal girls.

**Study III** was a multicenter study including data on FP counseling (n=610) and FP treatments (n=468) of women with breast cancer at six Swedish University Hospitals with programs for FP. In this study 380 cycles of controlled ovarian stimulation (COS) in gonadotropin-releasing hormone (GnRH) antagonist protocol were available for the final analysis. Three recently introduced approaches to COS in women with cancer aiming to increase the safety of the procedures were investigated: 1) the concurrent use of letrozole, 2) the random start initiation of stimulation, and 3) the use of gonadotropin-releasing hormone agonist (GnRHa) for ovulation trigger instead of hCG trigger. The efficacy of these approaches, measured as the number of cryopreserved oocytes and embryos, was observed to be at least non-inferior compared to the standard GnRH antagonist protocols. Overall survival did not differ between women with versus without FP, and women in letrozole versus non-letrozole group.

**Study IV**, a register-based nationwide cohort study, included 425 women with and 850 women without FP history, all of them diagnosed with breast cancer at reproductive age. Rates of live births (aHR: 2.3, 95%CI: 1.6-3.3) and ART-treatments (aHR: 4.8, 95%CI: 2.2-10.7) after breast cancer were higher among women that had undergone FP when compared to women without FP. FP exposure was not associated with any decrease in overall survival in this cohort.

In conclusion, the findings suggest that the use of ART for FP and fertility treatment in eligible young women with cancer seems to be both safe and efficacious. Yet, some caution is warranted when interpreting the findings, since these studies were not randomized and women with cancer who undergo FP might be healthier, on average, than the women who do not ("healthy FP effect"). In women with a previous history of cancer that achieve pregnancy with donor oocytes, individual assessment of pregnancy risks and potential increased obstetric surveillance may be indicated. Further research investigating the risk of breast cancer-relapse in women undergoing FP, with additional measures taken to overcome a possible "healthy FP effect", is of utmost importance.

# LIST OF SCIENTIFIC PAPERS

I. Marklund A, Nasiell J, Berger AS, Fagerberg A, Rodriguez-Wallberg KA. Pregnancy Achieved Using Donor Eggs in Cancer Survivors with Treatment-Induced Ovarian Failure: Obstetric and Perinatal Outcome. *J Womens Health (Larchmt). 2018;27(7):939-945. E-pub 2018 May 1.*

II. Rodriguez-Wallberg KA, Marklund A, Lundberg F, Wikander I, Milenkovic M, Anastacio A, Sergouniotis F, Wånggren K, Ekengren J, Lind T, Borgström B. A prospective study of women and girls undergoing fertility preservation due to oncologic and non-oncologic indications in Sweden-Trends in patients' choices and benefit of the chosen methods after long-term follow up. *Acta Obstet Gynecol Scand. 2019;98(5):604-615. E-pub 2019 Mar 18.*

III. Marklund A, Eloranta S, Wikander I, Kitlinski ML, Lood M, Nedstrand E, Thurin-Kjellberg A, Zhang P, Bergh J, Rodriguez-Wallberg KA. Efficacy and safety of controlled ovarian stimulation using GnRH antagonist protocols for emergency fertility preservation in young women with breast cancer-a prospective nationwide Swedish multicenter study. *Hum Reprod. 2020;35(4):929-938.*

IV. Marklund A, Lundberg FE, Eloranta S, Hedayati E, Pettersson K, Rodriguez-Wallberg KA. Reproductive Outcomes After Breast Cancer in Women With vs Without Fertility Preservation. *JAMA Oncol. 2020 Nov 19. doi: 10.1001/jamaoncol.2020.5957. Epub ahead of print. PMID: 33211089.*

ADDITIONAL PUBLICATIONS
(NOT INCLUDED IN THESIS)

Marklund A, Sjövall A, Blomqvist L, Carlson J, Rodriguez-Wallberg KA. Endometriosis, the great imitator - a successful case of fertility preservation in a woman receiving sterilizing treatment due to a diagnosis of rectosigmoid carcinoma. *Gynecol Endocrinol*. 2019;35(11):945-948. *E-pub 2019 Jun 28.*

# CONTENTS

# LIST OF ABBREVIATIONS

| | |
|---|---|
| AFC | Antral Follicle Count |
| AMH | Anti-Mullerian Hormone |
| ART | Assisted Reproductive Technologies |
| BC | Breast Cancer |
| BMI | Body Mass Index |
| CI | Confidence Interval |
| CLBR | Cumulative Live Birth Rate |
| COS | Controlled Ovarian Stimulation |
| ER | Estrogen Receptor |
| ESHRE | European Society of Human reproduction and Embryology |
| ET | Embryo Transfer |
| FET | Frozen Embryo Transfer |
| FP | Fertility Preservation |
| FSH | Follicle-stimulating hormone |
| GnRH | Gonadotropin Releasing Hormone |
| GnRHa | Gonadotropin Releasing Hormone agonist |
| Gy | Gray |
| hCG | Human Chorionic Gonadotropin |
| HER2 | Human Epidermal Growth Factor Receptor 2 |
| HR | Hazard Ratio |
| HR | Hormone Receptor |
| ICSI | Intracytoplasmic Sperm Injection |
| IVF | In Vitro Fertilization |
| LH | Luteinizing hormone |
| NGF | Non-growing follicle |
| OD | Oocyte Donation |
| OHSS | Ovarian hyperstimulation syndrome |
| OR | Odds Ratio |
| POI | Premature Ovarian Insufficiency |
| PPH | Postpartum hemorrhage |
| PTB | Preterm birth |
| RCT | Randomized Controlled Trial |
| RR | Risk Ratio |
| RT | Radiotherapy |
| SE | Standard Error |

# 1 INTRODUCTION

*"May your choices reflect your hopes, not your fears." - Nelson Mandela*

Fertility – what's in the word?
The state of being fertile.
From Latin *fertilis*, "bearing in abundance or fruitful".
Commonly understood as the ability to get pregnant, to produce offspring.

Something that we usually take for granted, when young.
Or may even suffer from, when unprepared for its power.
A potential source of joy and happiness.
Or a cause of existential crisis, when threatened or absent.
Something very complex and delicate.
Something worth to protect.

In young people diagnosed with cancer, anti-neoplastic treatments pose a risk of damage to fertility. Though not always an issue of primary concern at the time of diagnosis, the inability to get children later in life may cause stress and sorrow.

The field of fertility preservation (FP) is relatively new. For men, freezing of spermatozoa was described as early as the 18th century, but cryopreservation became feasible and practical first in the mid-1900s. For women, achievements of in-vitro fertilization (IVF) and the establishment of assisted reproductive technologies (ART) into clinical practice in the 1970s became an important milestone. These techniques were gradually implemented for FP, with tremendous development during the last three decades.

When this project started in 2015, the practice of FP for women with cancer was already established at six university hospitals in Sweden. According to the international and national guidelines, oncologists were encouraged to refer all young patients facing gonadotoxic therapies to fertility counseling. Available options of FP included cryopreservation of embryos, oocytes and ovarian tissue, with the latter still considered an experimental method. Women who developed ovarian failure as a side effect of cancer treatment could also have the option of attempting pregnancy with donated oocytes. Long-term follow-up reports on FP efficacy and safety profile in women with cancer and on their obstetrical risk following ART treatments using donor oocytes were lacking. Therefore, the studies of this thesis were designed with some specific questions in mind:
Is there any increased risk of adverse perinatal outcome in pregnancies achieved with donated oocytes in women previously treated for cancer? Have trends in FP-choices changed over time? Which protocols for ovarian stimulation in the population of women with breast cancer are most effective and safe? What are the post-diagnosis birth rates in women with and without FP indicated by BC diagnosis? Does the history of FP affect prognosis for survival in women with BC?

By providing answers to the questions above, the overall purpose of this thesis was to increase the knowledge on efficacy and safety of FP and fertility treatments in young women with cancer and, ultimately, to contribute to a better quality of life in this population. Well-defined strategies to secure a fair chance of biological parenthood in the future, after completing the treatments, may give strength and hope in difficult times of struggling with the disease. May knowledge and hope, not fear, guide choices regarding future fertility and motherhood for young women with cancer.

# 2 BACKGROUND

## 2.1 CANCER AND FERTILITY

Worldwide, about 3-10% of patients diagnosed with cancer are younger than 40 years of age (1, 2). Though the burden of cancer across all ages combined is higher in males than females, women are affected more often than men during the reproductive years (3).

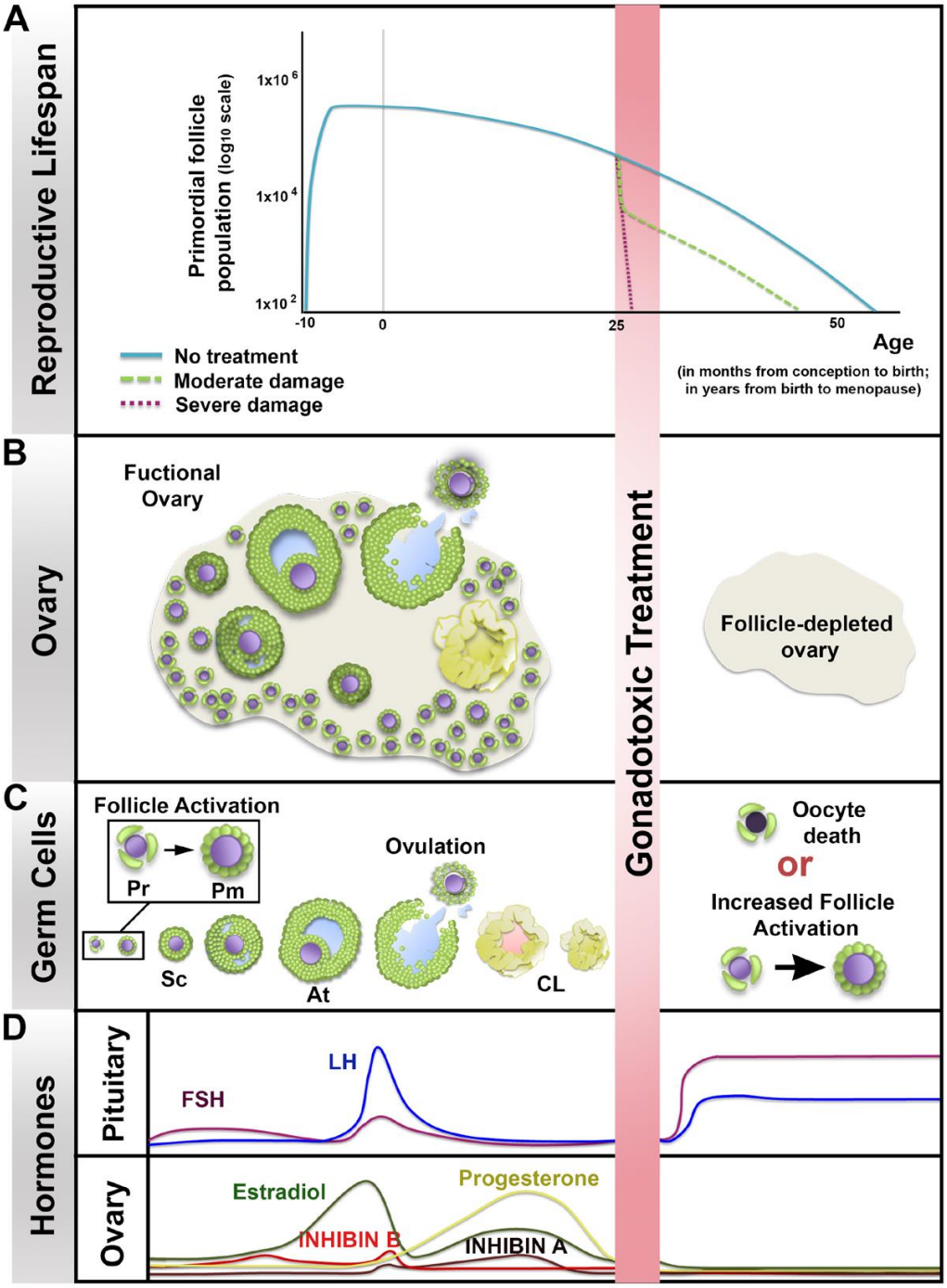
Advances in early detection and improved treatment efficacy have led to a growing number of survivors. Consequently, specific concerns and needs of these patients have acquired high priority. Research has shown that fertility and reproduction issues are important to young cancer survivors, and iatrogenic infertility may have profound negative effects on their quality of life (4, 5). Furthermore, there is evidence that most cancer survivors prefer biological parenthood to other options such as adoption or oocyte donation (6).

### 2.1.1 The effect of cancer disease and its treatment on female fertility.

Possible adverse effects of cancer disease and its treatment on female fertility depend on multiple factors (7):

- age of the patient and her pre-treatment fertility status
- type and stage of cancer disease
- anatomic or vascular changes in the pelvic region due to surgery.
- different modalities and combination of antineoplastic treatments:
  - dose and location of the fields of radiotherapy
  - type, dose and dose-intensity of chemotherapeutics
  - type and dose of hormonal therapy and targeted therapy

*Figure 1. Consequences of gonadotoxic treatments in women of fertile age.*



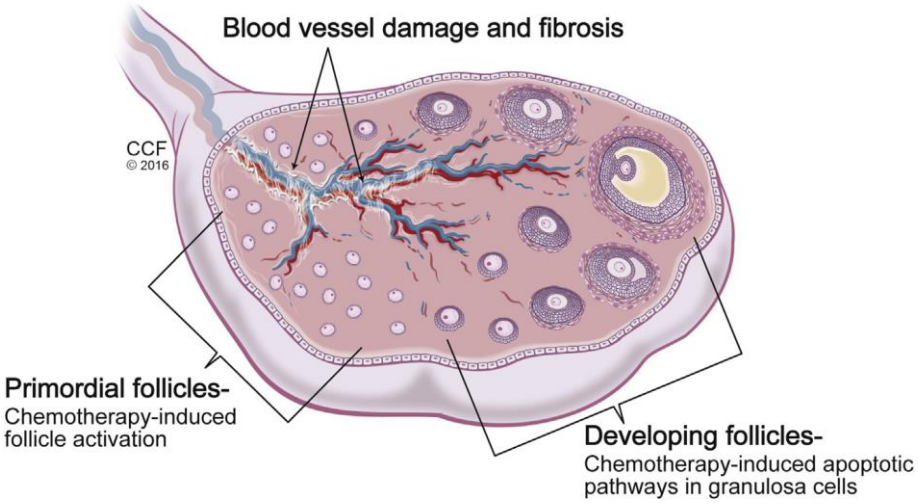

(A) The primordial follicle population—the ovarian reserve—can be represented by a parabolic curve across the female lifespan in which activation or death of primordial ovarian follicles occurs progressively with each menstrual cycle, from puberty to the menopause. Reprinted from Wallace and Kelsey (8). (B–D) The growth of follicles with each cycle maintains hormonal balance necessary for overall women's health. Gonadotoxic stress or treatment, such as chemotherapy or radiation therapy (red bar across all panels), induces a rapid decrease in the highly sensitive primordial follicles of the ovarian reserve (A, C), resulting in a follicle-depleted ovary (B) and premature ovarian failure (POF). Depletion of the ovarian reserve disrupts normal endocrine function and the production of hormones such as follicle-stimulating hormone (FSH), luteinizing hormone (LH), estradiol, progesterone, inhibin B, and inhibin A (D), leading to hormonal imbalance similar to that seen in postmenopausal women.

Reprinted with permission from J Gynecol Oncol. Kim SY, Kim SK, Lee JR, Woodruff TK. Toward precision medicine for preserving fertility in cancer patients: existing and emerging fertility preservation options for women. *J Gynecol Oncol*. 2016;27(2):e22.

*Chemotherapy*

The adverse effects of chemotherapy on ovarian function are well-recognized. During recent years, increasingly detailed data have become available on how different regimes may affect ovaries in short and long term (9-11). Although exact mechanisms behind gonadotoxicity are not yet fully understood (12), proposed hypotheses include (i) direct effect on the DNA of follicles inducing apoptosis, with dividing granulosa cells being particularly susceptible to damage; (ii) triggering a massive growth of dormant follicles, which are then destroyed; or (iii) inducing vascular damage and stromal fibrosis (11, 13-15). Short-term effects, causing temporary amenorrhea, depend mainly on the apoptosis of growing follicles. Long-term effects are usually explained by damage to primordial follicular reserve (11).

*Figure 2. Proposed mechanisms for chemotherapy-induced damage on ovarian follicles.*




Chemotherapy causes ovarian damage by both direct and indirect mechanisms.

Reprinted from Hickman LC, Valentine LN, Falcone T. Preservation of gonadal function in women undergoing chemotherapy: a review of the potential role for gonadotropin-releasing hormone agonists. Am J Obstet Gynecol. 2016;215(4):415-422, with permission from Elsevier.

Chemotherapeutic drugs can be divided into five categories: alkylating agents, antitumor antibiotics, platinum-based drugs, antimetabolites, and taxanes (11). Growing data indicate specific toxicity of alkylating therapy on the patients' reproductive function (16), with no consistent threshold for a safe dose existing at present. Classification of infertility risk induced by chemotherapy in females is presented in Table 1. Nonetheless, individual risk is highly variable and difficult to predict, as the total impact of treatments depends on many different factors.

*Table 1. Classification of infertility risk induced by chemotherapy protocols and radiotherapy in females. Adapted from (17).*

| Chemo- or radiotherapy | Degree of risk |
|---|---|
| Hematopoietic stem cell transplantation and total body irradiation<br>Radiotherapy to a field including the ovaries | High risk >80% |
| CAF, CMF, CEF x6 30–39 years of age ACx4 >40 years of age | Intermediate risk |
| ABVD, CHOP, CVP, AML, ALL CAF, CMF, CEF x6 <30 years of age; ACx4 <40 years of age | Lower risk (<20%) |
| Vincristine<br>Methotrexate<br>Fluorouracil | Very low or no risk |
| Taxanes<br>Irinotecan<br>Oxaliplatin<br>Monoclonal antibodies<br>Tyrosine kinase inhibitors | Unknown risk |

Abbreviations: C, cyclophosphamide 600–1200 mg/m2; A, adriamycin 25–60 mg/m2; F, fluorouracil 600 mg/m2; E, epirubicin 60 mg/m2; M, methotrexate 40 mg/m2; B, bleomycin 10 U/m2; V, vinblastine 6 mg/m2; D, dacarbazine 375 mg/m2; V (O), vincristine 1.2 mg/m2–2 mg; P, prednisolone 40 mg/m2; H, hydroxydaunorubicin 50 mg/m2.

