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PREGNANCY RELATED RISK FACTORS FOR BREAST CANCER

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Experience shows that disturbances in the womb
often lead to breast cancer, a form of cancer which is
more common among nuns than among other women.
This is not attributable, as I see it, to menstrual disorders,
but rather to a life in celibate.
(…)
Why are the breasts more sensitive to disturbances in the womb
than other parts of the body, which suffer less frequently?
Given time, it might be possible for the scientists to explain
this mysterious relationship. We have not yet conquered
the whole territory of truth.

Bernardo Ramazzini (1700)

SUMMARY

Pregnancies offer a life-long reduction in breast cancer risk. It has repeatedly been shown that both number of pregnancies and age at first birth affect the future breast cancer risk. The mechanisms for this protection are still not fully investigated. Promising results from animal experiments indicate that the placental hormone hCG could be involved, but data from human populations are lacking. Likewise, it is still unclear to what extent pregnancy characteristics influence the protection. These are the questions addressed by this thesis.

The first study describes the risk of breast cancer after molar pregnancies. Molar pregnancies involve exposure to high levels of hCG, without other characteristics of a normal pregnancy. All women with a diagnosis of molar pregnancy in the Swedish Cancer Registry (n=3371) between 1958 and 1993 were followed up for subsequent diagnoses of breast cancer. The observed number (59) was then compared to the expected number based on year of birth and follow-up period (46), yielding a SIR of 1.3 (95% confidence interval 1.0-1.7).

The second study uses another proxy variable for increased hCG exposure, namely hyperemesis. Breast cancer cases from the Swedish Cancer registry were compared to matched controls regarding diagnoses of hyperemesis in the Swedish In-Patient Care Registry. There was practically no difference between cases and controls (adjusted OR=1.05 for any versus no diagnosis of hyperemesis, 95% CI 0.86-1.27).

The third study focuses on duration of molar pregnancies and breast cancer risk. Women diagnosed with hydatidiform mole and a subsequent breast cancer diagnosis were identified from the Swedish Cancer Registry. They were compared to matched controls regarding the duration of molar pregnancy before evacuation. A slightly shorter period was noted for cases than for controls.

The fourth study investigates the possible relationship between abortions and breast cancer. Exposure information was abstracted from maternal care and birth records for 1759 cases and an equal number of matched controls. A history of at least one abortion was noted for 383 cases and 473 controls yielding an adjusted odds ratio of 0.84 (95% CI 0.72-0.99). Both induced and spontaneous abortions were associated with odds ratios below unity.

The last study investigates the impact of weight change during pregnancy, child weight and placental weight on maternal breast cancer risk, in the same material as study IV. A slightly increased risk was found with increasing child weight and placental weight, whereas no association was found with maternal weight change.

In conclusion, the presented studies do not support the theory of a protective effect of hCG on breast cancer risk. Further, abortions do not seem to increase breast cancer risk, at least not if followed by a childbirth. Finally, maternal risk of breast cancer seems to be modestly associated to offspring birth weight and placental weight, but not to maternal weight change during pregnancy.

CONTENTS

LIST OF PUBLICATIONS

This thesis is based on the following manuscripts, which will be referred to in the text by their roman numerals:

I. Erlandsson G, Weiderpass E, Lambe M, Ekbom A
Hydatidiform moles and the long-term risk of breast cancer (Sweden).
Cancer Causes and Control 2000 Feb;11(2):117-20

II. Erlandsson G, Lambe M, Cnattingius S, Ekbom A
Hyperemesis gravidarum and subsequent breast cancer risk.
British Journal of Cancer 2002 Oct 21;87(9):974-6.

III. Larfors G, Lambe M, Cnattingius S, Ekbom A
Duration of molar pregnancies and breast cancer risk – a case-control study.
Manuscript

IV. Erlandsson G, Montgomery SM, Cnattingius S, Ekbom A
Abortions and breast cancer: Record-based case-control study.
International Journal of Cancer 2003 Feb 20;103(5):676-9.

