Department of Medical Epidemiology and Biostatistics,
Karolinska Institutet, Stockholm, Sweden

Endogenous and Exogenous Hormonal Factors in Female Cancers – Studies of Risk and Prognosis

Chantal Claudine Orgéas

Karolinska Institutet

Stockholm 2009

Cover figure: Female cancers awareness ribbons within the female symbol: Breast cancer ribbon (pink), ovarian cancer ribbon (teal), and uterine/endometrial cancer ribbon (peach)

Published and printed by Universitetsservice US-AB
Nanna Svartz väg 4, 171 77 Solna, Sweden

ISBN 978-91-7409-328-5

This work is dedicated to my beautiful daughter Célia
and wonderful husband Jérôme

“I can calculate the motions of the heavenly bodies,
but not the madness of people.”

- Sir Isaac Newton

ABSTRACT

Extensive evidence points to hormonal influences which play a critical role in the development and progression of breast, ovarian and endometrial or uterine cancers. These cancers share many common hormonal features, which are crucial in the etiology and subsequent development of these cancers. Exposure to estrogens and progestogens, both endogenous and exogenous during a woman's life can influence the risk of cancer in these target organs. As certain hormonal and reproductive factors affect the risk of developing these cancers, similarly, it is plausible that they would also influence tumor-defined characteristics and survival in breast, ovarian and endometrial cancers.

My thesis work aims to further investigate the roles of endogenous and exogenous sex hormones on the etiology, risk and prognosis – defined by tumor characteristics and survival; in breast, ovarian and endometrial cancers. These aims were investigated through the following four studies.

Study I aimed to assess the impact of infertility treatment of clomiphene citrate (CC) and/or gonadotropins with causes of infertility on the incidence of breast cancer. We observed no overall increased risk for breast cancer with infertility treatment; however, women with non-ovulatory causes treated with high dose CC therapy may have an elevated risk for breast cancer.

Study II investigated 5-year survival in patients with ovarian cancer according to hormone replacement therapy (HRT) use before and after diagnosis. We found that HRT-use prior to diagnosis of epithelial ovarian cancer did not affect 5-year survival, except for a possible survival advantage in serous cancers. Women using HRT after diagnosis had a better survival than never users.

Study III looked at the effects of established menstrual risk factors on tumor characteristics and survival in postmenopausal breast cancer. We found an earlier age at menarche to have a significant impact on breast cancer prognosis and survival.

Finally, Study IV looked at the influence of menopausal hormone therapy (MHT) on tumor characteristics and relative survival in postmenopausal endometrial cancer. The findings indicated that users of MHT had a better survival than non-users.

The findings of these studies add new evidence in understanding the etiological mechanisms by which carcinogenesis may act to affect these cancers. What is clear from these findings is that these mechanisms are multifarious and complex and that no simple association exists between hormonal exposures and female cancers, since the influence appears contradictory for the incidence of the cancer and prognosis of the cancer. These patterns indicate the mosaic of mechanisms involved.

CONTENTS

ABBREVIATIONS

BMI	Body Mass Index
BOT	Borderline Ovarian Tumors
CC	Clomiphene-Citrate
CI	Confidence Interval
COCs	Combined Oral Contraceptives
dl	deciliters
EOC	Epithelial Ovarian Cancer
ER	Estrogen Receptor
FIGO	International Federation of Gynecology and Obstetrics
FSH	Follicle Stimulating Hormone
GnRH	Gonadoptropin Releasing Hormone
hCG	Human Chorionic Gonadotropin
hMG	Human Menopausal Gonadoptropin
HR	Hazard Ratio
HRT	Hormone Replacement Therapy
ICD	International Classification of Diseases
IU	International Units
IVF	In-Vitro Fertilization
LH	Luteinizing Hormone
mg	milligrams
MHT	Menopausal Hormone Therapy
ml	milliliters
mm	millimeters
n	number
ng	nanograms
OR	Odds Ratio
PCOS	Polycystic Ovary Syndrome
pg	petagrams
PR	Progesterone Receptor
RER	Relative Excess hazard Ratio
SHBG	Sex Hormone Binding Globulin
SIR	Standardized Incidence Ratio
TSH	Thyroid Stimulating Hormone
µg	micrograms

LIST OF PUBLICATIONS

This thesis is based on the following papers:

I. Orgéas CC, Sanner K, Hall P, Conner P, Holte J, Nilsson SJ, Sundfeldt K, Persson I, Chia KS, Wedrén S, Dickman PW, Czene K.
Breast cancer incidence after hormonal infertility treatment in Sweden: a cohort study.
American Journal of Obstetrics and Gynecology 2009; 200:72.e1-72.e7.

II. Mascarenhas C, Lambe M, Bellocco R, Bergfeldt K, Riman T, Persson I, Weiderpass E.
Use of hormone replacement therapy before and after ovarian cancer diagnosis and ovarian cancer survival.
International Journal of Cancer 2006; 119:2907-2915.

III. Orgéas CC, Hall P, Rosenberg LU, Czene K.
The influence of menstrual risk factors on tumor characteristics and survival in postmenopausal breast cancer.
Breast Cancer Research 2008; 10:R107

IV. Orgéas CC, Hall P, Wedrén S, Dickman PW, Czene K.
The influence of menopausal hormone therapy on tumor characteristics and survival in endometrial cancer patients.
Submitted.

INTRODUCTION

Modern women's lives are constantly in a dynamic state. The evolution of women's changing roles throughout time and even throughout daily living in today's society has brought with it pressure and stressors on the one hand, as women struggle to maintain traditional female roles, as well as choice and flexibility on the other hand, with contemporary roles of the modern day working career woman. In today's western world, women can be and do anything. At times, this is through our own choices – our career paths, relationships and the choice and timing of parenting. At other times, life situations dictate this to us – one's own chronic or acute illness, changes in family structure, or other significant events that shape and shade the rest of our lives.

Today's western society simply expects that women should handle these choices and roles with ease, composure and without any complaint. Partly attributable to the changing roles, lifestyles and choices of women, is firstly, the contemporary woman's lack of tolerance for any negative side effects of hormonal imbalances that may present throughout the lifetime. Secondly, with modern women being more educated and choosing first to establish careers and delay childbearing, women demand the flexibility to choose at which stage in their life they become mothers, rather than this role being dictated by their biology. Therefore, women are seeking both, immediate and long-term solutions. In consequence, today's western women increasingly rely on the use of exogenous hormones as solutions to cope with their own demands of their changing roles, lifestyles and choices in modern day society.

Extensive experimental, clinical and epidemiological evidence points to hormonal influences which play a critical role in the development and progression of breast, ovarian and endometrial or uterine cancers. These cancers share many common features; in particular estrogens and progestogens are crucial in the etiology and subsequent development of these cancers. The exposure to estrogens, both endogenous and exogenous during different phases of a woman's life can influence the risk of cancer in these target organs. Although the exact mechanisms for hormonal effects at the cellular and molecular levels remain unclear, there are certain hormonal and reproductive risk factors associated with each of these malignancies. Hormonal factors shown to increase breast cancer risk are late age at menopause, early age at menarche, hormonal replacement therapy, recent oral contraceptive use, parity, age at first full-term pregnancy, postmenopausal obesity, and adult weight gain [1]. Risk factors for endometrial cancer are an early age at menarche, ovarian dysfunction, infertility, nulliparity, late age at menopause, and postmenopausal estrogen therapy without added progestins [1]. Hormonal risk factors for ovarian cancer include nulliparity, infertility and use of hormone replacement therapy (HRT) or menopausal hormone therapy (MHT) [2]. Regarding prognosis and survival, it remains inconclusive as to the influence of endogenous and exogenous hormonal and reproductive factors in these female cancers. As hormonal and reproductive factors affect the risk of developing these cancers, similarly, it is plausible that they would also influence tumor-defined characteristics and survival in breast, ovarian and endometrial cancers. However, the effects on risk and prognosis in these cancers could be very different in terms of carcinogenesis.

My thesis work aims to further investigate the roles of endogenous hormones in addition to the increasing use of exogenous sex hormones present in modern day society, on the etiology, risk and prognosis in breast, ovarian and endometrial cancers. Overall, this work aims to deepen our understanding of possible hormonally derived carcinogenic mechanisms, and the effects of these hormones on tumor characteristics, progression and survival in these female cancers.

BACKGROUND

The epidemiological evidence on breast, ovarian and endometrial cancer is extensive and will only be reviewed as appropriate to the research questions of the studies comprising this thesis.

Descriptive Epidemiology

Breast cancer

Breast cancer is the most common female cancer worldwide [3]. Ten percent of all incident cancers diagnosed worldwide are attributable to breast cancer in both western and industrialized countries [3]. Globally, breast cancer incidence rates are highest in Northern and Western Europe, Australia, New Zealand, Northern America, Argentina and Uruguay. The lowest incidence rates are evident throughout Asia, Africa and Central and South America [4]. However, recent patterns in breast cancer incidence have been increasing rapidly in previously low incident geographical areas, as a consequence of these regions steadily adopting more 'westernized' lifestyles and childbearing patterns [5].

In 2006, there were 430,000 incident cases of breast cancer in Europe; 29% of all cancer cases in the same year. Breast cancer was the third most common cause of death in Europe in 2006 with 131,900 deaths or 18% of total cancer deaths in Europe [6].

Ten percent of all Swedish women will develop breast cancer sometime during her lifetime [7]. In Sweden, the age-standardized incidence rate (European standard) was 125.8 per 100,000 women in 2006 [6]. The proportion of all cancers attributable to breast cancer in Sweden is 28.7% [8].

Estimated age-standardized mortality rates (European standard) for breast cancer in Sweden in 2006 was 21.1 per 100,000 [6]. Approximately 14.5% of all cancer deaths in Sweden are attributable to breast cancer [8]. Since the start of the Swedish Cancer Register in 1958, the incidence of breast cancer has been on a steady incline, in contrast to the slight decline in mortality since 1975 [9, 10]. This decline in mortality is attributable to improvements and advances in treatment, as well as heightened efforts in breast cancer screening programs [7]. In Sweden, the 15-year relative survival is estimated at around 85%, and approximately 78,000 women survive a diagnosis of breast cancer [10].

Ovarian cancer

Each year more than 190,000 new cases of ovarian cancer are diagnosed worldwide, accounting for approximately 4% of all cancers diagnosed in women [11]. There is considerable variation in incidence rates of ovarian cancer, with the highest rates in Northern Europe and America and the lowest rates in Asia and Africa [11]. In Europe, approximately 43,000 incident cases occur each year. Within Europe, the lowest rates of ovarian cancer are evident in the Southern European countries of Greece, Portugal and Cyprus, whereas the highest rates are observed in the Northern and Eastern European countries of Denmark, Czech Republic, Estonia and Lithuania [11].

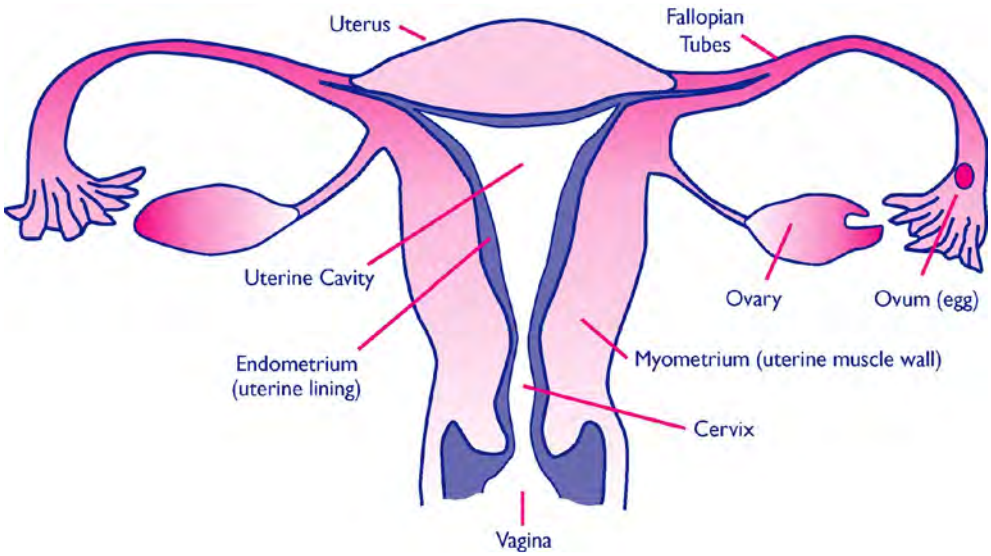

Figure 1: The female reproductive tract (source: [12])

In Sweden, ovarian cancer is the fifth most common cancer in women and the leading cause of death among all gynecological cancers [13]. Approximately 3.7% of all cancers in Sweden are attributable to ovarian cancer [8]. In high incidence areas, a woman's lifetime risk of ovarian cancer is 1-2% [14]. Despite therapeutic advances with radical surgery and paclitaxel based chemotherapy, ovarian cancer prognosis remains poor, with 5-year survival rates at approximately 40% [15]. Approximately 6.1% of all cancer deaths in Sweden are attributable to ovarian cancer [8]. To date, there exists no effective screening procedures available and almost all cases of ovarian cancer are diagnosed at clinically advanced stages [16, 17].

Endometrial cancer

Cancer of the corpus uteri, or uterine endometrial carcinoma is the seventh most common cancer in women worldwide [18]. Endometrial cancer's greater importance lies in the number of incident cases, rather than mortality (3.9% of incident endometrial cases, compared with 1.7% of endometrial cancer deaths) [18]. Incidence rates are highest in North America and Western Europe by 10%, compared with rural Africa or Asia. In areas of high incidence, endometrial cancer is the most common female gynecological malignancy. In migration studies from low-risk to high-risk areas, there is strong evidence supporting the role of environmental factors as opposed to genetic risk factors for endometrial cancer [18].

Endometrial cancer is most common in postmenopausal women, with more than 90% of cases diagnosed in women over 50 years of age and the highest incidence in women over 65 years. Survival of endometrial cancer is excellent, with approximately 86% according to SEER registries and 78% according to European registries [18].

In Sweden, 1.8% of women will develop endometrial cancer by the age of 75 years [8]. Approximately 6.1% and 1.5% of all Swedish cancers are attributable to incident endometrial cancers and endometrial cancer deaths respectively [8]. Age-standardized incidence rates in Sweden have been steadily increasing over the last 50 years and were 26 per 100,000 women in 2004 [7]. Contrastingly, mortality has been

at a steady decline over the past 40 years, with the current 5-year survival rate at 82% [19].

Female cancer comparisons in Sweden

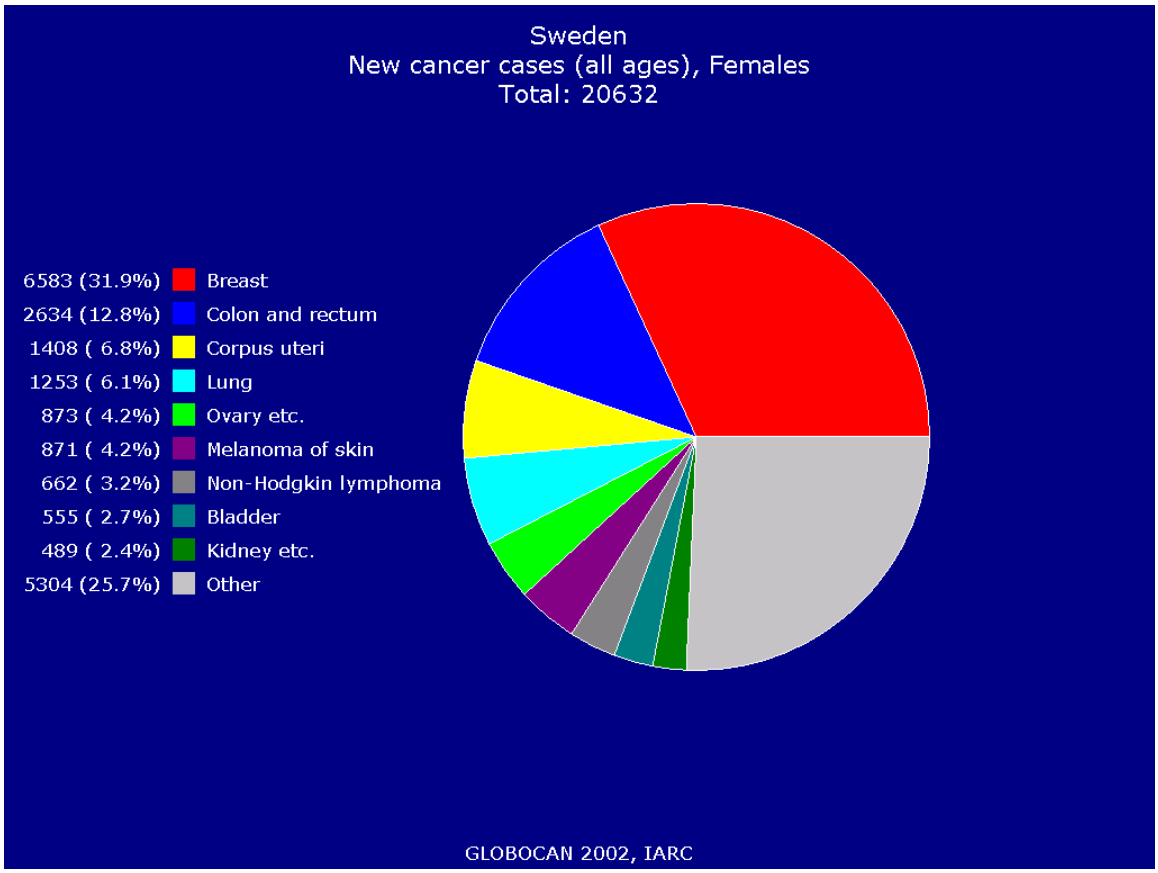

Figure 2: Incident cases for all female cancers in Sweden for all ages (source: [8])

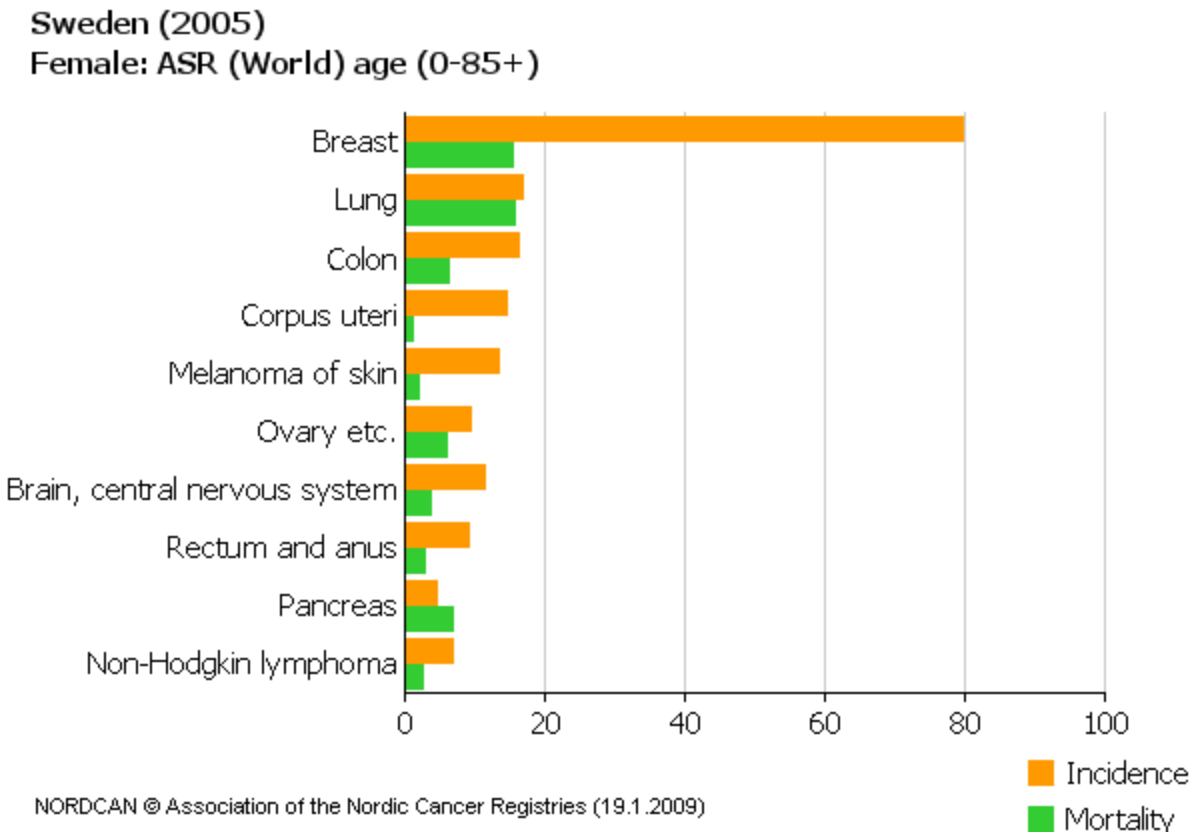

Figure 3: World age standardized incidence and mortality rates per 100,000 for all female cancers in Sweden (source: [8])

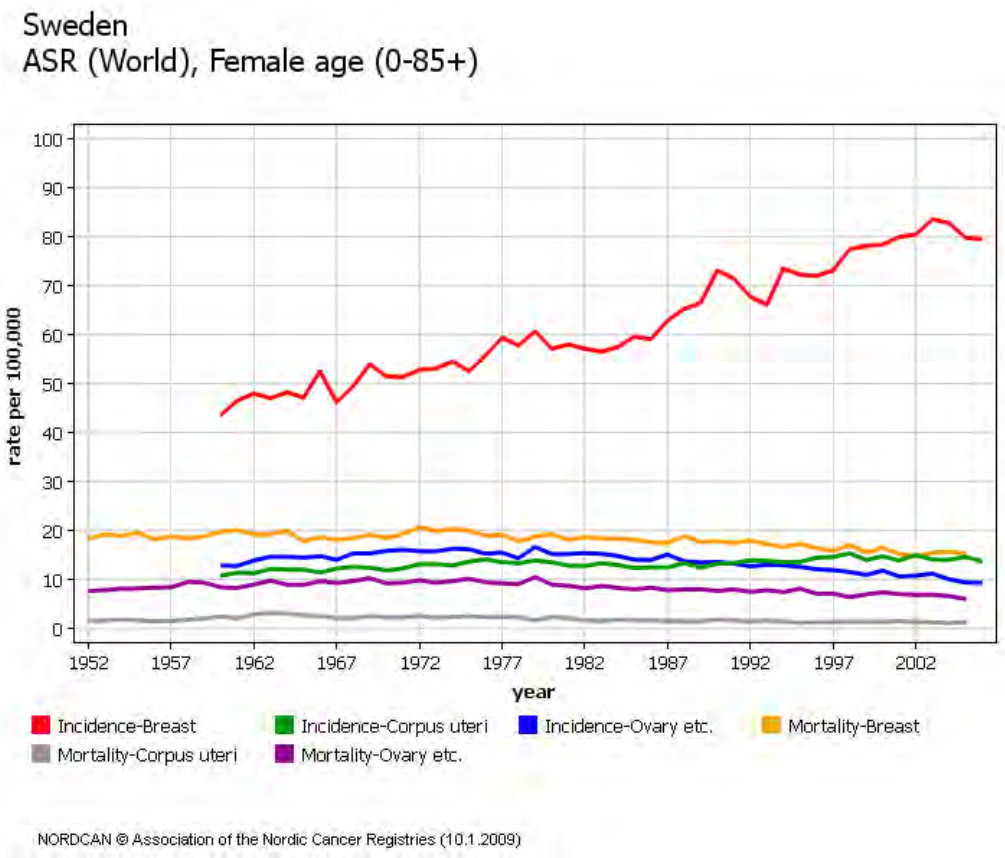

Figure 4: World age standardized incidence and mortality rates per 100,000 for breast, ovarian and endometrial cancers in Sweden (source: [8])

Endogenous Hormonal Risk Factors in Female Cancers

All reproductive and endogenous hormonal factors affect the risks of breast, ovarian and endometrial cancers differently. Where one particular factor may reduce the risk of one female cancer, the same factor may increase the risk in another female cancer. These endogenous factors will now be summarized for each cancer.