In recent years, increasing knowledge about possible mechanisms involved in chemotherapy-induced ovarian damage has been applied to develop the therapies with potential to protect follicular reserve (18, 19). Further research in this field, parallel to the further spread of FP practice, will hopefully result in even better chances to preserve fertility in young women with cancer.

*Radiotherapy*

When the irradiation field includes the ovaries, radiotherapy may cause permanent ovarian damage. Radiation therapy has a dose-related effect on primordial follicular reserve (20, 21). A dose of 2 Gray (Gy) represents the estimated dose required to destroy 50% of primordial follicles (22), confirming that oocytes are extremely sensitive to radiation. With increasing age, a gradual decrease in the non-growing follicle pool occurs, therefore a smaller dose of radiation may be sufficient to deplete the pool and to cause irreversible damage to the ovaries (23).

In female patients, radiotherapy may also cause adverse effects on reproductive function by damage to the uterus, resulting in increased risk of preterm delivery (24) and stillbirth (25), or by damage to hypothalamus and pituitary, resulting in subsequent ovarian dysfunction (26).

*Other antineoplastic therapies*

The effects of molecular targeted agents (such as monoclonal antibodies and kinase inhibitors) on female reproductive function are still largely unknown, though their use in treating different types of cancer is increasing (9).

Surgical therapy involving reproductive organs may have obvious detrimental effects on future fertility.

In women with hormone-positive BC, modern standards of treatment include adjuvant endocrine therapy for at least ten years. These adjuvant hormonal therapies (tamoxifen, aromatase inhibitors, GnRHa) have no direct irreversible effects on the ovarian function. However, an age-related reduction in the ability to conceive would inevitably occur due to the delay needed for completion of several years of endocrine treatment.

### 2.1.2 Markers of post-treatment fertility

Counseling young women on their risk of infertility after cancer treatments is a difficult task. Knowledge of the infertility rates after most current cancer treatments, and the impact of patients' individual factors such as age, pretreatment fertility status, and genetic susceptibility to the treatments on the degree of fertility impairment is still limited.

Damages to reproductive function in girls and young female cancer survivors result in hormone ovarian insufficiency, arrested puberty, premature ovarian insufficiency (POI), and infertility (23). According to the 2016 guidelines by the European Society of Human Reproduction and Embryology (ESHRE), POI can be defined as a clinical condition that develops in any adult female at an age younger than 40 years and is characterized by the absence of menstrual cycles for at least 4-5 months and two elevated serum follicle-stimulation hormone levels in menopausal range (27).

Persistence of regular menstruation after cancer treatment has been used to assess the residual ovarian function in many studies. In other words, amenorrhea has often been considered as a surrogate measure of infertility. Yet, maintenance or resumption of cyclic menses after treatment does not exclude compromised ovarian reserve and does not guarantee normal fertility (7). Thus, there is a need for accurate markers of ovarian function before and after treatment. Among those available today, the serum concentration of anti-Müllerian hormone (AMH) and the antral follicle count (AFC) by transvaginal ultrasound are considered to be the most useful (28). Still, natural conceptions may occur in women with very low AMH/AFC, and the value of these biomarkers in the prediction of early menopause remains uncertain. Therefore, they have been proposed in a research context, although not yet established in clinical practice (9).

### 2.1.3 Options to achieve motherhood after treatment of cancer

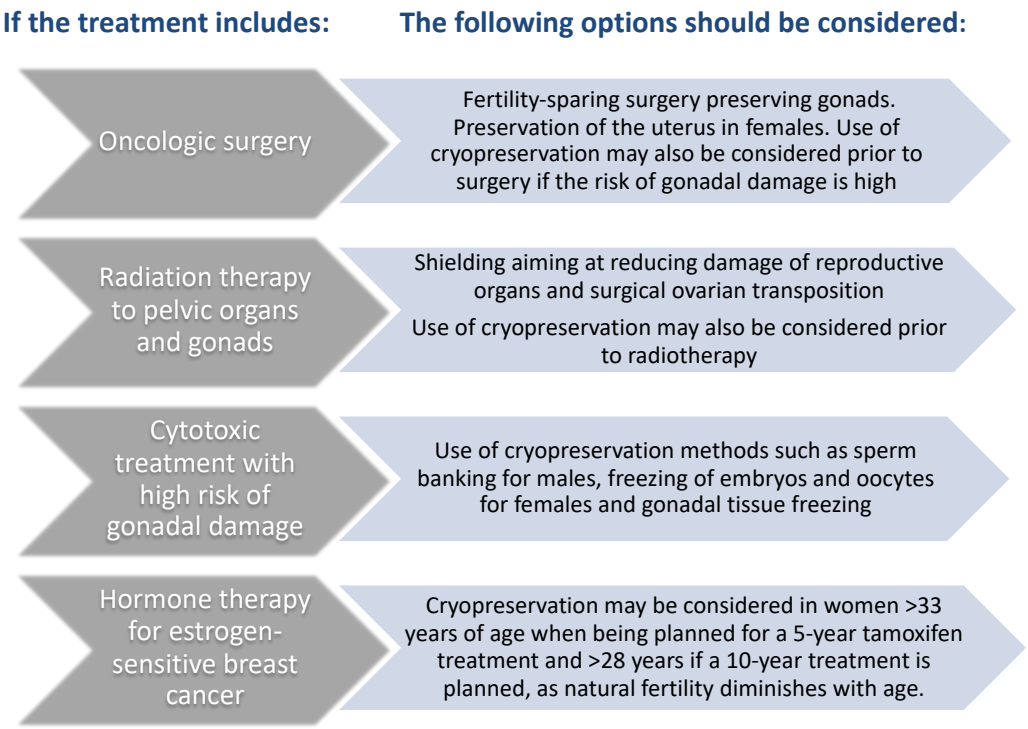
Historically, female cancer survivors had to cope with infertility due to ovarian insufficiency developed as a sequela of their cancer treatment. Today, professionals involved in oncologic healthcare of girls and women of reproductive age are recommended to refer them for counseling on available fertility preserving options before gonadotoxic treatments are started. In selected cases of gynecological cancers diagnosed at an early stage (29, 30), fertility-sparing surgical treatments have been developed and implemented (31). International guidelines are being regularly updated (32-38). For women who present with childbearing wish after having developed ovarian failure, attempts to pregnancy with ART treatments using donor oocytes can be offered. Additional options to achieve parenthood for women with uterine factor infertility include uterus transplantation and surrogacy, the last one though not legally allowed in Sweden. Adoption remains an important alternative to parenthood for many cancer survivors. However, adoption agencies may have policies that do not encourage cancer survivors as prospective adoptive parents, due to considerations toward their medical history and associated risks for relapse. Thus, challenges of adoption processes have been reported in survivors of cancer (39).

## 2.2 FERTILITY PRESERVATION METHODS

The last two decades have brought several options for women with cancer considering FP. FP has emerged as a new clinical discipline, with the goal to develop methods to restore reproductive function in young cancer survivors. In Europe the terms “Fertility Preservation” or “Reproductive Oncology” have been preferred, whereas in the U.S, the term “Oncofertility”, coined by Dr. Woodruff in 2006, has become widely accepted. These terms refer all to a field of medicine that connects oncology with reproductive health, encompassing the endocrine health and fertility management options in the context of cancer disease (40).

The first cryopreservation of embryos in a woman with BC was reported 1996. It was a result of a natural IVF-cycle prior to chemotherapy (41). A lot has happened since then. Today, cryopreservation of oocytes and/or embryos after controlled ovarian stimulation (COS) are considered to be the standard strategy for preserving female fertility when there is sufficient time (32), but cryopreservation of ovarian tissue has also been recently recognized as an established FP-method (42). Other strategies, such as pharmacological protection of the gonads, are generally considered experimental (33, 43).

*Figure 3. Fertility preservation strategies depending on the type of oncological treatment in females and males.*

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### 2.2.1 Embryo and oocyte cryopreservation

Embryo cryopreservation has been routinely used for storing surplus embryos after IVF for infertility treatment since the 1980's, thus it has been an established method for FP during many years (44). Reassuring data on pregnancy and live birth rates with thawed embryos have been reported. Therefore, it has previously been offered as a first-choice FP method for women of reproductive age who have a partner and sufficient time for COS (7, 45).

Cryopreservation of oocytes is another option for FP. It is particularly attractive for women without a partner and those not wishing to use donor sperms or to cryopreserve embryos due to religious, legal or ethical considerations. The newly developed technique of vitrification of oocytes has demonstrated a high efficacy, and an international consensus was reached in 2013 to recognize also oocyte cryopreservation as a clinically established method for FP (32).

Both the aforementioned methods require about two weeks' time for controlled ovarian stimulation (COS) with gonadotropins, to finally inducing oocyte maturation of large follicles with a hCG or GnRHa trigger. These treatments are monitored using transvaginal ultrasound and blood tests for hormonal determinations. After collection of the oocytes through ultrasound-guided transvaginal needle aspiration, these are cryopreserved,

currently by vitrification methods (46). Only mature oocytes are usually cryopreserved and immature oocytes are discarded. However, in vitro maturation methods have been proposed to increase the yields (47). If the oocytes are fertilized in vitro the resulting embryos can be cryopreserved at cleavage stage (day 2-3) or at blastocyst stage (day 5-6). For women with breast cancer, there is usually a gap of 4-6 weeks between surgery and the start of chemotherapy, and therefore sufficient time is available to undergo ovarian stimulation (48), which requires about two weeks to complete. Oocyte collection can also be performed without ovarian stimulation ("natural-cycle IVF"), aiming at obtaining the single oocyte that naturally would ovulate (49).

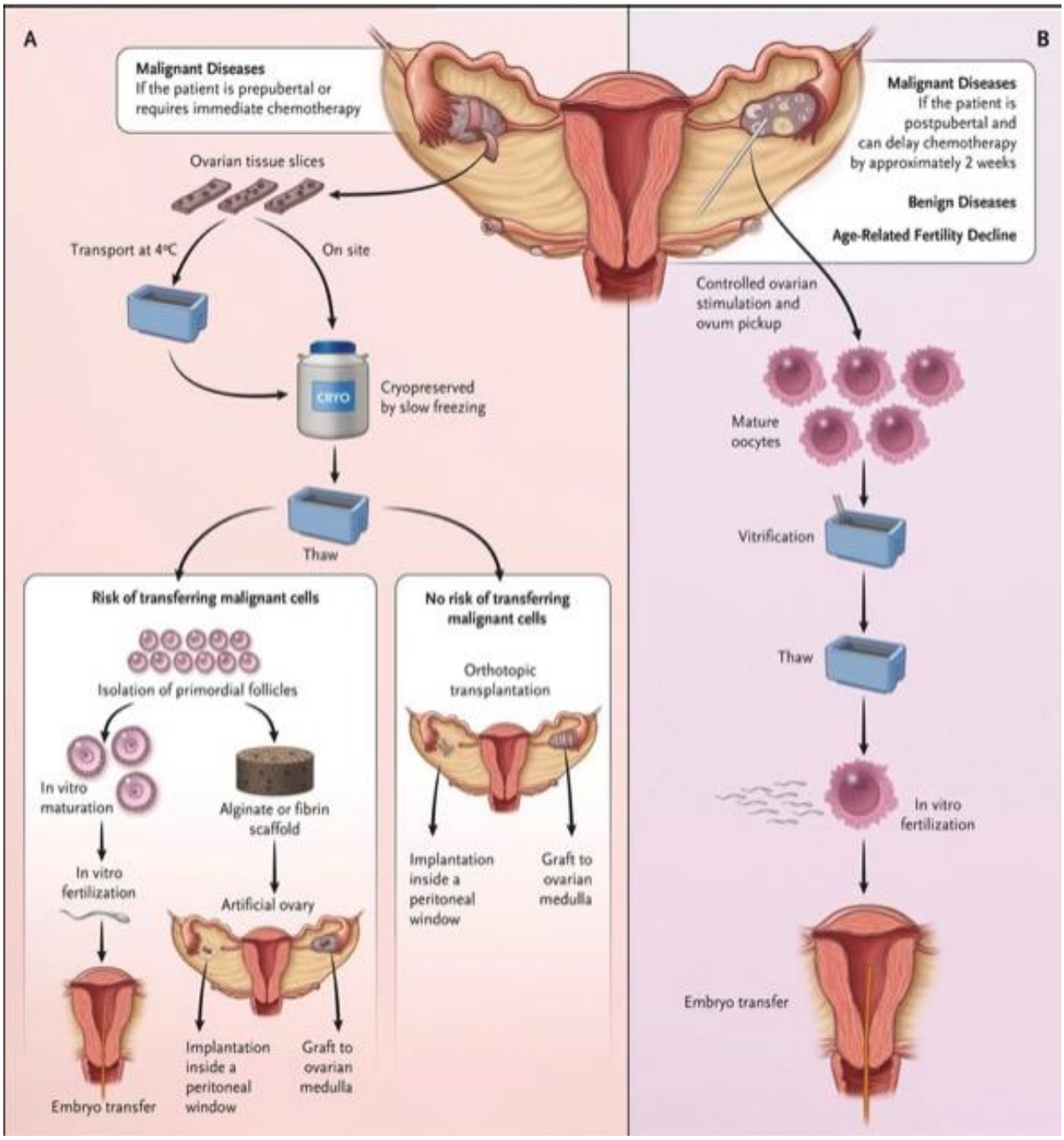
### 2.2.2 Ovarian tissue cryopreservation

Ovarian tissue cryopreservation does not require any hormonal stimulation, sperm donor or sexual maturation, and it is therefore a unique FP option for young girls with cancer, or for those patients who need to start chemo- or radiotherapy promptly. This method has been considered experimental for many years (32, 35, 50) until recently, when recognized as an established FP-technique by the American Society of Reproductive Medicine (42). Ovarian tissue is usually harvested laparoscopically and frozen to be later thawed and re-implanted. The best site for transplantation seems to be the orthotopic position, but subcutaneous areas such as forearm or lower abdomen have been described (51).

The first ovarian transplant procedure was reported in 2000 (52). Since then, re-implantation of ovarian tissue has resulted in 87 live births in 69 women, and a total of 93 children born (53). Taking into account not only peer-reviewed publications but also abstracts and congress proceedings, a recent review reported a total of 130 children born (54). In a meta-analysis from 2017, cumulative live birth and ongoing pregnancy rate per patient worldwide was evaluated to be 37% (55). However, according to newer data, with increased experience and improved surgical techniques, current success rates are likely to be even higher (56). By combining vitrification of oocytes and cryopreservation of ovarian tissue, a theoretical live birth rate of 50-60% was proposed to be possible. Therefore, this combined technique is suggested for young women who are at high risk for POI, when enough time for this is available (54).

Concerns that re-implantation of ovarian tissue in women previously treated for cancer may re-introduce cancer cells have been raised (57), and screening of ovarian tissue for malignant cells should always be performed when this FP method is used. In vitro maturation and cryopreservation of oocytes from harvested ovarian tissue could open new possibilities for the patients with high risk of ovarian involvement. Another potential to utilize cryopreserved ovarian tissue involves the development of so-called transplantable artificial ovary; with primordial follicles being isolated and transferred onto a scaffold, eliminating the risk of re-introducing malignant cells (58, 59). Today, both these techniques are only investigational and not in clinical use.

*Figure 4. Options for fertility preservation in females.*




If the patient is prepubertal or requires immediate chemotherapy (Panel A), ovarian tissue is removed in the form of multiple biopsy specimens (or an entire organ) and cut into cortical strips. The tissue is then cryopreserved by slow freezing on site (or transported to a processing site at a temperature of 4°C). After thawing, if there is no risk of transmitting malignant cells, the ovarian tissue can be grafted to the ovarian medulla (if at least one ovary is still present) or re-implanted inside a specially created peritoneal window. If there is a risk of transmitting malignant cells, ovarian follicles can be isolated and grown in vitro to obtain mature oocytes, which can then be fertilized and transferred to the uterine cavity. Isolated follicles may be placed inside a scaffold (alginate or fibrin), creating an artificial ovary that can be grafted to the ovarian medulla or peritoneal window. If the patient is post-pubertal and chemotherapy can be delayed for approximately 2 weeks (Panel B), mature oocytes can be removed after ovarian stimulation and vitrified on site. After thawing, they can be inseminated and transferred to the uterine cavity in the form of embryos. This technique can also be used in women with benign diseases or in those with age-related fertility decline. The techniques in Panels A and B can also be combined, with ovarian-tissue cryopreservation followed by controlled ovarian stimulation and vitrification of oocytes. The combined technique theoretically yields a 50 to 60% chance of a live birth.

### 2.2.3 Other options for fertility preservation

Other FP options include ovarian suppression and ovarian transposition.

Ovarian suppression through gonadotropin-releasing hormone analogues (GnRHa) is theoretically an attractive approach, as pharmacological prevention of ovarian depletion could be a better choice than invasive FP methods. Until now, there is still controversy regarding the efficacy of this method (60), and the latest guidelines on FP provided by the American Society of Clinical Oncology, ASCO, recommend to use it only when proven FP methods are not feasible (35). The relevant issue is that although randomized trials have been conducted, fertility has not been their primary outcome (61). Additionally, none of the trials has been blinded, thus the groups of women that received GnRHa in the studies have attempted pregnancy more frequently that the women in the control groups (62). Studies with improved design are needed, and if proven to be safe and effective, GnRHa can be a good alternative in patients interested in reducing the risk of infertility, additionally to post-treatment amenorrhea and menopausal symptoms (63).

Ovarian transposition means that the ovaries are surgically moved away from the radiation field and it can be practiced when pelvic radiation is a part of the antineoplastic treatment. This technique was first described in 1958 (64), but its effectiveness is still debated. In a systematic review from 2019, including 765 patients with ovarian transposition, the return of ovarian function was found in 20-100% of women receiving external beam radiation to the pelvis, in 64-100% of those receiving pelvic brachytherapy alone, and in 0-69% of those who also received concurrent chemotherapy (65).

Trachelectomy (removal of the cervix, upper vagina and parametrium instead of removal of the whole uterus), as well as other conservative surgical and radiation therapy approaches to specific pelvic cancers, are also available but they lay outside the scope of this review.