V. Larfors G, Cnattingius S, Ekbom A
Pregnancy characteristics and maternal breast cancer risk.
Manuscript

LIST OF ABBREVIATIONS

AFP	α-fetoprotein
CI	confidence interval
DMBA	7,12-dimethyl-benz-*a*-anthracene
FSH	follicle stimulating hormone
hCG	human chorionic gonadotropin
hPL	human placental lactogen
HRT	hormone replacement therapy
ICD	international classification of diseases
IGF	insulin like growth factor
LH	luteinizing hormone
NRN	national registration number (in Swedish: *personnummer*)
OR	odds ratio
RTP	registry of the total population
SIR	standardized incidence ratio

INTRODUCTION

It has been established that pregnancies offer a long-term protection against breast cancer. Many and early pregnancies lowers women's breast cancer risk substantially. By what mechanisms this protection acts, and whether different pregnancies offer different protection, are questions where the scientific evidence still is inconclusive. These questions have been the focus for this thesis, and the articles behind it.

In the summarizing part of this thesis ("kappan"), it has been my ambition to give a background to the work, as to how, and more importantly, why it has been performed. The background section contains notes, not only to the area of pregnancy related risk factors for breast cancer, but also brief notes on breast cancer epidemiology in general. Although such notes might seem very basic for a cancer researcher at first glance, I am sure that some of my opinions on breast cancer etiology are possible to debate. I have felt that these opinions are important to describe, as they form the platform for the later hypotheses. Further, for any reader who is not working in the field of breast cancer epidemiology, such notes could be useful.

BACKGROUND

TRENDS AND INCIDENCE OF BREAST CANCER

Breast cancer is a major cause of morbidity and mortality from cancer in the western world (1). In Sweden, more than 6,000 new cases are diagnosed yearly. This means that approximately 10 % of all women will suffer from breast cancer during their lifetime (2). This also makes breast cancer the most common cancer diagnosed among women and the second most common cancer type overall in Sweden (basaliomas not included). The incidence is unevenly distributed around the world. In Asia breast cancer is much less common. The highest incidence is found in white women in California (1). Migration studies suggest that life-style factors, probably early in life, account for this difference (3).

In Sweden, as in most of the western world, incidence has been increasing for more than fifty years. For the last 20 years, an increase of 1.5 % yearly has been noted. No thorough explanation has been provided for this increase, but life-style factors are believed to be crucial.

The mortality from breast cancer has been dramatically improved during the last decades. In spite of the increase in breast cancer incidence, fewer women die from the disease in Sweden today than did thirty years ago (4). Although no-one can say for sure, both earlier detection through mammography programs and better therapy have probably contributed to this mortality decrease. Still, breast cancer is a major cause of mortality. In 2001, 1,487 women died from breast cancer in Sweden, making it the fifth most common cause of cancer mortality this year (5).

Breast cancer is a disease in women, and in particular in older women. Breast cancer in men exists, but in a number not comparable to that of women. This thesis only concerns breast cancer in women. Male breast cancer needs separate studies, not only because of the relative rarity, but also due to a potentially different etiology.

Breast cancer is also rare in women less than 30 years old, and increases thereafter with age until the age of 65 by which the incidence flattens out on a level of 300 cases per 100,000 person-years (Figure 1). As is shown in the figure, no distinct change in breast cancer incidence occurs at the time of menopause. Still, breast cancers before and after menopause are different in terms of etiology. For example, obesity, which is a risk factor for post-menopausal breast cancer, has little influence, or even a protective effect on breast cancer before menopause (6).