Breast cancer

Extensive lines of evidence exist supporting an integral role for ovarian sex hormones, i.e. estrogen and progesterone, in the etiology of breast cancer. Supporting the role of hormones in breast carcinogenesis is the sharp increase in breast cancer incidence in women up to the age of around 50 years – the average age of menopause, after which the production of ovarian sex hormones stops and the increase in breast cancer incidence rates slows [20]. Any increased risk for breast cancer throughout the lifetime is generally accepted to be due to increased lifetime exposure to high levels of the endogenous sex hormones of estrogen and progesterone, particularly estradiol which increases breast cell proliferation and inhibits apoptosis of the breast epithelium [21].

During the past ten years, numerous epidemiological studies have examined the association between serum hormone concentrations and the risk of breast cancer. Among postmenopausal women, results have shown a positive association between serum estradiol concentrations and the risk of breast cancer; with postmenopausal women with high concentrations of serum estradiol having an increased risk of breast cancer of two-fold, compared to women with lower concentrations of serum estradiol [22].

Reproductive risk factors

The menstrual cycle and age at menarche

An older age at menarche is consistent with a decreased risk of breast cancer [23]. For every one year increase in the commencement of menarche, the risk of breast cancer decreases by approximately 5% [24]. Other menstrual factors such as cycle length and irregular menstruation have not been consistently associated with the risk of breast cancer [25-31].

Parity

Parity has a dual and opposing effect on the risk of breast cancer. Parity increases the risk of breast cancer immediately after a birth, however, this excess risk slowly declines and the long-term effect of parity becomes protective against breast cancer [32]. The increased risk following a birth is thought to be due to the effect of sex hormones promoting preclinical breast cancers [33], whereas the long-term protective effect could be attributable to the final differentiation of the breast during a full-term pregnancy [34]. Nulliparous women have been shown to have a 25% increased risk of the disease compared to women with at least one full-term pregnancy [35, 36]. The protection afforded by parity increases linearly with increasing parity [35, 36]. The age of a first full term birth affects the risk of breast cancer independent of parity and is greater with a younger age at first birth [23]. A meta-analysis of studies from the Nordic countries showed that an age at first birth younger than 20 years, conferred a 30% reduced risk of breast cancer compared with women with an age at

first birth older than 35 years [35]. Results from studies investigating incomplete pregnancies have shown to have no increased or decreased risk of developing the disease [23].

Breastfeeding

The effect of breastfeeding on the risk of breast cancer has been questionable, largely due to the small change in risk associated with the average breastfeeding duration [20]. A review of studies in developing countries where breastfeeding duration can be very long has concluded substantial protective effects [36, 37]. However, there exists conflicting results in developed countries [36].

Age at menopause

Women with an older age at menopause have an increased risk of breast cancer compared to those women who cease menstruating earlier [23, 38]. The risk increases by approximately 3% for each subsequent delayed year of menopause [39]. The reduced risk for women with a younger age at menopause is evident regardless of whether menopause occurs naturally or is surgically induced through bilateral oophorectomy [20]. Recently, surgically induced menopause before the age of 35 years has been shown to halve the risk of breast cancer [40].

Ovarian cancer

The main types of neoplastic ovarian tumors are derived from epithelial, germ, sex cord and stromal cellular components [41]. Epithelial ovarian tumors mainly comprise 80 to 90% of ovarian neoplasias and of these; approximately 15% are borderline ovarian tumors (BOT), whereas the rest are invasive epithelial ovarian cancers (EOC) [41]. The epithelial tumors are further subdivided into serous, mucinous, endometrioid, clear-cell and undifferentiated histologies. It remains unclear if BOT are precursors of EOC or a completely separate disease entity [42], and whether the differing histological types of EOC and BOT share common risk factors [43-45].

Ovarian cancer etiology is multifactorial with environmental, endocrinological and genetic factors directly or indirectly related to ovarian carcinogenesis. The exact causes of ovarian cancer remain ambiguous, however, based on epidemiological and pathophysiological evidence, several non-mutually exclusive theories regarding the etiology of this cancer have been proposed. Fathalla [46] proposed the theory of incessant ovulation, in which repetitive disruption and repair of the ovarian surface epithelium leads to a higher probability of spontaneous mutations and therefore, increases the risk for ovarian cancer. This hypothesis is supported by the consistently observed protection afforded by pregnancy, use of oral contraceptives, and breastfeeding; all of which inhibit ovulation. Further support for this concept was extended by Casagrande and colleagues [47] in which the risk of ovarian cancer was calculated according to ovulatory age. Results concluded that the total time spent in ovulation between menarche and menopause was more closely related to risk than either parity or oral contraceptive use alone. Stadel [48] proposed the gonadotropin hypothesis which states that high levels of circulating gonadotropins increases the risk of ovarian cancer, particularly present in the early postmenopausal years, and coincides with the increase in age-specific incidence rates of ovarian cancer. The retrograde transportation hypothesis states that any carcinogenic factors (endogenous or exogenous) that gain access to the ovaries through an intact reproductive tract increase the risk of ovarian cancer [49]. This is supported by several epidemiological

studies which have found that tubal ligation and hysterectomy have been associated with a 67% reduction in risk of ovarian cancer, which appears to last for at least 20 to 25 years after surgery [50, 51]. Other hypotheses propose that apoptosis acts to reduce risk, either through a pregnancy by the clearance of premalignant lesions [52], or through the high levels of progesterone in pregnancy [53].

Reproductive risk factors

Menstrual factors – age at menarche and age at menopause

Numerous epidemiological studies have addressed the associations of age at menarche and age at menopause with the risk of ovarian cancer. In a review by Riman and colleagues [41], these menstrual factors appeared weak predictors of ovarian cancer risk. Moderately elevated risks of EOC were observed among women with a menarche of younger than 12 years, compared to those older than 14 years, however, the majority of the odds ratios (OR) were not significant [41]. The authors reported no associations between older age at menarche and risk of EOC in all other studies reviewed.

The findings regarding age at menopause and risk of ovarian cancer appear conflicting. In their review, the authors reported findings of a positive association between an older age at natural menopause and EOC risk compared to a younger age at menopause, as well as conflicting findings of risk being unrelated to the age of menopause [41]. Similar findings were reported for BOT.

Parity

Consistent epidemiological evidence points to a lower risk of ovarian cancer in parous women compared to nulliparous women [27, 43, 45, 54, 55]. This risk decreases with increasing parity. Recently it has been shown that nulliparous women have double the risk of ovarian cancer compared to women with 3 or more children [56]. However, the effect of age at first birth and the risk of ovarian cancer remain conflicting and yet to be resolved [41, 55]. Studies investigating the effect of incomplete pregnancies and ovarian cancer risk report either slightly reduced risks [55], or no associations [57, 58]. Overall, incomplete pregnancies seem to afford some protection from EOC, although the protection is somewhat weaker than that of full-term pregnancies [41].

Breastfeeding

The majority of evidence indicates a protective effect of breastfeeding on the risk of ovarian cancer. The only prospective study to address this association found a significant reduction is risk among women who breastfed for 18 months or longer [59]. In a collaborative study, a 20% reduction in risk was apparent among women who ever breastfed [60].

Infertility

In a recent pooled analysis of eight case-control studies investigating the association between infertility and ovarian cancer risk, the findings reported were that nulliparous women attempting pregnancy for more than five years had a 2.67 fold increased risk, compared with women attempting to conceive for less than a year [61].

Endometrial cancer

More than 90% of endometrial cancers arise within the epithelium and are Type I endometrioid adenocarcinomas [62]. The second type of endometrial tumors is Type

II or non-endometrioid tumors. Type I endometrioid cancers can be subdivided into 4 distinct groups: (i) adenocarcinomas and adenoacanthomas (squamous metaplasia) and account for 80% of epithelial tumors; (ii) papillary adenocarcinomas; (iii) clear cell carcinomas; (iv) mixed adenosquamous carcinomas [62].

Endometrioid Type I tumors appear to be the result of excess exposure to estrogens unopposed by progestogens [62-64]. These tumors are primarily associated with endometrial hyperplasia, increased levels of serum estradiol [63] and also express estrogen and progesterone receptors [65]. These tumors are characterized by a favorable prognosis [64].

Reproductive risk factors

Menstrual factors – age at menarche and age at menopause

An early age at menarche and a late age at menopause have been shown to significantly increase the risk of endometrial cancer attributable to the prolonged years of estrogen exposure [66]. Other menstrual factors associated with an increased risk include irregular menstruation or annovulatory menstrual cycles and a greater duration of menstruating days [67, 68].

Parity

Pregnancy and parity have been shown to decrease the risk of endometrial cancer by up to 30% for a woman's first birth and by 25% for each successive birth [66]. A late age at last birth has also been shown to reduce risk [66]. The proposed mechanism by which pregnancy and childbirth acts in reducing risk is by the elimination of premalignant cells through the shedding of cells during delivery and/or the break in unopposed estrogen exposure during a pregnancy [69].

Infertility

Infertility or a three year period of unsuccessful attempts at conceiving has been associated with an up to three-fold increased risk of endometrial cancer [70-72].

Exogenous Hormonal Risk Factors in Female Cancers

Similarly as with endogenous risk factors, exogenous hormones affect the risk of breast, ovarian and endometrial cancers differently. These exogenous factors will now be summarized for each cancer.

Breast cancer

Oral contraceptives

Based on original data of a collaborative analysis of 54 studies in 25 countries with data on over 50,000 women with breast cancer, the findings of this re-analysis concluded that the use of oral contraceptives slightly increases the risk of breast cancer in current and recent users, however there appeared no significant excess risk ten or more years after cessation [73]. The main conclusions of these studies in respect to breast cancer etiology, is that whatever effects exists, the effect remains small. What can be concluded from this is that women using oral contraceptives have similar total breast cell proliferation as those women with normal cycles [74]. The various preparations of oral contraceptives since their introduction in the 1960s does not affect risk, and risk of breast cancer is similar in women irrespective of family history, ethnicity, education, age at menarche, menopausal status, height, weight and alcohol consumption [73]. Findings from this collaborative analysis indicate that use is associated with a large excess of localized cancers than metastatic breast cancers; which raises the possibility that the increase risk in recent users may be attributable to a surveillance bias.

Hormone replacement therapy (HRT)

The use of HRT for menopausal climacteric symptoms has been shown to increase the risk of breast cancer and reduce the sensitivity of mammography [75-77]. Current or recent users of HRT have been found to have an increase of 2% per subsequent year of use. For women using HRT for a minimum of 5 years, this risk was increased up to 35% [39]. The increased risks were greatest for users of combined estrogen-progestin regimes, than for estrogen only regimes. Risk increases with duration of use: the risk for current users of combined regimes for 10 or more years was 2.31 [Confidence Interval (CI): 2.08-2.56], compared to 1.74 (CI: 1.60-1.89) for 1 to 4 years of use [76]. Risk was found to decrease with cessation of use; with past users having similar risks to never users of HRT. The findings from a recent review concluded that the excess risk of breast cancer, stroke and pulmonary embolism in postmenopausal users of HRT for more than 5 years far outweighed the reduction in the incidence of colorectal cancer and hip fractures [78].

Hormonal infertility treatment

Hormone infertility treatment includes exposure to a variety of hormonally active drugs, including clomiphene citrate (CC), human menopausal gonadotropin (hMG) and gonadotropin-releasing hormone (gRH). The effects of infertility treatment on the risk of breast cancer remain uncertain, due to the fact that most studies investigating the association have been hampered by limited power and the inability to account for important confounding factors [79]. The results from a large study reported a significant and transient increase in the risk of breast cancer in the 12 months following ovarian stimulation for *in vitro* fertilization [80].

Pharmaceutical hormonal infertility therapies have changed over the more recent years and it should be noted that the described preparations here, may not reflect accurately those regimes in practice today. The commonly used pharmaceutical drugs for hormonal infertility treatment are:

1 Clomiphene-citrate (CC)
2 Human chorionic gonadotropin (hCG)
3 Human menopausal gonadotropin (hMG)
4 Follicle stimulating hormone (FSH)
5 Combined oral contraceptives (COCs)

1 Clomiphene Citrate

Mechanism of action of CC

Blockade of estrogen receptors in the hypothalamic arcuate nucleus, leads to an increase in gonadotropin-releasing hormone (GnRH) [81]. CC also increases the pituitary sensitivity to GnRH in a similar way to estradiol [82]. As a result, FSH and luteinizing hormone (LH) are released from the anterior pituitary leading to ovulation [83]. CC may also exert direct effects on the ovary, sensitizing the granulosa cell to pituitary gonadotropin [81]. Serum progesterone and estradiol concentrations are increased during the luteal phase of the cycle in a direct dose-response relationship [84].

The anti-estrogenic effects of CC clinically present as hot flushes and changes in cervical mucus with the endometrium, being significantly thinner prior to the LH surge compared with spontaneous cycles [85]. Other side effects of CC include abdominal discomfort, ovarian enlargement and blurring of vision [86].

Multiple pregnancies are less likely with CC than with gonadotropic preparations, however the incidence being higher than that following spontaneous ovulation [86].

Administration of CC for ovulation

For ovulation induction CC is administered daily for 5 days beginning on the third, fourth or fifth cycle day and is highly effective in inducing ovulation by competing with endogenous estrogen for hypothalamic estrogen receptors in patients with evident follicular dysfunction [86]. The effect of repeated administration of a single 50 mg tablet at 28-day intervals is cumulative; therefore CC may be more effective in inducing ovulation during the second and later cycles of treatment [81]. Additionally, due to the continuing presence of CC, ovulation may also occur in the cycle following discontinuation of treatment typically occurring 3-7 days following administration when the anti-estrogenic effects have diminished [81].

Effect of CC on the luteal phase – implications for breast tissue proliferation

Mid-luteal phase endometrial glycogen content in addition to serum estradiol and progesterone are increased in CC cycles in proportion to the dose of clomiphene administered [84]. Dickey [87] showed that serum progesterone concentrations during the mid-luteal phase averaged 2700 ng/dl in spontaneous cycles and 3200 ng/dl in CC cycles of pregnancies which went to term and that progesterone concentrations were elevated much higher in CC-induced pregnancy than in spontaneous pregnancy during earlier gestational weeks. Furthermore, serum estradiol is increased through the first 16 weeks of gestation and progesterone

concentrations are increased through the first 7 weeks in CC cycles compared to spontaneous cycles which continued to term [88].

2 Human menopausal gonadotropin (hMG)

Human menopausal gonadotropin (hMG) is a potent preparation used for ovarian stimulation either to induce ovulation or to effect follicle maturation [86]. The preparation is a purified form of gonadotropins extracted from pooled urine specimens obtained from post-menopausal women, consisting of 75 or 150 IU each of FSH and LH per ampule [86]. Until recently, the only source of gonadotropins for treatment of anovulation and use in ovarian stimulation was hMG with preparations like Pergonal, with this urinary-derived preparation containing both FSH and LH, which together accounted for only 5% of the protein content [89]. However, despite developments in the purification processes, hMG content is subject to inherent variability in the amounts of LH present [90].

Mechanisms of action of hMG

FSH initiates gonadal differentiation and maturation through its action on granulosa cells [91] and promotes estradiol production in combination with LH [89]. Additionally, FSH induces follicular LH receptors allowing ovulation and development of the corpus luteum in response to the mid-cycle LH surge [92]. Therefore, all women with pituitary insufficiency need both exogenous FSH and LH for follicular development and thus benefit from hMG-hCG therapy [93]. However, hMG does not require a functioning hypothalamus or pituitary to be effective [86].

hMG is highly effective provided anovulation is not due to primary ovarian failure, with approximately 90% of patients expected to respond with ovulation; pregnancy rates reach 70% [86]. Multiple birth rates are high, occurring in 15-30% of gonadotropin-induced pregnancies [86].

Administration of hMG for ovulation induction

hMG is administered via intramuscular injection, followed by appropriate timing of hCG; with hMG used to stimulate follicle development and hCG stimulating a preovulatory surge of LH [86].

The dosage of hMG required for follicular maturation needs individualized titration for each patient and even for different cycles within the same patient (Wallach, 1995). An initial dose of 75 to 150 IU/day is customary, with treatment usually being continued for 7 to 12 days, with each daily dose of gonadotropin determined by indirect parameters of follicular response, using a combination of monitoring by ultrasound and serial determinations of serum estrogen [86].

Adverse reactions to hMG include ovarian hyperstimulation, ovarian enlargement, abdominal discomfort, pain, bloating, pulmonary and vascular complications, multiple pregnancies and occasionally sensitive reactions to the preparation itself [86].

3 Human chorionic gonadotropin (hCG)

hCG is available under the trade names of: Pregnyl; A.P.L; Follutein; Profasi; [86] and recently developed recombinant versions of hCG such as Ovitrelle/Ovidrel; to overcome the issues of impurity and avoidance of disease transmission with urinary derivatives [94].

Mechanisms of action of hCG

Human chorionic gonadotropin (hCG) is the hormone secreted by the trophoblastic cells of the chorionic villi of placenta in pregnant women with especially marked secretion in the cytotrophoblastic Langhans cells [95]. hCG has biological actions similar to that of luteinizing hormone (LH) and is used primarily to induce final follicular maturation with subsequent ovulation [94, 95] and peri-ovulatory changes, such as granulosa cell luteinisation and corpus luteum function [94].

Administration of hCG for ovulation induction

hCG is generally used to achieve ovulation in patients whose ovaries have undergone preliminary stimulation with hMG to produce follicular maturation [86]. Dosage used is 5000 to 10000 IU administered via intramuscular injection approximately 24 hours after the last dose of hMG [86].

The timing of hCG administration is critical, with too early administration resulting in oocyte atresia or failure to provide mature eggs; and too late administration resulting in oocyte over-maturation [96]. Timing of hCG administration is predicated upon a combination of ultrasound monitoring and serum estradiol levels, based on the establishment of one or more mature, preovulatory follicles, usually between 15 to 20 mm in diameter [86], with the recently generally adapted procedure to administer hCG when the follicle size is approximately 18mm [94]. Higher serum levels of estradiol at hCG administration (greater than 4000 pg/mL) results in a significantly increased risk of ovarian hyperstimulation [86]. In the absence of ultrasound evidence of ovulation, a second dose of hCG may be administered [96].

4 Follicle stimulating hormone (FSH)

Purified FSH is also known as urofollitrophin, Follitropin or Metrodin. Purified FSH has been recommended for ovulation induction in patients with polycystic ovarian disease who have an elevated LH:FSH ratio and have failed to ovulate in response to CC [97]. Purified FSH is also used in conjunction with hCG or hMG and hCG to achieve follicle maturation in an in-vitro fertilization (IVF) cycle [86].

Mechanisms of action of purified FSH

Similar to hMG, the ovary is the direct target for purified FSH preparations. Elevated FSH levels are believed to play a role in recruitment of a cohort of follicles [86]. During transition of the granulosa cell auto-differentiation from squamous to cuboidal shapes, FSH receptors appear [86]. With advancement of follicular development to late secondary and early tertiary stage, receptors for estradiol, progesterone, testosterone and glucocorticoids appear with a single follicle dominating with enriched FSH receptors [86]. This developing follicle secretes low levels of estrogen and inhibin, further reducing pituitary FSH output [86]. As a critical level of estradiol is reached, the pituitary gland paradoxically responds with a gonadotropin surge of LH which subsequently triggers follicle rupture and ovulation occurs [86].

Administration of purified FSH for ovulation induction

Administration and monitoring measures of FSH is similar to those of hMG/hCG [86].

Adverse effects of purified FSH use include ovarian hyperstimulation syndrome with ovarian enlargement, ovarian cyst formation, multiple pregnancy, vascular effects and gastrointestinal symptoms [86].

5 Combined oral contraceptives (COCs)

Combined oral contraceptives may be used to treat menstrual disturbances, sub-fertility and infertility in women with polycystic ovary syndrome (PCOS). Histological features of polycystic ovaries are a thick smooth fibrotic pearly white capsule, multiple small peripheral follicles (2-8 mm) and theca cell hyperplasia [98]. The multiple follicles are inactive and arrested in the mid-antral stage of development; with the ovarian stroma consisting of luteinized theca cells [98].

Mechanism of action of COCs

The use of COCs to treat menstrual disturbances, sub-fertility and infertility is usually in the first line of treatment, with the main objective to primarily restore endogenous hormonal exposures to sex steroids, LH, FSH and sex hormone binding globulin (SHBG). It should be noted that COCs act in the suppression of endogenous gonadotropins, therefore, not exerting any direct effects on ovulation induction via FSH and LH. At least three cycles of an oral contraceptive regimen are required to reach an endocrine equilibrium as reflected in levels of LH, FSH, SHBG and sex steroids [98].

In investigations by Prevelic and colleagues [99], they found normalization of hormonal profile and ovarian volume in hirsute women with PCOS treated with Diane-35® (containing 2mg of cyproterone acetate and 35 µg of ethinyloestradiol); with reduction of enlarged ovaries documented in two-thirds of women with PCOS after 12 cycles of treatment. The authors also document the LH/FSH ratio and serum testosterone were normalized after the third cycle. Additionally, a beneficial effect was observed on future fertility, with several patients conceiving soon after discontinuation of Diane-35®; most probably the result of an improved hormonal milieu and ovarian changes during treatment. It would therefore be justified recommending several cycles of such treatment to improve the hormonal profile and hence chances of pregnancy in women with PCOS as the cause of subfertility/infertility, before proceeding to the more strenuous ovarian stimulant measures of CC, hMG, hCG and FSH [98].

Side effects include those of any COCs: breast tension, headaches, dizziness, nausea, nervousness and depression, with some patients reporting decreased libido and fluctuations in body weight [98].

Ovarian cancer

Oral contraceptives

The contraceptive effect of combined oral contraceptives containing estrogens and progestins is mediated by the suppression of the mid-cycle gonadotropin surge with a consequent inhibition of ovulation [41]. The most significant findings from numerous epidemiological studies are the consistently reported substantial and persistent protective effects against ovarian cancer. The most recent collaborative reanalysis of individual data from 23,257 ovarian cancer cases and 87,383 controls from 45 epidemiological studies in 21 countries shows that use of oral contraceptives confers long-term protection against ovarian cancer which persists for decades [100]. Results

conclude that the reduction in risk is greater the longer the duration of use, with a significant reduction in risk more than 30 years after cessation. This protective effect was not altered by ethnicity, education, age at menarche, parity, family history, use of HRT, body-mass index, height, or consumption of alcohol or tobacco. The study's findings suggested that oral contraceptives have already prevented around 200,000 ovarian cancers and 100,000 deaths from the disease and that over the next few decades the number of cancers prevented will increase to approximately 30,000 per year [100].

HRT

Previous epidemiological studies on the relation between use of HRT and the subsequent risk of ovarian cancer have been inconclusive [2, 101-111]. In the most recent meta-analysis, ever use of HRT was associated with a 19 to 24% increased risk of ovarian cancer, which was greater among users of estrogen-only therapy compared to combined estrogen-progestin therapy [112]. In another recent and large cohort study, use of HRT was association with a significantly increased risk of incident and fatal ovarian cancer [113]. The study found current users of HRT were at an increased risk with increasing duration of use; however, risk did not vary according to the hormonal constituents used, mode of administration or the type of HRT regimen. Risks did not vary by socioeconomic status, reproductive history, use of oral contraceptives, body-mass index, or alcohol or tobacco consumptions. The study also found that risk associated with current use varied by tumor histology, with the greatest risk evident among serous ovarian tumors. Women who stopped taking HRT had similar risks for ovarian cancer compared to women who were never users of HRT [113].

Hormone infertility treatment

Previous epidemiological evidence investigating the association between hormone infertility treatment and the risk of ovarian cancer has yielded conflicting results [114]. Overall, the evidence does not support a link between hormone infertility treatment and the risk of invasive EOC [61, 115]; however, an increased risk of BOT has been observed with the use of fertility drugs [60, 116-118]. A recent cohort study found an increased risk for invasive EOC with the use of gonadoptropins and an increased risk of BOT with the use of CC therapy [119]. However, limitations exist in the methodological considerations of most studies.