## 2.3 BREAST CANCER AND FERTILITY

Breast cancer (BC) is the most common malignancy affecting women during their reproductive years (66). About 15% of all invasive BC cases occur in women of reproductive age, with clustering between the ages of 30 and 40 years (66, 67). Early diagnosis and improved treatment options have remarkably improved survival rates over the last few decades: the 5-year relative survival in women diagnosed with BC prior to age of 45 has been estimated to 88% (2). In general, the predicted 5-year relative survival for patients with BC is now close to 90% (68).

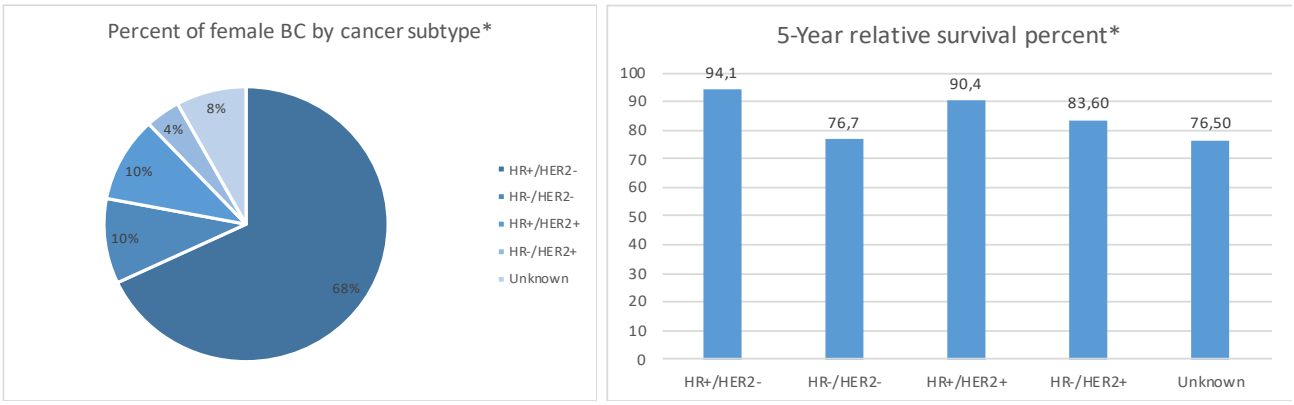
There are four main subtypes of female BC (1):

- HR+/HER2- ("Luminal A")
- HR-/HER2- ("Triple Negative")
- HR+/HER2+ ("Luminal B")
- HR-/HER2+ ("HER2-enriched")

HR stands for hormone receptor. HR + tumor cells have receptors for the hormones estrogen or progesterone so that these hormones can promote the growth of HR+ tumors. HER2+ means that tumor cells produce high levels of a protein called HER 2 (human epidermal growth factor), which is associated with the development and progression of certain aggressive forms of BC.

Treatment options and survival prognosis differ between the subtypes of BC. HR+/Her2- subtype has been reported to have the best survival pattern, followed by the HR+/Her2+ subtype and the HR-/Her2+ subtype.

*Figure 5. Subtypes of breast cancer in women and 5-year relative survival*




*Based on data from (1).

### 2.3.1 How do breast cancer treatments affect fertility?

*Chemotherapy*

In women below age of 40 years, BC often presents with biologically aggressive features, thus chemotherapy in combination with other treatments is a standard of care. Possible ovarian effects of chemotherapy have been described in this thesis. Approximately 5% of women with BC diagnosed at age 30, 32% diagnosed at age 35, and 80% diagnosed at age 40, experience post-treatment infertility despite return of their menses (69). Alkylating agents (e.g., cyclophosphamide) are particularly harmful depleting the follicle pool, and the gonadal toxicity is also dose-related and age-dependent, thus the damage is more evident in patients of older age with an already reduced follicle pool in their ovaries (32). A review by Oktay et al. reports that about 20-80% of survivors older and less than 20% of survivors younger than age 40 would experience ovarian failure after four cycles of doxorubicin plus cyclophosphamide (AC) (70). In xenograft models, apoptotic death of primordial follicles was shown to be the main mechanism of ovarian damage induced by AC and doxorubicin (71). AC followed by docetaxel was reported to result in the highest rate of amenorrhea 12 months after treatment (70%), followed by concurrent docetaxel plus AC (58%), and doxorubicin plus docetaxel (38%) (72). Adding the monoclonal antibody trastuzumab to the standard chemotherapy does not seem to have any further effect on the risk of treatment-related amenorrhea in premenopausal women (73).

*Radiotherapy*

During the standard whole-breast radiotherapy (50 Gy), the dose that reaches the pelvis via internal scatter is about 2.1-7.6 centigray (Gy x $10^{-2}$), which is unlikely to have any negative effects on the function of uterus and ovaries (74). Although radiation to the pelvis is low, pregnancy and oocyte harvest are not recommended during radiotherapy for BC, but are considered to be possible after the completion of treatment (75).

*Endocrine therapy*

While chemotherapy is often directly toxic for ovaries by destroying the ovarian follicles, endocrine treatments such as tamoxifen have also indirect effects on fertility in women of pre-menopausal age. Endocrine therapy *per se* can result in impaired ovulatory and endometrial function, including temporary amenorrhea (75). While on treatment with tamoxifen, pregnancy is contraindicated because of the increased risk of teratogenicity (75, 76). The treatment with tamoxifen is currently indicated for five to ten years in women with estrogen receptor (ER) positive BC, depending of the specific risk evaluation. Thus, there is an overall consequence of developing age-related infertility in women who need to complete their endocrine therapy prior to attempting pregnancy, as natural fertility declines with age. The standard chemotherapy regime for BC is assumed to result in approximately 10 years' worth of ovarian reserve loss (48). When there is a need for both chemotherapy and endocrine BC treatment in a woman of premenopausal age, the risk of subsequent infertility should be considered as very high (77).

### 2.3.2 Wish and chance of pregnancy after breast cancer

At the time of cancer diagnosis, approximately half of young BC patients wish to become pregnant after completing therapy (33). Some evidence suggests that women with BC may prefer a less toxic regimen of chemotherapy to avoid gonadal toxicity and risk of infertility if given a choice, even if this treatment is slightly inferior in terms of protection from recurrence (34). However, BC survivors have the lowest subsequent pregnancy rate among female cancer survivors, which is approximately 70% lower than expected in the general population (35, 36). This difference can be explained by the gonadal toxicity of chemotherapy, longer periods of endocrine therapy in patients with the hormone-sensitive disease, and also a concern that pregnancy could cause cancer relapse (27). There is growing evidence that the latter fear is not justified (78). In the most recent treatment period, the correlation between history of BC and subsequent reduced pregnancy rate appears to be less pronounced than in the former years, probably reflecting not only a change in treatments but also in how patients are advised by their health-care providers regarding post-cancer pregnancy (35).

Since young women with BC report great distress about the prospect of infertility (5), it is of utmost importance to address this concern and inform patients that FP can improve their chances of becoming pregnant after treatment.

## 2.4 FERILITY PRESERVATION IN WOMEN WITH BREAST CANCER

### 2.4.1 Safety

To date, studies have not shown any increased risk of BC recurrence after FP procedures, but the follow-up time in the most studies has been relatively short (79-81).

Cryopreservation of embryos and oocytes are the most widely used FP methods in women with BC; both these methods require COS. During ovarian stimulation, peak estradiol levels may increase several-fold compared to physiological peri-ovulatory levels (82, 83). Research has shown that estrogen and its metabolites may play a role in the propagation of breast cancer (84). Although the studies have so far not shown that short-term exposure to exogeneous estrogen may worsen prognosis in women with BC (81, 85), there has been a concern that hormonal stimulation may be potentially dangerous for women with ER-positive BC. Tamoxifen and letrozole as long-term adjuvant therapy improve prognosis for women with ER-positive BC (86). Based on this fact, potentially safer protocols for ovarian stimulation with the addition of these agents have been developed in recent years (87, 88). Addition of letrozole has been reported to have higher efficacy than tamoxifen, therefore the former is usually preferred (89). Additional improvements aiming at higher safety of the stimulation protocols using letrozol in women with BC include a maturation trigger using GnRHa (90).

In a matched cohort study including 148 women with BC, Rodriguez-Wallberg et al. reported that hormonal stimulation, with or without letrozole, was not associated with an increased risk of BC recurrence during a mean follow-up time of 6.6 years, when compared to age-matched unexposed controls (81). These findings support the practice of FP in women with BC, irrespective of the need of hormonal stimulation. Still, the question about the potential benefits of letrozole on the reproductive outcome and long-term safety remains open.

In women diagnosed with BC, the time window between surgery and start of chemotherapy usually provides a sufficient period for ovarian stimulation. Therefore, in the studies mentioned above, tumors were generally removed before the start of COS for FP. Data concerning safety of COS for FP prior to neoadjuvant therapy and/or surgery (i.e. without previous tumor surgery) are scarce (91, 92).

When neoadjuvant chemotherapy is indicated with prompt start, or in cases where ovarian stimulation is not desired, cryopreservation of ovarian tissue can be offered instead. This method eliminates potential risks of hormonal stimulation but the chance of future pregnancy relies on the functionality of the tissue regained after re-transplantation. Certain concern of reintroducing malignant cells with re-implantation of ovarian tissue has been articulated. However, to date, studies have not shown any evidence of malignant cells in cryopreserved ovarian tissue from women with non-metastatic BC (93).

### 2.4.2 GnRH antagonist protocols - which one to choose?

A Cochrane review from 2013, summarizing evidence regarding safety and efficacy of tamoxifen and letrozole addition to standard stimulation protocols in women with ER-positive BC, did not find any randomized controlled trials to refer to (94). Investigating possible impact of co-administration of letrozole on the oocyte yields, two studies reported no difference (87, 95), while two others found small but significant decrease in oocyte yields (96, 97).

A multicenter randomized open-label trial was started in the Netherlands and Belgium in 2014 with the aim to evaluate the effectiveness of ovarian stimulation with tamoxifen and letrozole compared to standard ovarian stimulation on the number of oocytes retrieved (98). The study planned to enroll 53 women in each group (letrozole vs tamoxifen vs standard ovarian stimulation), and it is ongoing. Hopefully, the results of this study will contribute to the understanding of the effect of different types of ovarian stimulation in women with BC. It would also pave the way for long term follow-up on the safety of this procedure.

The use of GnRHa instead of standard hCG for ovulation trigger is reported to reduce the risk of ovarian hyperstimulation. In women with BC, studies comparing the use of GnRH agonist vs hCG trigger in COS with co-administration of letrozole have found no difference in the total number of retrieved oocytes, but a higher proportion of mature oocytes with GnRH agonist (90, 99).

A more recent improvement has been the introduction of random-start stimulation protocols enabling cancer patients to undergo COS for yielding of oocytes irrespective of the phase of their menstrual cycle (100). A systematic review on the utility of random-start ovarian stimulation for FP was published 2017(101). It suggested that random start could shorten interval between ovarian stimulation and oocyte retrieval, with a yield of mature oocytes and embryos that was comparable to that of conventional stimulation protocols, albeit with a higher gonadotropin dose required in the former.

Most available studies on the efficacy and safety of these new approaches in the population of women with BC have been small and retrospective (77, 79, 87, 90, 102-105).

### 2.4.3 Efficacy

The short-term efficacy of FP can be measured as the number of oocytes or/and embryos cryopreserved, with respect to different COS protocols, in a specific patient population that determines the indication of FP. Long-term efficacy can be measured as pregnancy and live births rates post-diagnosis, with specific focus on pregnancies and live births achieved using cryopreserved specimens.

So far, data on utilization rate of oocytes and embryos and the rate of subsequent pregnancies and live births in cancer patients are scarce.

Regarding the outcomes of embryo cryopreservation in BC patients, Oktay et al have reported pregnancy rates comparable to those expected for IVF in general population (41). Summarizing outcomes of currently used FP procedures for oncological indications in a literature review from 2019, ter Welle-Butalid et al. report that 23% (range 13-63%) of 614 women underwent one or more embryo transfer, and 40% of those (range 9-75%) had a live birth (59). The proportion of patients returning for embryo transfer after prior oocyte preservation varied from 0 to 5%, and the live birth rate among those who returned was between 33 and 50%. BC-specific results were not reported. In a retrospective study of Cobo et al., the cumulative live birth rate (CLBR) after oocyte cryopreservation in 1073 women with cancer (whereof 64.4% had BC) was compared to that in 5289 healthy women with elective oocyte banking (15). Altogether, 80 cancer survivors attempted pregnancy after treatment, resulting in a CLBR of 35.2%. Age over 35 years and/or oncological indication for oocyte vitrification were found to negatively impact pregnancy and live birth rates in that study (15).

In 2018, Moravek et al. compared long-term outcomes of cancer patients who pursued FP (n=204) and those who did not (n=293), with a live birth rate of 6.4 % vs 5.5% after spontaneous pregnancies (17). Twenty-one women returned to use cryopreserved specimens, resulting in 16 live births. Of 497 women included in that study, 52.7% had BC. Further, in a recently published cross-sectional study of Vriens et al., a cohort of 118 women with early-stage BC received FP counseling and 34 of them chose FP (18). The 5-year live birth rate was 27% in total; 10 women in FP group gave birth to 12 babies, and 16 women in non-FP group gave birth to 20 babies, with a high rate of spontaneous pregnancies. Only 3 women applied for the transfer of their cryopreserved embryos, two of them also asked for pre-implantation genetic diagnosis of the frozen embryos because they were carriers of a BRCA1-mutation.

Most of the aforementioned studies have been published within the last 3 years. Data on the efficacy of FP at the start of this project were even scarcer. As the reports on long term reproductive outcomes after FP with oncological indications are mostly of retrospective character, with relatively small numbers of patients included, there is still an obvious need to conduct prospective studies of large scale.

## 2.5 PREGNANCY AFTER CANCER

### 2.5.1 Pregnancy rates

In general, the pregnancy rate in cancer survivors is expected to be lower than that of the general population, with female patients being more severely affected than male (106-109).

Several large studies have been published providing invaluable information related to the likelihood of pregnancy and live birth in survivors of childhood cancers, in relation to both diagnoses and treatments (109-113). Meanwhile, data on survivors of cancer in adulthood and BC in particular are limited. A pooled pregnancy rate of 3% has been estimated from population-based studies on women who had received treatment for BC (altogether 711

women included, median age 31-33 years) (114). In a large cohort study by Nichols et al, the 10-year cumulative incidence of postdiagnosis live birth in women with BC was 8% (4,445 women included), while it was 30% and 29% from melanoma and Hodgkin lymphoma, respectively (115).

In a Norwegian register-based study, female cancer survivors had a 39% lower pregnancy rate than the general population during a median observation time of 6 years. This observation was highly dependent on the cancer type, with survivors of melanoma or thyroid cancer having pregnancy rates highly comparable with the general population, while BC survivors had the lowest rates of post-cancer motherhood (108). To further study the true pregnancy deficit in the population of female cancer survivors, Anderson et. al recently conducted a large cohort study based on data from the Scottish Cancer register (116). They concluded that cancer survivors achieved fewer pregnancies across all cancer types (overall reduction of 38%), with marked reduction in women with breast, cervical and brain/central nervous system tumors, and leukemia. The history of treatment with chemotherapy and radiotherapy were both shown to contribute to the reduction, highlighting the importance of appropriate fertility counseling at the time of diagnosis. Overall for different cancer forms, higher rates of pregnancies have been reported with more recent treatment period (112, 116), possibly reflecting an increase in risk-adapted use of gonadotoxic treatment methods, higher rates of FP, and a change over time in how the patients are advised regarding post-cancer pregnancy.

Even though research has shown an increased risk of emotional distress due to iatrogenic infertility (117, 118), it is important to keep in mind that pregnancy rate in the population of cancer survivors reflects far more than only fertility or infertility rate. Other factors than gonadotoxic effects of antineoplastic treatments can affect pregnancy rates among cancer survivors. Partnership, desire for having children, potential concerns about how the pregnancy may affect the patient's own health and prognosis, or fear of recurrence during the eventual offspring's childhood, are all relevant variables that may influence the decision to become or not to become pregnant.

### 2.5.2 Safety of pregnancy after cancer

Regarding the safety of pregnancy following cancer, there are no apparent reservations. Still, concerns have been articulated when it comes to safety of pregnancy after BC and ER-positive BC in particular, as pregnancy could provide potentially detrimental endocrine stimulation of residual cancer cells (119, 120). So far, results of studies on the impact of subsequent pregnancy on long-term BC outcomes have been reassuring, also in women with ER-positive disease (121-127). In this context, selection bias, known as the "healthy mother effect", has often been discussed. Valachis et al. performed a meta-analysis of published articles that have tried to overcome the "healthy mother effect" bias. The results of that study indicate that in early breast cancer patients, pregnancy that occurs at least 10 months after diagnosis does not jeopardize prognosis and may actually confer significant survival benefit (126). Similarly, in a meta-analysis by Hartman et al., a subanalysis controlling for "healthy mother effect" included only studies that have been controlled for

the aforementioned bias by matching for nodal status, ER status, disease-free interval and treatment details. A total of 17 049 women were included in the subanalysis. Reduced risk of death, pHR 0.65 (95%CI 0.52-0.81), was observed in women who became pregnant after treatment of BC, as compared to women who did not become pregnant (128).

In clinical practice, the timing of subsequent pregnancy is a challenge. Most experts find it reasonable to postpone pregnancy for 2 years following diagnosis, mainly to overcome the period associated with a relatively high risk of recurrence (33). Lambertini et al. reported in 2018 that in a cohort of 333 women with pregnancy after BC and 874 matched nonpregnant controls, no difference in disease-free survival was observed after a median follow-up of 7.2 years; time to pregnancy had no impact on patients' outcomes (124). In a cohort of Taiwanese women (249 exposed matched to 4 unexposed controls), Chuang et al. reported lower mortality in women who became pregnant after BC diagnosis, with a more pronounced association for those who became pregnant more than 3 years after diagnosis (121). Today, most clinicians adopt an individualized approach, taking into consideration such parameters as risk of recurrence, given treatment and patients fertility status. For women with ER-positive BC, the need of 5-10 years of endocrine therapy may make the situation particularly complicated. Temporary interruption of endocrine therapy to allow pregnancy has been proposed (129). The ongoing POSITIVE study (NCT02308085) aims to evaluate the safety of this approach in women who have received 18 to 30 months of endocrine therapy (129).