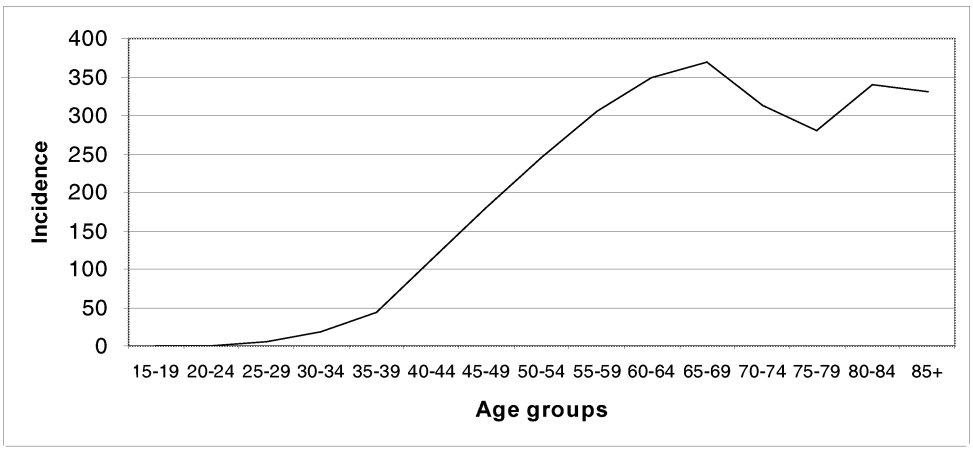

Figure 1. Incidence of breast cancer in different age groups in Sweden 2000 (2).

WHAT CAUSES BREAST CANCER?

In spite of an intense research in the field, the exact etiology of breast cancer is still not clarified. Nevertheless, it is a misconception to think of the cause of breast cancer as a mystery, where the single answer is still lacking. Instead, the research resembles a gigantic jig-saw puzzle, where the pattern is visible but several pieces are still missing. A major drawback so far is that very few of the known breast cancer risk factors are avoidable, and that practically no primary prevention is possible.

Cancer is a heterogeneous group of diseases

In spite of the frequent media reports on "the cause of cancer", cancers are far from equal in terms of etiology. For example, smoking, which is the major cause of lung cancer (7), has little or no impact on breast cancer incidence (8). Likewise, chronic inflammation, which is a major cause of cancer in liver (9), has probably no significant part in breast cancer etiology.

Cancer is a condition in which a cell line's potential to grow, reproduce, infiltrate surrounding tissue and form distant metastases is only restricted by the cellular environment and in which internal control systems are knocked out. Basically, any cell in the body, which has kept the ability to grow and reproduce, also has the potential to turn into cancer. In breast cancer, this cell is usually an epithelial cell in the ducts or lobuli of a mammary gland. In order to become cancerous, a cell line has to gain a number of genetic changes. These changes turn off the cell line's own control systems. These systems normally reduce the cellular power to grow and spread, and forces the cell line to apoptosis. Some of the genetic changes may be present from conception, others are gathered during fetal, infant and adult life. In this thesis, the former ones are called hereditary factors, whereas the latter are called environmental

factors[*]. This multi-step process may seem determinated, but most carcinogenic mutations occur in fact at random. They result from the fact that our body is constantly regenerated through cell divisions, and in each division some minor mutations occur. Most mutations are repaired, but some persist. The number of persisting mutations increase when a tissue is rapidly growing or in a state of stress such as inflammation. Some chemicals, known as carcinogens, also increase the number of persisting mutations. In some instances, retroviruses or other organisms may also induce carcinogenic changes in order to improve their own conditions.

The difference in ways of inducing cellular changes is the explanation to the differences in cancer etiology mentioned before. Lung tissue is heavily exposed to carcinogens from tobacco smoking or irritation from asbestos while these chemicals hardly reaches the breast glands. Chronic inflammation, which is a common cause of liver cancer due to hepatitis or primary biliary cirrhosis, is also rare in the breast epithelium. The major cause of breast cancer is instead the continuous hormone dependent growth of the epithelial cells which proceeds throughout life (10). Among cancers with a hormone dependent etiology are also cancers of the ovaries, cervix and prostate.

Hereditary factors

To what degree breast cancer is a hereditary disease is under debate. Traditionally, 10% of the breast cancer incidence has been explained by hereditary factors, but a large study on twins in the Nordic countries estimated the proportion to be as high as 30% (11). Although hereditary factors account for a minor portion of the breast cancer incidence than the environmental, the known breast cancer genes are among the strongest known risk factors for breast cancer, both in terms of relative risk of carriers versus non-carriers and in absolute number of attributable cases (12). This is due to the fact that the limited number of mutations discovered so far account for a large part of the risk attributable to hereditary factors. These include BRCA1 and BRCA2, two breast cancer specific genes and mutations in p53, a gene linked to many cancer forms (13). Hereditary factors do not cause a separate form of breast cancer, but contribute to the individual risk together with environmental factors. A little simplified, one might say that the environmental threshold is lower for women who bear hereditary risk factors from conception. Still, since risk factors depend on each other, the environmental risk panorama may differ in women with a hereditary risk from those in women without such.