Endometrial cancer

Oral contraceptives

Mitotic activity rates are lower during days 1 to 4 of the menstrual cycle, then increase rapidly and remain steady until day 19, after which mitotic rates drop to zero for the remainder of the cycle [120]. As such, mitotic activity is at its highest when estrogen is unopposed, and almost negligible in the presence of progesterone. As would be expected then, the use of continuous combined oral contraceptives is associated with a significant reduction in the risk of endometrial cancer, as users have fewer days of unopposed estrogen exposure every month. The continuous addition of a synthetic progestin is believed to accord this protective effect. For every year of use, the reduction in risk is approximately 10% [66] and has been purported to persist for up to 20 years after cessation of use [121].

HRT

The first hormone replacement introduced to provide relief from climacteric symptoms was estrogen only therapy, which was found to increase the risk of endometrial cancer substantially [122]. The increased risk of endometrial cancer with estrogen only therapy led to the introduction of combined estrogen-progestin therapy [123]. Recent results from the Million Women Study concluded an increased risk of 50% among current users of estrogen only therapy and 80% in those users of tibolone preparations [124]. The authors additionally conducted a meta-analysis of previous studies and found a non-significant reduced risk for ever users of continuous combined HRT compared to never users and a small, however significant increased risk among users of cyclic estrogen-progestin therapy. The Million Women Study did not have sufficient past-users of HRT to calculate risks after cessation, however a Swedish study reported a significant ongoing risk among previous users of estrogen only therapy but had stopped 5 or more years previously [125]. Body-mass index (BMI) potentially may modify the effects of HRT; in the Million Women Study estrogen only and tibolone therapies were only assessed among women with a BMI lower than 25, whereas there was negligible increased risk among obese women [124]. The risk of developing endometrial cancer with HRT is dose dependent; the results from one study indicate a 32-fold increased risk among those women taking 1.25 mg per day of unopposed estrogen for 2 years, compared with those women taking 0.3 mg per day [126].

Summary: Endogenous and Exogenous Hormonal Factors and their Effects on Female Cancers

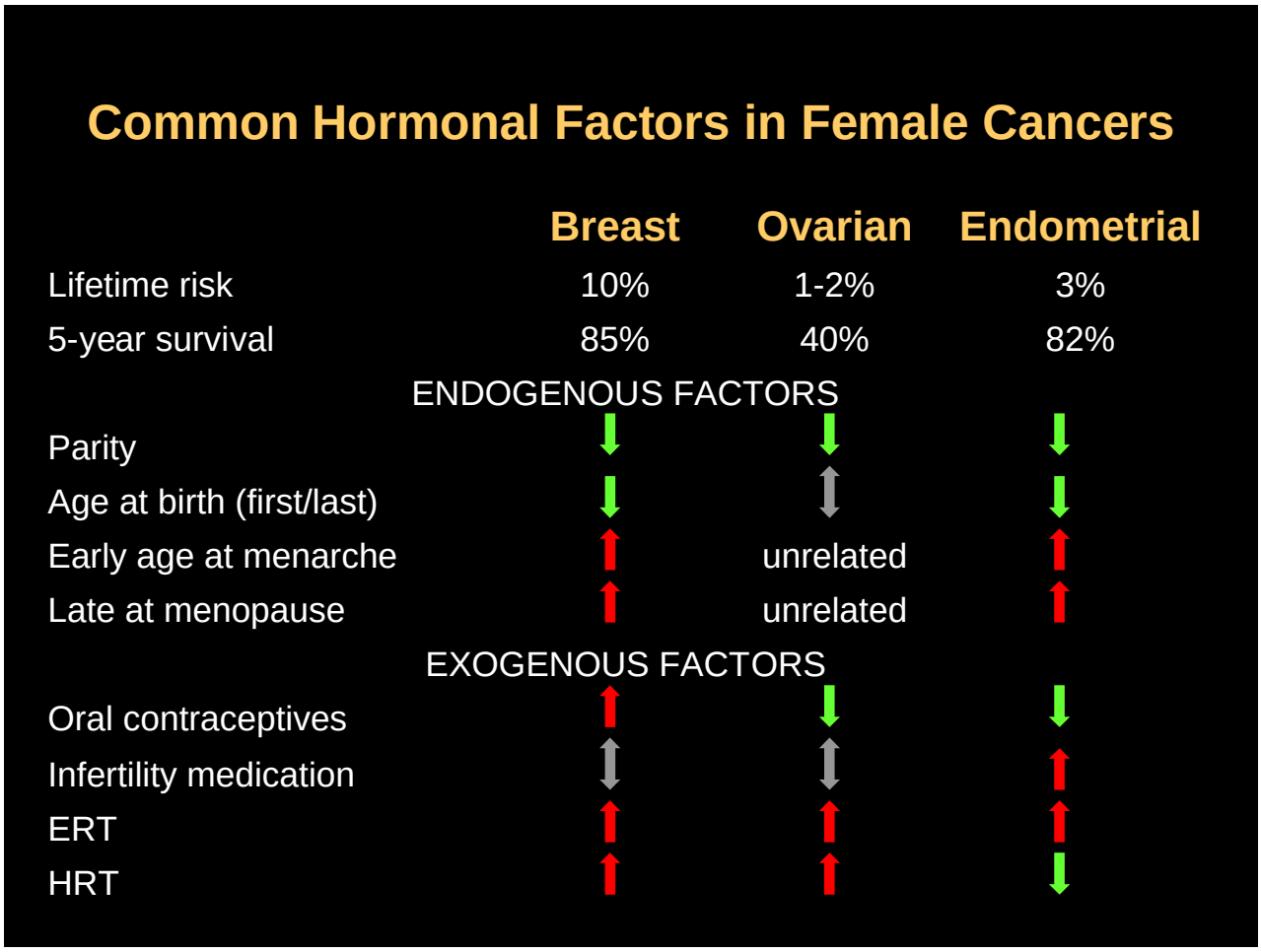

Figure 5: A summary of the common endogenous and exogenous hormonal factors in female cancers of breast, ovarian and endometrial cancer.

Legend: In green: reduced risk; in red: increased risk; in grey: inconclusive findings of increased and decreased risks. For breast cancer: age at first birth is the more important factor; for endometrial cancer: age at last birth is the more important factor.

The Association of Hormonal Risk Factors with Tumor Characteristics and Survival

Breast cancer and menstrual risk factors

Breast cancer is a morphologically and clinically heterogeneous disease. It remains less clearly understood how established epidemiological risk factors relate to tumor characteristics and survival. Haenszel hypothesized that factors influencing breast cancer induction, also affect prognosis [127]. Estrogen promotes growth in breast cancer cell lines [128] and lower estrogen levels have been correlated with improved disease-free survival in postmenopausal breast cancer [129]. Tumor characteristics are important in determining survival [130]; however, they only explain a fraction of the variation observed in survival [131].

Several studies have generated support for Haenszel's hypothesis, with the confirmed association between obesity and poorer breast cancer prognosis [132-135]. To date, conflicting results exist on whether menstrual risk factors for breast cancer influence tumor progression and survival in patients [132, 136-145]. This may be partially due to variations in age categorizations for age-dependent risk factors, such as age at menarche, and age at menopause; making interpretation difficult, since there may be a critical time window of susceptibility during adolescent and puberty development that influences tumor initiation and progression.

One study investigated hormone related breast cancer risk factors and breast tumor proliferation, measured by the protein Ki-67 and mitotic count [142] and found no significant association between tumor proliferation or mitotic count and age at menarche. Age at menarche has previously been inconsistently associated with survival. Three studies found an association between early age at menarche and reduced survival [136, 138, 145]. Caleffi and colleagues [136] studied women treated with modified radical mastectomy and found a significantly poorer survival with early age at menarche. In the study by Juret and colleagues [138], a proposed hypothesis suggested that the association may reflect a correlation with axillary nodal involvement. The most recent study by Trivers and colleagues [145], found poorer survival with a younger age at menarche, however, this study was restricted to women less than 55 years of age, and the association was only evident in premenopausal women when stratified by menopausal status. Most other studies found no association with age at menarche and survival [132, 137, 141, 143, 144, 146-149], or older age at menarche being associated with worse survival [139]. Possible explanations for the discrepancies in findings could be a difference in distribution of age at diagnosis, lack of adjustment for potential confounders, adjustment for tumor characteristics that are intermediates in the causal pathway of the association being addressed, and different categorizations of age at menarche.

All previous studies investigating the association between age at menopause and survival have found no association [132, 137, 141, 143, 147, 148], with the exception of one study [139] which found that early or late age at menopause was associated with a poorer survival, compared to women with menopause between the ages of 46 and 54 years.

Ovarian cancer and HRT

Hormonal factors are known to influence ovarian carcinogenesis, however little is known about the effects on survival. The presence of hormone receptors and production in ovarian tumors indicate that hormonal factors may additionally influence the growth of ovarian cancers [150-157]. Thus, it is of prime importance to examine whether hormone-related risk factors are associated with clinical tumor characteristics and prognosis in women who develop ovarian cancer. Of recent and controversial interest, is the use of HRT and its association with an increased risk for ovarian cancer. Furthermore, the use of HRT has been hypothesized to affect epithelial ovarian cancer prognosis.

To date only a few studies [110, 158-161] have investigated the possible association between use of HRT and ovarian cancer mortality or survival. Based on limited sample sizes, the results of these studies have been inconsistent. In the only large prospective study [110, 161], postmenopausal estrogen use for 10 or more years before cohort enrolment (and cancer diagnosis) was associated with an increased risk of ovarian cancer mortality that persisted up to 29 years after cessation of use. The most recent study to investigate HRT use prior to diagnosis of ovarian cancer found a slight improvement in survival, albeit non-significant [162]. All studies investigating HRT use following diagnosis of ovarian cancer found it to be unrelated to survival [158-160].

Endometrial cancer and HRT

Exogenous hormones are well established factors in endometrial carcinogenesis. The association of HRT with a better prognosis in endometrial cancer has been hypothesized to be due to the development of less aggressive tumors among HRT users [163]. On the other hand, it has been reasoned that findings of improved survival among users of estrogens could be attributed to earlier detection of tumors due to increased medical surveillance among hormone users [163, 164], or users being from higher socio-economic classes with better access to health care [164].

Most of the previous studies investigating HRT and endometrial tumor characteristics have shown that HRT users have less aggressive tumors, despite methodological differences [163, 165-169], with the exception of two studies [101, 168]. One example of a study showing the better prognosis of HRT users, is the large study by Collins and colleagues [163], where estrogen use was associated with earlier stage, lower grade of tumor and less frequent myometrial invasion. On the other hand, the most recent study, the Women's Health Initiative Randomized Trial [101], found no ascertainable differences in the distribution of tumor histology, stage or grade of endometrial cancer between users and non-users of HRT.

Previous studies investigating HRT and endometrial cancer survival have been conflicting. The majority of studies have found significantly better survival among users of HRT [163-165, 169-171]. Contrastingly, some studies found an increased mortality with HRT use [172, 173]. The study by Schairer and colleagues [173] found that mortality from endometrial cancer was not related to the prescription of weak estrogens with or without progestins; however mortality was reportedly 40% higher in women prescribed more potent unopposed estrogens. In the recent study by Khan and colleagues [172], a significant increased risk of endometrial cancer mortality was found for ever users of sex hormones, however, this study was severely hampered by a small number of deaths.

AIMS

Overall, the aim of this thesis was to further investigate the roles of endogenous and exogenous hormonal factors on the etiology, risk and prognosis in breast, ovarian and endometrial cancers. Specific aims of each paper are as follows:

Paper I

To assess the impact of hormonal infertility treatment, together with the underlying causes of infertility on the incidence of breast cancer.

Paper II

To assess the effects of use of hormone replacement therapy before and after a diagnosis of invasive epithelial ovarian cancer or borderline ovarian tumors, on the impact of 5-year survival.

Paper III

To investigate the effects of established menstrual risk factors on tumor characteristics and 5-year survival in postmenopausal breast cancer.

Paper IV

To investigate the effects of ever use of menopausal hormone therapy (MHT) before diagnosis on tumor grade and depth of myometrial invasion, and 5-year relative survival in postmenopausal endometrial cancer patients.

STUDY PARTICIPANTS AND METHODS

Sweden could be considered an epidemiological utopia for medical research. With its population based registers, equitable health care system and National Registration Number (NRN) assigned to all Swedish residents, the quality of research and possibilities are excellent.

The NRN is a unique personal identification number that has been assigned to all Swedish residents since 1947. The first six numbers comprising a NRN are composed of the date of birth, followed by numbers indicating place of birth, sex and a control digit. This unique number makes personal identification possible and facilitates record linkage between population and health registers.

PAPER I

The Study Cohort

The cohort comprised 1135 women treated for sub-fertility associated disorders who attended the major clinics of obstetrics and gynecology in Stockholm, Gothenburg and Uppsala in Sweden between 1961 and 1976 (Figure 6).

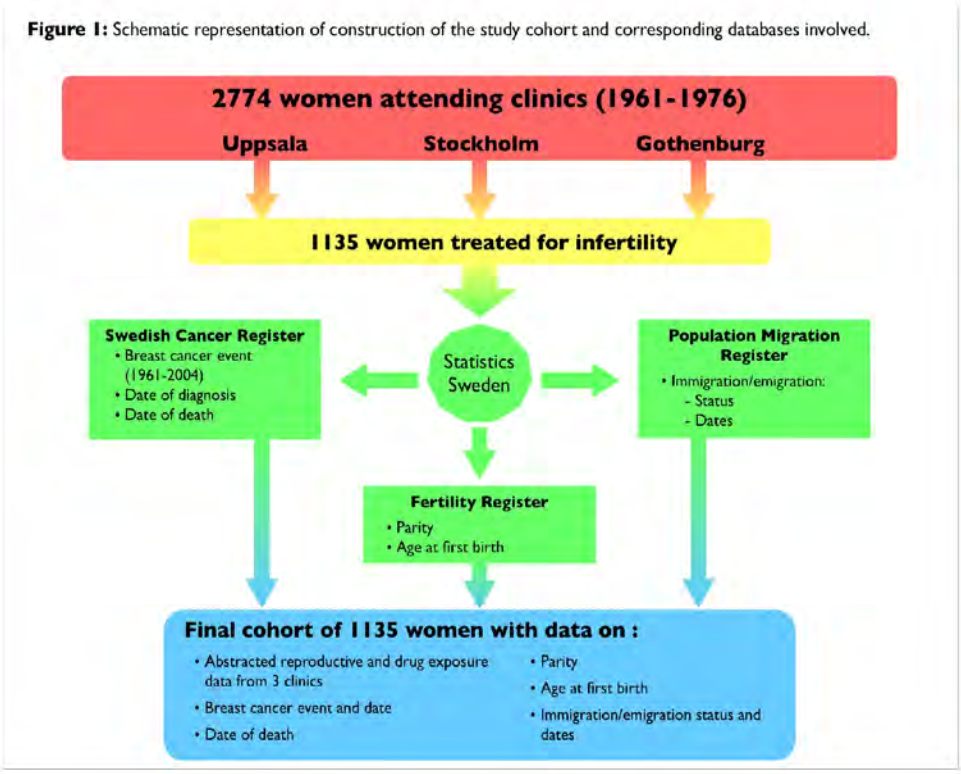

Figure 6: A schematic representation of construction of the study cohort and corresponding databases involved.

Legend: In red: all women seeking treatment for any type of menstrual disturbances, not necessarily only infertility, and attending one of three major clinics of obstetrics and gynecology; in yellow: women eventually desiring pregnancies with difficulty conceiving retained in the cohort; in green: cohort data sent to Statistics Sweden for record linkage with three registers held by the bureau; in blue: final cohort of 1135 women with clinical and registry based data.

All women with any history of menstrual problems, irregular periods or absence of periods during the lifetime were identified. These women were not necessarily seeking treatment for infertility, however, sought medical help for any menstrual disturbances through their physicians. Of all of the women with menstrual problems, those that did eventually wish to conceive were retained in our cohort. Record linkage was performed by Statistics Sweden in 2006, using the individual unique NRN assigned to all Swedish residents. The cohort was linked with the national Swedish Cancer Register, Population Migration Register, and the Fertility Register to comprise the final cohort. Strictly speaking, a woman whom eventually conceives and delivers is only sub-fertile, however, for simplicity; we will refer to all fertility related conditions as infertility. All patients were either referred by their primary physician or attended the clinics independently.

Exposure Information

Pharmaceutical drugs used for hormonal infertility treatment in this study cohort were; CC, hCG, hMG and FSH.

Other abstracted data included; reasons for referral to infertility treatment, age at registration, menstruating pattern, bleeding disorders, pregnancies, abdominal/pelvic surgeries and x-ray and use of oral contraceptives. Information on parity and age at first birth was obtained from the Fertility Register.

Classification of Exposures

In the analyses we defined CC as an exclusive exposure group, while hCG, hMG and FSH were combined as exposure to gonadotropins collectively. In clinical practice, if FSH or hMG are prescribed, hCG is often given concurrently. As the biological potency varied between different gonadotropins used in the study cohort, we chose to state the number of menstrual cycles during the hormonal infertility treatment administered, as a measure of exposure dosage. For exclusive users of CC or gonadotropins, we classified dosage as low, comprising one to three treatment cycles; and high, comprising four or more treatment cycles.

Causes of infertility were assessed by a gynecologic endocrinologist after a complete assessment of the patient's medical history, full blood tests of sex hormones, temperature charts, sperm analyses, laparoscopy, and x-ray if required. Reasons for referral to the infertility clinics were categorized into; suspected ovulatory factors, mechanical factors, and other factors. For simplicity, herein we will refer to all suspected ovulatory factors as ovulatory factors. Ovulatory factors were defined as a diagnosis of anovulation or amenorrhea (more than three months between cycles); and oligomenorrhea (35-90 days between cycles) or polycystic ovary syndrome/Stein-Leventhal syndrome and hirsutism. Mechanical factors included cervical competence factors and abnormalities of the fallopian tubes or uterus. Other factors included

endometriosis, habitual abortions, immunological factors, and unexplained infertility. Further, we dichotomized these into those presenting with ovulatory factors, and those presenting with non-ovulatory factors; comprised of mechanical and other factors combined, based on women with non-ovulatory factors presenting with intrinsic baseline hormonal levels similar to the hormone levels in normally ovulating women.

Follow Up

Follow up commenced on the date of first hormonal treatment. Information obtained from the Swedish Cancer Register used to ascertain all cases of breast cancer from 1961 through to 31 December 2004, included the date of cancer diagnosis with breast cancers coded according to the *International Classification of Diseases.* End of follow up was recorded as the date of diagnosis of primary breast cancer, date of death, date of emigration or 31 December 2004; whichever occurred first.

Information on parity and age at first birth was obtained from the Fertility Register, established in 1971.

Statistical Methods

Standardized incidence ratios (SIRs) – the ratio of the observed number of breast cancer cases to those expected number of breast cancer cases, according to breast cancer rates observed in the total population of Swedish women– were calculated, as an estimate of the relative risk. Population breast cancer rates taking parity and maternal age at first birth into consideration were derived from linkage between the Swedish Cancer Register and the Multi-Generation Register. The Multi-Generation Register provides links to all parents of children born from 1932 onwards and registered at anytime since 1961. Information pertaining to age at first birth and parity from the Multi-Generation Register is identical to the Fertility Register, as these two registers are constantly updated against each other in Sweden. The expected number of breast cancer cases was derived by multiplying the observed number of person-years contributed by all individuals in the cohort with incidence rates dependant on age (5-year intervals), calendar periods (5-year intervals commencing in 1961 to 2004), parity (nulliparous, 1, 2, 3, and 4 or more children) and age at first birth (5-year intervals). The SIR's and their corresponding 95% confidence intervals were based on the assumptions that the observed number of cancer cases follows a Poisson distribution.

Written informed consent was obtained from all women who agreed to participate in the study after being contacted by their treating physicians in Uppsala, Göteborg and Stockholm. The Institutional Review Board of the Karolinska Institutet, Sweden, approved the study.

PAPERS II-IV

Papers II through to IV are extensions of case-control studies conducted among all Swedish residents born in Sweden and aged 50 to 74 years and diagnosed with incident invasive epithelial ovarian cancer or borderline ovarian tumors, primary invasive breast cancer, or incident histopathologically confirmed endometrial cancer.

Overall, the data collection was based on a 20 page self-administered questionnaire, mailed to all consenting participants. The questionnaire covered detailed information concerning age, body type, leisure time physical activity at various stages throughout life, education, employment, smoking, alcohol consumption, coffee drinking habits, hereditary factors, medical history, reproductive history and detailed information on the use of exogenous hormones.

Further information regarding the inclusion criteria and detailed information of the exposures assessed, will be outlined with each subsequent paper.

PAPER II

This study was based on a follow-up of patients who previously participated in a nation wide population-based case-control study in Sweden [2] in which we observed an about 50% increased ovarian cancer risk among women who used estrogens without progestins, or with progestins added cyclically for half or less of the monthly treatment period.

Founding case-control study

In short, women were 50-74 years of age at study enrolment, born in and residents of Sweden, and had at least one intact ovary (women with bilateral oophorectomy were excluded). The recruitment period extended from October 1, 1993, to December 31, 1995. Eligible case patients were previously free of ovarian malignancies and presented with a newly diagnosed, histologically confirmed, invasive or borderline epithelial ovarian tumor. Patients were identified through reports to six regional cancer registries that together provide a complete nationwide cancer registration [13].

After being informed about the study by their physicians, case patients agreeing to participate signed an informed consent form before study enrolment. A total of 1205 women with incident ovarian tumors of any histological type were reported to the regional cancer registries, and 914 patients (76%) initially agreed to participate. Of these, eight women declined participation in the present follow-up survival study, and one was excluded due to the physicians' denial of access to patient records. Of the 905 remaining cases, 68 had non-epithelial ovarian cancer; 13 other gynecologic malignant tumors; one record revealed relapse of a previous ovarian cancer diagnosed in 1991; five were intestinal cancers; two were benign tumors; and 17 were described as cancers of the abdomen and peritoneum, according to pathological re-evaluation during the patient's treatment in the clinics. The final study population consisted of 799 women with ovarian cancer, of which 150 had BOT, and 649 invasive EOC. The histological classification was based on pathology reports alone.

Exposure data were collected through mailed self-administered questionnaires that covered demographic, medical, gynecological, reproductive and lifestyle factors including questions on height, weight, diet, physical activity, heredity, previous disease, gynecological surgery, pregnancies, births, menstruations and number of visits to gynecologists (prior to diagnosis). In 50% of the cases, the requested information was supplemented with a telephone interview to further enhance the accuracy of information attained. Detailed information pertaining to HRT and oral contraceptives was obtained. To facilitate the recall of oral contraceptives and HRT use, the questionnaire showed pictures of all the HRT brands commercially available in Sweden beginning in the 1950s.

Present cohort study and follow-up

All information on HRT use before cancer diagnosis was obtained through the initial questionnaire pertaining to exposure data, answered at enrolment in the founding case-control study [2]. The HRT exposure variables were classified as estrogen only (ERT – medium potency estrogens, i.e. conjugates estrogens, estradiol, and other synthetic estrogens without added progestins); estrogens with progestins combined cyclically (<16 days/cycle, most commonly 10 days/cycle); or continuously (≥19 days/cycle, most commonly 28 days/cycle). Information was also obtained on low potency estrogens (oral or vaginal estriol, dienestriol, or low dose estradiol [25 μg/day]). In addition, information was available on progestin only therapy used in the treatment of perimenopausal bleeding irregularities and for the alleviation of symptoms related to benign appearing ovarian cysts. For HRT treatment, women were categorized as never users, exclusive users of only one HRT regimen, and non-exclusive users who over time had taken more than one type of HRT. We calculated duration and recency of HRT use separately for each type of regimen (i.e. estrogens only, estrogens combined with progestins cyclically, estrogens combined with progestins in a continuous way, and overall estrogens combined with progestins) and overall duration and recency of use for any type(s) of HRT taken. We categorized duration of use as never users; less than or equal to 3 years of use; and greater than 3 years. All exposures were censored after an index date, which was defined as 3.0 months before the date of diagnosis for each patient. Women that used any type of HRT at the index date were defined as current users. Former users were all other users that were not current users.

Finally, we looked at the combined effects of duration and recency of any HRT treatment received by patients. This combined variable was classified as never users at baseline; current users and former users of shorter or longer duration (≤3, > 3 years). We could only consider these combined overall effects for women who had complete information for both, duration and recency of HRT use. Missing information was encountered when some women recalled HRT use, but not the specific duration or recency of use.