### 2.5.3 Pregnancy outcomes

The rate of spontaneous pregnancy loss in cancer survivors is comparable to siblings and the general population (130, 131). The only exception with higher rates is the group of women with the history of cranial or abdominopelvic radiation, who are also more frequently affected by second-trimester losses (110, 130).

Higher rates of preterm birth have been reported in cancer survivors (1.5 to 2-fold compared to the general population or siblings); the elevation of risk was related to abdominopelvic radiation in a dose-dependent fashion (24, 132). The risk to have a low-birth-weight baby (<2500 g) has also been observed to be higher among female cancer survivors compared to controls. However, higher rates of small for gestational age offspring were only observed in women with a history of abdominopelvic radiation (24, 132). This suggests that preterm birth rather than intrauterine growth restriction lies behind those elevated rates of low-birth-weight babies among female cancer survivors.

Regarding other potential pregnancy-related complications, an increased risk of gestation diabetes (RR 2.62, 95% CI 2.22-3.04), pre-eclampsia (1.32, 1.04-1.87), post-partum hemorrhage (2.83, 1.92-4.67), cesarean delivery (2.62, 2.22-3.04), and maternal postpartum hospitalization >5 days (3.01, 1.72-5.58) in survivors of adolescent and young adult cancer survivors in comparison to females with no cancer history has been reported in a large population-based study (133). These data emphasize the importance of adapted pre-

conceptional counseling and adapted pregnancy surveillance in female cancer survivors, with involvement of both obstetric and oncologic expertise.

Health risks in the offspring of female cancer survivors have been studied relatively extensively during recent years, and the results are reassuring. No significantly increased risks of congenital malformations (134, 135), chromosomal abnormalities (136), or cancer (except for rare events of familial cancer syndrome) (137) have been reported.

## 2.6 PREGNANCY WITH DONOR OOCYTES IN FEMALE CANCER SURVIVORS

For women who develop iatrogenic ovarian failure, fertility treatment with donor oocytes may provide a valid option to experience pregnancy and biological parenthood.

In 2016, Luke et al reported in a population-based cohort study that the likelihood of a live birth after ART was reduced among female cancer survivors when using autologous oocytes, while it was similar to women without cancer when donor oocytes were used (138). These results are encouraging, although the previously mentioned concerns regarding potential pregnancy risks related to cancer treatments' adverse effects remain.

Current evidence suggests that oocyte donor treatment in itself is a significant and independent risk factor for pregnancy complications (139-141). Some studies indicate a 2 to 3-fold increased risk of gestational hypertension and preeclampsia (PE) (142-144), a higher incidence of gestational diabetes (144), preterm delivery (145), placental abnormalities (145) and first trimester bleeding (143, 144, 146). Oocyte donation is often associated with advanced maternal age, which is itself a known risk factor of adverse obstetric and perinatal outcomes. However, even in women of younger age, increased pregnancy complications such as hypertension have been reported in pregnancies using donor oocytes (147).

In Sweden, ART using donor oocytes became approved in 2003. These treatments have been exclusively provided by university hospitals that cover large healthcare regions. The costs are financed by the tax-funded healthcare system available to the whole population. Treatments with donor oocytes require thorough physical and psychosocial screening of the oocyte recipient candidates, and they are restricted to women 25-40 years of age who are healthy at the time of attempting pregnancy. A strict policy of single embryo transfer is also practiced in Sweden to reduce additional pregnancy risks of multiple births (148, 149).

There is a particular lack of data on obstetric and perinatal outcomes of pregnancies achieved using donor oocytes in women previously treated for cancer. Swedish strict policies regulating treatments with donor oocytes minimize potential differences in health status between women with and without a history of cancer undergoing these treatments, providing a possibility to study the true effects of cancer history on obstetric and perinatal outcome in this population.

# 3 AIMS OF THE THESIS

The overall aim of this thesis has been to explore the efficacy and safety of different methods for fertility preservation and fertility treatment in young women with cancer. Its main focus has been on women with breast cancer (BC) as this is the most common malignancy affecting women of reproductive age.

**Specific aims:**

| | |
|---|---|
| **Study I:** | To investigate whether there is a higher risk of obstetric and perinatal complications after treatment with donor oocytes in women with cancer history compared to women without prior cancer. |
| **Study II:** | To explore the trends over time in women's decisions after standardized fertility preservation counseling.<br><br>To evaluate the efficacy of the FP methods by assessing the return rates and utilization rates of cryopreserved specimens after long-term follow up. |
| **Study III:** | To investigate the efficacy and safety of current approaches to controlled ovarian stimulation aimed at fertility preservation in women with breast cancer. |
| **Study IV:** | To compare and evaluate long-term reproductive outcomes after breast cancer in women with vs without fertility preservation. |

# 4 MATERIALS AND METHODS

## 4.1 EPIDEMIOLOGICAL STUDY DESIGN AND ANALYSIS

Designs used for quantitative research can be broadly categorized into two groups: analytical and descriptive. The goal of any analytical study is to test a hypothesis, to find a possible association/causality between exposure and outcome. Analytical studies may be further divided into experimental and observational. In experimental studies, the researcher, in one way or another, affects the exposure. Observational studies, on the other hand, do not involve any form of practical experiment: the researcher collects the data, but does not manipulate the exposure. Observational studies can be descriptive and/or analytical.

*Table 2. The most common types of epidemiological study design*

| | Experimental | Observational |
|---|---|---|
| Descriptive | | Case report, case report series, incidence study |
| Analytical | Randomized Clinical Trials<br>Interventional studies | Cohort studies, case-control studies |

For assessing medical evidence, different hierarchies have been proposed over time (150), where the studies are graded according to their ability to accurately represent "the truth", mainly dependent on their design and quality. RCTs (Randomized Clinical Trials) and meta-analyses of RCTs are considered the most reliable evidence. Due to the nature of randomization, they are able to control for potential bias by creating an equal distribution of prognostic variables (both known and unknown) within a sample population (151).

However, randomized studies are not feasible in certain scenarios, or may be simply unethical. Thus, observational studies are sometimes the only option to acquire valuable data on specific scientific questions. Although observational studies generally cannot prove causality, they provide tools for estimating associations between variables and risks of outcomes occurring. Studies in this thesis utilize observational epidemiological design. More precisely, all of them are cohort studies. Some of the basic characteristics of this design are discussed below.

**Cohort studies**

The study population in a cohort study is identified based on exposure, so that the groups of individuals included are similar in many ways but differ by a certain characteristic (exposure). Both groups (exposed and unexposed) are followed over time, and the outcome(s) are recorded continuously.

The advantages of a cohort study include the opportunity to study associations between multiple outcomes and multiple exposures. When randomization to a certain exposure is not possible due to ethical or practical reasons, the cohort design is a useful alternative. In addition, inclusion of large study samples, for instance, through the use of national registers, makes it possible to study rare exposures. Compared to RCTs, cohort studies are usually less expensive to conduct, and may allow longer follow-up time. The disadvantage

is that the studies with this design may suffer from confounding by indication and selection bias. If the groups are not truly comparable at the baseline, differences in the outcome may reflect other factors rather than a measure of the association to the study exposure.

Cohort studies can be prospective or retrospective, typically depending on the time of the occurrence of the outcome in relation to the time at which the study was initiated.

In prospective studies, the cohort is followed forward in time, and specific outcomes and other related variables are documented. At the time when subjects are enrolled, baseline exposure information is collected, but the important thing is that none of the subjects have yet developed any outcome of interest. The variable of interest is measured in advance of the outcome of interest (152).

Retrospective design, on the other hand, means looking at historical data. The sample and the outcome are defined, and then the researcher looks back in time to collect data about factors believed to be related to already existing outcome, for instance, by sending questionnaires to the study participants (152).

While retrospective studies may provide opportunity to acquire data from existing sources (such as hospital notes), being less costly and more quickly implemented than prospective designs, that usually require time and funding for follow-up, they may be limited by accuracy and comprehensiveness of previously recorded data such as recall bias in the case of self-reported information (153).

As previously mentioned, cohort studies can be used to investigate possible association between variables, but generally not for establishing causation. Still, by measuring the strength of associations and the consistency of associations across a range of studies, inferences about causation can be drawn.

**Different measures of association and statistical methods to estimate them**

Different measures of estimating the strength of the association between exposure and outcome are used in different settings. A brief description of such measures and the relevant statistical methods for their estimation used in the studies of this thesis follows below.

The probability of the occurrence of an event, or the *risk* of an event, can be calculated by dividing the number of events by the number of people at risk. The *risk ratio* (RR), often referred to as *relative risk*, is the risk among exposed subjects divided by the risk among unexposed subjects. Similarly, the *odds of an event* can be calculated by dividing the number of times an event happened by the number of times it did not happen within the same population. The *odds ratio (OR) is the* odds among the exposed divided by the odds among the unexposed. The OR is a close approximation of the risk ratio when the outcome is rare. A *rate* is defined as the number of times that an event happens in a population over a fixed period of time. The rate takes person-time into account, which is important when the risk changes over time or when the follow up time differs between exposed and unexposed subjects. *The hazard rate* is the instantaneous hazard of an outcome event. It is calculated

as number of events divided by the person-time at risk. The *hazard ratio (HR)* is the ratio of the hazard rate in the exposed group divided by the hazard rate in the unexposed group. HRs can be interpreted differently depending on the outcome of interest, for instance, as an incidence rate ratio when estimating differences in incidence rates, or as a mortality rate ratio when studying mortality.

Regression modeling is one of the most important statistical methods used in analytical epidemiology. Regression models are often applied to investigate the association between an exposure and an outcome, while allowing for adjustment for other factors that may influence both the exposure and the outcome called confounders. Regression models can be used for exposures and confounders on any measurement scale, i.e. binary, continuous, or categorical. Depending on the measurement scale of the outcome and the study's design, different regression models can be used (154). *Linear regression* is suitable for continuous outcomes and can be used to estimate the mean difference in outcome between exposed and unexposed individuals or the expected change in the outcome for a one-unit change in a continuous exposure. The fundamental assumption of linear regression models is that the association between exposure and outcome is linear. *Logistic regression* is used to estimate ORs for binary outcomes (155). *Cox proportional hazards regression* is commonly used for survival analysis to analyze time-to-event data. Cox regression estimates HRs for binary outcomes without making any assumptions about the underlying hazard rates of the exposed and unexposed individuals (154).

## 4.2 ERRORS IN EPIDEMIOLOGICAL RESEARCH

Epidemiological studies measure characteristics of populations; they measure the frequency of an outcome or aim to investigate associations between exposure and outcome. Since these studies are being carried out by human beings on human beings, they have both practical and ethical constraints, and are almost invariably subjected to errors. To achieve accurate estimates of possible associations, both the risk of systematic and random errors needs to be addressed.

### 4.2.1 Systematic error

Systemic error can be understood as any difference between the true value and the value obtained in the study that cannot be explained by random error, i.e. any systematic tendency to under- or overestimate an association because of deficient design or execution. Systematic error may negatively affect the validity of the study, both internal (how well the study is conducted) and external (generalizability of the findings). *Confounding, selection bias and information bias* are often considered to be the main sources of systematic error.

Since the exposure is not randomly assigned in observational studies, the subjects in the exposed and unexposed group may differ systematically due to other reason than exposure. When exposure and outcome share common cause(s), a spurious association termed *confounding* will be induced between them. Age and gender may act as confounding factors

in many studies but in general, confounding is unique for each studied association, and requires detailed knowledge of the respective scientific field.

There are methods for preventing confounding at the design level (e.g., matching and restriction) and at the analysis stage (e.g., stratified analysis or statistical regression models). Distortion that remains after controlling for all known confounders in the design and/or analysis is known as *residual confounding*. Thus, awareness of the risk for confounding is important at all stages of conducting a study, and conclusions should not only be based on results of statistical analyses and characteristics of the study design, but should incorporate the existing knowledge about the studied phenomena.

*Selection bias* is another source of systematic error. It occurs when there is a systematic difference between those who are enrolled in the study and those who are not (affecting generalizability), or those exposed and those who are not (affecting comparability between the groups). As the association between exposure and outcome among those who do not participate in the study is usually unknown, eventual selection bias can in most cases only be inferred rather than observed.

Thirdly, *information bias* results from systematic differences in classifying subjects regarding their exposure and outcome status. For continuous variables, information bias is referred to as *measurement error*, while for categorical values it is called *misclassification*, and it can be further divided in *non-differential* and *differential* misclassification. According to Rothman (156), these two types of misclassification can be defined as follows:

*"For exposure misclassification, the misclassification is nondifferential if it is unrelated to the occurrence or presence of disease; if the misclassification of exposure is different for those with and without disease, it is differential. Similarly, misclassification of disease [outcome] is nondifferential if it is unrelated to the exposure; otherwise, it is differential."*

### 4.2.2 Random error

Random error is associated with variations resulting from chance. It occurs because estimates in epidemiological studies are based on samples, and there are different degrees of accuracy in how each single sample reflects the population at large. Random error is usually associated with three factors:

- the sample size
- the degree of inter-individual variability in the sample
- the magnitude of observed differences (when the observed difference in outcome between the groups increase, the likelihood of it being caused by chance decreases)

Random error is unpredictable but it can be reduced by using larger sample sizes and efficient statistical analyses. Estimation of random error can be done through two methods: *p-value*, or hypothesis contrast test, and *confidence intervals*.

*P-value* indicates the probability of observing differences between the groups, even if the null hypothesis of no difference between them was true. Hence, the smaller the p-value, the lower probability that the observed difference was a result of random error. Most commonly, differences with a p-value <0.05 are considered statistically significant. However, this criterion is arbitrary, as a p-value of 0.04 means there is a 4% chance of finding the observed difference due to sampling variability, and 0.06 indicates a probability of 6%. Practically, the difference between 4% and 6% probability is very small, but only the first value would be considered statically significant.

*Confidence intervals (CIs),* just as p-values, are derived from the standard error (SE), and the two of them are naturally related. CIs can be used regardless of the type of outcome measure, and give us a range of values within which we are reasonably confident that the true population difference lies. The point estimate is the most likely value based on observed data, and a 95% CI can be interpreted such that we are 95% confident that the interval contains the true value of the population parameter.

The SE is inversely related to the sample size. As the sample increases in size, the SE decreases and, if there is an association, the CI becomes narrower. Based on this, the use of CIs when considering whole population data (such as the national cancer register) has been debated, since the study population is not a sample of the national population. However, even national populations can be considered samples from the world population, and the registers are not always 100% complete. Therefore, the use of CI is, as a rule, motivated (157).

Both p-values and CI constitute measures of *precision* in the study There is an ongoing debate regarding the advantages and disadvantages of using CIs and p-values, but this discussion is complex and lies outside the scope of this thesis. In the included studies we have generally reported CIs for estimates from regression analyses and p-values for comparisons in descriptive tables.

## 4.3 INTERNAL AND EXTERNAL VALIDITY

Precision and validity are two important concepts when evaluating the quality of any observational study. As previously described, precision is a lack of random error. Validity, on the other hand, refers to a lack of systemic error.

*Internal validity* can be explained as the strength of the inferences from the study, i.e. if the study does estimate what it aims to estimate. Are the observed changes in the outcome between the groups attributed to the exposure and not to other possible causes? Lack of a control group, or a control group that is not comparable to the exposed group in measurable or unmeasurable ways, would compromise the internal validity.

*External validity* refers to the ability to generalize results of a study to a more universal population. External validity mirrors the degree to which the conclusion s of a study would be true for other populations, in other places and at other times. High internal validity is

considered to be a pre-requisite for high external validity, but is not a guarantee for it. If the sample is not representative, the external validity would be low. Thus, the most common loss of external validity in observational studies is usually referred to the fact that studies involve small samples from a single geographic location or facility (158).

## 4.4 OVERVIEW OF STUDY DESIGNS AND METHODS IN THE THESIS

*Table 3. Overview of study designs and methods.*

*BC – breast cancer, COS – Controlled Ovarian Stimulation, FP – fertility preservation.*

| Study | I. | II. | III. | IV |
|---|---|---|---|---|
| **Design** | Prospective cohort study | Prospective cohort study | Prospective nationwide multicenter cohort study | Prospective nationwide register-based matched cohort study |
| **Population** | Women undergoing donor oocyte treatment | Girls and women undergoing FP | Women with BC undergoing COS for FP | Women of reproductive age diagnosed with BC |
| **Intervention Or Exposure** | Exposed to antineoplastic treatments that resulted in iatrogenic ovarian failure | Oncologic indication for FP | COS with antagonist protocols adapted to women with BC (addition of letrozole, a random start day of COS, use of a GnRHa trigger) | Use of different FP methods |
| **Comparison** | Women with ovarian failure from other causes than antineoplastic treatments | FP indicated by benign deseases or conditions | Use of standard COS with antagonist protocols | No FP |
| **Outcome** | Obstetric and perinatal outcome | Pregnancy and live birth rates. Trends in choices of FP methods over time. | Number of oocytes and embryos cryopreserved | Post-BC live births, ART-treatments and all-cause mortality |
| **Time** | 2003-2015 (donor oocyte tretment) | 1st October 1998- 1 December 2018 (FP counseling) | 1st January 1995 – 30th June 2017 (FP-counseling) | 1994 – 30th June 2017 (BC diagnosis) |
| **Number of participants** | 31 women with a previous history of cancer (25 pregnancies) and 212 women without history of cancer (244 pregnancies) | 1254 female patients (852 with oncologic indication and 402 with non-oncologic reasons for FP) | 610 women that underwent FP counseling<br><br>380 cycles with GnRH antagonist | 1275 women (450 exposed to FP and 850 unexposed to FP) |
| **Analysis method** | Logistic regression | Student t-test, chi-square, logistic regression | Complete case linear regression models | Delayed-entry Cox proportional hazard models |

As shown in the table above, all four studies of this thesis are observational studies.

**Study I** is a cohort study including women who underwent oocyte donor treatments at Karolinska University hospital between 2003 and 2015, where information on exposure, outcome and other variables of interest had been continuously recorded in the clinical registry. The study investigates association between history of cancer as a cause of infertility and adverse obstetric or perinatal outcome among women treated with donor oocytes.