Radiation

Ionizing radiation is in absolute numbers a miniscule cause of breast cancer, but I find it worth to mention as this is the only certain cause of breast cancer beside

[*] Hereditary risk factors are frequently called "genetic". Although hereditary factors are genetic, I try to avoid the term, since cancer is a entirely genetic disease and both environmental and hereditary risk factors ultimately result in genetic changes.

hereditary and hormonal factors. From our general understanding of cancer etiology, we can deduct that exposure to ionizing radiation would increase the risk of gathering carcinogenic mutations. An increased risk of breast cancer has also been noted after radiotherapy in the thoracic region (14), and among survivors of the nuclear bombings in Japan (15).

Hormonal carcinogenesis

Although other mechanisms have been proposed, sex hormones cause cancer primarily by acting as normal growth factors in the body (10, 16). Since increasing cell growth also means increasing risk of persisting mutations, the life-long exposure to endogenous and exogenous hormones can influence the risk of developing cancer.

Estrogens

It was discovered already in the late 19th century that oophorectomy lead to a dramatic regression of some human breast cancers (17). Together with the knowledge that breast cancer almost exclusively occurs in women, and the later finding that administration of exogenous estrogens in men could lead to male breast cancer, these studies lead to the estrogen hypothesis; the more exposure to estrogens during a woman's life, the higher her breast cancer risk. The theory has gained support both from direct measurements of endogenous hormones (18), from indirect measures of life-time exposure as age at menarche and age at menopause (16), and from studies on exogenous administration of estrogen during menopause (19).

Progesterone

Earlier, exogenous administration of estrogens in hormone replacement therapy was hypothesized to be less harmful for breast cancer risk if combined with progesterone, as is the case for endometrial cancer. Later studies show, however, that for breast cancer this is a misunderstanding. Combined progesterone and estrogen administration results in a higher risk of breast cancer than estrogen alone (19-21). The potential role for endogenous progesterone in breast cancer etiology is less certain (22).

PREGNANCIES AND BREAST CANCER

The pregnancy paradox

The first known author to observe a protective effect of parity on breast cancer risk was probably Bernardino Ramazzini, an Italian pioneer in occupational medicine. He noted already 1700 in his "Di morbis artificum" that nuns had breast cancer more often than did other women, and accounted this to their lack of childbearing (23). In modern medicine, numerous authors have confirmed his hypothesis that childbearing protects against breast cancer (24). This is a paradox since pregnancies constitute virtual estrogen baths, and would according to our previous knowledge of estrogen effects grossly increase breast cancer risk. Hence, the relationship between sex

hormones and breast cancer need to be modified when considering the effect of pregnancies.

A dual effect

Whereas the first authors only noted a protective effect on a life-long basis following pregnancies, the theory has been modified since. More detailed studies have revealed a pattern where the risk increases for a few years following pregnancy, after which the risk again decreases to settle on a lower level than the original (25). This could explain the strong impact on breast cancer risk of age at first pregnancy, and the moderate effect of age at subsequent pregnancies (26, 27). An earlier pregnancy contributes to the life-long protection for a longer period and has a relatively shorter period of initial risk increase. Moreover, this initial period occurs at an age when breast cancer is a rare disease.

Possible mechanisms

A number of theories have been put forward to explain the relationship between pregnancies and breast cancer and won more or less scientific acceptance. The answer is probably a combination of some of these theories.

It could be argued that pregnancies mean interruptions of the menstrual cycles, and less menstrual cycles mean less risk of breast cancer. In order for this mechanism to explain the relationship, however, menstrual cycles must increase breast cancer risk in another way than through estrogen exposure. Further, the protection conceived by pregnancies is much stronger than what could be expected only through fewer menstrual cycles on a life-time basis. Still, the interruption of menstrual cycles could possibly account for the protection against breast cancer conceived by breast-feeding (10). This protection is much smaller and has a clear time-dependent relationship to breast cancer risk (28).

Among less likely (although not altogether unbelievable) ideas could also be mentioned the hypothesis that women, during pregnancy, would eliminate lipophilic carcinogens to the fetal fat and vernix caseosa (29), or that exposure to fetal antigens would induce an immunological protection against so far unidentified carcinogens (30).