Other relevant data obtained from the initial questionnaires included socioeconomic status, use of and duration of any type of oral contraceptives, body mass index (BMI – defined as weight in kilograms divided by height in meters squared), smoking status one year prior to diagnosis, parity, age at menarche and menopause, history of tubal ligation, and a family history of ovarian cancer in the mother or sister of the patient.

Additional patient data required for the present follow-up survival study included information on use and duration of HRT *after* diagnosis, and detailed clinical information on tumor characteristics, treatment modalities, recurrence and progression of the disease.

Exposure information pertaining to HRT use *after* diagnosis was recorded on ever/never use, start and stop dates of treatment and if HRT treatment was ongoing at the time of data abstraction. If nothing was specifically stated in the patients' medical records about prescriptions of any type of HRT, it was recorded in the abstracting form as "not stated". After consultation with local gynecological oncologists, we reclassified ''not stated'' as ''not users'', since HRT are only sold or used under medical prescription in Sweden (except for low potency estrogens), and the absence of a prescription in the medical record of a cancer patient means with great certainty that HRT was not used. Information was *not* available for all patients about specific types of HRT prescribed *after* diagnosis, or if patients changed types of HRT. We categorized duration of use of HRT *after* diagnosis as never users; less than 1 year; 1 to 2 years; and greater than 2 years. Information on prognostic factors included FIGO (International Federation of Gynecology & Obstetrics) stage (I, II, III, IV), WHO grade of differentiation (well differentiated, moderately differentiated and poorly differentiated), tumor size at diagnosis, residual tumor size, the presence of multiple simultaneous primary tumors, histological subtype (serous, mucinous, endometrioid, clear cell, undifferentiated, others), reasons for primary diagnosis (gynecological routine examination, the presence of symptoms, and other reasons) and treatment.

We considered as outcomes overall mortality (death from any cause) and cause specific mortality (death from ovarian cancer or related causes).

Date and cause of death information was obtained through record linkage with a nationwide Cause of Death Register updated through December 31, 2002, using the individually unique national registration number.

Causes of ovarian cancer deaths were defined as women dying from ovarian cancer (ICD-9 codes 183.0 – 183.9) and C56 (ICD-10) or having 'malignant tumor in the ovary' as the underlying cause of death. Related causes of death were considered as death from possibly metastatic tumors, such as: unspecified location of malignant tumor in the peritoneum (n = 1); several malignant tumors with different points of origin (n = 1); tumor of uncertain nature in the ovary (n = 1); and malignant tumor in the uterus except isthmus uteri (n = 2).

The Ethics Committees of the Karolinska Institutet, Sweden, approved the study.

Statistical methods

Overall survival time was defined as the time interval from the date of ovarian cancer diagnosis to the date of death from any cause. Cause-specific survival was defined as the time interval from the date of diagnosis to the date of death from ovarian cancer or related causes. All patients were followed for five years or until death. The end of follow up for the analyses presented here was set to December 31, 2002.

STATA® Version 8.2 was used for data analyzes. Contingency tables and univariate summary measures were produced to describe the patients at the beginning of follow-up, in term of the hormone exposure variables and prognostic factors. Kaplan-Meier estimates and graphs were produced to describe the overall and stratified survival distribution. The log rank test was used to assess whether there was any statistical difference and those variables with a *p*-value less than *0.25* were considered eligible to be included in the multivariate analysis [174].

In an initial step prior to multivariate analyses, graphical assessments were performed for all covariates to assess the proportionality assumptions.

The Cox proportional hazard regression model was subsequently fit to estimate the effect of HRT and its derived variables, adjusted by variables found to be important in the first part of the analysis: age, FIGO stage, WHO grade of differentiation, and histological subtype of tumor. We used the likelihood ratio test based on the partial likelihood to assess the independent effect of the explicative variables as well as the interaction terms. Appropriated goodness-of-fits and diagnostic measures, together with graphic methods, based on the Schoenfeld and Martingales residuals [175] were ultimately produced to assess the appropriateness of the models chosen, such as the proportionality assumption underlying the Cox model.

Tests of association used in the analyses to test significance between groups were the likelihood ratio test and Pearson's Chi-square tests.

PAPER III

Study design

This study is an extension of a population-based case-control study among all Swedish women born in Sweden and aged 50 to 74 years of age between October 1, 1993 and March 31, 1995 and described in detail elsewhere [38, 176]. We used a case-case design in which we obtained odds ratios and estimated hazard ratios, as measures of relative risk comparing breast cancer cases' categories of menstrual factors; to investigate the relationships between menstrual factors, tumor characteristics, and 5-year breast cancer survival.

Participants

Women with incident primary invasive breast cancer were identified through the six Swedish Regional Cancer Registries and contacted by their doctors. Out of 3979 women with a primary diagnosis of invasive breast cancer, 3345 women (84%) participated in the study. The primary reasons for non-participation were patient's or doctor's refusal due to patient ill-health. Excluded patients had previous or other cancers (151 cases), noninvasive breast cancer according to patients' records from the regional cancer registry (58 cases), a diagnosis outside the study period (19 cases), lack of patient consent (58 cases), premenopausal status (198 cases) being younger than 55 years with an unknown age at menopause (202 cases), missing age at first birth (5 cases), and missing height or recent weight (14 cases). The study included 2640 eligible postmenopausal breast cancer patients of European descent.

Protocol

The ethical review board at the Karolinska Institute and the six ethical review boards in other regions of Sweden approved the study. Prior to participation via a mailed questionnaire, written consent was obtained from all patients. The mean interval between diagnosis and data collection was 4.3 months (standard deviation 1.5 months).

Data collection and classification

With the exception of clinical data on tumor characteristics and follow-up data for survival outcomes, exposure and covariate data used in this study were derived from the case-control study questionnaire. In brief, data on sociodemographic, anthropometric, reproductive, and menstrual factors, use of oral contraceptives, and medical history (1 year prior to data collection) were collected by means of a postal questionnaire. Detailed information pertaining to use of HRT, including timing and type of hormones for each treatment episode, were requested, along with a color chart displaying all preparations ever marketed in Sweden, included with the questionnaire to facilitate recall. Additionally, approximately 50% of cases were contacted by telephone to complete missing or ambiguous responses, mainly on the use of HRT.

Menstrual factors assessed were age at menarche, age at menopause, irregular menstruation, cycle length, and lifetime number of menstrual cycles. Age at menarche was classified as 11 years or younger, older than 11 and younger than or at 13 years, older than 13 and younger than or at 14 years, and older than 14 years. Menopause was defined as the age at last menstrual period, or age at bilateral oophorectomy, if one year or more prior to data collection. Analyses for age at menopause were firstly restricted to women with known natural or surgical age at menopause that had not used HRT prior to menopause, to assure an accurate classification of the true age at menopause. Due to the large percentage of women with a missing true age at menopause (39%), it was decided to use all women with an age at menopause and adjust our analyses for use and type of HRT. Age at menopause was grouped as: earlier than 50 years, 50 years to 55 years, and older than 55 years. Irregular menstruation was either absent or present during the lifetime. Cycle length was classified as 27.5 or fewer days per cycle, 28 days, or more than 28 days. Lifetime number of menstrual cycles was a created variable, derived from all women with known values for age at menarche, age at menopause, parity, and cycle length; and did not include those women with irregular menstruation, or miscarriages and/or abortions. Lifetime number of menstrual cycles was classified as 423 or fewer cycles per lifetime, more than 423 but less than or equal to 500 cycles, and more than 500 cycles.

Information regarding tumor characteristics was retrieved from the medical records of all participants from surgical and oncological units throughout Sweden. Data pertaining to tumor characteristics included: tumor size; grade, classified according to the Nottingham histological grade or Bloom-Richardson scale; estrogen receptor (ER) and progesterone receptor (PR) status; and lymph node involvement. Information on grade was not in routine use in Sweden during the study period, and is therefore missing in 33% of patients.

The Swedish NRN was used to link the cohort with the Swedish National Population Register, and the Swedish Cause of Death Register, to obtain data on emigrations, and the date and causes of death respectively.

Statistical analyses

Tumor presentation

The significance of differences between tumor characteristics and menstrual factors were evaluated using frequencies with Chi-square tests of association. All probability values of $P<0.05$ were considered significant. Odds ratios (OR) with 95% confidence intervals (CI) were calculated using polytomous multiple logistic regression with tumor characteristics as the dependent variables, with the category of each tumor characteristics having the best prognosis as the reference group, and the remaining categories as the outcome. Potential confounders were included in the models in a step-wise approach based on established biological knowledge of confounders particular to the associations of interest between menstrual risk factors and prognostic tumor characteristics, rather than solely based on a 10% percentage shift in the estimates.

Survival analysis

Follow-up time commenced on the date of breast cancer diagnosis to the date of death, emigration or date of study truncation – 5-years following the date of diagnosis; whichever occurred earlier. The outcome was breast cancer specific deaths (ICD-9: 174.9; and ICD-10: C50.9). One woman emigrated, 264 died from breast cancer, and 383 died from other causes, during 12290 person-years of follow-up.

Breast cancer mortality rates were calculated by menstrual factors as the number of breast cancer deaths per 100 person-years. Cumulative 5-year survival rates were calculated using the Kaplan-Meier method and the significance of differences in survival were evaluated using the log rank test. The Cox proportional hazards regression model was used to quantify the effects of menstrual factors on 5-year survival. The covariates chosen for multivariate analyses adjustments were based on biological associations deemed important in assessing menstrual factors and survival.

The menstrual prognostic factors in our study all occurred prior to the diagnosis of breast cancer. Therefore, any effects on tumor progression will be mediated through the biological characteristics of the tumor itself. Hence, adjusting for any tumor characteristic variables in the Cox regression model would be incorrect as they are not confounding the association between menstrual factors and breast cancer survival; rather, acting as intermediates in the causal pathway.

STATA® Version 9.2 was used for data analyses.

PAPER IV

Study design

This study is an extension of a population-based case-control study of all Swedish women born in Sweden, aged 50 to 74 years of age between January 1, 1994 and December 31, 1995 and described in detail elsewhere [121, 125, 177]. To investigate the relationships between use of MHT, tumor characteristics and endometrial cancer

relative survival, we used a case-case design; in which we obtained odds ratios and estimated excess hazard ratios as measures of relative risk comparing endometrial cancer cases' ever and never use of MHT.

Parent study

During the study period, all endometrial cancer cases were identified through the six Regional Cancer Registries in Sweden, which provides complete information on incident cancers. The study was restricted to women who had not undergone hysterectomy or who had a previous diagnosis of breast or endometrial cancer. Eligible patients were those women with a histopathologically confirmed endometrial cancer as reported to the cancer registry. Of all eligible cases, 802 (76%) women participated in this initial questionnaire-based study. The primary reasons for non-participation were patient and physician refusal. Detailed information on the use of MHT, including the brand, dose and dates of use for each type of treatment were collected. Recall of MHT was facilitated by color picture charts of all brands commercially available throughout Sweden during 1950 to 1995. Other relevant information collected covered data on medical, reproductive, lifestyle and anthropometric factors, including age at diagnosis, age at menarche, total parity, age at first and last births, age at natural menopause, body mass index, and smoking. Findings from this study have been previously published [121, 125, 177-181].

Present study – participants

For our current study, we performed additional linkage with the Swedish Cause of Death Register. In order to confirm previous endometrial cancers from the parent study, we linked the cohort of 802 cases to the national Swedish Cancer Register for confirmed endometrial cancers with an ICD code 172 (*7th edition*). Of the original 802 cases, two had missing personal identification numbers, making record linkage impossible, whilst 19 cases did not have a primary diagnosis of endometrial cancer, and 10 cases were benign. After restricting our analyses to postmenopausal women, the final cohort comprised 683 endometrial cancer patients born and resident in Sweden. Out of 1055 eligible women in the parent study, the participation rate in the current study was 65%.

Protocol

The study was approved by the ethical review board at the Karolinska Institute. Prior to participation via a mailed questionnaire, written consent was obtained from all patients. The mean interval between diagnosis and data collection was 8.4 months (standard deviation 4.6 months).

Classification of MHT

MHT use was categorized as having ever used the therapy, or never. Ever use of any particular MHT was not mutually exclusive; meaning a woman could be considered an ever user of more than one type of hormone therapy. The classification of MHT was as follows:

1) Any form of medium potency MHT
2) Any form of medium potency conjugated or synthetic estrogens (estradiol or other synthetic estrogens); with or without progestins
3) Combined medium potency conjugated or synthetic estrogens and progestins (progesterone-like progestins, i.e. 17-hydroxy-progesterone derivatives; or testosterone-like progestins, i.e. 19-nor-testosterone derivatives):

a. In cyclic form (cyclic progestins added to estrogens for less than 16 days per cycle, mostly for 10 to 14 days per cycle)
b. In continuous form (progestins added to estrogens for 19 or more days per cycle, typically daily)

4) Low potency vaginal estrogens (estriol 0.5 mg, dienoestrol 0.5 mg, or estradiol 0.25 µg) applied daily during the initial two to three weeks of treatment, and followed by bi-weekly applications
5) Low potency oral estrogens consisting of one or two milligrams per day

All hormone exposures were censored after an index date; defined as six months before the date of diagnosis.

Histopathological classification

Information regarding tumor characteristics was retrieved from all 35 pathology departments in Sweden and reviewed and reclassified by the study pathologist, who was blinded to hormone use and other exposures. The histological specimens of patients were reclassified as: endometrioid adenocarcinoma (*n* = 624 or 91%), or non-endometrioid adenocarcinoma comprising; seropapillary carcinoma (*n =* 36 or 5%), clear-cell carcinoma (*n =* 8 or 1%), adenoacanthoma (*n* = 3 or 0.4%), or adenosquamous carcinoma (*n* = 12 or 2%). Endometrioid adenocarcinomas were further classified as Grade 1 (well differentiated, *n =* 230 or 37%), Grade 2 (moderately differentiated, *n =* 281 or 45%), or Grade 3 (poorly differentiated, *n =* 113 or 18%). All endometrioid adenocarcinomas were analyzed separately to non-endometrioid adenocarcinomas. However, due to low power among the specific sub-types of non-endometrioid adenocarcinomas, we analyzed these carcinomas as one entity. Hysterectomy specimens were obtainable for 525 women (77%). Among these women, the depth of myometrial invasion was classified as none or less than 50% (*n* = 348 or 51%), and 50% or more of myometrial thickness or penetration through the serosa (*n* = 177 or 26%).

Follow-up data on survival

The Swedish NRN was used to link the cohort with the Swedish National Population Register, and the Swedish Cause of Death Register, to obtain data on emigrations, and the dates of death respectively. The latter register covers all residents in Sweden. Patients were followed up to five years after the date of diagnosis of endometrial cancer. One woman was found to have emigrated during follow-up and was consequently censored at the date of emigration.

Statistical analyses

Tumor presentation

The significance of differences between tumor characteristics and use of MHT was evaluated using frequencies with Chi-square tests of association. All probability values of $P<0.05$ were considered significant. Odds ratios (OR) with 95% confidence intervals (CI) were calculated using polytomous multiple logistic regression [182] with tumor characteristics as the dependent variables; with the reference group being the category of tumor characteristic with the best prognosis, and the remaining categories as the outcome. Potential confounders were included in the models in a step-wise approach based on established biological knowledge of confounders particular to the associations of interest between MHT and prognostic tumor characteristics, rather than solely based on a 10% percentage shift in the estimates.

Relative survival analyses

Relative survival ratios, defined as the observed survival among patients divided by the expected survival of a directly comparable group from the general Swedish population and assumed to be free of endometrial cancer, were used to estimate excess mortality. The calculation of relative survival ratios accounts for competing causes of death. Observed survival for the cohort was based on deaths from all causes. The expected survival proportion was estimated from the Swedish population's life tables stratified by age, sex, and calendar time. Estimates of the expected survival proportions are based on tables of annual probabilities of all-cause mortality in the general Swedish population. We used the Ederer II method [183] for estimating expected survival, in which the matched individuals were considered to be at risk until the corresponding endometrial cancer patient died or was censored. Estimated relative excess hazard ratios (RER), a measure of excess mortality, were modeled in the structure of generalized linear models using Poisson regression, and adjusted for age and calendar time of diagnosis, with never users of MHT as the reference group.

RESULTS

PAPER I

Of all 1135 women exposed to any hormonal treatment, 24% remained nulliparous and 76% were parous following the end of treatment. A total of 67% of women presented with ovulatory dysfunction; 5% for mechanical factors; and 28% for other sub-fertility related factors. The median age at diagnosis was 53 years.

Overall, we observed 54 cases of breast cancer in the study cohort (5%), which did not statistically significantly exceed those expected numbers derived from the general population of Swedish women after adjustment for attained age, calendar period of breast cancer diagnosis, age at first birth and parity [SIR = 1.01 (95% CI: 0.77-1.31)] (Table 1). All rates where only attained age and calendar period of diagnosis were adjusted for yielded higher incidence ratio estimates in all groups compared with the fully adjusted rates for additional parity and age at first birth. Predominantly, exclusive users of the CC only therapy and users of combined CC and gonadotropins therapy had slightly elevated risks, albeit non-significantly, [SIR = 1.15 (95% CI: 0.73-1.80)]; and [SIR = 1.28 (95% CI: 0.87-1.88)] respectively. Users of gonadotropins only therapy had a 47% decreased risk of breast cancer which was significant. When investigating the effects of CC by dose, users of high dose CC had an almost two-fold increased risk of breast cancer [SIR = 1.90 (95% CI: 1.08-3.35)]. In women treated with any hormonal treatment, the absolute risk of developing breast cancer overall was 7.6% by age 70 years.

Overall women referred for non-ovulatory reasons had a 32% non-significant excess risk of breast cancer associated with any exposure to hormonal treatment, compared with a 18% non-significantly reduced risk among women referred for ovulatory reasons. All estimates for type of hormonal treatment and dose cycles were consistently higher among women referred for non-ovulatory factors compared to those referred for ovulatory factors. Women referred for non-ovulatory factors had a 3 fold increased risk of breast cancer after four or more cycles of CC only [SIR = 3.00 (95% CI: 1.35-6.67)]. Women referred for ovulatory factors had a 64% reduced risk of breast cancer with exclusive treatment of gonadotropins [SIR = 0.36 (95% CI: 0.14-0.97)] (Table 2).

TABLE 1:

Standardized incidence ratios and 95% CI for breast cancer among women undergoing hormonal infertility treatment in Sweden between 1961 and 1976, for total follow-up period to Dec. 31, 2004, by exposure to hormonal treatment

Variable	Observed cases (n)	SIR (95% CI)[a]
Any exposure to hormonal treatment	54	1.01 (0.77-1.31)
Type		
CC only	19	1.15 (0.73-1.80)
Gonadotropins only	9	0.53 (0.28-1.00)
Both (CC and gonadotropins)	26	1.28 (0.87-1.88)
Dose cycles (n)		
CC only, low (1-3)	7	0.80 (0.38-1.68)
CC only, high (4+)	12	1.90 (1.08-3.35)
Gonadotropins only, low (1-3)	4	0.49 (0.18-1.32)
Gonadotropins only, high (4+)	5	0.63 (0.26-1.51)

CC, clomiphene citrate; *CI*, confidence interval; *SIR*, standardized incidence ratios.

[a] Rates adjusted for attained age, calendar period of breast cancer diagnosis, total parity, and age at first term birth.

TABLE 2:

Breast cancer among women undergoing hormonal infertility treatment in Sweden between 1961 and 1976

Variable	Ovulatory factors		Nonovulatory factors	
	Observed cases, n[a]	SIR (95% CI)[a,b]	Observed cases, n[c]	SIR (95% CI)[b,c]
Any exposure to hormonal treatment	28	0.82 (0.57-1.19)	26	1.32 (0.90-1.94)
Type				
CC only	9	0.96 (0.50-1.85)	10	1.38 (0.74-2.57)
Gonadotropins only	4	0.36 (0.14-0.97)	5	0.85 (0.35-2.04)
Both (CC and gonadotropins)	15	1.10 (0.66-1.82)	11	1.68 (0.93-3.06)
Dose cycles (n)				
CC only, low (1-3)	3	0.70 (0.22-2.16)	4	0.90 (0.34-2.41)
CC only, high (4+)	6	1.39 (0.62-3.10)	6	3.00 (1.35-6.67)
Gonadotropins only, low (1-3)	2	0.49 (0.12-1.96)	2	0.50 (0.12-1.99)
Gonadotropins only, high (4+)	2	0.31 (0.08-1.24)	3	1.99 (0.64-6.16)

CC, clomiphene citrate; *CI*, confidence interval; *SIR*, standardized incidence ratios.

[a] Ovulatory factors include: diagnosis of anovulation or amenhorrhea, oligomenorrhea or polycystic ovary syndrome/Stein-Leventhal syndrome, and hirsutism.
[b] Rates adjusted for attained age, calendar period of breast cancer diagnosis, total parity, and age at first term birth.
[c] Nonovulatory factors include: endometriosis, abnormalities in the fallopian tubes or uterus, habitual abortions, immunologic factors, and other cervical factors with unexplained fertility.

PAPER I I

Among the 799 patients studied, 347 died from ovarian cancer or related causes and 22 died for other reasons after 5 years of follow-up. There were 649 cases of EOC and 150 cases with BOT, and they were analyzed separately.

Invasive Epithelial Ovarian Cancer (EOC)
After 5 years of follow-up, 290 (45%) of the 649 women with EOC were alive and 359 dead: 344 deaths were due to ovarian cancer and 22 were due to other causes. In the following only results from the *cause-specific analyses* will be reported in detail, as they did not differ substantially from the overall mortality.

As expected, elderly women had a poorer survival, while use of oral contraceptives, BMI before diagnosis, smoking, age at menarche and menopause, parity, family history of ovarian cancer, and tubal ligation were unrelated with survival.

A significantly better survival was evident in women who were diagnosed through their routine gynecological examination [hazard ratio (HR) = 0.47, 95% CI = 0.29 to 0.76)], compared to women that were diagnosed primarily through the presentation of symptoms. The highest probability of death was observed in women with a FIGO stage IV tumor (HR = 13.82, 95% CI = 8.99 to 21.26) relative to those presenting with a FIGO stage I tumor. Compared to women with well differentiated tumors (according to the WHO grade of differentiation classification), women with moderately and poorly differentiated tumors had a worst survival (HR = 2.46, 95% CI = 1.49-4.06; and HR = 3.94, 95% CI = 2.46 to 6.31 respectively). For residual tumor size after primary surgery, women with tumors greater than two centimeters had 1.43 (95% CI = 0.99 to 2.08) times the probability of dying from ovarian cancer compared to women with a residual tumor size less than two centimeters. However, the greatest probability of death was observed in women whose tumors were non-measurable due to difficulties in quantifying the residual tumor mass at time of surgery (HR = 2.32, 95% CI = 1.57 to 3.44). The majority of the ovarian tumors was of serous subtype (n=326), followed by endometrioid (n=168), mucinous (n=62) and other types (n=79). Women with mucinous type of ovarian tumor had a slightly better survival than women with other histological types of EOC.

Use of HRT before epithelial ovarian cancer diagnosis
In total, HRT was used by 166 women (26%) before EOC diagnosis. Overall, there were no clear differences in EOC survival between women that had used any type of HRT compared to never users (multivariate adjusted HR 0.83; 95% CI = 0.65 to 1.08) (Table 3).

Use of different types of HRT before diagnosis (exclusive users of estrogen, estrogens with cyclically added progestins, estrogens with continuously added progestins and combined estrogens and progestins) was not associated with EOC survival. Duration or recency of use of HRT before diagnosis – considered separately or in combination – were not associated with survival. The majority of women (68%) who had ever used HRT had done so in the year preceding ovarian cancer diagnosis.

There was no clear difference in risk of death between exclusive and nonexclusive users of any type of HRT. However, the patterns observed for estrogen only (non-exclusive use), estrogen with continuously added progestins (both for exclusive and non-exclusive use) and combined estrogen-progestin (non-exclusive use) are suggestive of better survival in users -albeit non significant.

Use of estriol (administrated orally or vaginally) before diagnosis was rare, and not associated with EOC survival. In the following we will present results on HRT use overall and according to different combinations disregarding use of estriol.