**Study II** is a cohort study of girls and women referred for FP to the Reproductive Medicine Clinic of Karolinska University Hospital between 1 October 1998 and 1 December 2018. This study summarizes the results of 20 years of FP counseling at the center. It assesses return rates and utilization rates of cryopreserved specimens during long-term follow-up in relation to oncologic vs non-oncologic indications for FP. It also investigates how trends in decisions regarding preferred FP-methods have changed over time.

**Study III** is a prospective multicenter cohort study with national coverage including 610 women with BC counseled on FP between 1 January 1995 and 30 June 2017 at six Swedish regional FP programs. Efficacy of newly introduced potential improvements to COS for FP (addition of letrozole, random start of COS, use of GnRHa trigger) was investigated in comparison to standard GnRH antagonist protocols. We also report long-term overall survival and reproductive outcome in relation to FP-status.

**Study IV** is a register-based nationwide matched cohort study, where long-term reproductive outcome, defined as post-BC live births and performance of ART-treatments, were compared among 425 women who had undergone FP at one of Swedish University hospitals and 850 comparators with BC but without history of FP, matched on age-group, time-period and health care region.

### 4.4.1 Study I

**Study population and data sources**

Study I included all cases of oocyte donor treatment performed at Karolinska University Hospital between 2003 and 2015 to the women with iatrogenic infertility due to previous cancer diagnosis (n women = 31). For the analysis of main outcome, only women with deliveries following OD treatments were included (20 women, 25 pregnancies). A group of comparators consisted of women who underwent similar fertility treatment at the same period of time (212 women, 244 pregnancies) but did not have any history of cancer. For the subanalysis, all OD treatments in the post-cancer group (31 women, 102 treatment cycles) were included.

In Sweden, OD treatment has been available since 2003, restricted to university hospitals only. Just like other ART-treatments, they are provided within tax-funded health care

system to couples with primary infertility, female age < 39 years and male partner <56 years. Until 2019, OD treatment could only be offered to couples, not to single women. It required a strict psychosocial evaluation process, where both potential parents needed to prove their good health and potential to take care of the future offspring. To reduce maternal and perinatal complications associated with multiple conceptions (159), the rule of single embryo transfer is applied to OD-treatments.

Demographic characteristics, data on cancer treatments, OD-treatments and pregnancy outcome were collected from the medical records. Data on treatments using OD were prospectively entered to the Reproductive Medicine's electronic database (LinneFiler®, Fertsoft AB, Uppsala, Sweden). Data on pregnancy and delivery were prospectively registered in Obstetrix® (Cerner Sweden AB, Stockholm).

**Variables**

Exposure: history of antineoplastic treatment.

Co-variates considered as possible confounders: maternal age at embryo transfer (ET), maternal body mass index (BMI, $kg/m^2$), and smoking at the first antenatal visit.

Primary outcome: obstetric and perinatal outcomes defined by corresponding ICD-10 code or record in medical files of Obstetrix®: preeclampsia (PE), eclampsia, HELLP syndrome (hemolysis, elevated liver enzymes, and a low platelet count), pregnancy-induced hypertension, placental abruption, preterm premature rupture of membranes (PPROM), postpartum hemorrhage of >1000mL, placenta previa, Gestational Diabetes Mellitus, small for gestational age, preterm birth (birth before a gestational age of 37 complete weeks) further subdivided into extreme (<28 weeks), very preterm (28 - <32 weeks), and moderate (32 - <37 weeks), mode of delivery (Cesarean or vaginal), APGAR less than 7 at any point during the first 10 minutes after birth, and pH <7 at umbilical arterial blood.

In the subanalysis, exposure was defined as history of radiotherapy to abdomen/ pelvis/ total body irradiation. Outocme was defined by corresponding record in LinneFiler® or/and Obstetrix®: thickness of endometrium (measured by ultrasonography on the day of embryo transfer), pregnancy rate (defined as positive U-hCG per treatment), delivery rate (defined as delivery after gestational age of 22 weeks and 6 days), mean gestational age at birth, and mean weight at delivery.

**Statistical analysis**

The associations between the history of antineoplastic treatment and adverse outcome in OD pregnancies were calculated using logistic regression, estimating ORs and 95% CIs. As there were very few smokers in the cohort, smoking was not included as a confounder. We performed complete case analysis, i.e. only observations with complete information on the included variables were included in the regression model. Further adjustment for intrasibling correlation with a robust estimator of the standard error was performed in order to account for the independent data structure (one woman could contribute with more than

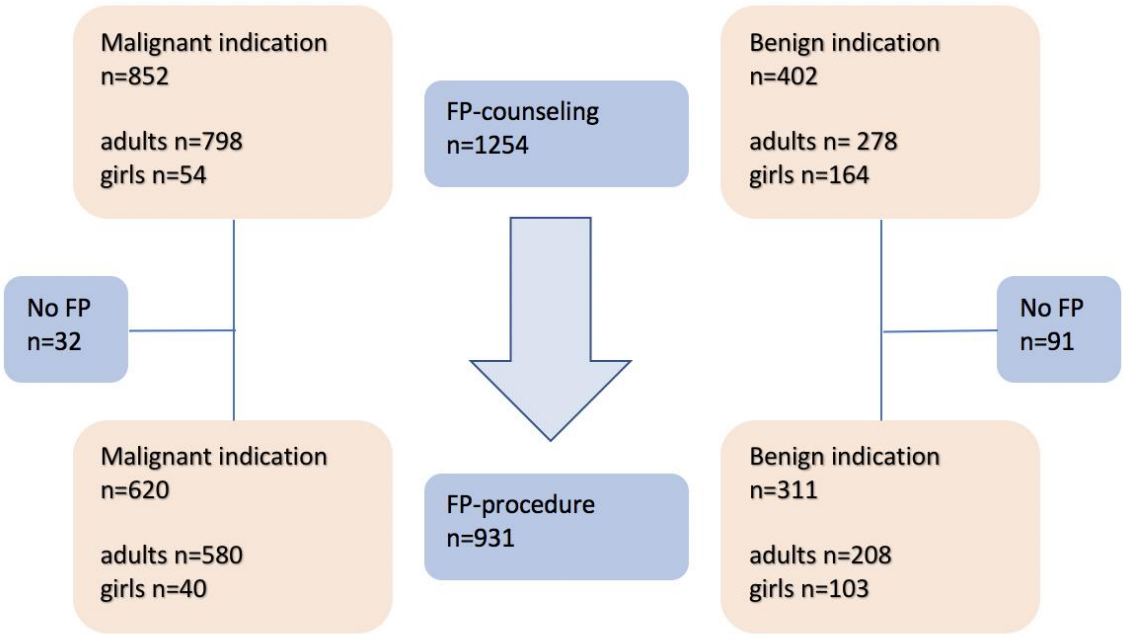
one observation if she had more than one child). For the subanalysis, mean values and rates were compared. P-value was calculated using Pearson's chi-squared test for factor variables and t-test for the continuous variables.

### 4.4.2 Study II

**Study population and data sources**

In Study II we used the data collected prospectively in the electronic database at the Reproductive Medicine center (LinneFiler®) regarding clinical characteristics, details of ART treatment, and eventual utilization of cryopreserved cells/tissues among women and girls referred for FP to Karolinska University Hospital between 1 October 1998 and 1 December 2018. Using unique Swedish personal identification number, each person in the cohort was linked to the Total Population Registry of the Swedish Tax Agency to assess overall survival in the entire cohort as of 1 December 2018.

*Figure 6. Flowchart of the study population, Study II. FP – fertility preservation.*




**Variables**

Exposure: oncologic indication for FP as compared to benign indications for FP, and age >18 years at the time of FP as compared to the age of 1-17 years.

Detailed data on diagnosis that indicated FP counseling, and data on demographic and medical characteristics at the time of FP counseling were presented.

Primary outcome: return rate for counseling or pregnancy attempt as well as long-term reproductive outcome defined as pregnancy and live birth rate (for benign vs malignant indications). Trends in preferred FP methods over study period (adults vs children and adolescents) were analyzed.

Secondary outcome: outcome of FP cycles aiming at oocyte or/and embryo cryopreservation.

Survival in the entire cohort was presented as a descriptive component.

**Statistical analysis**

The proportion of women who returned for counseling and fertility treatment was calculated out of all the women who had undergone FP, were 18-45 years at the end of follow-up, and were alive and living in Sweden at least 1 year following FP. The return rates of women with malignant and benign indications for FP were compared using logistic regression (ORs and 95% CIs) with adjustment for attained age. Pregnancy and live birth rates were calculated among women who returned for fertility treatment, and compared between malignant and benign indications using chi-square tests.

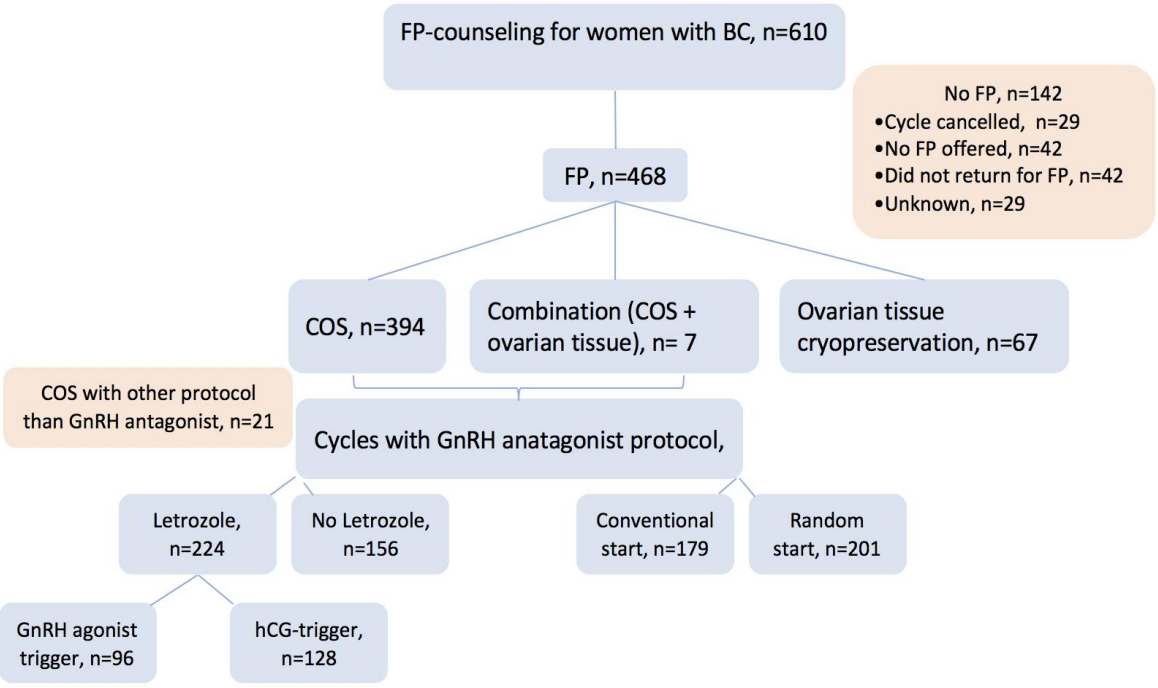
### 4.4.3 Study III

**Study population and data sources**

The study population included women with diagnosis of BC, referred for FP counseling. The study subjects were identified from the electronical medical records at six Swedish university hospitals with programs for FP, each covering a healthcare region.

Data on demographical and clinical characteristics, as well as the details related to FP-procedures, were collected from the electronic medical records. The system of electronic medical records has been implemented in Sweden since 1997, and the data are registered prospectively at the clinical treatment registry at each hospital. Data on overall survival was obtained from the Total Population Register of the Swedish Tax agency, using unique identification numbers assigned to the Swedish citizens.

*Figure 7. Flowchart of the study population, Study III. BC - breast cancer, COS - controlled ovarian stimulation, FP - fertility preservation.*




## Variables

Exposure was defined as:
1. Use of GnRH antagonist protocols with potential improvements (i.e. co-administration of letrozole, random start of COS and use of GnRHa trigger) as compared to standard GnRH antagonist protocols;
2. FP treatment as compared to FP-counseling only;
3. Return for FP counseling/utilization of cryopreserved specimen among women who received FP as compared to women who have not returned yet.

Number of oocytes and embryos cryopreserved were defined as primary outcomes. Secondary outcomes included return for new FP counseling, treatment, eventual deliveries and overall survival during follow-up.

## Statistical analysis

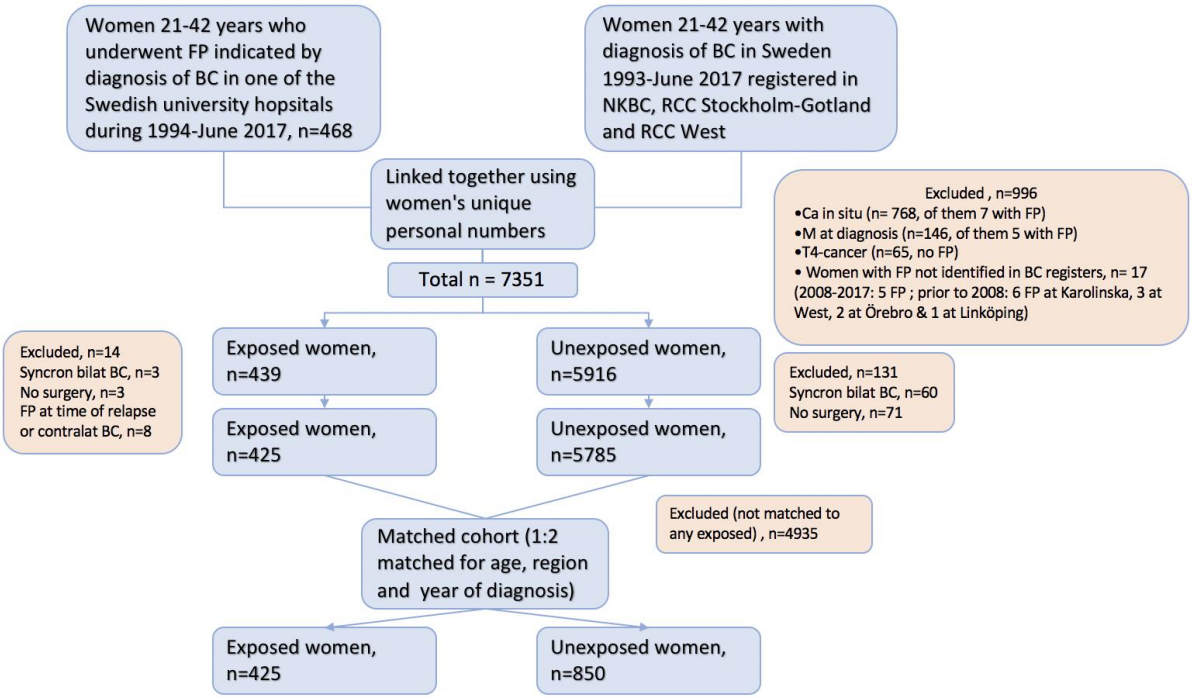
Complete-case linear regression models were used to estimate associations between exposure and primary outcome. Overall survival was compared between the groups using Kaplan-Meier estimates of all-cause survival and log rank tests.

### 4.4.4 Study IV

**Study population and data sources**

The study population included women of reproductive age diagnosed with BC. Women who underwent FP at one of seven Swedish university hospitals between January 1, 1994 and June 30, 2017 were identified in the medical records of each hospital and, using their unique personal number assigned to all Swedish residents, further identified in the Swedish quality registers for BC. Two matched comparators unexposed to FP were sampled for each exposed woman (Fig 8). Data on FP procedures for women with FP were obtained from the electronic medical records of each hospital. Additional variables of interest could be retrieved by linking the cohort to Swedish population registers (Table 4).

*Figure 8. Flowchart of the study population, Study IV. BC - breast cancer, FP - fertility preservation.*




**Variables**

Exposure was defined as receiving FP treatments.

The primary outcome was live birth after the diagnosis of BC. Secondary outcomes included reception of ART treatments after BC diagnosis. All-cause mortality was also included as a secondary outcome since it is a competing risk in the studied associations. Perinatal outcomes were included as a descriptive component.

*Table 4. Swedish population registers used to obtain information on cohort.*

| Register | Description | Years | Information retrieved |
|---|---|---|---|
| Swedish National Cancer Register | Since 1958, six regional cancer centers report on all cancer cases, and the data includes histological type, site and stage of tumor, date of diagnosis, eventual date and cause of death, with the coverage rate of 96%. | 1994-2017 | date of BC diagnosis, age at diagnosis, tumor characteristics, treatment details for BC |
| Swedish National Quality Register for BC | A population-based register, with information on tumor characteristics, treatment, and relapse occurrence in patients diagnosed with invasive BC since 2008, with the coverage rate of 99%. | 2008-2017 | |
| Regional quality registers for BC, for regions West and Stockholm-Gotland | Prior to 2008, data on tumor characteristics, treatment details and relapse occurrence for all cases of invasive BC were reported to the regional quality registers. | 1994-2007 | |
| The Total Population Register | It contains data on life events including birth, death, marital status and migration; maintained by the government agency Statistics Sweden. Updates are transmitted daily from the Tax Agency to the TPR. | 1994-2018 | date of death, immigrations and emigrations |
| The Swedish Multi-Generation Register | It contains connections between index persons (registered in Sweden at some time since 1961 and born in 1932 or later) and their biological parents. | 1994-2018 | year of live births |
| Longitudinal integrated database for health insurance and labor market studies (LISA) | It is maintained by the government agency Statistics Sweden and holds annual registers since 1990, integrating existing data from the labor market, educational and social sector. | 1994-2017 | educational level and country of birth |
| The Medical Birth Register | It includes data on all births in Sweden since 1973, reported information comes from medical records from the prenatal, delivery and neonatal care. | 1994-2017 | date, perinatal and obstetric outcomes of post-diagnosis live births |
| The National Quality Registry for Assisted Reproduction | It was started 2007 and contains data on all ART-treatments in Sweden, in both public and private IVF clinics, with 100% coverage rate of care providers. | 2007-2017 | treatment details and outcome of all ART cycles |
| Abbreviations: ART, assisted reproductive technology; BC, breast cancer. | | | |

## Statistical analysis

Delayed-entry Cox proportional hazard models, with the time since diagnosis as the underlying time scale were used to estimate HR and 95% CI for live births, ART and all-cause mortality. The cumulative incidence of post-BC childbirths and ART treatments in exposed and unexposed women were estimated non-parametrically in the presence of the competing risk of death.