A theory, which seems biologically plausible, is that the massive exposure to estrogens during pregnancies results in a down-regulation of estrogen receptors in the breast epithelium. Somewhat in support of this theory, one ecological study has found higher estrogen levels during pregnancy in women in a low risk area for breast cancer (Shanghai) compared to women in a high risk area (Boston) (31). However, another study found that breast cancer cases had higher levels of estrone and equally high levels of estradiol during pregnancy compared to healthy controls (32).

The theory that has won the greatest acceptance is that pregnancies lead to a structural change of the breast epithelium. This change includes both proliferation and differentiation, which in turn leaves the epithelium less susceptible for carcinogenic mutations (33). The suggested reason for the differentiation is to prepare the breasts for

lactation. The theory is supported by results from experiments on rats (34). If this theory is correct, it could explain the dual effect of pregnancies. The initial phase would then be the result of the pregnancy induced proliferation, resulting in a higher short-term risk that cancer precursors evolve into cancers.

Still, this theory of a structural change does not explain the hormonal mechanisms for the protection. As previously discussed, estrogens alone are generally known to simply increase proliferation, and thereby breast cancer risk. One hypothesis is that another hormone is responsible for the induction of differentiation. This hormone should then be pregnancy specific, and have receptors present on the breast epithelium. One suggested hormone is hCG, further discussed below (35).

Effects on mother and child

The pregnancy is not only important for the mother's future breast cancer risk, but also for the fetus' (given that the fetus is a girl, of course) (36). Some of the hormones that could affect the mother's risk also has an impact on the daughter. The effects should not to be mixed since the mechanisms are totally different. However, the research performed on intrauterine and reproductive risk factors for breast cancer have influenced each other.

PREGNANCY CHARACTERISTICS

It is of importance to investigate the relationship between pregnancies and breast cancer further, with regard to pregnancy characteristics. Since specific characteristics are associated with specific changes in pregnancy hormone levels, such studies could give etiological insights to the relationship between pregnancies and breast cancer risk. Furthermore, if specific pregnancy characteristics constitute risk factors for breast cancer, the identification of these could be a mean of primary or secondary prevention against breast cancer. Of course, there are numerous studies which in any way address these questions, so I will just briefly summarize some of the investigated pregnancy characteristics and their relationship to breast cancer here.

Gender of fetus

Whether the fetus is a girl or boy has little impact on maternal breast cancer risk (37-39). However, one study focusing on this question has found a risk decrease for women who had given births to two or more boys only compared to those who had given birth to two or more girls only (40), indicating that hormones which vary with gender of offspring may be involved in the protection against breast cancer. Girl pregnancies are associated with increased levels of hCG (41), and decreased levels of α-fetoprotein in maternal blood (42), whereas maternal androgen levels are reported not to differ depending on gender of fetus (43).

Preeclampsia

Preeclampsia means a rise in maternal blood pressure during pregnancy, in combination with proteinuria. It has in a number of studies been linked to a decrease in maternal breast cancer risk (38, 44, 45). Preeclampsia is associated with alterations in several pregnancy hormone levels. Preeclamptic pregnancies are associated with increased levels of hCG, progesterone and androgens, whereas levels of estrogens, SHBG and IGF-1 are decreased (although the data on estrogens are conflicting, especially concerning estradiol)(46, 47).

Emesis

Nausea during pregnancy and subsequent breast cancer risk has been investigated in at least two previous studies. One found an increase in breast cancer risk (48), whereas the other found a decrease (38). The reason for the discrepancy is unclear, but there are several differences between the two studies. The first study investigated the effect of treatment for nausea, while the other used self-reported nausea or vomiting as exposure. As hyperemesis is used as a proxy variable in study II, the hormonal alterations in pregnancies complicated by emesis are discussed later.

Twinning

The data on twinning and maternal breast cancer risk are also somewhat conflicting. Twinning has in earlier studies not been found to influence breast cancer risk (44, 49, 50), or provide a moderate decrease in breast cancer risk (51-53). In a more recent study it has been linked to an increased risk instead (39). However, this increase was confined to the first five years following a multiple birth, the period where pregnancies in general increase breast cancer risk. The question of a differential effect of multiple births compared to singleton on breast cancer risk is of great importance from an etiological perspective, since many pregnancy hormones are increased in twin pregnancies compared to singleton (54-56). Clinically, the data do not suggest any important influence on maternal breast cancer risk.