The proportion of HRT users and non-users before diagnosis was similar among women being diagnosed with different tumor FIGO stages (stage I=29% users, 28% non users; stage II=13% users,11% non users; stage III=46% users, 46% non users; stage IV=12% users, 14% non users) WHO grade of differentiation (well differentiated=13% users, 14% non-users; moderately differentiated=26% users, 26% non users; poorly differentiated=54% users, 51% non users, not stated=8% users, 9% non users) and histological ovarian tumor subtypes. Diagnosis through routine gynecological examination was more frequent among users of HRT compared to never users (13.9% and 7.1% respectively), notably for diagnosis of highly differentiated FIGO stage I tumors.

Except for an indication of better 5-year survival among users of HRT diagnosed with serous tumor (HR = 0.69, 95% CI = 0.48 to 0.98 after controlling for FIGO stage and WHO degree of differentiation at diagnosis) no evidence of better survival was observed. When we added an indicator variable for HRT use after diagnosis in this analysis, the confidence intervals of the HR included unity (HR 0.74, 95% CI 0.52-1.08). The analysis of histological subtypes – including a detailed analysis of serous tumors -according to duration of use of HRT before diagnosis (never, < 3 years, 3 or more years of HRT use) and recency of use – analysed separately or in combination - did not reveal any clear patterns of association We also added an indicator variable for histological type in the models for all ovarian cancer together in relation to all types of HRT grouped, and the results were basically unchanged.

Use of HRT after diagnosis and EOC

Women who were prescribed HRT *after* tumor diagnosis (44%) were all below 60 years of age. Users of HRT *after* an EOC diagnosis were at a significantly lower risk of dying compared to never users after diagnosis (multivariate HR = 0.57, 95% CI 0.42-0.78 when adjusting for age at diagnosis, tumor stage and grade of differentiation). Results did not change substantially when an indicator variable for the histological type was added in the models of all invasive ovarian cancers considered together (HR=0.61; 95% CI=0.45-0.84). The better survival was observed for women with serous tumors (multivariate HR 0.65; 95% CI 0.44-0.96) and other tumors (0.23, 95% CI 0.06-0.91) but not clearly for women with mucinous or endometrioid tumors (Table 3).

The finding of a significantly better survival was observed both amongst women who were current users and former users of HRT at time of data abstraction from medical records.

Combined use of HRT before or after diagnosis

We also compared never users of HRT both before and after diagnosis with:

a. Users before diagnosis, never users after diagnosis,
b. Never users before diagnosis, users after diagnosis and
c. Users before and after diagnosis.

The Kaplan Meier five-year survival curves for the combination of use of HRT before and after EOC diagnosis are presented in Figure 7.

Women who were users of HRT after diagnosis had a lower risk of death, regardless of use of HRT before diagnosis. We repeated these analyses for the different histological subtypes of ovarian tumors. Women diagnosed with a serous tumor that had used

HRT both before and after diagnosis had a lower risk of dying within 5 years of diagnosis. The use of HRT both before and after diagnosis did not entail survival advantage for women with mucinous, endometrioid, and other ovarian tumors. However, the number of patients in each subgroup was relatively small, making estimates unstable in some subgroups.

The mean age of women who never used HRT (63,72 years; SD 7,02) was slightly higher than of users of HRT before diagnosis only (61,58 years; SD 7,24), after diagnosis only (58,81 years, SD 7,75), and both before and after diagnosis (58,11; SD 6,26). There was no difference in the proportion of women using chemotherapy among these groups of women. Use of HRT before EOC diagnosis was more common among white-collar workers (above 40%) than among blue-collar workers (about 25%), but use of HRT after diagnosis did not differ substantially after diagnosis according to social class (19% among blue-collar workers and 25% among white-collar workers). However, the addition of indicator variables for socioeconomic status in the models already including age (as a continuous variable) and multivariate models with FIGO stage and WHO grade did not alter risk estimates for survival according to HRT use after diagnosis at all.

BOT and use of HRT before and after diagnosis

Among 150 women with BOT, 140 (93%) survived at least 5 years: 10 women died, 3 of then of ovarian cancer and 7 by other causes. Information on use of HRT before diagnosis was available for 141 women: 64 (45%) never used HRT before or after diagnosis; 29 (21%) used HRT before diagnosis, 72 (51%) used HRT after diagnosis. There were 24 (17%) women who used HRT both before and after diagnosis, 48 women (34%) used after diagnosis only; and 5 women (4%) used HRT before diagnosis only. Of the 10 deaths for any cause occurring among women with BOT, only one had used HRT before diagnosis. The 3 deaths due to ovarian cancer among women diagnosed of BOT none had used HRT before or after diagnosis. The overall mean survival time for women with BOT was above 5 years.

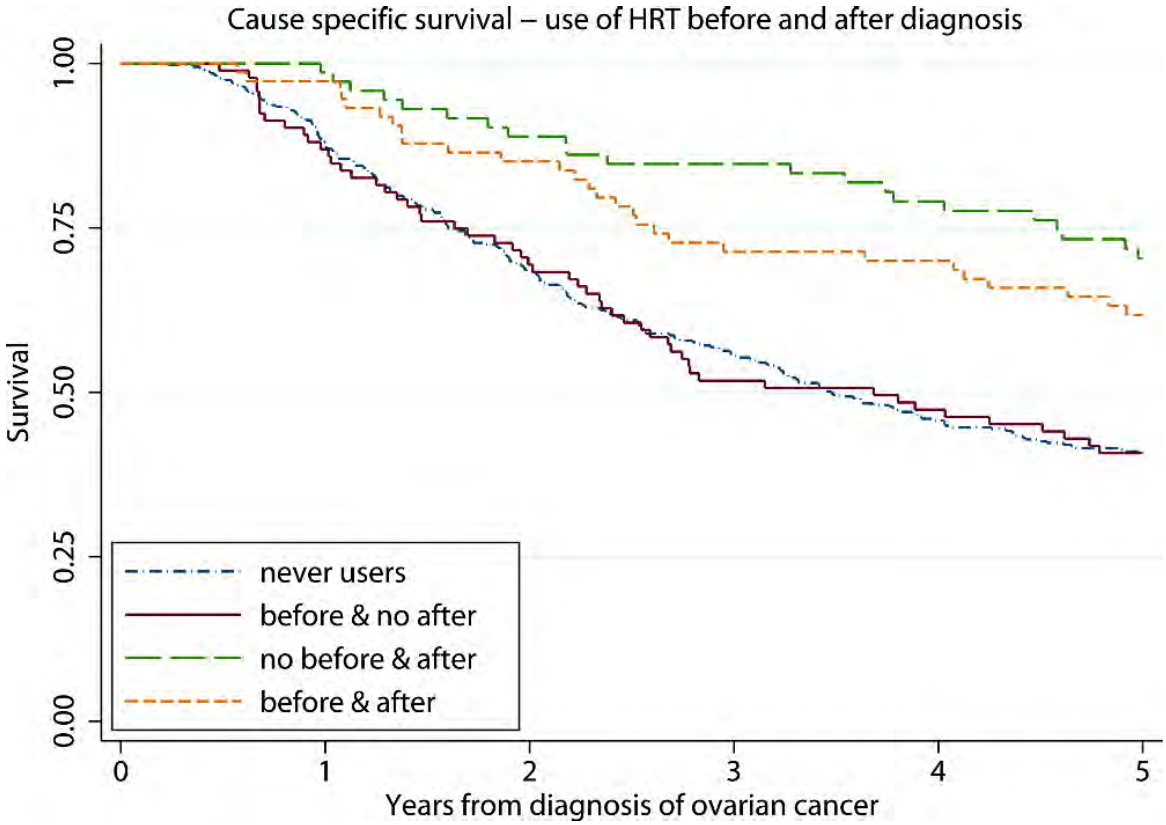

Figure 7: Five-year EOC cause-specific survival according to use of HRT before and after ovarian cancer diagnosis.

TABLE 3:

Use of HRT before and after diagnosis of invasive EOC and survival

HRT use	Cases *n*	Deaths *n* (%)	HR (95%CI)
		Any HRT BEFORE diagnosis:	
Ever	166	82 (49)	0.83 (0.65-1.08)
Serous			
Ever	87	42 (48)	0.69 (0.48-0.98)
		Any HRT AFTER diagnosis:	
Ever	150	51 (34)	0.57 (0.42-0.78)
Current	120	36 (30)	0.55 (0.35-0.87)
Former	30	15 (50)	0.75 (0.62-0.89)

PAPER III

All menstrual factors were analyzed in relation to tumor characteristics and 5-year survival. Cycle length, total lifetime number of menstrual cycles, irregular menstruation, and age at menopause showed no significant trends, with most estimates close to unity. As such, only the results of survival analyses will be presented for these menstrual factors.

The majority of cases experienced menarche between the ages of 11 and 13 years of age and older than 13 and younger than or at 14 years of age (989 and 698 cases respectively), with 9% missing age at menarche. In total, 264 deaths from breast cancer occurred, with 14% of deaths occurring in women with the earliest age at menarche, 9% and 10% with an intermediate age at menarche (an age at menarche of between 11 and 13 years; and older than 13 and younger than or at 14 years, respectively), and 8% with the oldest age at menarche.

Age at menarche and tumor characteristics

Age at menarche was significantly associated with grade and lymph node involvement. Only adjusted models are presented, as unadjusted estimates were virtually unchanged. Women with an age at menarche at 11 years or younger had a greater than 2-fold increased risk for tumors of medium [OR=2.05 (95% CI: 1.00-4.19)] and high [OR=2.04 (95% CI: 1.01-4.16)] grade compared to women with the oldest age at menarche with a low tumor grade. Similarly, women with intermediate ages at menarche had significantly increased risks for medium [OR=1.47 (95% CI: 1.00-2.15) in those older than 11 and younger than or at 13 years; and OR=1.74 (95% CI: 1.15-2.62) in those older than 13 and younger than or at 14 years]; and high grade tumors [OR=1.55 (95% CI: 1.06-2.26) in those older than 11 and younger than or at 13 years; and OR=1.45 (95% CI: 1.00-2.19) in those older than 13 and younger than or at 14 years]. Women with earlier ages at menarche were also at a significant increased risk for having tumors with lymph node involvement [OR=1.49 (95% CI: 1.02-2.19)]; and [OR=1.29 (95% CI: 1.02-1.65)] for the earliest age at menarche, and menarche older than 11 and younger than or at 13 years respectively, compared to those oldest at menarche with no nodal involvement (Table 4).

Menstrual risk factors and survival

Kaplan-Meier survival curves showed significant differences in survival between the youngest and oldest ages at menarche (p-value for log-rank test = 0.0466) Figure 8). Using a Cox-model, survival was poorest in women with the earliest age at menarche, with a 72% increased risk of dying within five years of diagnosis [HR=1.72 (95% CI: 1.02-2.89)]. Cycle length, total lifetime number of menstrual cycles, irregular menstruation, and age at menopause showed no significant trends in survival using Kaplan-Meier method or Cox-modeling. Only adjusted estimates for survival are presented, as unadjusted values were virtually identical (Table 5).

TABLE 4:

Relation of age at menarche to tumor-defined characteristics of breast cancer

	Age at menarche, years Odds ratio (95% CI)[a, b]		
Tumor characteristic	**≤11**	> 11 and **≤13**	> 13 and **≤14**
Grade			
Low			
Medium	2.05 (1.00–4.19)	1.47 (1.00–2.15)	1.74 (1.15–2.62)
High	2.04 (1.01–4.16)	1.55 (1.06–2.26)	1.45 (1.00–2.19)
Lymph node involvement			
Absent			
Present	1.49 (1.02–2.19)	1.29 (1.02–1.65)	1.22 (0.95–1.58)

[a]Reference group: age at menarche of more than 14 years, with the category of best prognosis within each tumor characteristic. [b]Odds ratio estimates adjusted for body mass index at 18 years of age, age at first birth, age at diagnosis, and ever use and type of menopausal hormone therapy (never users, exclusive estrogen therapy, and combined estrogen-progestin therapy). ER, estrogen receptor; PR, progesterone receptor.

TABLE 5:

Breast cancer-specific five-year survival in relation to menstrual factors

Menstrual factor	Deaths	Mortality rate[a]	Hazard ratio (95% CI)[b]
Age at menarche, years[c]			
> 14	46	1.81	1.00 (reference)
> 13 and ≤14	71	2.19	1.26 (0.86–1.84)
> 11 and ≤13	89	1.93	1.14 (0.79–1.65)
≤11	23	2.99	1.72 (1.02–2.89)

[a]Breast cancer deaths per 100 person-years. [b]All menstrual factor hazard ratio estimates adjusted for age at first birth, age at diagnosis, ever use and type of menopausal hormone therapy (never users, exclusive estrogen therapy, and combined estrogen-progestin therapy).[c]Age at menarche hazard ratio estimates additionally adjusted for body mass index at 18 years of age.

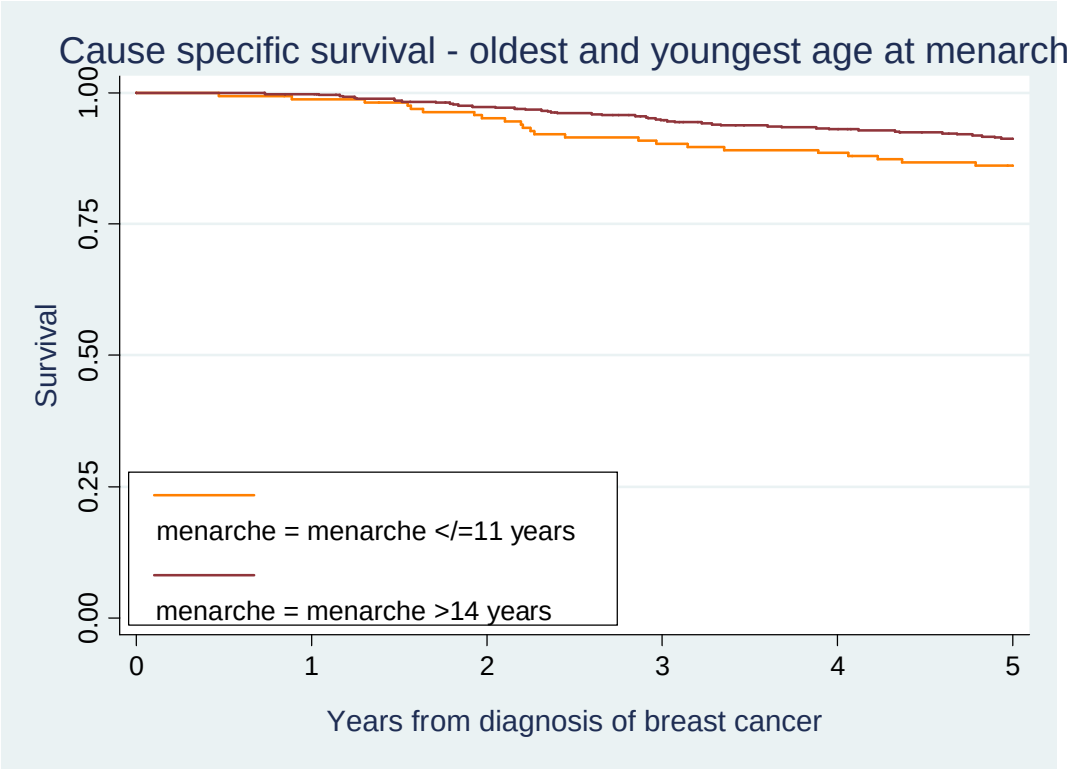

Figure 8: Five-year breast cancer cause-specific survival according to the youngest and oldest ages at menarche.

PAPER IV

Age at diagnosis was significantly associated with use of all forms of MHT, with the exception of estrogens. Ages at menarche, last birth and natural menopause were not associated with use of any MHT. A low body mass index was also significantly associated with increased use of all MHT, except low potency vaginal estrogens. Medium potency MHT was more commonly used by non-smokers than smokers.

Ever use of MHT and tumor characteristics

We found ever use of any form of MHT was significantly associated with tumor grade (*p*-value = 0.02). Specific subtypes of MHT showed no significant associations with tumor grade. The depth of myometrial invasion was significantly associated with ever use of any form of MHT (*p*-value = 0.001); estrogens (*p*-value = 0.002); estrogens and progestins (*p*-value = 0.001); and in particular estrogens with cyclic progestins (*p*-value = 0.001).

Overall, ever use of any MHT entailed lower risks of having tumors of moderate and poorly differentiated grade compared to never use. After multivariate adjustment, we found ever users of cyclic estrogens and progestins, and low potency oral estrogens to have significantly lower risks of having the poorest differentiation of tumor grade [OR = 0.23 (95% CI = 0.07-0.73)]; and [OR = 0.44 (95% CI = 0.21-0.91)]; respectively (Table 6).

The protective effect of ever use of MHT against tumors with a potential poor prognosis was observed for the depth of myometrial invasiveness. After multivariate adjustment, we found ever users of any form of MHT; in particular, users of any form of estrogens, combined estrogens and progestins, and cyclic use of estrogens and progestins; to have significantly lower risks of having tumors with the deepest myometrial invasion (Table 6).

MHT and relative survival

Overall, we observed 96 deaths during 3179 person-years at risk during five years of follow-up. We found that all never users of any MHT had slightly lower relative survival ratios at five years. Analyses of the adjusted estimated relative excess hazard ratios revealed significantly improved survival for ever users of any form of MHT [RER = 0.40 (95% CI = 0.16-0.97)]; in particular ever users of any form of estrogens [RER = 0.38 (95% CI = 0.15-0.99)]. All estimates for specific forms of MHT were below unity. We additionally conducted analyses of the adjusted excess hazard ratio model excluding non-endometrioid Type II tumors, and the significance of results remained unchanged (Table 7).

TABLE 6:

Five-year relative survival for postmenopausal women diagnosed with endometrial cancer in relation to MHT use

	TUMOR GRADE Adjusted OR (95% CI)∫▲		MYOMETRIAL INVASION Adjusted OR (95% CI)Σ▲
Use of MHT|	2	3	**≥50% thick/** through serosa
Any form of MHT¶			
No			
Yes	0.82 (0.47-1.45)	0.52 (0.23-1.20)	0.34 (0.17-0.71)
Any form of estrogens¶†∞			
No			
Yes	0.74 (0.42-1.32)	0.59 (0.27-1.31)	0.46 (0.23-0.91)
Estrogens and Progestins¶∞ǂ∫Ω			
No			
Yes	0.89 (0.50-1.57)	0.55 (0.22-1.33)	0.34 (0.15-0.76)
Estrogens with cyclic progestins¶∞∫Ω			
No			
Yes	0.64 (0.34-1.18)	0.23 (0.07-0.73)	0.27 (0.10-0.73)
Low potency vaginal estrogens§			
No			
Yes	0.91 (0.50-1.64)	1.00 (0.48-2.07)	0.67 (0.36-1.23)
Low potency oral estrogens~			
No			
Yes	0.74 (0.45-1.20)	0.44 (0.21-0.91)	0.60 (0.34-1.03)

For details of footnote legend, please refer to tables 3 and 4 of paper IV

TABLE 7:

Five-year relative survival for postmenopausal women diagnosed with endometrial cancer in relation to MHT use

	5-YEAR FOLLOW-UP	
Use of MHT|	Observed number of deaths	Relative Excess Hazard Ratio (95% CI)▲
All 683 cases with 3179 person-years at risk for 5-year follow-up	96	-
Any form of MHT¶		
No	75	1.00 (reference)
Yes	21	0.40 (0.16-0.97)
Any form of estrogens¶†∞		
No	76	1.00 (reference)
Yes	20	0.38 (0.15-0.99)
Estrogens and Progestins¶∞ǂ∫Ω		
No	84	1.00 (reference)
Yes	8	0.17 (0.01-1.96)
Estrogens with cyclic progestins¶∞∫Ω		
No	86	1.00 (reference)
Yes	5	0.23 (0.04-1.47)
Low potency vaginal estrogens§		
No	83	1.00 (reference)
Yes	13	0.95 (0.39-2.29)
Low potency oral estrogens~		
No	78	1.00 (reference)
Yes	18	0.76 (0.34-1.71)

For details of footnote legend, please refer to table 5 of paper IV

DISCUSSION

METHODOLOGICAL CONSIDERATIONS

Study design

In this thesis the epidemiological study designed used throughout was the cohort study. Generally, a cohort comprises a group of people who share a common condition. Cohort studies measure and compare the incidence of disease among exposed and unexposed groups within a cohort. Within a cohort are a group of people exposed to the condition or treatment of interest under investigation and the other group would not be exposed to the condition or treatment of interest; both groups followed up over a period of time to ascertain outcome. If a positive association exists between the exposure and the disease of interest, we would expect the proportion of the exposed group in whom the disease develops i.e., the incidence in the exposed group, would be greater than the proportion of the non-exposed group in whom the disease develops i.e., the incidence in the non-exposed group. The hallmark of cohort studies are these exposed and unexposed groups and within every cohort study there exists two potential ways to generate these two groups:

1 By creating a study population or selecting groups for inclusion in the study based on whether or not they were exposed
2 By selecting a defined population prior to any of its members becoming exposed or prior to the exposures of interest being identified

In my first study the study population was created by selecting two groups of women seeking treatment for infertility; those who were treated with infertility drugs (exposed) and those who did not receive treatment (unexposed). In my second, third and fourth papers the method of generating the cohorts used was by selecting a pre-defined population, in all three studies, this being a population-based selection of all women aged 50 to 74 years with either breast, ovarian or endometrial cancers respectively and the exposure information relating to HRT exposure being collected after these populations were defined.

Cohort studies are generally very expensive and time-consuming for rare diseases. The major problem with the design of cohort studies is that the study population must often be followed up for a long period of time to determine whether the outcome of interest has developed. This was particularly the case with paper I, in which the study began in 1961 with follow up was ending in 2004 to be able to accumulate enough cases with breast cancer. Papers II, III and IV used exposure data from case-control studies and follow up data linked from national Swedish registries. This method of obtaining follow up data was very efficient and involved less time.

Validity

The validity of any epidemiological study is essential if we are to be able to assess the associations under investigation and arrive at conclusions. Validity of a study is the absence of any systematic error that may distort an association under investigation. Generally these systematic errors can be considered bias and confounding.

Selection bias

This bias relates to systematic errors in the selection and participation of the participants under study. In all cohort studies selection bias mainly occurs when participants lost to follow-up have differing risks of developing the disease compared to those whose full follow-up could be determined and when the frequency of loss to follow-up differs across the exposures being studied.

In paper I, a possibility of a selection bias would have been introduced if women seeking treatment for infertility may not have been included in the study due to them seeking treatment outside of the three major infertility clinics in Sweden during the time period and were treated with a different protocol or pharmaceutical drug regimes that differed vastly from those centres included in the study. In papers II and IV, a possibility of selection bias may have been introduced in the parent studies due to non-participation in these parent studies. Non-participation was related to patient's refusal and physician's refusal and it is highly probable that patients with advanced stages of disease may be less likely to participate. However, all comparisons made in these two studies were made among all women who did agree to participate, therefore could be considered internally valid. In paper III, the possibility of selection bias was minimal as the exposures being addressed were all prior to any knowledge of breast cancer, especially with age at menarche, since women who participated in the study could not have differed systematically in their age at menarche and agreement to participate. In paper II, for our results for HRT use after diagnosis, a subtle selection bias may have occurred in which patients with the best overall health status and prognosis as perceived by the treating physician, for example those with a radical and complete surgery including hysterectomy and bilateral salpingo-ophorectomy with omentectomy, were more likely to be prescribed HRT. Finally, in all four studies, very few participants were lost to follow up which would have had a negligible influence on our estimates.

Surveillance bias

If a population with certain exposures in monitored specifically over a period of time, and this monitoring consequently affects the probability of being diagnosed with the disease under investigation, this may introduce a surveillance bias; leading to erroneous estimates of the relative risk. In three of the four studies comprising this thesis which especially address the use of medication for treatment this may be likely. If those women prescribed either HRT or hormone infertility treatment were more closely monitored for future hormonally related cancers, thus leading to an earlier diagnosis of the cancer this would introduce a surveillance bias. This may be possible as women visiting their gynecologists regularly for HRT prescriptions may have regular check ups including manual and palpitation examinations, thus having the possibility of any tumors being diagnosed at an earlier stage. With hormone infertility treatment prescription this may be less likely, considering the age at which women seek treatment for infertility and the relatively short period that infertility treatment may span, coupled with the long interval between seeking hormone infertility treatment and the age at which the incidence of breast cancer usually peaks.