# 5 RESULTS

## 5.1 STUDY I

Study I examined the association between history of cancer treatment and risk for pregnancy complications in a cohort of 232 women treated with OD at Karolinska University Hospital. The main results of this study were:

- 20 women with the history of cancer (exposed) and 212 women without cancer history (unexposed) achieved 25 vs 244 live births after treatment with donated oocytes, respectively; there were no statistically significant differences in the baseline characteristics between these two groups.

- In multivariable adjusted analysis, women with the history of cancer had increased incidence of preterm births (aOR 5.54, 95% CI 2.01-15.31). The mean gestational week at delivery was 36.9 in the exposed group and 39.3 in the unexposed group. Stratifying for extreme (<28 weeks), very (<32 weeks), and moderate (<37 weeks) prematurity, the risk for very preterm birth was observed to be several fold increased in the exposed group (aOR: 17.4, 95% CI: 3.99–75.79), while the difference in incidence of extreme and moderate preterm birth was not significant between the groups (OR: 3.35, 95% CI 0.33-33.93 and aOR: 2.92, 95% CI 0.88-9.66). For extreme preterm birth, only unadjusted analysis was performed (due to only 1 case in the exposed group).

- The incidence of PPROM did not differ significantly between the groups (aOR 3.85, 95% CI 0.96-15.42).

- Compared to women without cancer history, survivors of cancer had a higher incidence of PE (aOR 2.79, 95% CI 1.07-7.34). However, the risk for hypertensive disorder of pregnancy (including both PE and pregnancy-induced hypertension) did not differ significantly between the groups (aOR 1.8, 95% CI 0.69-4.69).

- Cesarean was the mode of delivery in 48% of women in the exposed group and 43% in the unexposed group, compared to 17% of all deliveries in Sweden (160).

- PPH, defined as bleeding over 1000 mL, occurred in 32% vs 27% of women in the exposed vs the unexposed group, compared to a general rate of about 10% in Sweden (160).

- The incidence of Apgar score below seven during first 10 minutes after births and the need of care at the Neonatal Intensive Care Unit (NICU) did not differ between the groups (aOR 2.40, 95% CI 0.24-24.46 and aOR 1.14, 95% CI 0.36-3.61).

In a subanalysis in Study I, differences in reproductive outcome among women with and without history of radiotherapy (RT) (to abdomen, pelvis/total body irradiation) were

investigated in a cohort of 31 women with oocyte donation treatment indicated by iatrogenic infertility after cancer treatments. The main results of this subanalysis were:

- 15 women with the history of RT have undergone 47 treatment cycles with donated oocytes (26 fresh embryo transfers (ET) and 21 frozen embryo transfers, FET) compared to 16 women without history of RT, that have undergone 55 cycles, 26 ETs and 29 FETs.

- Among women with the history of RT, mean thickness of endometrium at treatment cycle was 7.4 mm (range 4-12 mm) compared to 9 mm (range 5-14.2 mm) among women without history of RT, p-value < .001.

- There was no difference regarding pregnancy rate (23% vs 36%, p = 0.2), live birth rate (19% vs 29%, p = 0.3) or delivery per pregnancy rate (82% vs 80%, p = .9) in in women with vs without history of RT.

## 5.2 STUDY II

Study II examined trends over time in choices of FP methods in women and girls with oncologic and non-oncologic indications for FP at Karolinska University Hospital. It also investigated return rates and long-term reproductive outcome in relation to benign vs malignant indications. The main results of this study were:

- Between 1 October 1998 and 1 December 2018, 1254 females (1076 adults and 178 girls under age of 18) received FP counseling for either oncologic (n=852) or benign (n=402) indications, and were included in the study. Of those, 592 vs 285 females with malignant vs benign indications proceeded to FP treatments.

- The majority of adult women elected cryopreservation of embryos and/or oocytes (n=538, 73%) as opposed to cryopreservation of ovarian tissue (n=221, 27%). More than a half of women with partner (53%) elected to either cryopreserve unfertilized oocytes or to cryopreserve both oocytes and embryos.

- Patients' choices of FP method have changed over time during the study period. For adolescents, cryopreservation of ovarian tissue was the preferred method in the 2000s, while an increasing number of postmenarchal girls have elected to undergo controlled ovarian stimulation for cryopreservation of oocytes in recent years. Also, since 2014, cryopreservation of oocytes has replaced cryopreservation of embryos as the most popular method of FP among women with partner.

- By December 2018, the mean time of follow-up was was 6.1 ± 4.8 (0-19) years since FP consultation. Ninety-seven patients had died (91 in the group with malignant FP indications, and 6 in the group with benign FP indications), 21 had emigrated from Sweden and 32 were still younger than 18 years.

- During the follow-up, 27% of women aged 18-45 who had undergone FP for oncologic vs non-oncologic indications, and were alive and living in Sweden at least 1 year following FP, returned for a new reproductive counseling, additional FP or to attempt pregnancy. Patients with malignant FP indications had a slightly lower likelihood of returning for counseling (OR 0.72, 95% CI 0.51-1.0) and a significantly lower likelihood of returning for pregnancy attempt (OR 0.41, 95%CI 0.27-0.62) when compared to patients with benign indications.

- Overall, utilization rates among women who were alive and of childbearing age, and had a follow-up of at least one year after FP, were 29%, 8% and 5% for embryos, oocytes and ovarian tissue, with pregnancy rates of 66%, 54% and 25%, and live birth rates of 54%, 46% and 7% respectively.

- Among women who returned for fertility treatment during follow-up (53/402 vs 75/592 of women with benign vs malignant FP indication), overall live birth rate was 66% vss 34% ($p < .001$), with live birth rate after use of cryopreserved specimen 47% vs 21% ($p = .002$).

## 5.3 STUDY III

Study III examined efficacy of certain novel approaches to controlled ovarian stimulation aiming at oocyte and/or embryo banking in the setting of FP programs for young women with BC, and investigated long-term outcomes including reproductive outcome and overall survival in women who did vs did not proceed to FP after initial FP counseling.
The main results are presented below:

- Of 610 who received FP counseling indicated by BC diagnosis, 468 women proceeded to FP treatment (FP group): 41% of them aimed at oocyte banking, 28% at embryo banking, 15% at shared oocyte and embryo banking, and 14% at cryopreservation of ovarian tissue. In some few cases, a combination of FP methods was practiced.

- Women in the FP group were significantly younger and had lower parity than those who did not proceed to FP after counseling.

- Of 401 COS cycles performed, 380 used a GnRH antagonist and were included in the main analysis. Protocols with concurrent use of letrozole were applied in 59% of the cases (n=224), and in 43% of these cycles final oocyte maturation was induced with GnRHa as opposed to a standard hCG trigger. As opposed to conventional start, random start was practiced in 53% of all GnRH antagonist cycles.

- In letrozole vs no-letrozole group, the number of total and mature oocytes retrieved as well as the number of oocytes and embryos cryopreserved were similar.

- When GnRHa was used as a maturation trigger in letrozole-cycles, number of oocytes retrieved ($p<.05$) and embryos cryopreserved ($p<.005$) was higher than in the group with conventional hCG trigger. As women in the group with GnRHa trigger had significantly higher AFC and AMH (confounding by indication), we performed an additional complete case analysis adjusted for AFC and AMH; association between GnRHa trigger and higher number of retrieved oocytes was not confirmed, but the one between GnRHa trigger and higher number of cryopreserved embryos was still statistically significant ($p=.04$).

- The number of retrieved oocytes, as well as the number of cryopreserved oocytes and embryos were similar in COS cycles with random vs conventional start, but higher total dose of gonadotropins was required in women undergoing random-start COS ($p<.001$).

- Women who proceeded vs did not proceed to FP after counseling had similar overall survival, with a 5-year survival proportion of 0.94 (95%CI: 0.87-0.97) vss 0.94 (95% CI 0.91-0.96). No difference in overall survival was found between women in COS vs non-COS group either.

- Among women who underwent COS, 5-year overall survival in letrozole vs non-letrozole group was similar, 0.96 (95%CI:0.91-0.98) vs 0.95 (95%CI:0.90-0.97). As the majority of women in letrozole group had ER-positive BC, additional Cox regression models adjusted for letrozole alone (Yes/No) and subsequently adjusted for ER-positivity (Yes/No) as a complete case analysis (data on ER-status available for 280 of 280 women) were tested. There was no difference in overall survival between the women in no-letrozole group (reference) and letrozole group (HR 0.96, 95%CI: 0.27-3.34).

- One-fifth of women who underwent FP returned during follow-up time to either use cryopreserved specimens or with a wish of a new fertility counseling, with regard to completed BC treatment. Of these women, 26% delivered at least one baby by the time of our report, compared to 5% among women who had not (yet) returned and 5% among women from the no-FP group.

## 5.4 STUDY IV

Study IV investigated associations between long-term reproductive outcomes and FP in a matched cohort of 1275 women, diagnosed with BC in Sweden 1994 - 2017. The main results were:

- Among women with the history of FP at the time of BC diagnosis, 22.8% (97 of 425) had at least one post-BC live birth after a mean follow-up of 4.6 years, compared to 8.7% of women (74 of 850) without history of FP, and with a mean follow-up of 4.8 years.

- Rate of post-BC live births was higher among women who had undergone FP, when compared to women without FP history (aHR: 2.3, 95%CI: 1.6-3.3).

- The five-year cumulative incidence of post-BC live births was 19% vs 9% among women exposed vs unexposed to FP. After ten years, the cumulative incidence of post-BC live births was 41% vs 16%, respectively.

- ART-rates after BC were higher in the FP group, compared to no-FP group (aHR: 4.8, 95%CI: 2.2-10.7).

- Compared to women who had not received FP treatments at the time of BC diagnosis, women with the history of FP had a lower rate of all-cause mortality (aHR:0.4, 95%CI:0.3-0.7), with five-year cumulative incidence of death of 5.3% vs 11.1% in FP vs no-FP group.

- Mean time from diagnosis to live birth was 4.5 ± 2.5 years (range 1-13) in the exposed and 4.5 ± 2.3 years (range 1-13) in the unexposed group.

- Among the women who gave birth after BC, 77.1% in the exposed group and 33.9% of those in the unexposed group were nulliparous before their cancer diagnosis ($p<.001$). Women with FP were also more likely to have more than one child after diagnosis (37.3%) compared to women with no FP (17.7%, $p=.04$).

# 6 DISCUSSION

## 6.1 STUDY I

### 6.1.1 Main findings and interpretation

Results of Study I suggest that in pregnancies achieved using donated oocytes, women with a history of cancer have a higher risk of perinatal complications, particularly preterm birth and PE, than women without cancer history. In a subanalysis of reproductive outcome after OD cycles, the history of RT to abdomen/pelvis/total body was associated with thinner endometrium, when compared to the history of treatment with other modalities. These findings suggest that already known increased risk for adverse perinatal outcome in pregnancies with donated oocytes may potentially be amplified in women with previous cancer treatments.

### 6.1.2 Methodological considerations and validity

To our knowledge, that was the first study investigating specific maternal and perinatal risks in OD pregnancies among women with a history of cancer. With that said, it was not without limitations.

As this was an observational study, lacking randomization, participants in the exposed and the unexposed group could differ systematically due to other reasons than exposure. The two groups were comparable at baseline (first antenatal visit) as regards to age, BMI and smoking habits. The treatments were performed at the same medical unit. Like all other candidates for OD treatments in Sweden, all the participants in this study went through the same procedure of physical and psychosocial screening to ensure that they did not have any apparent contraindications for pregnancy. Though the deliveries occurred at more than one hospital, all of them were tertiary hospitals in Sweden with the similar standard of care, and theoretically, there should not be any systematic difference between the exposed and unexposed group regarding the choice of the hospital. All these facts would support the comparability of the groups at baseline.

Logistic regression models were used to analyze the main outcome. The confounders considered a priori for inclusion in multiple logistic regression models were maternal age, maternal BMI, and smoking status at the first antenatal visit. Smoking status was missing in 6/25 vs 0/244 cases in the exposed vs unexposed group, and among cases with smoking information there were very few smokers in either group (5.3% vs 2.9%). Our analyses were complete case analyses, and missing data on smoking in 24% of cases in the exposed group would result in losing of 24% of exposed participants. We therefore adjusted only for age and BMI. Post-publication sensitivity analysis, adjusting for smoking, maternal BMI and age among the cases with complete data, made the observed increased risk for PE among women with previous cancer non-significant, aOR 2.46 (0.89-6.80), while the results for preterm birth were more robust, with OR 4.70 (1.51-14.63) after this additional

adjustment. Similarly, stratifying on the smoking status, and including only non-smokers with complete data in the analysis (22 cases among women with previous cancer and 216 among comparators), results for PE were no longer significant, aOR 2.60 (0.93-7.25), while the difference in risk for preterm birth remained, aOR 5.03 (1.61-15.74).

Information bias, or more precisely non-differential misclassification, could have occurred as we did not have data on the age of oocyte donors or the oocyte quality – variables with the potential impact on outcome.

Women undergoing OD treatments in Sweden have to fulfill certain requirements on their physical and psychosocial condition, and be under the age of 40 at time of starting the treatment. This fact increases the internal validity of the study, making the exposed and unexposed group similar to each other on other characteristics than exposure status. At the same time, it may also introduce selection bias and reduce generalizability/external validity of our results to other settings, not having the same rigorous procedure of selecting candidates for OD treatments.

The major limitation of this study was beyond any doubt its small sample size and few events. As it has been previously mentioned, random error is usually associated with three factors: the sample size, the degree of inter-individual variability in the sample, and the magnitude of observed differences (when the observed difference in outcome between the groups increases, the likelihood of it being caused by chance decreases). The wide CIs reflect a large random error and indicate that the precision is low.

The sample available for subanalysis of reproductive outcome after OD in cancer survivors with and without history of RT was even smaller. Data were missing on certain important variables, such as detailed information on cancer treatments. Due to the small sample size, adjustment for possible confounders could not be performed, and groups with observed statistical difference in age, BMI and endometrial thickness were directly compared. Therefore, the observed differences in endometrial thickness should be interpreted with caution, although they were consistent with results of previous studies in the field.

### 6.1.3 Clinical and scientific context

An increased risk of PTB in OD pregnancies is well-documented (161-163). In our cohort, the incidence of PTB was 9% among women with a history of cancer and 36% among women without cancer history. Stratifying for extreme, very preterm and moderate preterm births, statistically significant differences in outcome were found for very preterm births. It is difficult to find plausible physiological explanation for an increase in very preterm births only, and not in other types of PTB. This finding is probably related to the problem of stratifying outcome in already a small sample, and the observed difference in OR for all preterm births is more robust. In general, spontaneous preterm births (i.e. births that follow spontaneous preterm labor and PPROM) are often regarded as a syndrome, with the most common causes being infection or inflammation, vascular disease, and uterine overdistension (164). For indicated preterm births, common reasons include pre-eclampsia

or eclampsia, and/or intrauterine growth restriction (164). Theoretically, increased level of anxiety for complications among women with history of cancer and their obstetricians could make them more prone to opt for planned preterm delivery to avoid further potential risks, but no cases of iatrogenic PTB without clear indication or pathology were found in the cohort, and most cases of PTB were related to PE and PPROM. Difference in PPROM between the groups did not reach statistical significance, but it would be of value to test for it in a larger cohort. In a recently published cohort study from Sweden, being to our knowledge the largest study on this topic so far, significantly higher risk of being born preterm has been observed among children of female cancer survivors (OR 1.48, 95% CI 1.39-1.59) (165). The reason for increased risk of PTB in this population is most probably multifactorial. As correctly noted by Carg et al., much of the research in the field of oncofertility has so far focused on gonadotoxic effects of anti-neoplastic treatment on ovaries (166). Meanwhile, injury to a highly mitotic tissue like endometrium may also affect fertility and obstetric outcomes, as endometrium can be central to embryo implantation and utero-placental exchange. Compromised blood supply to endometrium and myometrium and/or fibrosis of uterus as sequalae of radiation, resulting in deficient placentation and remodeling during pregnancy, are among proposed explanations to PTB and other pregnancy complications in cancer survivors.

In a meta-analysis from 2016, risk of developing hypertensive disorders of pregnancy in OD pregnancies compared to ART-pregnancies with autologous oocytes was found to be significantly increased (OR3.92, 95% CI 3.21-4.78) (167), consistent with results of other studies reporting on increased rate of pregnancy-induced hypertensive complications compared to both IVF/ICSI and spontaneously conceived pregnancies (141, 161, 168). Complex immunological interaction in the fetal-placental unit is often discussed as a possible explanation (169, 170). Swedish population-based matched cohort study from 2019 found significantly increased risk of pre-eclampsia in survivors of childhood and adolescent cancers, when compared to an age-matched comparison group without cancer history (OR 3.46, 95% CI 1.58-7.56) (171). Results of previous studies in this area have been conflicting, with association observed in some studies (133), but not in others (172, 173). Possible explanations for increased risk of PE in cancer survivors include the fact that survivors of childhood cancers more often suffer from obesity (174), with obesity being one of the important risk factors for PE (175). Besides, injury to endometrium as a result of chemo- and/or radiotherapy, discussed in the previous paragraph, could also affect implantation and development of utero-placental unit, potentially resulting in increased risk of pre-eclampsia.

The findings of this study add to the existing knowledge on increased perinatal complications of pregnancies with donated oocytes, suggesting an amplified risk for PE and PTB in women previously treated for cancer when compared to women without cancer history. Still, perinatal outcome in the studied group in general were reassuring, and the use of OD to achieve pregnancy in women with iatrogenic ovarian failure related to history of cancer should be encouraged. As the sample size was small, future studies to confirm observed associations are needed, and if confirmed, potential clinical implications could include update of guidelines for surveillance of pregnancies in this specific group. Timely initiating of available measures to reduce risk of PE and PTB, combined with

individualized screening routines aiming at early detection of elevated blood pressure, proteinuria, and warning signs for PTB could be some of the possible steps to reduce morbidity.

## 6.2 STUDY II

### 6.2.1 Main findings and interpretation

This study presents over a 20-year long experience of a FP program covering Stockholm's healthcare region, with around 2 million inhabitants. In recent years, cryopreservation of unfertilized oocytes became an increasingly popular FP-method both among post-pubertal girls, and among women in committed relationships. Previously, the former group most often elected cryopreservation of ovarian tissue, while the majority in the latter group opted for cryopreservation of embryos. This trend reflects recognition of oocytes' cryopreservation as a clinically established method in 2013, as its efficacy has been proved in FP programs worldwide (176). Moreover, it highlights the reproductive autonomy this FP method provides to females, as Swedish law states that the partners must be in a relationship at the time of using cryopreserved embryos.