Weight of child and placenta

One study has found a statistically significant positive association between the offspring's ponderal index and subsequent maternal mortality in breast cancer (57). A positive association, albeit not significant, was also noted for increasing birth weight. Another study has also linked increasing birth weight to maternal incidence of breast cancer (39). A low weight or a small size of the placenta has in one study also been linked to a decreased risk of breast cancer (58). Higher birth weight has been linked to higher pregnancy levels of estrogens in maternal circulation (59-61).

Length of gestation

At least two studies have shown increased risks of maternal breast cancer following premature deliveries (before week 37 and 32, respectively) (62, 63), although length of gestation within normal intervals has not been found to be associated with subsequent breast cancer risk (44).

Abortions and breast cancer

Slightly related to length of gestation is the question whether induced or spontaneous abortions affect breast cancer risk. Possibly due to the political importance of the question it has gained much more attention, and engaged several of the world's leading cancer epidemiologists. Aborted pregnancies are assumed not to give the protection of a full-term pregnancy, but the question is whether they even increase breast cancer risk compared to not having a pregnancy at all. To my knowledge, the first to note such a risk increase was Segi et al. in a Japanese case-control study on risk factors for breast cancer (64). I will not attempt to review all studies performed since, but I will exemplify the different study designs by which the question has been addressed. Here, I will only report the results on induced abortions, which have been the most discussed. Spontaneous abortions have generally not been associated with any change in breast cancer risk (65, 66). Further, all reported relative risks reflect having an aborted pregnancy compared to not having a pregnancy. The choice of this exposure variable could be discussed, but it is the most frequently used (66).

Experimental studies on pregnancy interruption and breast cancer risk have been performed on Sprague-Dawley rats (67). Using an animal model has the advantage that the result can be directly studied in the breast epithelium, instead of just measuring breast cancer rates. After an induced abortion, the rats showed significantly less differentiated terminal end buds, indicating a higher long-term breast cancer risk.

A small number of cohort studies have been published. In 1989, a follow-up of the Swedish abortion registry was published (68). The authors found no evidence of an increased risk of breast cancer, but the study was criticized for its lack of control of confounders, especially reproductive history. In 1997, a cohort study based on the Danish abortion registry was published (69). This study, which did not find any relationship in any direction, had better control of confounders, and is the largest study published so far on the subject. Three years later, the so far only cohort study, which found a positive relationship between abortions and breast cancer, was published (70). A non-significant 10% increase in breast cancer risk was noted for any versus no history of induced abortions, but due to the low prevalence of exposure, the authors warranted a careful interpretation of their data. Recently, results from a Chinese cohort study were published, which found no significant increase in breast cancer risk following induced abortions, although the risk estimates were slightly above unity (71).

Most studies on abortions and breast cancer risk have used a case-control design with retrospective data, usually based on interviews. Although some studies have been small, with little control of confounding and generally adding little new knowledge, this design has also been used by some of the most ambitious studies in the

field. Most studies with this design have found risk estimates above unity (72-79), but not all (38, 80, 81).

Some recent studies have also used a case-control design, but with prospectively recorded data, often from medical records (82-84). All these studies have instead found slightly decreased breast cancer risks following one or more induced abortions, although the authors have not acknowledged anything but the lack of positive associations.

The different results from retrospective case-control studies compared to studies with prospective designs have by some authors been referred to reporting bias in the retrospective design. Women with breast cancer are supposedly more willing to answer sensitive questions about abortions than community controls. Whether or not this reporting bias exists is under debate. A Dutch case-control study found a positive association between abortions and breast cancer in catholic areas but not in protestant, a difference, which the authors concluded was due to reporting bias in the catholic areas (85). An earlier evaluation in Sweden found that cases were more prone to report abortions, when answers were compared to registry data (86). However, a more recent comparison in the US did not detect any significant differences in answers between cases and controls (87).