Confounding

A problem in most epidemiological studies is that an association may be observed and the researcher may be tempted to jump to conclusions about a causal inference when, in fact, the relationship may not be causal. The exposure and the outcome may be associated with another factor which gives a false impression of a true association. This

additional factor could be considered a distortion of effects and needs to be taken into account in the design and analyses of epidemiological studies. For a factor to be considered a confounder of an association between exposure and outcome it needs to be associated with the exposure independently, associated with the outcome independently and not an intermediate in the causal pathway. To be able to examine if an association is confounded by other factors, one needs a priori knowledge of disease etiology and associated factors. If this is known and measured in the design of a study, it can be adjusted for in the analyses. Finally, it should be understood that confounding is by no means an error, rather, a true phenomenon that is identified in a study. However, failure to take into account the measurement and control in the interpretation of the findings is a sure error and will certainly bias any conclusions drawn from a study.

In paper I, confounding the association between infertility treatment and the subsequent risk of breast cancer were parity and age at first birth, since the estimates changed significantly once adjustment had been made for these confounders. However, in this study we were unable to account for the confounding effects of obesity and the use of HRT; both known to be associated with the use of hormone infertility drugs and independently associated with breast cancer. In the second study confounding the association between the use of HRT and ovarian cancer survival were the factors of age at diagnosis, stage and grade of ovarian tumors. Hence, controlling for these factors was carried out in the analyses. We additionally controlled for the histological subtypes of tumors as we believed these to potentially confound our assocation; however, the estimates remained virtually unchanged implying that the effect of histology was not confounding our findings. In paper III, confounding the association between menstrual factors and breast cancer survival were the effects of age at first birth, age at diagnosis, ever use and type of HRT for all menstrual factors; as well as body mass index at 18 years of age which we used as a proxy for childhood obesity in the association between age at menarche and breast cancer prognosis. In this study, we cannot exclude the possibility that age at menarche could be an intermediary in the association between childhood BMI and prognosis. However, controlling for BMI at diagnosis did not attenuate the observed effects of age at menarche on tumor characteristics and survival. Similarly we were able to adjust our analyses for known confounding effects in paper IV; investigating the association between MHT use and endometrial cancer survival.

Random error

Precision is fundamental in epidemiological studies if we are to make accurate conclusions. Random error in any study occurs by chance alone and can lead to false associations and incorrect conclusions. In any epidemiological study there will always be the possibility that significant associations could occur purely by chance. To reduce the probability of spurious associations by chance a factor contributing to minimizing the risk of such random error is a large sample population. Firstly, a study should be sufficiently large to provide a certain degree of confidence in the estimates generated. Likewise, for all sub-group analyses within any study population, there should be sufficient numbers to test associations.

In all of the studies in this thesis and to various degrees, we encountered the problem of having insufficient numbers to assess the associations with further stratification in subgroups. However, the problem was particularly present in papers I and IV. In paper I, we observed only 54 cases of breast cancer which severely limited our conclusions

within the subgroup analyses of dosage, as measured by cycles of treatment when stratifying by the underlying cause of infertility. In paper IV, the problem of insufficient numbers was evident in subgroup analyses of particular regimes of HRT use, limiting the conclusions that could be drawn based on duration and recency of use of HRT.

External validity

In epidemiological research, given that a study is internally valid, the ultimate objective is to be able to generalize the results beyond that of just the study population; to other heterogeneous external populations.

In study I, despite its' population based approach; the external validity of the findings is somewhat limited. The findings among exclusive users of CC and gonadotropins therapy may not be directly generalizable to the currently used methods for treating infertility today, with advanced assisted reproductive technologies, with regard to the preparation, cumulative dosage, concurrent surgical treatment and timing of administration. Studies II, III and IV were all population based and as such could be considered representative of the Swedish population. However, caution should be taken when extending the results of these findings, particularly with the interpretation of the use of HRT and ovarian and endometrial cancer tumor characteristics and survival. Compared to studies from the US, European studies have found greater risk estimates which may be partly explained by the differences in HRT preparations in the two regions, additionally, interactions may be present with genes, lifestyle and environment, which may differ vastly depending on geographical and economical factors among countries. Similarly, in paper III which assessed menstrual factors and breast cancer prognosis, it is known that women from developed countries have an early menarche than those from developing countries due to nutrition and lifestyle patterns, as well as the genetic variation present globally for age at menarche.

FINDINGS AND IMPLICATIONS

Hormone infertility treatment and the risk of breast cancer

In Study I, we observed no overall increased risk for breast cancer in the 1135 women exposed to hormone infertility treatment. However, we consistently observed significantly increased risks in breast cancer incidence among those women who were administered high doses of CC exclusively. Stratification by reasons for referral concluded a significant excess risk among exclusive users of high dose CC therapy referred for non-ovulatory factors. We additionally observed a reduced risk of breast cancer among those women who were exclusive users of gonadotropins therapy; however, these results had limited statistical power.

The effects of CC have been reported to increase serum estradiol and progesterone levels in stimulated menstrual cycles [184]. Based on the finding of an excess risk of breast cancer with CC treatment in this and other studies [185-187], we hypothesize that high dose CC has the potential to increase oestrogen, and ultimately progesterone in normal ovulating women, resulting in a possible increased risk of breast cancer.

The notion of fertility drugs and underlying causes of infertility being two independent risk factors for breast cancer [79, 188-192] is supported by our findings. Hhigher risks observed among women referred for non-ovulatory reasons compared with ovulatory reasons, indicates that the drug therapies may be acting on normal intrinsic hormone levels for women referred with non-ovulatory factors. Any elevation in estrogen and progesterone levels attributable to drug treatment could hypothetically further increase oestrogen and progesterone proliferative action on breast tissue [193]. On the other hand, women referred for ovulatory factors have had irregular or absent menstrual cycles, and consequently reduced cumulative exposure of oestrogen and progesterone on breast proliferation [193] Thus, any increase in oestrogen and progesterone levels attributable to drug therapy in these women could possibly mimic normal breast proliferative activity. Therefore, despite all women receiving similar hormonal treatment for infertility, it must be highlighted that not all women were similar endocrinologically; as women with ovulatory disturbances may have a hormonal milieu very different to that of women referred for non-ovulatory factors.

A reduction in breast cancer risk among exclusive users of gonadotropins therapy was another finding of this study. These findings are consistent with those first reported by Russo and colleagues in their series of studies on mammary carcinogenesis in rodents [194-196]. They established that a term pregnancy resulted in significant protection against chemically carcinogenic induced malignant transformation [34]. Human chorionic gonadotropins, administered in virgin rats has shown a dose-dependent reduction in tumor incidence and number of tumors; leading to the authors' implication of a protective role in mammary carcinogenesis and a hormonal approach to prevention of breast cancer [197].

The results of this study indicate that multiple cycles of CC hormonal infertility treatment may have the possibility to induce hormone levels high enough to potentially affect subsequent breast cancer risk depending on the underlying cause of infertility. Given the current prevalence and intensity of infertility treatments, breast cancer risk should be a consideration in follow up women treated with multiple cycles of CC therapy.

HRT use before and after ovarian cancer diagnosis and survival

Study II found no clear differences in EOC survival between ever users of any type of HRT and never users. There was an indication of better survival among ever users of HRT diagnosed with serous EOC, although without any clear patterns according to duration and recency of use. For endometrioid EOC – for which results from a few studies have suggested a causal association with HRT [41] – we found no evidence of an association between HRT use before diagnosis and survival. Similarly, no indication of better survival was observed for any other histological subtypes of EOC with HRT use before diagnosis.

Our results do not corroborate findings from previous studies [110, 161] suggesting a worsened prognosis among women using estrogens alone before ovarian cancer diagnosis. We observed no effect, regardless of duration or recency of estrogen use before ovarian cancer diagnosis. Our findings of no association between use of HRT prior to diagnosis and survival appear novel.

Among gynecologic oncologists in Sweden and elsewhere, estrogens without progestins have been the most common type of hormone therapy prescribed among women diagnosed with ovarian cancer [198]. We found an indication of better survival among women who used HRT after diagnosis, particularly among patients with serous and endometrioid histological types. Despite accounting for a younger age distribution in our analyses, we cannot exclude that our results may reflect a subtle selection process that could not be accounted for in our analysis.

The biological mechanisms through which HRT used after ovarian cancer diagnosis may act to influence tumor growth, and ultimately survival remains unclear.

Our findings on use of HRT after ovarian cancer diagnosis contradict a few previously published studies. In one study investigating HRT use after ovarian cancer surgery, no significant difference in disease free survival was found between HRT users and non-users [159]. Similarly, a smaller prognosis study [158] found HRT after diagnosis not to influence progression of EOC. In a small randomized controlled trial no significant differences were found in the disease free interval or overall survival according to use of estrogens after surgery [160].

In conclusion, given that ovarian cancer mortality rates are decreasing while incidence rates seem to have stabalized [199], more women are surviving for many years with a diagnosis of ovarian cancer. Therefore, assessing the risk-benefit and safety of use of HRT after diagnosis is of relevance from the patients' perspective.

Menstrual risk factors and breast cancer prognosis

In Study III we found age at menarche to be significantly associated with tumor grade, and lymph node involvement. Consistently, an age at menarche of 11 years or younger had the poorest survival. We did not find any associations for cycle length, irregular menstruation, lifetime number of menstrual cycles, and age at menopause, with tumor characteristics or survival.

This study is the first study to investigate menstrual factors of age at menarche, cycle length, irregular menstruation, lifetime number of ovulatory cycles, and age at menopause with tumor characteristics as the outcome. Our findings of significantly

greater risks of higher grade tumors, and the presence of lymph node involvement with earlier ages at menarche appear novel.

Early age at menarche is a well established risk factor for breast cancer [200]. This may be linked to a greater exposure to estrogens, which are promoters of breast cancer [201], as women with an early age at menarche have long-term increases in serum estradiol and lower serum sex hormone binding globulin (SHBG) concentrations than women with a late age at menarche [202]. These hormone levels prevail throughout the second and third decades of life [202]. We hypothesize possible similar mechanisms acting on breast carcinogenesis at earlier ages of menarche to influence the development and programming of tumors.

It should be emphasized that menarche is only the culmination of a series of hormonal, somatic and anthropometric changes [203] and that early puberty, breast development and menarche follow a naturally occurring process, predetermined by a biological clock that once initiated, turns on a rather independent process of breast development and maturation [204]. Puberty is a critical period in breast carcinogenesis [205] possibly explained by a very high number of terminal duct lobular units, that is, the functional units of the breast with the greatest proliferative activity [206], and the origin of most cancers [207]. Frazier, hypothesized that rapid physical growth during adolescence gives less time for repair of DNA, and thereby, permanent DNA damage with a carcinogenic potential [208]. A recent study by Ahlgren and colleagues [209] showed that after accounting for the growth patterns during childhood and adolescence, age at menarche was not related to risk of breast cancer. Similarly, we hypothesize that we cannot rule out the effects of puberty and growth during childhood and adolescence on the impressionable breast, and that it is during this critical time window of susceptibility not only breast carcinogenesis is initiated, but tumor biology and prognosis determined.

Furthermore, our findings of no association for the total number of lifetime menstrual cycles a woman experiences with tumor characteristics and survival, lends further support to the hypothesis of a critical time window of susceptibility acting on breast carcinogenesis and prognosis; as opposed to the “estrogen augmented by progesterone” hypothesis [193] of cumulative lifetime exposure to estrogens and progesterone through regular ovulatory cycles, since all estimates were close to unity.

In conclusion, we found age at menarche to be significantly associated with grade and lymph node involvement, and survival to be poorest in women with the earliest age at menarche. The finding of this study is timely due to the decreasing age at menarche in most developed countries, and emphasizes the importance of potential early life influences on breast cancer tumor characteristics and survival.

MHT and endometrial cancer prognosis

The findings from study IV indicate that ever use of any MHT seemed to induce less aggressive tumors with consequently better survival observed among ever users compared to never users. We found stronger associations of increased 5-year survival among medium potency MHT users than among low potency vaginal and oral estrogens.

Our study’s findings showing that MHT users have less aggressive tumors are in agreement with all previous studies investigating MHT and endometrial tumor characteristics, despite methodological differences [163, 165-169], with the exception of

two studies [101, 168]. Similarly, our findings of significantly better survival among users of MHT are consistent with the majority of other studies [163-165, 169-171]. Contrastingly, some studies found an increased mortality with MHT use [172, 173].

Previously, it has been argued that the findings of improved survival among users of MHT could be attributable to earlier detection of tumors due to increased medical surveillance among ever users of hormone therapies [163, 164], or hormone therapy users being from more socio-economically advantaged classes with greater accessibility to health care [164]. In our study, we utilized data from Sweden with the unique equitable nature of the Swedish health care system, which would be less prone to inequalities in health and health care evident in other European countries [210-212]. In recent comparisons of 23 European countries using the EUROCARE study investigating cancer patient survival, Sweden had the second highest 5-year relative survival for breast and ovarian cancers [210, 212]. Similarly, in a recent study investigating the socioeconomic inequalities in health in 22 European countries, Sweden is one of the few countries in Europe with the lowest average rate of death from any cause, and one of the lowest due to causes amenable to medical intervention [211]. Consequently, the improved survival observed in our study would be unlikely to be due to earlier detection of tumors as a result of increased medical surveillance among ever users of MHT.

In this study, we have been able to show findings consistent with the interpretation of a better prognosis for both tumor characteristics and survival with ever use of MHT. Furthermore, when comparing the particular MHT compounds used in this study, for use of any type of medium potency ingested MHT; the estimates for survival are fairly similar and well below unity. In comparison, low potency oral and vaginal estrogens are closer to unity, with low potency vaginal estrogens showing almost no effect. This is biologically plausible given that expected effects would be weaker for lower potency preparations, and the weakest with topical vaginal applications. This additionally provides further evidence of our findings not being due to detection and surveillance bias.

Cancers associated with prior MHT use may result in less aggressive tumors, thereby resulting in potentially improved survival [163]. This hypothesis is supported by the bulk of recent evidence for both breast [213-216] and ovarian [217] cancers, but contrasted by a few studies [113, 218]. Despite the extent of evidence reporting favorable tumor characteristics and survival among users of MHT for endometrial, breast and ovarian cancers, this does not imply that MHT use is safe, simply because of an illusive improvement in survival [163].

In conclusion, the findings of this study show that ever users of MHT have significantly favorable tumor characteristics and improved survival compared to never users of MHT with endometrial cancer. Notwithstanding the bulk of evidence whereby users of MHT have been shown to have improved tumor characteristics and survival for endometrial, breast, and ovarian cancers, evidence to the contrary from large studies has been reported and continues to be cause for concern. Caution is imperative in the decision to prescribe MHT to women seeking relief for climacteric symptoms, and should be based on a thorough work up of female cancer risk factors, as well as the overall health of patients seeking MHT treatments.

CONCLUSIONS

- No overall increased risk for breast cancer was shown with infertility treatment. Women with non-ovulatory causes treated with high-dose CC therapy may have an elevated risk for breast cancer.

- HRT use prior to diagnosis of EOC does not affect 5-year survival, with the exception of a possible survival advantage among women with serous EOC. Women using HRT after diagnosis had a better survival than women who were never users.

- Age at menarche has a significant impact on breast cancer prognosis and survival. It remains to be established if the associations are attributable to age at menarche directly, or associated with the early life physiological events of breast development and carcinogenesis also taking place during childhood, and puberty; as menarche is only the culmination in this series of events.

- Endometrial cancer patients who were ever users of MHT had more favorable tumor characteristics and better survival compared to never users of MHT. These findings support the notion that MHT induces endometrial cancer with less aggressive characteristics.

FINAL REMARKS AND FUTURE RESEARCH

My thesis has evaluated endogenous and exogenous hormonal factors on the risk of breast cancer, survival in ovarian cancer, and tumor characteristics and survival in breast and endometrial cancers. The findings of these studies add new evidence in understanding the etiological mechanisms by which carcinogenesis may act to affect these cancers. What is clear from the findings of this thesis is that these mechanisms are multifarious and complex and that no simple association exists between hormonal exposures and female cancers, since the influence is greatly varied for the incidence of cancer and prognosis of the cancer. Recent findings of HRT in relation to risk provide convincing evidence for an increased risk of breast cancer, ovarian cancer and depending on the type of HRT; increased or decreased risk of endometrial cancer. However, the evidence in relation to HRT and prognosis in these cancers appears to have a favourable impact on tumor characteristics and survival in these cancers. These apparently contradictory patterns indicate the mosaic of mechanisms concerned.

Weighting heavily on today's clinicians' conscience, is the challenging task of treating the woman in her reproductive years seeking help for an inability to conceive naturally, or the postmenopausal patient seeking relief for climacteric symptoms. What is a practising clinical supposed to do? The decision whether or not to prescribe her therapy requires a careful weighing up of the risks and benefits of therapy with specific attention to the individual patient's reproductive and family history of all hormonally dependent female cancers. At present, replacement of ovarian hormones is a declining practice attributable to current credible evidence linking HRT to breast cancer. Until future hormonal therapies are developed and tested, clinicians will continue to face this dilemma.

The findings of this thesis bring with it new and unanswered questions. Specifically, gene expression studies have identified and validated the existence of intrinsic breast cancer subtypes. Future studies on endogenous and exogenous hormonal factors and the interaction with specific breast cancer subtypes, breast cancer oncogenes and proto-oncogenes would shed further light on the molecular pathways of how infertility treatment drugs and HRT acts on breast cancer. Similar studies would be beneficial for ovarian cancer considering we found a significant survival advantage with serous EOC tumors. Genetic causes of endometrial cancer are uncommon; however, having a first-degree relative with endometrial cancer has been associated with double the risk of developing the disease [219]. Studies investigating the effects of HRT on prognosis within these subgroups would be of interest. To assess the effects of hormonal interactions with genetic on molecular cancer susceptibility, large studies with replication are essential. Finally, whether or not the improvements seen in survival with the use of HRT is attributable to surveillance and earlier detection of tumors, a preferred selection of patients with more favourable health characteristics, or hormonally derived mechanisms to result in better tumor biological characteristics and survival, needs to be assessed in large randomized clinical trials for breast, ovarian and endometrial cancers.

ACKNOWLEDGEMENTS

The road to this thesis has been particularly challenging, which without the help, support, guidance and friendship of many people, directly or indirectly, this thesis would have simply been impossible. I would like to thank all the women who participated in my studies, for your willingness and taking the time in your lives to help us in getting one step closer to understanding and hopefully one day, eradicating your cancer. These women are a humbling reminder of the reality of cancer. Particularly, I wish to express my deepest and sincere gratitude to the following people:

Kamila Czene my principal supervisor, for believing in me 100% all the way throughout this journey. Thank you for teaching me about science and epidemiology and to always view things critically and ensure tenacity in the search for scientific truth. Thank you for all your hard work and efforts that you have put in over the years; without you this thesis simply would not be possible. You are truly my inspiration. Most importantly, thank you for your warmth, honesty and wonderful friendship and our non-scientific chats about anything and everything.

Per Hall my co-supervisor. Thank you for believing in me and my scientific competence and your unfailing support and guidance through the years. You have certainly shown me what creativity and enthusiasm can do in science! Thank you for coordinating such an excellent GAME program and giving me fantastic opportunities to learn epidemiology in Singapore and Sweden.

Paul Dickman my co-supervisor, for welcoming me to MEB and introducing me to the possibilities of a PhD. You are a true statistician genius and excellent teacher, making the most difficult of statistical concepts seem so easy to understand with your pedagogical talent. Thank you for believing in me and your unfailing support throughout, not to mention your great friendship and all the fun times in Singapore, Italy and Stockholm.

Sara Wedrén my co-supervisor, also known as “boss lady”, for your profound knowledge in breast and endometrial cancer and your encouragement, support, guidance and friendship in Singapore. Your ability to view things from a critical perspective continues to incite me. Thank you for welcoming me into your home in Sweden and feeding me lots of yummy Swedish food!

Ann-Sophie Andersson for taking the time to help me understand the CAHRES ovarian study and always answering my questions with a smile.

Mats Lambe, Rino Bellocco, Kjell Bergfeldt, Tomas Riman, Ingemar Persson and Elisabete Weiderpass for valuable experience and collaboration in the ovarian cancer study.

Peter Conner for taking the time to help me to understand the HIT cohort and for valuable comments on the paper.

Jan Holte, Staffan Nilsson and Karin Sundfeldt for your excellent comments and collaboration on the HIT paper.

Sandra Eloranta and Therese Andersson for your remote assistance with teaching me about the practicalities of relative survival and answering my questions so efficiently.

Lena Rosenberg for your deep knowledge and help with all things breast cancer and CAHRES.

Hans-Olov Adami, Nancy Pedersen, and Henrik Grönberg for creating and maintaining an excellent epidemiological empire and giving me the most valuable opportunity to learn from the best.

Katarina and Vahid Shahedi, my best friends in Sweden. Thank you so much for sharing so much with me and for always being there for me in difficult times with your excellent advice and good spirits. Thank you for the wonderful friendship and honesty and for sharing such memorable times in Singapore and Umeå - ice-fishing, saunas, and serving us gourmet restaurant dishes at every meal!

Denny Rönngren loyal and trusted true friend, thank you for all the great fun times in Stockholm, ice-skating trips, biking trips all over Stockholm, ice-cream sessions, lunches and dinners and for all your help and support with just about everything. Thank you for your honesty and our reflections about life and spirituality.

Michaela Prochazka for sharing laughs and friendship.

Kristjana Einarsdóttir and Anthony Gunnell, for your valuable friendship and advice and our recent catch ups and chats in Perth. I will certainly be seeing more of you in the not too distant future!

Linda Lindström for your positive outlook on life, good humor and always offering support and encouragement, especially with good advice on dissertation.

Juhua Luo, Junmei Miao Jonasson, Anna Svensson, Shahram Bahmanyar, Kazem Zendehdel, Kenji Kato, Bo Ding, and Edyta Bajak; fellow GAME pioneers, for sharing the fun times and memorable experiences in Singapore and Sweden.

Åsa Odenbro for friendship and fun times in Stockholm and Singapore and welcoming me to your home and showing me wonderful Swedish hospitality. Especially with your köttbullar and macaroni!

Monica Rundgren for friendship and chats about life and for all your help with doctoral administration, especially from a distance.

Ann Almqvist for corridor chats and help with all grant applications.

Pär Sparén for friendship and fun skating sessions in Stockholm.

Marie Reilly and Yudi Pawitan for sharing exquisite culinary experiences in Stockholm.

Olle Hansson, Gunnar Petersson, Gastón Nuñez and Torbjörn Gustafsson for offering so much support remotely and solving all my IT problems so efficiently.

Marie Dokken and Camilla Ahlqvist for much help with the dissertation process from a distance.

Ove Strind for your understanding and help with all administration tasks.

Endre Kobold for your friendliness and help with all administration.

All fellow colleagues, past and present at MEB, particularly: Christina Hultman, Sven Cnattingius, Elinor Fondell, Anastasia Iliadou, Alex Ploner, Anna Johansson, Sanna Green, Marie Krushammar, Stefano Calza, Camilla Björk, Stefan Johansson, Gunilla Sonnebring, Alexandra Ekman, Niklas Bergvall, Bosse Rydh, Pia Fernberg and Dariush Nesheli who in one way or another have enhanced my time at MEB and made it extremely memorable.

Kee-Seng Chia my boss in Singapore, for your kindness and generosity and coordinating an excellent GAME program from which I have benefited immensely.

Aileen Ng, Eng-Jee Lim, Chee-Wee Ong, Sharon Wee, Xueling Sim, and Chuen Seng Tan for fun and friendship during my time at CME in Singapore.

Therese Shaw, Kay Sauer and Professor Collin Binns at Curtin University for introducing me to the field of epidemiology and biostatistics and arousing enough curiosity in me to pursue a PhD.

Stefan Czene for allowing me to be the “other husband” and taking up so much of your wife at home and on weekends, for much help with IT technicalities and for making me delicious cakes and cookies.