During a long-term follow up, return rates for attempting pregnancy and live birth rates, both overall and related to FP, were lower in women with previous oncologic FP indications compared to women with benign FP indications. That could be related to the need to postpone pregnancy attempts for several years after certain cancer diagnoses, and also to an increased level of concern in both women and their doctors that pregnancy may adversely affect prognosis for disease-free survival. However, the need for FP counseling and FP procedures has been high over years in both groups. Summarizing experiences from a 20-year period in one of the Scandinavian largest FP programs, this study makes particularly clear the importance of clinical and scientific achievements in the field of oncofertility for the patients' choices of FP methods, and for their opportunities to experience biological motherhood.

### 6.2.2 Methodological considerations and validity

Study II was a prospective cohort study, with certain weaknesses and strengths related to its design.

Its large sample size, broad spectrum of FP indications included, long follow-up and use of cross-link data on overall survival and migration from a population-based registry makes it in many ways unique in comparison to other studies evaluating outcome of FP-programs.

The standardized counseling procedure, clear guidelines for referral to FP available on institutional and internal webpages, standard operating procedures following national guidelines for FP can all be considered as measures to ensure comparability between the exposed and unexposed group, i.e. females with malignant vs benign FP indications. Still, a

potential cause of selection bias could have occurred during the referral process, where FP-counseling and FP-treatments were not offered to the sickest patients, resulting in a so-called “healthy FP” selection bias. That could affect the exposed group more than the unexposed, as it lies in the nature of malignant diagnoses that the course of the disease can be dramatic with quick deterioration of the health status, thus affecting comparability of the groups. It could also affect the cohort in general, reducing generalizability of the results.

Factors associated both with oncologic indications for FP and with the return rate to FP-clinic could bias the study results if not adjusted for (confounding). Women with oncological FP-indication were significantly older at baseline, and also older at the time of return for fertility treatment. Therefore, ORs for return rate were adjusted for age. Other potential confounders that we could not adjust for include parity at FP-counseling, smoking status and socioeconomic characteristics.

Information bias could have occurred at collecting of data on reproductive outcome. Data could be missing for patients who conceived spontaneously, or chose to proceed to fertility treatments at other centers or abroad. This bias would be by its nature non-differential, resulting in underestimating pregnancy and live birth rates in the cohort.

Precision of the study can be regarded as high in the context of other studies of FP-outcome due to the relatively large sample size and relatively high prevalence of outcome, reflected by the narrow CIs. However, adjustment to several potential confounders was not possible, as mentioned above.

External validity, on the other hand, reflects ability to generalize results of a study to a more universal population. As previously mentioned, possible “healthy FP” selection bias could theoretically affect generalizability of our results. Moreover, in countries where medically motivated FP-procedures are not included in the public tax-funded healthcare system, economical barriers would result in different characteristics of the populations referred for FP-counseling compared to Sweden, therefore reducing generalizability of our results to these populations. Another factor that may affect external validity is that surrogacy with use of a gestational carrier is not allowed in Sweden, in contrast to some other countries, where return rate for pregnancy attempts may thus be higher than those observed in our study, and differ less from those in women with benign FP-indications.

With all that said, with the study being an analytical observational study, some of its value in the clinical context comes from its descriptive components. The field of oncofertility is still young, and some questions that may arise during FP-counseling can yet be answered only by referring to certain experiences described in the literature.

### 6.2.3 Clinical and scientific context

The importance of providing patients with center-specific information on FP experience has been emphasized in other studies (177), mainly with regard to the fact that the published results are usually coming from the centers with the highest pregnancy rates, and therefore

can be difficult to generalize to centers with less experience. First and most, this study provides important data for FP-counseling of girls and women in Sweden, where FP programs follow national guidelines. However, with certain reservations, they can also be extrapolated to other milieus.

A trend over increasing popularity of cryopreservation of oocytes as the preferred FP method, both among post-pubertal girls and among women in committed relationships, was observed in this study. The main advantage of cryopreservation of mature oocytes when compared to embryos is that this method guarantees woman's reproductive autonomy in the future, preserving her ability to choose a partner for procreation at the time she decides to attempt pregnancy (178). The efficacy of re-transplantation of ovarian tissue was compared to the use of vitrified oocytes in a prospective cohort study from 2018 (179); no statistically significant differences were found in pregnancy and live birth rates. In a recently published study, involving 60 patients after transplantation of ovarian tissues, pregnancy rate of 50% and live birth rate of 41% were reported (180). Evaluating oocyte vitrification outcomes in the largest series today, Cobo et al. reported a cumulative live birth rate of 68.8% vs 42.1% in women ≤35 years of age in women with elective FP vs oncologic FP indications (181). Caution should be taken when comparing these rates, as the populations and follow-up time may be different. However, in general, outcomes of both methods are reassuring, as also confirmed by the fact that since 2019, American Society for Reproductive Medicine considers ovarian tissue cryopreservation to be an established, no longer experimental, FP method (42). In our study, we also hypothesize that women who return for re-transplantation of ovarian tissue may require longer time from FP to pregnancy attempt, as they usually are younger at the time of FP, and the procedure in itself requires more time and recourses than a pregnancy attempt with thawed oocytes or embryos.

Lower return-, pregnancy- and live birth rates in women with oncologic FP-indications, as compared to the women with benign indication in our study, are of interest. In a large cohort study of women who had their oocytes vitrified for FP, return rate to attempt pregnancy, implantation rate, ongoing pregnancy- and live birth rate were lower for onco-FP patients, when compared to patients with elective FP (181). The effect of sole presence of cancer on live birth rates could though not be confirmed by the adjusted OR in a binary logistic regression analysis, while age was found to strongly affect outcome. Still, the authors argued that the lack of confirmation of association between previous cancer diagnosis and poorer reproductive outcome in that analysis could be explained by a smaller number of cancer patients returning to use their oocytes. With reference to the hypothesis from the 1970s, that cancer could be viewed as a systemic disease (due to altered equilibrium for homeostasis in the whole organism), rather than a local disease (182), the authors emphasized the importance of further studies analyzing possible association between history of cancer and IVF outcome (181). In our cohort, reproductive outcome after FP with oncological indications was observed to be not as good as that after FP performed for benign indications, which could be consistent with the theory that reproductive outcome after FP may be affected by the underlying malignancy at the time of FP. Difference in long-term reproductive outcome could theoretically also be explained by the effects of anti-neoplastic treatments (116). However, as the evidence in that fields is so

far scarce, possible correlation between previous cancer and long-term reproductive outcome after FP needs further investigation.

## 6.3 STUDY III

### 6.3.1 Main findings and interpretation

Results of Study III support the premise that recently introduced approaches to COS protocols for FP in eligible women with BC are efficacious and safe. Overall survival was used as a proxy of safety in this study, and the observed rates did not differ between women with vs without history of FP treatments. However, as the survival rates for BC are high, and hopefully will continue to increase, the rate of BC recurrence would be a better measure of safety. It should be investigated in relation to FP in future studies.

### 6.3.2 Methodological considerations and validity

Just as the previous studies discussed in this thesis, this was a prospective cohort study. It included data from 6 university hospitals with FP programs in Sweden, covering large healthcare regions, together covering the whole country.

The national coverage and large sample size were the main strengths of this study. Due to the hormone responsiveness of even the ER-negative BC (183), it would not be ethical to randomize women to letrozole vs non-letrozole group; therefore, prospective cohort design was chosen for the study.

The risk of selection bias in this study was low because FP in Sweden is provided within tax-funded healthcare system, with full-population coverage and equal access. On the other hand, when it comes to FP, there is always a risk of "healthy FP" selection bias – that is, that women who proceed to FP are healthier or feel healthier, and therefore are more likely to proceed to FP after counseling. In contrast, women who are more affected by the disease choose - or get advised by their medical providers - to skip additional medical procedures not related to the treatment of their disease.

As a rule, all potential confounders included in the regression modeling were selected a priori based on their known association with both the exposure (type of COS protocol; or FP) and the outcome (number of oocytes and embryos cryopreserved; live birth or overall survival).

There were certain problems with missing data in the study. Important possible confounding variables for the main analysis of the efficacy of different approaches to COS had substantial proportions of missing values; BMI (21%), AFC (58%), AMH (56%).

Building a regression model to control for possible confounding in our analysis of reproductive outcome in Table 2 (in relation to the type of COS-protocol), we only adjusted

for age as a clear confounder. Including BMI, AFC and AMH would have resulted in a loss of a huge proportion of study subjects. We reasoned that there were no differences in BMI between the groups at baseline, regarding available data, and assumed that the observations were missing completely at random.

Type of BC is a variable that could have affected exposure (type of COS protocol, at least when it comes to letrozole vs non-letrozole group), but not the outcome (number of oocytes and embryos cryopreserved), and therefore, cannot be considered to be a confounder for this analysis. Concerning the overall survival, there is nevertheless a possibility that ER-status is associated with overall survival and that ER-status potentially confound the (non-significant) association between letrozole usage and overall survival.

To rule this out, we have estimated a series of Cox regression models adjusted for letrozole (Yes/No) alone and subsequently adjusted for ER-positivity (Yes /No). The results from the models are shown below:

*Table 5. Cox regression models adjusted for letrozole (Yes/No) alone and subsequently adjusted for ER-positivity (Yes/No).*

| | **Number of women** | **Model 1 HR (95% CI) unadjusted** | **Model 2 HR (95% CI) complete case unadjusted** | **Model 3 HR (95% CI) adjusted** |
|---|---|---|---|---|
| **letrozole** | | | | |
| No | 156 | 1.00 (ref) | 1.0 (ref) | 1.00 (ref) |
| Yes | 224 | 0.64 (0.23-1.78) | 0.67 (0.20-2.26) | 0.96 (0.27-3.34) |
| p-value | | 0.40 | 0.52 | 0.95 |
| **ER Status** | | | | |
| Neg | 83 | - | - | 1.00 (ref) |
| Pos | 197 | - | - | 0.26 (0.08-0.79) |
| missing | 100 | | | |
| p-value | | | | 0.018 |

Because the data on ER-status were not available for 100 women (out of the 380 included in the overall survival analysis presented in the manuscript), a complete-case analysis for women with non-missing information on ER-status was first conducted (model 2) for comparison with the adjusted results (model 3). There was no significant difference in overall survival between women in the letrozole-group (HR 0.96, 95%CI: 0.27-3.34), compared to women who underwent COS without letrozole (reference).

"Confounding by indication" is a term used when a variable is associated with the exposure status and may also affect the outcome. AMH and AFC differed significantly between the group with GnRHa- vs hCG-trigger, and could indeed be considered to be confounders by indication (GnRHa is often preferred when there is a higher risk of OHSS, e.g. in patients with high AFC or AMH). In clinical setting, the use of GnRHa or hCG had mainly depended on the preference of the physician that planned the day of oocyte retrieval. Some of the physicians are today familiar with the GnRHa-trigger, but some are not yet at that point, and they still prefer hCG. To address this issue, we have performed an additional analysis adjusted for AMH and AFC for the women for whom these data were available

(complete case analysis, as the data were missing at random). The difference in the mean number of cryopreserved oocytes was still significant.

Data on the stage of BC and details of oncological treatment were not available, and adjustment for these potential confounders in the survival analysis could not be performed.

Data on pregnancy and delivery rates could be incomplete as some women might have moved to other health care regions or searched fertility or pregnancy care abroad. That plausibility could have constituted information bias. As the possibility of missing data among women unexposed to FP is higher, that would be a differential misclassification to consider when interpreting the results, and when designing future studies in that population.

Precision of the study can be regarded as high due to the large sample size compared to other similar studies. Our hypothesis was that the new approaches to COS in the setting of FP indicated by BC are at least non-inferior in their efficacy when compared to standard approaches. The only significant difference we found was a higher number of cryopreserved embryos in the group with GnRHa trigger as compared to conventional hCG trigger. This result was still robust in a sensitivity analysis adjusted for AFC and AMH. Besides that, there were no significant differences in outcome between the groups. But as the p-values in some cases were low (cryopreserved embryos in letrozole vs no-letrozole group 4.0 vs 5.3, p-value =.075; cryopreserved oocytes in conventional vs random start 10.6 vs 9.0, p-value =.067), the results should be still interpreted with caution, as the probability that observed difference was due to sampling variability was only about 7% - not statistically significant, but still worth consideration.

As this was a multicenter study with nationwide coverage, its external validity may be regarded as high, at least when it comes to the analysis of efficacy of modified approaches to COS. For the analysis of survival and long-term reproductive outcome, a larger and more relevant comparison group would be desirable, with complete data on potential confounders, in order to increase both internal and external validity.

### 6.3.3 Clinical and scientific context

Concurrent use of letrozole is aimed to maintain low systemic estrogen levels during COS, and the results of the previous studies examining its impact on oocyte yield were inconsistent, with both increased (103), decreased (96, 97) and unaffected (87, 95) oocyte yield reported. This inconsistency could depend on relatively small sample sizes and differences in COS protocols. Results of our study were consistent with the conclusions from the systematic review by Rodgers et al, where addition of letrozole was not associated with any decrease in total oocyte yield, with acknowledgement of the limitations of the existing literature on COS for FP in women with BC (79).

The finding of increased number of cryopreserved embryos in letrozole group with GnRHa trigger (as opposed to hCG-trigger) is consistent with the results of previous studies, that indicated higher yield of mature oocytes (82, 83, 159) and embryos(184) in addition to significant reduction of OHSS risk, when utilizing GnRHa-trigger.

Opportunity to initiate COS irrespective of the phase of menstrual cycle is of particular importance in the setting of FP for oncological indications, as the time window for FP, prior to the start of antineoplastic treatments, may sometimes be very small. A systematic review from 2017 concluded, that random start in such settings are associated with shorter interval between start of stimulation and oocyte pick-up, and resulted in similar number of mature oocytes and cryopreserved embryos (101). We didn't find any difference in the length of COS comparing the two groups, but the number of cryopreserved embryos was indeed similar, 4.75 vs 4.78, p=.9.

Even though data on long-term reproductive outcome and overall survival after FP in the setting of BC in young women were reassuring in this cohort, and are generally consistent with previous studies, these results should be interpreted with caution due to the previously discussed methodological considerations, and the fact that existing scientific literature in this field is scarce (79, 81). While randomized trials in this context are not feasible, population-based data from nationwide registers, including long-term reproductive and oncologic outcome, could hopefully help to provide more evidence in this complex and important area.

## 6.4 STUDY IV

### 6.4.1 Main findings and interpretation

A higher likelihood of post-BC live births and ART-treatments, and non-inferior all-cause survival, was observed in women who underwent FP at the time of BC diagnosis compared to women who did not have FP. Successful pregnancy after BC was shown to be possible both in women with and without history of FP at BC diagnosis. Future research evaluating long-term reproductive outcomes in young women with BC should ideally adjust for childbearing intent among those who undergo vs do not undergo FP.

### 6.4.2 Methodological considerations

As randomized studies of FP vs no FP in women with cancer facing infertility risk are not feasible, population-based studies investigating long-term real-world outcomes in unselected patient populations can provide valuable information in this context.

This study's key strength was its prospective design and the use of national and population registers with essentially complete coverage. It enabled identifying a relevant and well-defined matched comparison group, and ensured reliable information on reproductive outcome, survival, disease-related variables, and some other important confounding factors.

Matching aimed to balance the groups with respect to some key variables that could influence the outcome, minimizing variability caused by extraneous factors. The matching criteria included age-group at diagnosis (five-year periods), time of diagnosis (three-year

periods) and health care region. Still, comparison of baseline characteristics in the cohort reveals significant difference in age distribution between the groups, that can be explained by the differences between the groups within each five-year age-group since age was strongly related to the likelihood of opting for FP. We were unable to perform a tighter matching on age or to include additional matching variables, such as tumor characteristics, as the number of eligible comparators were too few. Instead, we took care of other confounding variables in our adjusted Cox proportional hazard models. These adjusted models included age at diagnosis (21-29, 30-34, 35-42), calendar period (1994-2007, 2008-2017) and parity at diagnosis (0, 1, ≥2), country of birth (Nordic, non-Nordic), educational level (compulsory school, secondary school, higher education), tumor size (T0,T1, T2,T3), lymph node metastases (0, 1-3, >3), ER-status (positive, negative), and a binary indicator for chemotherapy (neoadjuvant and/or adjuvant vs neither). Including age as a continuous variable did not change the results. In addition, there were no statistically significant interactions between FP and age (in three categories) when tested using the likelihood ratio test. Calendar period was included to indirectly adjust for unmeasured factors that have changed over time, such as COS protocols and BC treatment protocols. Similarly, educational level and country of birth were included not as confounders per se but as markers of other factors that could have influenced the outcomes, such as social inequality and health-seeking behavior.

Lack of data on childbearing intent or wish at the time of BC diagnosis was an important limitation of our study. It would be logical to assume that women who wish to have children after completion of BC treatment are more prone to opt for FP, potentially leading to confounding by indication. Additional adjustment of our results for childbearing intent would probably rend the difference in birth rate between the two groups smaller.

In studies based on population registers, selection bias can be caused by differential loss to follow-up. In our cohort, that was not a case, as 0.7% vs 0.9% of women in FP vs noFP have emigrated from Sweden during the study years. Access to FP, cancer treatment, ART-treatments, pregnancy- and delivery care is ensured to the population in Sweden within the tax-funded healthcare services. This fact further increases comparability between two groups, and minimizes the risk of selection bias. Still, discussing the finding of lower cumulative incidence of death following BC in women that had undergone FP we argue that there could be a “healthy FP effect” - a form of selection bias similar to “healthy mother effect” (185) in studies showing better prognosis in women who become pregnant after BC diagnosis (122, 123). Even though we have adjusted our analysis for disease-related and socioeconomic variables, there still could be some prognostic factors that we could not capture. As always, to generalize based on a certain outcome, the conclusions must integrate representativeness of the sample, completeness of data, statistical methods applied, but also existing knowledge about the phenomenon under study (186). Thus, to hypothesize that FP itself would provide a survival benefit, would not be logical.

For the first half of the study period (1995-2006), there was no information on ART treatments that did not lead to live births. Therefore, it was not possible to study the uptake of post-BC ART-treatments for the whole study period – an information bias, more specifically, a non-differential misclassification of outcome.