Finally, some meta-analyses and several reviews have been published. Although meta-analyses often are considered as the highest level of evidence, I consider this area a typical example of where a meta-analysis give very little new knowledge. Generally, meta-analyses have two main advantages. Firstly, by grouping data they can achieve statistical power in areas where no single study can do this by its own. In the area of abortions and breast cancer risk, however, statistical power has not been an issue for a long time. There is enough studies to achieve high statistical significance both for a positive relationship, for a null result and for a negative relationship. Secondly, meta-analyses can dilute systematic errors by grouping studies with different designs. However, the vast majority of the earlier studies used the same design, and the potential source of error from reporting bias would accordingly be present also in a meta-analysis. Not surprisingly, the meta-analysis performed by Brind and colleagues (before the Danish cohort) found a relative risk of 1.3 for ever having an abortion compared to never (88).

Among reviews, Michels and Willett's review from 1996 (66) still is a good introduction to the field, although it does not cover the studies from the last years. Several professional organizations have recently updated their recommendations in the area. In the US, NCI published an updated review after a summit in February 2003 (89), and ACOG published an updated guideline in August this year (90).

HCG AND BREAST CANCER RISK

A placental hormone

hCG is a dimerous glycoprotein, consisting of a α-chain and a β-chain. It has a molecular weight of 39 kDa (91). It is very similar to a number of other glycoprotein hormones as thyroid stimulating hormone, luteinizing hormone (LH) and follicle-

stimulating hormone (FSH). The α-chains in hCG and LH are almost identical and is the part that binds to the receptor, which accordingly is identical for these hormones. The β-chains differ (although they are similar), and specify the function of the different hormones. Because of the similarities, the hormones can cross-react and cause unintended effects. For example, morning sickness is believed to be caused by a cross-reaction by hCG on thyroid receptors (92). In some instances, hCG and LH are even considered to be functionally identical. This exchangeability can not be taken for granted when considering new roles for hCG, as the β-chains are only 80% identical.

hCG is a placental hormone, produced by the syncytiotrophoblasts. It is to 99% secreted into the maternal compartment. What makes it interesting in the area of pregnancies and breast cancer risk is that it is in physiological conditions very pregnancy specific. The highest hCG levels are produced in the first pregnancy, whereas parous women have lower pregnancy levels (93). Thus, it could serve as a candidate for the role of differentiator of the breast epithelium. Its main function is probably to maintain pregnancy by replacing LH and increasing estrogen and progesterone levels.

Experimental data

Animal experiments

Russo and co-workers have made several experiments on virgin Sprague-Dawley rats. Mammary cancer was induced in the rats by administration of DMBA (7,12-dimethyl-benz(a)anthracene), and the rats were treated with hCG before or after this administration. The studies have consistently showed beneficial effects of hCG on mammary cancer, both as a preventive and as a therapeutic agent (94). Further studies has also suggested that the effect is due to a hCG induced regulation of IGF, both through decreased expression of IGF genes in mammary cells and through increased expression of IGF binding protein (95). IGF has previously been demonstrated to be a potent mitogen in human breast cells (96). Investigations from another group on human breast cells implanted in mice support the previous findings (97). In this study, the effect of hCG was dependent on functioning ovaries, indicating a role for female sex hormones in the protective process.

Human in-vitro experiments

Human breast epithelial cells have been studied in a number of experiments. LH/hCG receptors are present in human breast epithelial cells, indicating a role for such hormones in physiological conditions (98). Studies have also demonstrated an inhibitory effect of hCG administration on growth of human breast epithelial cells *in vitro* (99, 100). LH/hCG receptors have been demonstrated also in human breast cancer cell lines (101). Results from the same group also indicates that LH/hCG receptor status is correlated to a higher differentiation and better prognosis of these tumors (102). hCG can also bind to prolactin receptors, which are also present in human breast cancer cell lines (103).

Epidemiological data

The only study so far which has considered exogenous administration of hCG and future breast cancer risk, was a cohort study of women who had used hCG in a weight-loss regimen or as an infertility treatment (104). The investigators found a decrease in breast cancer risk. However, the study had little control of potential confounding factors and especially confounding by indication could have influenced the results.