My wonderful friends in Perth, Ania Simonow, Frank Argiroffi, Neil Drouet, Adam Simonow, Simon Ingleson, Hemah Sakarapani, Roshan Perera and Priya Krishnan for true friendship and guaranteed fun times everytime I come back home.

My UK family, Aunty Pat, Uncle Basil, Aunty Gertie, Brian, Christine, June, Andrew and your lovely families, for giving me a much needed break and so much fun and laughter on my trips to the UK. In particular, my cousin Brian for so much help, our wonderful conversations and being such an inspiring person for me.

My parents, Ian and Judy Mascarenhas for all the encouragement and support over the years.

My dear brother Viv, for your unconditional love, support and friendship, for all our conversations about life and love and continuing to keep my mind open in a philosophical and spiritual realm, allowing my continued growth and learning.

Finally, my husband Jérôme and my daughter Célia; Jérôme, I am so thankful for your unconditional love, never-ending patience, support and for showing me the meaning of true love. You are a wonderful father to our daughter and make me so immensely proud of you. Célia, I have been truly blessed to have a daughter like you. Your lovely personality and sweet ways just shine through everyday. Thank you for being such a patient and easy going child, which let me work from home throughout and get things finished on time. When it all got a bit much, your smile and hearty laughs simply put this thesis in perspective.

RÉSUMÉ EN FRANÇAIS

L'état de l'art met en évidence le rôle fondamental des hormones sur le développement et la progression des cancers du sein, des ovaires et de l'endomètre ou de l'utérus. Ces cancers partagent plusieurs caractéristiques hormonales qui sont essentielles pour l'étiologie et les cancers qui en découlent. L'exposition aux oestrogènes et progestérones, tous deux endogènes et exogènes durant la vie de la femme, peut influencer le risque de cancer dans ces organes cibles. Certains facteurs hormonaux et reproductifs influencent le risque de développer ce type de cancers, comme il est également possible qu'ils agissent sur les caractéristiques des tumeurs et de la probabilité de rémission. Cependant, les effets sur le risque et le pronostic relatif à ces cancers peuvent être très différent sur le plan de la cancérogénèse.

Mon travail de thèse a pour objectif d'examiner les rôles des hormones sexuelles endogènes et exogènes sur l'étiologie, le risque et le pronostic (définis par les attributs des tumeurs et le taux de survie) des cancers du sein, des ovaires et de l'endomètre. Il s'articule autour des quatre études suivantes.

La première étude propose d'évaluer l'impact des traitements contre l'infertilité à base de clomiphene citrate (CC), et/ou de gonadotropines sur la fréquence et l'occurrence du cancer du sein. Nous n'avons observé aucune augmentation du risque de cancer avec le traitement pour l'infertilité; en revanche, les femmes présentant des symptômes non ovariens et ayant reçu de fortes doses de CC semblent présenter un risque élevé de cancer du sein.

La seconde étude met en avant le taux de survie à 5 ans chez des patientes ayant un cancer ovarien en fonction du traitement aux hormones de remplacement (HRT) utilisé avant et après le diagnostic. Nous avons mis en évidence que l'utilisation du HRT avant le diagnostic du cancer épithélial des ovaires n'avait aucun effet sur le taux de survie à 5 ans, hormis pour un taux de survie avantageux chez les cancers séreux. Les femmes utilisant le HRT après le diagnostic ont eu de meilleures chances de survie que celles qui ne l'ont jamais reçu.

L'étude III vise à évaluer les effets des facteurs de risque induits par les paramètres menstruels sur les caractéristiques des tumeurs et le taux de survie aux cancers du sein post-ménopause. Nous avons observé que l'âge précoce de la première menstruation est un facteur significatif influençant le diagnostic et le taux de survie.

Enfin, l'étude IV s'est intéressée à l'influence du traitement hormonal de la ménopause (MHT) sur les caractéristiques de la tumeur et le taux de survie relatif pour les cancers post-ménopause de l'endomètre. Les résultats ont montré un meilleur taux de survie chez les patientes traitées que chez les non traitées.

Les résultats majeurs de ces études apportent de nouvelles éléments à la compréhension des mécanisme étiologiques par lesquels la cancérogénèse peut affecter ces cancers. Il ressort clairement que ces mécanismes sont variés et complexes et qu'il n'existe pas de simple association entre l'exposition aux hormones de traitement et les cancers développés par ces femmes, notamment grâce à l'influence contradictoire de l'incidence du cancer et de son pronostic. Ces tendances indiquent donc l'existence une mosaïque de mécanismes impliqués.

REMERCIEMENTS

A mes fabuleux beaux-parents, papa Robert et maman Monique Orgéas. Vous êtes simplement les meilleurs beaux-parents que je puisse souhaiter. Merci de votre affection et soutien inconditionnels dans ces années difficiles, merci de votre aide pour la garde de Célia, pour la maison et pour avoir su rendre les jours plus faciles et confortables. Le plus important, merci de m'aimer comme votre propre fille. Sans vous, cette thèse n'aurait pas été réalisable.

Sébastien Balocco, mon dévoué et patient cousin. Merci de ton amitié et de m'avoir fait me sentir bien en France. Merci également d'être un excellent génie de l'informatique ce qui m'a aidé à résoudre bon nombre de problèmes techniques me permettant de finir cette thèse. Et en plus, tu m'as convertie au monde Mac Apple.

Ma "Mama" Mimi Balocco, merci pour ton amour, ta douceur, ton soutien, ton amitié et tout ce que tu as fait pour m'aider à m'intégrer en France.

Philippe et Maïté Fournier pour votre aide précieuse, votre hospitalité et votre amitié. Vous m'avez aidé dans certaines tâches administratives nécessaires à mon installation en France.

Un grand merci à Michèle, Coco et Romuald Tosato pour leur aide, leur dévouement, leur sympathie et leur amitié. Ils ont été les acteurs précieux de nos moments heureux.

Christine Giner et Lucie Sterling pour votre amitié et pour avoir toujours été présentes et volontaires pour m'aider, quelle que soit la tâche.

Monsieur le Professeur Francis Giraud, soyez remercié de votre soutien et de vos encouragements à finir cette thèse et pour l'aide que vous me proposez dans la recherche d'un emploi dans la recherche médicale.

Cédric et Caroline Banchelin, pour le plaisir, l'amitié et pour avoir partagé avec nous la grande aventure d'être parents. Votre considération et gentillesse m'ont réconforté et j'espère, maintenant que ma thèse est finie, avoir plus de temps à vous consacrer ainsi qu'à Jules.

Franck, Marie, Johanna et Orianne Morgillo. Vous êtes une famille modèle inspiratrice pour nous et merci de la fantastique amitié et les fous-rires que nous avons vécus à Lyon, Marseille et en Corse.

Tati Gisou, Cécile, Dany, Céline, Eric, Enola et Mauge, merci d'être une formidable belle famille, merci de l'amour, du soutien, de l'amitié et de la gentillesse que vous m'avez témoignés depuis toutes ces années.

REFERENCES

1. Persson, I., *Estrogens in the causation of breast, endometrial and ovarian cancers - evidence and hypotheses from epidemiological findings.* J Steroid Biochem Mol Biol, 2000. 74(5): p. 357-64.
2. Riman, T., et al., *Hormone replacement therapy and the risk of invasive epithelial ovarian cancer in Swedish women.* J Natl Cancer Inst, 2002. 94(7): p. 497-504.
3. Parkin, D.M., et al., *Estimating the world cancer burden: Globocan 2000.* Int J Cancer, 2001. 94(2): p. 153-6.
4. IARC, *World Cancer Report 2008*, P. Boyle, Levin, B., Editor. 2008, World Health Organization, International Agency for Research on Cancer: Lyon, France.
5. Chia, K.S., et al., *Profound changes in breast cancer incidence may reflect changes into a Westernized lifestyle: a comparative population-based study in Singapore and Sweden.* Int J Cancer, 2005. 113(2): p. 302-6.
6. Ferlay, J., et al., *Estimates of the cancer incidence and mortality in Europe in 2006.* Ann Oncol, 2007. 18(3): p. 581-92.
7. *Cancer Incidence in Sweden 2004*. 2006, The National Board of Health and Welfare, Centre for Epidemiology, Official Statistics of Sweden.
8. Engholm, G., Ferlay, J., Christensen, N., Bray, F., Gjerstorff, M.L., Klint, Å., Køtlum, J.E., Ólafsdóttir, E., Pukkala, E., Storm, H.H. *NORDCAN: Cancer Incidence, Mortality and Prevalence in the Nordic Countries, Version 3.3.* . 2008 [cited 2009 January 2]; Available from: http://www.ancr.nu.
9. Socialstyrelsen. *Mortality in Sweden*. 2003 [cited; Available from: http://www.socialstyrelsen.se/.
10. Socialstyrelsen. *Cancer Incidence in Sweden 2004*. 2005 [cited; Available from: http://www.socialstyrelsen.se/.
11. Ferlay, J., Bray, F., Pisani, P., Parkin, D.M., *GLOBOCAN 2002: Cancer Incidence, Mortality and Prevalence Worldwide*, in *IARC CancerBase No. 5. version 2.0*. 2004, IARC: Lyon, France.
12. *Cancer Research UK*. 2009 [cited 2009 January 2]; Available from: http://info.cancerresearchuk.org/cancerstats/types/uterus/incidence/.
13. The National Board of Health and Welfare, O.S.o.S. Cancer incidence in Sweden 2000 2002 [cited.
14. Parkin, D.M., P. Pisani, and J. Ferlay, *Estimates of the worldwide incidence of eighteen major cancers in 1985.* Int J Cancer, 1993. 54(4): p. 594-606.
15. Pettersson, F., *Annual Report on the Results of Treatment in Gynecological Cancer*. 1995, International Federation of Gynecology and Obstetrics: Stockholm.
16. Crayford, T.J., et al., *Benign ovarian cysts and ovarian cancer: a cohort study with implications for screening.* Lancet, 2000. 355(9209): p. 1060-3.
17. Petricoin, E.F., et al., *Use of proteomic patterns in serum to identify ovarian cancer.* Lancet, 2002. 359(9306): p. 572-7.
18. Parkin, D.M., et al., *Global cancer statistics, 2002.* CA Cancer J Clin, 2005. 55(2): p. 74-108.
19. Socialstyrelsen. *Cancer i Siffror*. 2005 [cited; Available from: http://www.socialstyrelsen.se/.
20. Key, T.J., P.K. Verkasalo, and E. Banks, *Epidemiology of breast cancer.* Lancet Oncol, 2001. 2(3): p. 133-40.
21. Yager, J.D. and N.E. Davidson, *Estrogen carcinogenesis in breast cancer.* N Engl J Med, 2006. 354(3): p. 270-82.

22. Key, T.J. and P.K. Verkasalo, *Endogenous hormones and the aetiology of breast cancer.* Breast Cancer Res, 1999. 1(1): p. 18-21.
23. Kelsey, J.L., M.D. Gammon, and E.M. John, *Reproductive factors and breast cancer.* Epidemiol Rev, 1993. 15(1): p. 36-47.
24. Hunter, D.J., et al., *Non-dietary factors as risk factors for breast cancer, and as effect modifiers of the association of fat intake and risk of breast cancer.* Cancer Causes Control, 1997. 8(1): p. 49-56.
25. Adami, H.O., et al., *The effect of female sex hormones on cancer survival. A register-based study in patients younger than 20 years at diagnosis.* JAMA, 1990. 263(16): p. 2189-93.
26. den Tonkelaar, I. and F. de Waard, *Regularity and length of menstrual cycles in women aged 41-46 in relation to breast cancer risk: results from the DOM-project.* Breast Cancer Res Treat, 1996. 38(3): p. 253-8.
27. Kvale, G. and I. Heuch, *Menstrual factors and breast cancer risk.* Cancer, 1988. 62(8): p. 1625-31.
28. Michels-Blanck, H., et al., *Menstrual patterns and breast cancer mortality in a large U.S. cohort.* Epidemiology, 1996. 7(5): p. 543-6.
29. Olsson, H., M. Landin-Olsson, and B. Gullberg, *Retrospective assessment of menstrual cycle length in patients with breast cancer, in patients with benign breast disease, and in women without breast disease.* J Natl Cancer Inst, 1983. 70(1): p. 17-20.
30. Parazzini, F., et al., *Lifelong menstrual pattern and risk of breast cancer.* Oncology, 1993. 50(4): p. 222-5.
31. Whelan, E.A., et al., *Menstrual cycle patterns and risk of breast cancer.* Am J Epidemiol, 1994. 140(12): p. 1081-90.
32. Beral, V. and G. Reeves, *Childbearing, oral contraceptive use, and breast cancer.* Lancet, 1993. 341(8852): p. 1102.
33. Lambe, M., et al., *Transient increase in the risk of breast cancer after giving birth.* N Engl J Med, 1994. 331(1): p. 5-9.
34. Russo, J., et al., *The protective role of pregnancy in breast cancer.* Breast Cancer Res, 2005. 7(3): p. 131-42.
35. Ewertz, M., et al., *Age at first birth, parity and risk of breast cancer: a meta-analysis of 8 studies from the Nordic countries.* Int J Cancer, 1990. 46(4): p. 597-603.
36. Layde, P.M., et al., *The independent associations of parity, age at first full term pregnancy, and duration of breastfeeding with the risk of breast cancer. Cancer and Steroid Hormone Study Group.* J Clin Epidemiol, 1989. 42(10): p. 963-73.
37. Lipworth, L., L.R. Bailey, and D. Trichopoulos, *History of breast-feeding in relation to breast cancer risk: a review of the epidemiologic literature.* J Natl Cancer Inst, 2000. 92(4): p. 302-12.
38. Magnusson, C.M., et al., *The role of reproductive factors and use of oral contraceptives in the aetiology of breast cancer in women aged 50 to 74 years.* Int J Cancer, 1999. 80(2): p. 231-6.
39. *Breast cancer and hormone replacement therapy: collaborative reanalysis of data from 51 epidemiological studies of 52,705 women with breast cancer and 108,411 women without breast cancer. Collaborative Group on Hormonal Factors in Breast Cancer.* Lancet, 1997. 350(9084): p. 1047-59.
40. MacMahon, B., *Epidemiology and the causes of breast cancer.* Int J Cancer, 2006. 118(10): p. 2373-8.

41. Riman, T., S. Nilsson, and I.R. Persson, *Review of epidemiological evidence for reproductive and hormonal factors in relation to the risk of epithelial ovarian malignancies.* Acta Obstet Gynecol Scand, 2004. 83(9): p. 783-95.
42. Riman, T., et al., *Risk factors for epithelial borderline ovarian tumors: results of a Swedish case-control study.* Gynecol Oncol, 2001. 83(3): p. 575-85.
43. Cramer, D.W., et al., *Determinants of ovarian cancer risk. I. Reproductive experiences and family history.* J Natl Cancer Inst, 1983. 71(4): p. 711-6.
44. Risch, H.A., et al., *Differences in risk factors for epithelial ovarian cancer by histologic type. Results of a case-control study.* Am J Epidemiol, 1996. 144(4): p. 363-72.
45. Wittenberg, J., et al., *Reproductive risk factors for mucinous and non-mucinous epithelial ovarian cancer.* Epidemiology, 1999. 10(6): p. 761-3.
46. Fathalla, M.F., *Incessant ovulation--a factor in ovarian neoplasia?* Lancet, 1971. 2(7716): p. 163.
47. Casagrande, J.T., et al., *"Incessant ovulation" and ovarian cancer.* Lancet, 1979. 2(8135): p. 170-3.
48. Stadel, B.V., *Letter: The etiology and prevention of ovarian cancer.* Am J Obstet Gynecol, 1975. 123(7): p. 772-4.
49. Cramer, D.W. and H. Xu, *Epidemiologic evidence for uterine growth factors in the pathogenesis of ovarian cancer.* Ann Epidemiol, 1995. 5(4): p. 310-4.
50. Hankinson, S.E., et al., *Tubal ligation, hysterectomy, and risk of ovarian cancer. A prospective study.* JAMA, 1993. 270(23): p. 2813-8.
51. Miracle-McMahill, H.L., et al., *Tubal ligation and fatal ovarian cancer in a large prospective cohort study.* Am J Epidemiol, 1997. 145(4): p. 349-57.
52. Adami, H.O., et al., *Parity, age at first childbirth, and risk of ovarian cancer.* Lancet, 1994. 344(8932): p. 1250-4.
53. Risch, H.A., *Hormonal etiology of epithelial ovarian cancer, with a hypothesis concerning the role of androgens and progesterone.* J Natl Cancer Inst, 1998. 90(23): p. 1774-86.
54. Hankinson, S.E., et al., *A prospective study of reproductive factors and risk of epithelial ovarian cancer.* Cancer, 1995. 76(2): p. 284-90.
55. Riman, T., I. Persson, and S. Nilsson, *Hormonal aspects of epithelial ovarian cancer: review of epidemiological evidence.* Clin Endocrinol (Oxf), 1998. 49(6): p. 695-707.
56. Granstrom, C., J. Sundquist, and K. Hemminki, *Population attributable fractions for ovarian cancer in Swedish women by morphological type.* Br J Cancer, 2008. 98(1): p. 199-205.
57. Purdie, D., et al., *Reproductive and other factors and risk of epithelial ovarian cancer: an Australian case-control study. Survey of Women's Health Study Group.* Int J Cancer, 1995. 62(6): p. 678-84.
58. Risch, H.A., L.D. Marrett, and G.R. Howe, *Parity, contraception, infertility, and the risk of epithelial ovarian cancer.* Am J Epidemiol, 1994. 140(7): p. 585-97.
59. Danforth, K.N., et al., *Breastfeeding and risk of ovarian cancer in two prospective cohorts.* Cancer Causes Control, 2007. 18(5): p. 517-23.
60. Whittemore, A.S., R. Harris, and J. Itnyre, *Characteristics relating to ovarian cancer risk: collaborative analysis of 12 US case-control studies. II. Invasive epithelial ovarian cancers in white women. Collaborative Ovarian Cancer Group.* Am J Epidemiol, 1992. 136(10): p. 1184-203.
61. Ness, R.B., et al., *Infertility, fertility drugs, and ovarian cancer: a pooled analysis of case-control studies.* Am J Epidemiol, 2002. 155(3): p. 217-24.

62. Southcott, B.M., *Carcinoma of the endometrium.* Drugs, 2001. 61(10): p. 1395-405.
63. Sherman, M.E., et al., *Risk factors and hormone levels in patients with serous and endometrioid uterine carcinomas.* Mod Pathol, 1997. 10(10): p. 963-8.
64. Shiozawa, T. and I. Konishi, *Early endometrial carcinoma: clinicopathology, hormonal aspects, molecular genetics, diagnosis, and treatment.* Int J Clin Oncol, 2006. 11(1): p. 13-21.
65. Lax, S.F., et al., *Clear cell carcinoma of the endometrium is characterized by a distinctive profile of p53, Ki-67, estrogen, and progesterone receptor expression.* Hum Pathol, 1998. 29(6): p. 551-8.
66. Pike, M.C., C.L. Pearce, and A.H. Wu, *Prevention of cancers of the breast, endometrium and ovary.* Oncogene, 2004. 23(38): p. 6379-91.
67. Hale, G.E., C.L. Hughes, and J.M. Cline, *Endometrial cancer: hormonal factors, the perimenopausal "window of risk," and isoflavones.* J Clin Endocrinol Metab, 2002. 87(1): p. 3-15.
68. Xu, W.H., et al., *Menstrual and reproductive factors and endometrial cancer risk: Results from a population-based case-control study in urban Shanghai.* Int J Cancer, 2004. 108(4): p. 613-9.
69. Kvale, G., I. Heuch, and S. Nilssen, *Re: "Endometrial cancer and age at last delivery: evidence for an association".* Am J Epidemiol, 1992. 135(4): p. 453-5.
70. Brinton, L.A., et al., *Reproductive, menstrual, and medical risk factors for endometrial cancer: results from a case-control study.* Am J Obstet Gynecol, 1992. 167(5): p. 1317-25.
71. Hardiman, P., O.C. Pillay, and W. Atiomo, *Polycystic ovary syndrome and endometrial carcinoma.* Lancet, 2003. 361(9371): p. 1810-2.
72. Henderson, B.E., et al., *The epidemiology of endometrial cancer in young women.* Br J Cancer, 1983. 47(6): p. 749-56.
73. *Breast cancer and hormonal contraceptives: collaborative reanalysis of individual data on 53 297 women with breast cancer and 100 239 women without breast cancer from 54 epidemiological studies. Collaborative Group on Hormonal Factors in Breast Cancer.* Lancet, 1996. 347(9017): p. 1713-27.
74. Pike, M.C., et al., *Estrogens, progestogens, normal breast cell proliferation, and breast cancer risk.* Epidemiol Rev, 1993. 15(1): p. 17-35.
75. Banks, E., *Hormone replacement therapy and the sensitivity and specificity of breast cancer screening: a review.* J Med Screen, 2001. 8(1): p. 29-34.
76. Beral, V., *Breast cancer and hormone-replacement therapy in the Million Women Study.* Lancet, 2003. 362(9382): p. 419-27.
77. Chlebowski, R.T., et al., *Influence of estrogen plus progestin on breast cancer and mammography in healthy postmenopausal women: the Women's Health Initiative Randomized Trial.* Jama, 2003. 289(24): p. 3243-53.
78. Beral, V., E. Banks, and G. Reeves, *Evidence from randomised trials on the long-term effects of hormone replacement therapy.* Lancet, 2002. 360(9337): p. 942-4.
79. Venn, A., D. Healy, and R. McLachlan, *Cancer risks associated with the diagnosis of infertility.* Best Pract Res Clin Obstet Gynaecol, 2003. 17(2): p. 343-67.
80. Venn, A., et al., *Risk of cancer after use of fertility drugs with in-vitro fertilisation.* Lancet, 1999. 354(9190): p. 1586-90.
81. Dickey, R.P. and D.E. Holtkamp, *Development, pharmacology and clinical experience with clomiphene citrate.* Hum Reprod Update, 1996. 2(6): p. 483-506.