For the studied phenomenon, the sample size may be considered large, with precision of estimates reflected in relatively narrow 95% CIs. Thus, the risk that the results were influenced by random error is relatively low.

Generalizability of the results may be considered as high for the countries with health care system standards similar to those in Sweden. Difference in the eligibility criteria for FP and/or ART-treatments would naturally affect the ability to extrapolate our findings. Thus, the opportunity of surrogacy, allowed in certain countries but not in Sweden, could result in a higher rate of post-BC live births, both in women with and without FP history.

### 6.4.3 Clinical and scientific context

While the practice of FP is spreading, data on pregnancy wish and outcome after cancer treatment in women that received FP are yet scarce. What proportion of these women would conceive spontaneously, undergo fresh IVF-cycles, use cryopreserved specimens or donated oocytes and finally achieve live births has not been yet evaluated in large prospective studies with register-based data. In June 2020, a search of PubMed for scientific literature written in all languages including the terms “breast cancer”, “fertility preservation”, and “reproductive outcome” or “live birth”, from January 1, 2000, until March 1, 2020, was performed by the author of this thesis. In a largest reported cohort of 118 women counseled on FP at the time of BC diagnosis, five-year live birth rate of 27% was reported; 29.4% among those who proceeded to FP and 19% among those who declined it (187). No nationwide population-based studies comparing long-term reproductive outcome in women with vs without FP indicated by BC diagnosis were found.

In general, no relevant control groups have been previously used when evaluating long-term reproductive outcome in women with a history of FP indicated by anti-neoplastic treatments. Long-term reproductive outcomes after FP indicated by cancer have been compared to those after elective FP for age-related fertility decline (181), or to those in women that received FP-counseling but did not proceed to FP treatments (187, 188), or reported as descriptive results in a cohort of women that underwent COS for FP with concurrent use of letrozole for cryopreservation of embryos (77).

In one of the first studies of real-life experience in centers for FP, Hulsbosch et al report that about one third of patients in remission within five years following the end of treatment attempted to become pregnant, with a pregnancy rate of 55% mostly after spontaneous conception (189). In 2018, Moravek et al. compared retrospectively long-term outcomes of cancer patients who pursued FP (n=204) and those who did not (n=293), with a live birth rate of 6.4 % vs 5.5% after spontaneous pregnancies. In addition, 10.3% of women with a history of FP returned to use cryopreserved specimens, whereof 51.7% had a live birth (190). Reproductive outcomes in relation to the type of cancer were not presented in these studies. In a review from 2019, ter Welle-Butalid et al. summarized outcomes of currently used FP procedures for oncological indications (63). The reported return rate for embryo transfer was on average 23% (range 13-63%). Of women who returned, on average 40%

(range 9-75%) had at least one live birth. The proportion of patients returning for embryo transfer after prior oocyte preservation varied from 0 to 5%. Those who returned had a live birth rate between 33 and 50%. The reported rates differed greatly between the studies included in the review, reflecting small numbers of patients and different length of follow-up.

Study IV, to our knowledge, presents long-term reproductive outcome in to date the largest cohort of women with diagnosis of BC at reproductive age, with and without FP at time of diagnosis, using prospectively collected data from population-based registers. There is an obvious clinical value in reporting long-term reproductive outcome in this population, as counseling and decision-making in the settings of FP services for women with BC should be based on accurate information regarding chances of post-BC pregnancy and live birth, both with and without help of FP. As mentioned, the finding of the improved all-cause survival in the FP group should be interpreted with caution. It should not be interpreted as a proof of survival benefit of FP in the setting of BC among young women, as any biological explanation to such would not be plausible. Still, previous studies using different research approaches have indicated at least non-inferior disease-free and overall survival in women with BC who undergo FP (79, 190, 191). In this context our findings could be interpreted as additional evidence for the results of these previous studies. Further research, preferably of prospective design and involving data from population-based registers, including not only overall survival but also BC-relapse as a more accurate proxy for safety, is important.

## 6.5 ETHICAL CONSIDERATIONS

Ethical approval has been obtained for all the studies included in the project. There are no economic interests in the studies for responsible persons. The results of all the studies have been peer-reviewed. Still, there are several ethical considerations to reflect upon.

Study I and II are prospective cohort studies using the clinical registry of the Reproductive Medicine Clinic of Karolinska University Hospital. Study III is a multicenter prospective cohort study. Study IV is a nationwide register-based cohort study. Have we chosen correct design to our studies? In the hierarchy of research designs, the results of randomized, controlled trials are considered to be evidence of the highest grade. In none of the studies of this project could we consider this design to be possible because of ethical considerations. Even though the advantages of certain stimulation protocols and safety and efficacy of different FP methods are still to be examined further, the choice of method / type of stimulation today is based on existing state of knowledge about possible risks and benefits, and on patients' preferences. Thus, while choosing the study design, we have tried to balance ethical considerations with attempts to increase existing evidence.

**Potential risks vs benefits for the women included in the studies**

Analysis of unidentified collected data gives no medical risks to the patients included. On the other hand, the patients who have already undergone FP treatments would not receive any medical benefits either. In further perspective, benefits for the entire patient group may

occur from the results of this project (e.g. evidence for efficacy and safety of different FP methods).

**Reflection on the matter of informed consent**

None of the studies clears identity of any woman. Data were analyzed unidentified, and only groups were analyzed and compared. Patient consent was obtained at time of FP, orally until 2008 and thereafter in writing. None of the patients counselled before 2008 has been contacted to obtain written informed consent to participate in the studies, and none of the women in the control group, i.e. women without history of FP at the time of diagnosis, has been contacted to obtain consent.

Would it be better from an ethical perspective to send information about the planned project and request written informed consent? It is difficult to ascertain how high or low the response rate would be in such case. Some patients have passed away because of their disease. Others that have survived their disease may have more important things in their lives than to fill in forms for permission to use their medical data for research that would not benefit them personally.

Sending the information about the project and obtaining written consent would mean greater work effort for the research group and higher costs. In a world where resources are limited, we must prioritize what they are used to. Is it ethical to obtain consent to each study that does not involve any medical risks to included individuals when we know that the necessary work effort and cost could instead be spent on research? Can potential benefit for the entire patient group be related to the fact that some patients may get upset that their medical data were included in the study - or in other words - that researchers have entered their records without explicitly asking for consent to this in relation to the current study? Would they still be sad about it if the results of the research would be of direct benefit to them? Would they prefer to regularly receive a bundle of information sheets and consent forms for different studies in their mailboxes? Should it be ethical to refrain from research if the potential participants would finally refuse to take a stand for each individual study?

Experience from previous studies suggests that women who undergo fertility treatments are generally very positive to the fact that their unidentified patient data are used for projects aimed at further development and improvement of treatments for infertility. Patients with malignant diseases are also motivated to contribute to the research that leads to improved diagnosis, treatment and quality of life.

Considering all these arguments, a refrain from collecting individual written informed consent from each woman included in the project could hardly be assumed as unethical, in the opinion of our research group. The ethics committee was approached to solve this issue. Permission was granted for this project, even for the patient groups that had not given informed consent previously to use their clinical and registry data for research purpose. A main argument in favor of not requesting informed written consent was that it would be difficult to contact women who had not received FP at the time of cancer diagnosis with the information about the current project. We cannot know the reason why some women were

referred for FP counseling while other were not; maybe they were not interested but it is also plausible that they received less information about fertility risks related to their cancer treatments, and were not aware of available FP options. Information about the current project would thus pose a risk of raising feelings of sadness and loss in those women who wish that they had been offered FP options.

**Further ethical reflections**

Utility and generalizability of the research results are two other important ethical issues. Potential usefulness of the results of these studies may be considered high as the practice of FP and fertility treatments in young cancer survivors is spreading rapidly. With some modifications, results of the included studies may be extrapolated to worldwide populations of young cancer survivors planning fertility treatment with donor oocytes (*Study I*), or females planning FP indicated by oncological or non-oncological diseases threatening their fertility (*Study II*), or women of reproductive age diagnosed with BC and concerned about if to go for FP or not, and which method of FP and COS in particular to opt for (*Study III and IV*).

Final ethical reflection is that it could be unethical not to perform studies on safety and efficacy of different methods of fertility preservation/treatment in young cancer survivors when it is known that issues of fertility and reproduction are of high importance to this group while the state of knowledge in this area is limited.

# 7 CONCLUSIONS

⇒ In pregnancies achieved using donated oocytes, women with a history of cancer may have a higher risk of perinatal complications, particularly preterm birth and possibly also pre-eclampsia, compared to the women without a history of cancer undergoing the same fertility treatment.

⇒ In females with FP indicated by the risk of infertility due to treatment of benign or malignant diseases, oncologic indications for FP are associated with lower return rates for attempting pregnancy and lower live birth rates.

⇒ Cryopreservation of oocytes has become increasingly popular among women and girls consulted for FP during recent years, over available FP options. This trend reflects accumulated evidence on the efficacy of this method, and also its' role in protecting female reproductive autonomy.

⇒ Recently introduced approaches to COS for FP in women with cancer are aimed to increase the safety and include concurrent use of letrozole, random start of stimulation, and use of GnRHa-trigger. Efficacy of these approaches, measured as the number of cryopreserved oocytes and embryos, was observed to be at least as good as for standard GnRH antagonist protocols.

⇒ FP at the time of BC diagnosis is associated with a higher likelihood of post-BC live births and ART-treatments and non-inferior all-cause survival compared to no FP in women diagnosed with BC at reproductive age.

⇒ In summary, the use of ART for FP and fertility treatments in eligible young women with cancer should be encouraged, as the results, in general, seem to be reassuring.

# 8 FUTURE PERSPECTIVES

In my attempt to explain to my 16-year old son what this thesis was about, he asked: "Why don't you just develop an artificial ovary so that you can drip some DNA on it – and voila, out comes an egg?" Today, this approach to FP would be more appropriate for science fiction, not for a PhD thesis. But, citing Saint Augustine: "Miracles are not contrary to nature but only contrary to what we know about nature." Certain approaches to FP may indeed sound highly experimental today, but who knows which of them would become clinically established in the years to come? Here are some to keep an eye on:

- Activation, growth, and maturation of follicles in vitro. Ongoing research aiming to optimize each culture step required for different transitional stages of the follicles may result in the development of a new effective option for FP in young female cancer patients, and for treatment of POI (192).

- Fresh ovarian tissue transplantation. Immunosuppression, with its inherent side effects, would make the procedure ethically and medically questionable. Still, when performed in monozygotic twins discordant for ovarian failure (193, 194), or from a person who has previously donated bone marrow to the patient (195, 196), it may become a good option.

- Artificial ovary. A transplantable artificial ovary's development requires finding a suitable matrix to isolate, encapsulate, and graft preantral follicles inside. This step would eliminate the risk of reseeding malignant cells at re-transplantation (58, 197). Recently, the growth of antral follicles could be observed in a fibrin scaffold with autografted human primordial follicles placed in a mouse model – a promising finding (198).

- Ovarian stem cells. In recent years, both germline stem cells and somatic ovarian stem cells have been reported, questioning the theory that female mammals stop producing germ cells after birth (199). Though there are numerous obstacles on the way of developing stem cell-based strategies for FP and restoration of endocrine function, ongoing research may bring them step-by-step closer to fruition.

While translational research in the area of FP has the potential to offer some mind-blowing solutions in the years to come, epidemiological research, and observational studies, in particular, will continue to be important in this field of research. They allow investigation of long-term outcomes, ensuring the safety and efficacy of new approaches in the settings where clinical trials would not be possible due to practical and ethical reasons.

Sweden has, in many ways, a unique system of healthcare, available to the whole population, and healthcare registers and population-based registers. It is possible to link the data from several registers and build up large cohorts with extensive data on potential confounders using the personal identity number assigned to all Swedish residents. Further, Nordic countries have similar health care systems as well as similar health and population

registers. The use of pooled data from Nordic countries opens even better opportunities to overcome the lower power problem that many smaller cohorts may suffer from.

As FP's safety is of utmost importance, the risk of BC relapse after FP, and COS, in particular, needs to be investigated further. We plan to examine disease-specific mortality and BC-relapse rate in the cohort used for Study IV. An important reservation is that the data on relapse are only available for the part of our cohort, as relapse as a variable in the National Breast Cancer Register has until recently been reported in only one region in Sweden; Stockholm-Gotland. For future studies, the model proposed by Rosenberg et al. may be used, with a coding template for relapse in BC based on typical patterns of ICD diagnoses in the National Patient Register (200).

The observed association between cancer history and increased risk of PE and PTB in women treated with OD, reported in Study I, should be further investigated in larger cohorts. Here, register-based data, ideally not only from Sweden but from all Nordic countries, would provide perfect opportunities for a study of sufficient power. A possible association between specific types of cancer/cancer treatments and increased risk of adverse perinatal outcome should also be investigated in this setting.

ESHRE has recently taken an important initiative to increase the quality of FP care. The ESHRE Female Fertility Preservation Guideline – the most updated and complete document in this field to date – has been published September 2020 (201). Through the ESHRE IVF monitoring scheme (EIM), data on FP's short and long-term outcomes are collected in an optional module. The registered parameters would include the number of interventions, the reason for FP (being medical- or non-medical), and the outcomes (number stored and number used). In the long run, these data would provide an excellent source for research.

Another essential trace to follow in research related to FP is how young women with cancer diagnosis experience fertility-related communication and FP procedures. Here, quantitative and qualitative research methods are applied to identify existing gaps and potential for improvement in providing FP-services. Short and long-term psychological effects of undergoing vs not undergoing FP represent important soft outcomes to investigate in future studies.

# 9 POPULÄRVETENSKAPLIG SAMMANFATTNING

Hos kvinnor och flickor kan cancerbehandling med cellgifter, strålning eller kirurgi skada äggstockar. Det kan leda till förlorad eller nedsatt fertilitet, dvs förmågan att bli gravid. Forskning har visat att möjligheten att skaffa barn är mycket viktig för många unga kvinnor som har behandlats för cancer.

Det finns olika vägar att gå för att öka chansen till graviditet efter avslutad cancerbehandling. Fertilitetsbevarande åtgärder för kvinnor idag inkluderar möjligheten att frysa ner obefruktade ägg, befruktade ägg (embryon) eller små bitar av äggstocksvävnad. För att få ett bra antal ägg behövs en stimulering av äggstockar med hormoner. Det tar i regel cirka 2 veckor. Därefter, om äggstockarna svarat på stimuleringen, kan äggen plockas ut, ev. befruktas och frysas ner. Bitar av äggstocksvävnad kan utförskaffas i samband med titthålsoperation och frysas ner. Vid framtida graviditetsönskan kan de sedan re-transplanteras (återinföras) i kroppen. För kvinnor som har förlorat funktionen i sina äggstockar, och inte har några ägg eller äggstocksvävnad i frysen, kan behandling med donerade ägg erbjudas i syfte att åstadkomma graviditet.

Det övergripande syftet med denna avhandling var att undersöka effektivitet och säkerhet av de ovanbeskrivna metoderna.

I **Studie 1** har vi undersökt utfall av 25 graviditeter bland 20 kvinnor med tidigare cancerdiagnos och 244 graviditeter bland 212 kvinnor utan cancerdiagnos, samtliga efter behandlingar med donerade ägg. Ökad risk för graviditetskomplikationer i form av för tidig födsel och pre-eklampsi (havandeskapsförgiftning) observerades bland kvinnor som tidigare behandlats för cancer.

**Studie II** inkluderade 1254 flickor och kvinnor som har fått rådgivning om fertilitetsbevarande åtgärder på Karolinska Universitetssjukhuset mellan 1998–2018. Samtliga hade antigen onkologiska (cancer) eller icke-onkologiska (godartade) sjukdomar vars behandling skulle innebära risk för äggstocksskada. Bland de olika fertilitetbevarande metoderna har nedfrysning av obefruktade ägg blivit den mest populära på senare år, både bland flickor som kommit in i puberteten och bland vuxna kvinnor. Kvinnor som hade gjort fertilitetsbevarande åtgärd p.g.a. cancer återvände till reproduktionskliniker i lägre utsträckning, och hade lägre förekomst av graviditeter och födslar, jämfört med kvinnor med icke-onkologiska indikationer.

**Studie III** baserades på uppgifter från reproduktionskliniker vid sex universitetssjukhus i Sverige. Sammanlagt 610 kvinnor har fått fertilitetsrelaterad rådgivning i samband med sin bröstcancerdiagnos mellan januari 1995 och juni 2017; 468 av dem valde att gå vidare med fertilitetsbevarande åtgärd, och 380 stimuleringscykler kunde inkluderas i vår analys. Effektiviteten av alternativa protokoll, utvecklade för att öka säkerheten av hormonella stimuleringar av äggstockar vid cancersjukdom, visade sig vara minst lika bra som effektiviteten av standardprotokoll, mätt i antal obefruktade och befruktade ägg som kunde

frysas ner. Den totala överlevnaden skilde sig inte mellan kvinnor som har och inte har fått fertilitetsbevarande åtgärder.

**Studie IV** använde information från svenska befolknings- och hälsodataregister. Barnafödslar och behandlingar med assisterad befruktning efter avslutad behandling av bröstcancer var betydligt vanligare i gruppen som hade genomgått fertilitetsbevarande åtgärd i samband med sin bröstcancerdiagnos (425 kvinnor), jämfört med en matchad kontrollgrupp utan fertilitetsbevarande åtgärd (850 kvinnor). Efter 10 år av uppföljning, har 41 % av kvinnor i den första gruppen och 16% i den andra fått minst ett barn. Det återstår att undersöka i fall dessa skillnader kan till viss del bero på att önskan, och inte bara förmågan, att skaffa barn skilde sig mellan grupperna. Något negativt samband mellan fertilitetsbevarande åtgärder och chansen att överleva har inte observerats.

Användande av IVF-tekniker för fertilitetsbevarande åtgärder och fertilitetsbehandlingar av unga kvinnor med cancer, under de förutsättningar som gäller i Sverige idag, förefaller vara både säkert och effektivt, enligt våra resultat. Fortsatta studier får undersöka vidare risken för återkomst av bröstcancer bland kvinnor med fertilitetsbevarande åtgärder. Koppling mellan fertilitetsbevarande åtgärd och prognos för andra typer av cancer behöver också studeras.

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