82. Hsueh, A.J., G.F. Erickson, and S.S. Yen, *Sensitisation of pituitary cells to luteinising hormone releasing hormone by clomiphene citrate in vitro.* Nature, 1978. 273(5657): p. 57-9.
83. Adashi, E.Y., *Ovulation indution: clomiphene citrate.*, in *Reproductive Endocrinology, Surgery, and Technology*, E.Y. Adashi, Rock, J.A. and Rosenwaks, Z, Editor. 1996, J.B.Lippincott: Philadelphia, USA. p. 1181-1206.
84. Fukuma, K., et al., *A graduated regimen of clomiphene citrate: its correlation to glycogen content of the endometrium and serum levels of estradiol and progesterone in infertile patients at the midluteal phase.* Fertil Steril, 1983. 39(6): p. 780-4.
85. Randall, J.M. and A. Templeton, *Transvaginal sonographic assessment of follicular and endometrial growth in spontaneous and clomiphene citrate cycles.* Fertil Steril, 1991. 56(2): p. 208-12.
86. Wallach, E.E., *Chp 30: Induction of ovulation - General concepts*, in *Reproductive Medicine and Surgery*, E.E. Wallach, Zacur, H.A., Editor. 1995, Mosby-Year Book, Inc.: St Louis, Missouri. USA. p. 555-568.
87. Dickey, R.P., *Evaluation and management of threatened habitual first trimester abortion.*, in *Advances in Clinical Obstetrics and Gynecology*, H. Osofsky, Editor. 1984, Yearbook Medical Publishers: Chicago. p. 329-388.
88. Dickey, R.P. and J.F. Hower, *Effect of ovulation induction on uterine blood flow and oestradiol and progesterone concentrations in early pregnancy.* Hum Reprod, 1995. 10(11): p. 2875-9.
89. Baer, G. and E. Loumaye, *Comparison of recombinant human luteinising hormone (r-hLH) and human menopausal gonadotropin (hMG) in assisted reproductive technology.* Curr Med Res Opin, 2003. 19(2): p. 83-8.
90. Stokman, P.G., et al., *Human chorionic gonadotropin in commercial human menopausal gonadotropin preparations.* Fertil Steril, 1993. 60(1): p. 175-8.
91. Hsueh, A.J., et al., *Hormonal regulation of the differentiation of cultured ovarian granulosa cells.* Endocr Rev, 1984. 5(1): p. 76-127.
92. Hillier, S.G., *Current concepts of the roles of follicle stimulating hormone and luteinizing hormone in folliculogenesis.* Hum Reprod, 1994. 9(2): p. 188-91.
93. Lunenfeld, B.a.L., E., *Chp 30: Ovulation induction with human menopausal gonadotropin*, in *Infertility - A Comprehensive Text*, M.M. Seibel, Editor. 1997, Appleton & Lange: Stamford, CT. USA. p. 507-524.
94. Ludwig, M., K.J. Doody, and K.M. Doody, *Use of recombinant human chorionic gonadotropin in ovulation induction.* Fertil Steril, 2003. 79(5): p. 1051-9.
95. Lunenfeld, B., *Historical perspectives in gonadotrophin therapy.* Hum Reprod Update, 2004. 10(6): p. 453-67.
96. Seibel, M.M., *Chp 31: Ovulation induction with follicle stimulating hormone*, in *Infertility - A Comprehensive Text*, M.M. Seibel, Editor. 1997, Appleton & Lange: Stamford, CT. USA. p. 525-536.
97. Seibel, M.M., et al., *Ovulation induction in polycystic ovary syndrome with urinary follicle-stimulating hormone or human menopausal gonadotropin.* Fertil Steril, 1985. 43(5): p. 703-8.
98. Prelevic, G.M., *Chp 5: Hirsutism and the polycystic ovary syndrome*, in *Drug Therapy in Reproductive Endocrinology*, J. Ginsburg, Editor. 1996, Arnold: London. p. 67-85.
99. Prelevic, G.M., Z. Puzigaca, and L.A. Balint-Peric, *Effects of an oral contraceptive containing cyproterone acetate (Diane-35) on the symptoms, hormone profile,*

and ovarian volume of hirsute women with polycystic ovarian syndrome. Ann N Y Acad Sci, 1993. 687: p. 255-62.
100. Collaborative Group on Epidemiological Studies of Ovarian, C., et al., *Ovarian cancer and oral contraceptives: collaborative reanalysis of data from 45 epidemiological studies including 23,257 women with ovarian cancer and 87,303 controls.* Lancet, 2008. 371(9609): p. 303-14.
101. Anderson, G.L., et al., *Effects of estrogen plus progestin on gynecologic cancers and associated diagnostic procedures: the Women's Health Initiative randomized trial.* JAMA, 2003. 290(13): p. 1739-48.
102. Bosetti, C., et al., *Relationship between postmenopausal hormone replacement therapy and ovarian cancer.* JAMA, 2001. 285(24): p. 3089; author reply 3090.
103. Hempling, R.E., et al., *Hormone replacement therapy as a risk factor for epithelial ovarian cancer: results of a case-control study.* Obstet Gynecol, 1997. 89(6): p. 1012-6.
104. Kaufman, D.W., et al., *Noncontraceptive estrogen use and epithelial ovarian cancer.* Am J Epidemiol, 1989. 130(6): p. 1142-51.
105. La Vecchia, C., A. Liberati, and S. Franceschi, *Noncontraceptive estrogen use and the occurrence of ovarian cancer.* J Natl Cancer Inst, 1982. 69(6): p. 1207.
106. Lacey, J.V., Jr., et al., *Menopausal hormone therapy and ovarian cancer risk in the National Institutes of Health-AARP Diet and Health Study Cohort.* J Natl Cancer Inst, 2006. 98(19): p. 1397-405.
107. Lacey, J.V., Jr., et al., *Menopausal hormone replacement therapy and risk of ovarian cancer.* Jama, 2002. 288(3): p. 334-41.
108. Purdie, D.M., et al., *Hormone replacement therapy and risk of epithelial ovarian cancer.* Br J Cancer, 1999. 81(3): p. 559-63.
109. Risch, H.A., *Estrogen replacement therapy and risk of epithelial ovarian cancer.* Gynecol Oncol, 1996. 63(2): p. 254-7.
110. Rodriguez, C., et al., *Estrogen replacement therapy and ovarian cancer mortality in a large prospective study of US women.* Jama, 2001. 285(11): p. 1460-5.
111. Weiss, N.S., et al., *Noncontraceptive estrogen use and the occurrence of ovarian cancer.* J Natl Cancer Inst, 1982. 68(1): p. 95-8.
112. Zhou, B., et al., *Hormone replacement therapy and ovarian cancer risk: a meta-analysis.* Gynecol Oncol, 2008. 108(3): p. 641-51.
113. Beral, V., et al., *Ovarian cancer and hormone replacement therapy in the Million Women Study.* Lancet, 2007. 369(9574): p. 1703-10.
114. Mahdavi, A., T. Pejovic, and F. Nezhat, *Induction of ovulation and ovarian cancer: a critical review of the literature.* Fertil Steril, 2006. 85(4): p. 819-26.
115. Kashyap, S., et al., *Assisted reproductive technology and the incidence of ovarian cancer: a meta-analysis.* Obstet Gynecol, 2004. 103(4): p. 785-94.
116. Brinton, L.A., et al., *Ovarian cancer risk after the use of ovulation-stimulating drugs.* Obstet Gynecol, 2004. 103(6): p. 1194-203.
117. Harris, R., A.S. Whittemore, and J. Itnyre, *Characteristics relating to ovarian cancer risk: collaborative analysis of 12 US case-control studies. III. Epithelial tumors of low malignant potential in white women. Collaborative Ovarian Cancer Group.* Am J Epidemiol, 1992. 136(10): p. 1204-11.
118. Rossing, M.A., et al., *Ovarian tumors in a cohort of infertile women.* N Engl J Med, 1994. 331(12): p. 771-6.
119. Sanner, K., et al., *Ovarian epithelial neoplasia after hormonal infertility treatment: long-term follow-up of a historical cohort in Sweden.* Fertil Steril, 2008.

120. Key, T.J. and M.C. Pike, *The dose-effect relationship between 'unopposed' oestrogens and endometrial mitotic rate: its central role in explaining and predicting endometrial cancer risk.* Br J Cancer, 1988. 57(2): p. 205-12.
121. Weiderpass, E., et al., *Use of oral contraceptives and endometrial cancer risk (Sweden).* Cancer Causes Control, 1999. 10(4): p. 277-84.
122. Grady, D., et al., *Hormone replacement therapy and endometrial cancer risk: a meta-analysis.* Obstet Gynecol, 1995. 85(2): p. 304-13.
123. Bernstein, L., *The risk of breast, endometrial and ovarian cancer in users of hormonal preparations.* Basic Clin Pharmacol Toxicol, 2006. 98(3): p. 288-96.
124. Beral, V., D. Bull, and G. Reeves, *Endometrial cancer and hormone-replacement therapy in the Million Women Study.* Lancet, 2005. 365(9470): p. 1543-51.
125. Weiderpass, E., et al., *Risk of endometrial cancer following estrogen replacement with and without progestins.* J Natl Cancer Inst, 1999. 91(13): p. 1131-7.
126. Genant, H.K., et al., *Low-dose esterified estrogen therapy: effects on bone, plasma estradiol concentrations, endometrium, and lipid levels. Estratab/Osteoporosis Study Group.* Arch Intern Med, 1997. 157(22): p. 2609-15.
127. Haenszel, W.M., *Contributions of End Results Data to Cancer Epidemiology.* Natl Cancer Inst Monogr, 1964. 15: p. 21-33.
128. Schneider, J., et al., *Antiestrogen action of 2-hydroxyestrone on MCF-7 human breast cancer cells.* J Biol Chem, 1984. 259(8): p. 4840-5.
129. Lonning, P.E., et al., *Influence of plasma estrogen levels on the length of the disease-free interval in postmenopausal women with breast cancer.* Breast Cancer Res Treat, 1996. 39(3): p. 335-41.
130. Dillon, D.A., *Molecular markers in the diagnosis and staging of breast cancer.* Semin Radiat Oncol, 2002. 12(4): p. 305-18.
131. Graf, E., et al., *Assessment and comparison of prognostic classification schemes for survival data.* Stat Med, 1999. 18(17-18): p. 2529-45.
132. Barnett, G.C., et al., *Risk factors for the incidence of breast cancer: do they affect survival from the disease?* J Clin Oncol, 2008. 26(20): p. 3310-6.
133. Dawood, S., et al., *Prognostic value of body mass index in locally advanced breast cancer.* Clin Cancer Res, 2008. 14(6): p. 1718-25.
134. Hede, K., *Fat may fuel breast cancer growth.* J Natl Cancer Inst, 2008. 100(5): p. 298-9.
135. Sellahewa, C., P. Nightingale, and A.R. Carmichael, *Obesity and HER 2 overexpression: a common factor for poor prognosis of breast cancer.* Int Semin Surg Oncol, 2008. 5: p. 2.
136. Caleffi, M., I.S. Fentiman, and B.G. Birkhead, *Factors at presentation influencing the prognosis in breast cancer.* Eur J Cancer Clin Oncol, 1989. 25(1): p. 51-6.
137. Ewertz, M., et al., *Survival of breast cancer patients in relation to factors which affect the risk of developing breast cancer.* Int J Cancer, 1991. 49(4): p. 526-30.
138. Juret, P., et al., *Age at menarche as a prognostic factor in human breast cancer.* Eur J Cancer, 1976. 12(9): p. 701-4.
139. Korzeniowski, S. and T. Dyba, *Reproductive history and prognosis in patients with operable breast cancer.* Cancer, 1994. 74(5): p. 1591-4.
140. Lees, A.W., et al., *Risk factors and 10-year breast cancer survival in northern Alberta.* Breast Cancer Res Treat, 1989. 13(2): p. 143-51.
141. Mohle-Boetani, J.C., et al., *Body size, reproductive factors, and breast cancer survival.* Prev Med, 1988. 17(5): p. 634-42.

142. Oestreicher, N., et al., *Hormonal factors and breast tumor proliferation: do factors that affect cancer risk also affect tumor growth?* Breast Cancer Res Treat, 2004. 85(2): p. 133-42.
143. Schouten, L.J., et al., *Prognostic significance of etiological risk factors in early breast cancer.* Breast Cancer Res Treat, 1997. 43(3): p. 217-23.
144. Tartter, P.I., et al., *The prognostic significance of Gail model risk factors for women with breast cancer.* Am J Surg, 2002. 184(1): p. 11-5.
145. Trivers, K.F., et al., *Association between reproductive factors and breast cancer survival in younger women.* Breast Cancer Res Treat, 2007. 103(1): p. 93-102.
146. Fisher, E.R., *Prognostic and therapeutic significance of pathological features of breast cancer.* NCI Monogr, 1986(1): p. 29-34.
147. Kyogoku, S., et al., *Survival of breast-cancer patients and body size indicators.* Int J Cancer, 1990. 46(5): p. 824-31.
148. Nair, M.K., et al., *Overall survival from breast cancer in Kerala, India, in relation to menstrual, reproductive, and clinical factors.* Cancer, 1993. 71(5): p. 1791-6.
149. Wang, D.Y., et al., *Influence of reproductive history on age at diagnosis of breast cancer and prognosis.* Int J Cancer, 1985. 36(4): p. 427-32.
150. Brandenberger, A.W., M.K. Tee, and R.B. Jaffe, *Estrogen receptor alpha (ER-alpha) and beta (ER-beta) mRNAs in normal ovary, ovarian serous cystadenocarcinoma and ovarian cancer cell lines: down-regulation of ER-beta in neoplastic tissues.* J Clin Endocrinol Metab, 1998. 83(3): p. 1025-8.
151. Galli, M.C., et al., *The occurrence of multiple steroid hormone receptors in disease-free and neoplastic human ovary.* Cancer, 1981. 47(6): p. 1297-302.
152. Holt, J.A., et al., *Estrogen and progestin binding in cytosols of ovarian adenocarcinomas.* Obstet Gynecol, 1979. 53(1): p. 50-8.
153. Karlan, B.Y., et al., *Steroid hormone effects on the proliferation of human ovarian surface epithelium in vitro.* Am J Obstet Gynecol, 1995. 173(1): p. 97-104.
154. Langdon, S.P., et al., *Oestrogen receptor expression and the effects of oestrogen and tamoxifen on the growth of human ovarian carcinoma cell lines.* Br J Cancer, 1990. 62(2): p. 213-6.
155. Lau, K.M., S.C. Mok, and S.M. Ho, *Expression of human estrogen receptor-alpha and -beta, progesterone receptor, and androgen receptor mRNA in normal and malignant ovarian epithelial cells.* Proc Natl Acad Sci U S A, 1999. 96(10): p. 5722-7.
156. Nash, J.D., et al., *Estrogen and anti-estrogen effects on the growth of human epithelial ovarian cancer in vitro.* Obstet Gynecol, 1989. 73(6): p. 1009-16.
157. Toppila, M., et al., *Steroid receptors in human ovarian malignancy. A review of four years tissue collection.* Br J Obstet Gynaecol, 1986. 93(9): p. 986-92.
158. Bebar, S. and M. Ursic-Vrscaj, *Hormone replacement therapy after epithelial ovarian cancer treatment.* Eur J Gynaecol Oncol, 2000. 21(2): p. 192-6.
159. Eeles, R.A., et al., *Hormone replacement therapy and survival after surgery for ovarian cancer.* Bmj, 1991. 302(6771): p. 259-62.
160. Guidozzi, F. and A. Daponte, *Estrogen replacement therapy for ovarian carcinoma survivors: A randomized controlled trial.* Cancer, 1999. 86(6): p. 1013-8.
161. Rodriguez, C., et al., *Estrogen replacement therapy and fatal ovarian cancer.* Am J Epidemiol, 1995. 141(9): p. 828-35.
162. Nagle, C.M., et al., *The influence of reproductive and hormonal factors on ovarian cancer survival.* Int J Gynecol Cancer, 2008. 18(3): p. 407-13.

163. Collins, J., et al., *Oestrogen use and survival in endometrial cancer.* Lancet, 1980. 2(8201): p. 961-4.
164. Robboy, S.J. and R. Bradley, *Changing trends and prognostic features in endometrial cancer associated with exogenous estrogen therapy.* Obstet Gynecol, 1979. 54(3): p. 269-77.
165. Chapman, J.A., et al., *Estrogen replacement in surgical stage I and II endometrial cancer survivors.* Am J Obstet Gynecol, 1996. 175(5): p. 1195-200.
166. Mittal, K.R. and K.W. Barwick, *Endometrial adenocarcinoma involving adenomyosis without true myometrial invasion is characterized by frequent preceding estrogen therapy, low histologic grades, and excellent prognosis.* Gynecol Oncol, 1993. 49(2): p. 197-201.
167. Nyholm, H.C., A.L. Nielsen, and P. Norup, *Endometrial cancer in postmenopausal women with and without previous estrogen replacement treatment: comparison of clinical and histopathological characteristics.* Gynecol Oncol, 1993. 49(2): p. 229-35.
168. Shapiro, J.A., et al., *Menopausal hormone use and endometrial cancer, by tumor grade and invasion.* Epidemiology, 1998. 9(1): p. 99-101.
169. Smith, D.C., R.L. Prentice, and D.E. Bauermeister, *Endometrial carcinoma: histopathology, survival, and exogenous estrogens.* Gynecol Obstet Invest, 1981. 12(4): p. 169-79.
170. Persson, I., et al., *Cancer incidence and mortality in women receiving estrogen and estrogen-progestin replacement therapy--long-term follow-up of a Swedish cohort.* Int J Cancer, 1996. 67(3): p. 327-32.
171. Suriano, K.A., et al., *Estrogen replacement therapy in endometrial cancer patients: a matched control study.* Obstet Gynecol, 2001. 97(4): p. 555-60.
172. Khan, M., et al., *Risk of endometrial cancer mortality by ever-use of sex hormones and other factors in Japan.* Asian Pac J Cancer Prev, 2006. 7(2): p. 260-6.
173. Schairer, C., et al., *Cause-specific mortality in women receiving hormone replacement therapy.* Epidemiology, 1997. 8(1): p. 59-65.
174. Bendel, R.B., Afifi, A.A., *Comparison of stopping rules in forward regression.* J Am Stat Assoc, 1977. 72: p. 46-53.
175. Therneau, T.M., Grambsch, P.M., Fleming, T.R., *Martingale-based residuals for survival models.* Biometrika, 1990. 77: p. 147-60.
176. Rosenberg, L.U., et al., *Menopausal hormone therapy and other breast cancer risk factors in relation to the risk of different histological subtypes of breast cancer: a case-control study.* Breast Cancer Res, 2006. 8(1): p. R11.
177. Weiderpass, E., et al., *Low-potency oestrogen and risk of endometrial cancer: a case-control study.* Lancet, 1999. 353(9167): p. 1824-8.
178. Moradi, T., et al., *Physical activity and postmenopausal endometrial cancer risk (Sweden).* Cancer Causes Control, 2000. 11(9): p. 829-37.
179. Weiderpass, E., et al., *Organochlorines and endometrial cancer risk.* Cancer Epidemiol Biomarkers Prev, 2000. 9(5): p. 487-93.
180. Weiderpass, E., et al., *Body size in different periods of life, diabetes mellitus, hypertension, and risk of postmenopausal endometrial cancer (Sweden).* Cancer Causes Control, 2000. 11(2): p. 185-92.
181. Weiderpass, E., et al., *Estrogen receptor alpha gene polymorphisms and endometrial cancer risk.* Carcinogenesis, 2000. 21(4): p. 623-7.
182. Begg, C. and R. Gray, *Calculation of polychotomous logistic regression parameters using individualized regressions.* Biometrika, 1984. 71: p. 11-18.

183. Ederer, F., L.M. Axtell, and S.J. Cutler, *The relative survival rate: a statistical methodology.* Natl Cancer Inst Monogr, 1961. 6: p. 101-21.
184. Sovino, H., T. Sir-Petermann, and L. Devoto, *Clomiphene citrate and ovulation induction.* Reprod Biomed Online, 2002. 4(3): p. 303-10.
185. Brinton, L.A., et al., *Breast cancer risk associated with ovulation-stimulating drugs.* Hum Reprod, 2004. 19(9): p. 2005-13.
186. Lerner-Geva, L., et al., *Infertility, Ovulation Induction Treatments and the Incidence of Breast Cancer-a Historical Prospective Cohort of Israeli Women.* Breast Cancer Res Treat, 2006. 100(2): p. 201-212.
187. Potashnik, G., et al., *Fertility drugs and the risk of breast and ovarian cancers: results of a long-term follow-up study.* Fertil Steril, 1999. 71(5): p. 853-9.
188. Burkman, R.T., et al., *Infertility drugs and the risk of breast cancer: findings from the National Institute of Child Health and Human Development Women's Contraceptive and Reproductive Experiences Study.* Fertil Steril, 2003. 79(4): p. 844-51.
189. Dor, J., et al., *Cancer incidence in a cohort of infertile women who underwent in vitro fertilization.* Fertil Steril, 2002. 77(2): p. 324-7.
190. Garland, M., et al., *Menstrual cycle characteristics and history of ovulatory infertility in relation to breast cancer risk in a large cohort of US women.* Am J Epidemiol, 1998. 147(7): p. 636-43.
191. Klip, H., et al., *Cancer risk associated with subfertility and ovulation induction: a review.* Cancer Causes Control, 2000. 11(4): p. 319-44.
192. Moseson, M., et al., *The influence of medical conditions associated with hormones on the risk of breast cancer.* Int J Epidemiol, 1993. 22(6): p. 1000-9.
193. Key, T.J. and M.C. Pike, *The role of oestrogens and progestagens in the epidemiology and prevention of breast cancer.* Eur J Cancer Clin Oncol, 1988. 24(1): p. 29-43.
194. Russo, I.H., M. Koszalka, and J. Russo, *Comparative study of the influence of pregnancy and hormonal treatment on mammary carcinogenesis.* Br J Cancer, 1991. 64(3): p. 481-4.
195. Russo, J., Saby, J., Isenberg, W. and Russo, I.H., *Pathogenesis of mammary carcinomas induced in rats by 7,12-dimethylbenz(a)anthracene.* Journal of the National Cancer Institute, 1977. 59: p. 435-445.
196. Russo, J. and I.H. Russo, *DNA labeling index and structure of the rat mammary gland as determinants of its susceptibility to carcinogenesis.* J Natl Cancer Inst, 1978. 61(6): p. 1451-9.
197. Russo, I.H. and J. Russo, *Hormonal approach to breast cancer prevention.* J Cell Biochem Suppl, 2000. 34: p. 1-6.
198. DiSaia, P.J., *Hormone Replacement Therapy in the Gynecologic and Breast Cancer Patient.* Cancer Control, 1996. 3(2): p. 101-106.
199. Bray, F., P. McCarron, and D.M. Parkin, *The changing global patterns of female breast cancer incidence and mortality.* Breast Cancer Res, 2004. 6(6): p. 229-39.
200. Clavel-Chapelon, F., *Differential effects of reproductive factors on the risk of pre- and postmenopausal breast cancer. Results from a large cohort of French women.* Br J Cancer, 2002. 86(5): p. 723-7.
201. Kuller, L.H., *The etiology of breast cancer--from epidemiology to prevention.* Public Health Rev, 1995. 23(2): p. 157-213.
202. Apter, D., M. Reinila, and R. Vihko, *Some endocrine characteristics of early menarche, a risk factor for breast cancer, are preserved into adulthood.* Int J Cancer, 1989. 44(5): p. 783-7.

203. Velie, E.M., S. Nechuta, and J.R. Osuch, *Lifetime reproductive and anthropometric risk factors for breast cancer in postmenopausal women.* Breast Dis, 2005. 24: p. 17-35.
204. Apter, D., *Hormonal events during female puberty in relation to breast cancer risk.* Eur J Cancer Prev, 1996. 5(6): p. 476-82.
205. Anbazhagan, R., et al., *Growth and development of the human infant breast.* Am J Anat, 1991. 192(4): p. 407-17.
206. Rudland, P.S., *Epithelial stem cells and their possible role in the development of the normal and diseased human breast.* Histol Histopathol, 1993. 8(2): p. 385-404.
207. Russo, J., et al., *Comparative study of human and rat mammary tumorigenesis.* Lab Invest, 1990. 62(3): p. 244-78.
208. Berkey, C.S., et al., *Adolescence and breast carcinoma risk.* Cancer, 1999. 85(11): p. 2400-9.
209. Ahlgren, M., et al., *Growth patterns and the risk of breast cancer in women.* N Engl J Med, 2004. 351(16): p. 1619-26.
210. Berrino, F., et al., *Survival for eight major cancers and all cancers combined for European adults diagnosed in 1995-99: results of the EUROCARE-4 study.* Lancet Oncol, 2007. 8(9): p. 773-83.
211. Mackenbach, J.P., et al., *Socioeconomic inequalities in health in 22 European countries.* N Engl J Med, 2008. 358(23): p. 2468-81.
212. Verdecchia, A., et al., *Patient survival for all cancers combined as indicator of cancer control in Europe.* Eur J Public Health, 2008. 18(5): p. 527-32.
213. Collins, J.A., J.M. Blake, and P.G. Crosignani, *Breast cancer risk with postmenopausal hormonal treatment.* Hum Reprod Update, 2005. 11(6): p. 545-60.
214. Daling, J.R., et al., *Association of regimens of hormone replacement therapy to prognostic factors among women diagnosed with breast cancer aged 50-64 years.* Cancer Epidemiol Biomarkers Prev, 2003. 12(11 Pt 1): p. 1175-81.
215. Gapstur, S.M., M. Morrow, and T.A. Sellers, *Hormone replacement therapy and risk of breast cancer with a favorable histology: results of the Iowa Women's Health Study.* JAMA, 1999. 281(22): p. 2091-7.
216. Rosenberg, L.U., et al., *Menopausal hormone therapy in relation to breast cancer characteristics and prognosis: a cohort study.* Breast Cancer Res, 2008. 10(5): p. R78.
217. Mascarenhas, C., et al., *Use of hormone replacement therapy before and after ovarian cancer diagnosis and ovarian cancer survival.* Int J Cancer, 2006. 119(12): p. 2907-15.
218. Rossouw, J.E., et al., *Risks and benefits of estrogen plus progestin in healthy postmenopausal women: principal results From the Women's Health Initiative randomized controlled trial.* JAMA, 2002. 288(3): p. 321-33.
219. Amant, F., et al., *Endometrial cancer.* Lancet, 2005. 366(9484): p. 491-505.