A number of studies have looked at conditions related to high hCG expression, which accordingly could work as surrogate markers of exposure to hCG. Twinning has, as mentioned earlier, in some studies been linked to a moderate decrease in breast cancer risk (51-53). There are, however, too many differences between multiple births and singletons to draw any distinct conclusions on hCG and breast cancer risk from this result (105). The same could be said for gender of offspring as a surrogate marker.

Three studies have looked at the same surrogate markers as in studies I and II. One study have investigated breast cancer risk following hydatidiform moles, and found a slightly elevated risk. As mentioned earlier, two studies have studied hyperemesis and breast cancer risk with conflicting results. I will further discuss the findings of these three studies in relationship to my own later.

All in all, the epidemiological data supporting a relationship between exposure to hCG and breast cancer risk is weak. This is worrying since hCG according to a recent article is already used in clinical trials on advanced breast cancer, based on the results from animal and *in vitro* experiments (102).

Other possible hormones

Several hormones could be candidates. The most promising is perhaps α-fetoprotein (AFP), a pregnancy specific hormone produced by the fetus, which to some degree enters the mothers' vascular system. A Californian case-control study found a significant negative association between high AFP during pregnancy and subsequent breast cancer risk in pregnancies in younger ages (106). According to a large Danish cohort, having levels of AFP above median compared to having levels below median is associated with a 41% risk decrease in subsequent breast cancer risk (107). The findings are also supported by ecological data that Chinese women have higher AFP levels during pregnancy than American women have (108).

Proxy variables used in this thesis

Three of the studies in this thesis (I-III) investigate possible relationships between breast cancer risk and histories of hydatidiform moles or hyperemesis. Although answering these questions could have a clinical value, they primarily act as surrogate markers for increased exposure to hCG.

Hydatidiform mole

Hydatidiform moles, or molar pregnancies, are conditions in which the placenta grows inappropriately, has cystic structural changes and produces large quantities of hCG. Hydatidiform mole is the benign form in a group of diseases called gestational trophoblastic diseases. In its classic form, complete mole, the placenta exists without the presence of a fetus. The etiological mechanism has been described as the result of a merge between a fertile sperm with an infertile egg (109). This explanation does not hold, however, for partial moles, where only a part of an otherwise normal placenta presents with molar characteristics. This condition results from a triploid fertilization, with the extra chromosome of paternal origin. Partial hydatidiform moles can coexist with a fetus, which in that case normally has growth retardation and congenital malformations (110).

Hydatidiform moles produce levels of hCG substantially higher than in normal pregnancies (111), making it a suitable model to study hCG exposure.

Hyperemesis

The word hyperemesis simply means "excessive vomiting", but usually refers to a severe nausea during pregnancy. In my studies, it is defined as nausea during pregnancy severe enough to require hospital care. The exact etiology of this nausea is still unknown (in spite of the interest pregnant women would have in its clarification). Several mechanisms have been proposed, both hormonal and others (112). The mechanism is, however, not crucial for the use of hyperemesis as a proxy variable for hCG exposure. Hyperemesis is correlated to increased levels of hCG, in a dose-dependent manner (92, 113). When considering hyperemesis as a proxy for hCG exposure, it is important to keep in mind that hyperemesis may also be correlated to other alterations in hormones, for example higher estradiol or lower prolactin levels, although findings regarding these associations are inconclusive (114-116).

AIMS OF THE THESIS

The overall aim of this thesis is to evaluate how pregnancies influence the future long-term breast cancer risk. As has been discussed, the dual effect of term pregnancies with a transient risk increase followed by a life-long risk decrease has been fairly well described. In this thesis, two questions are addressed. Firstly, through which biological mechanisms do pregnancies influence breast cancer risk, and more specifically, is hCG involved? Secondly, do different pregnancies have different effects on future breast cancer risk? In the articles the following specific issues have been addressed:

Is breast cancer incidence influenced by a history of a molar pregnancies? (Study I)

Do pregnancies complicated with hyperemesis have a different impact on future breast cancer risk than other pregnancies? (Study II)

Is the potential influence by a history of a molar pregnancies dependent on the duration of the molar pregnancy? (Study III)

Do pregnancies terminated by an induced or spontaneous abortion have an impact on future breast cancer risk? (Study IV)

Do pregnancy characteristics as maternal weight gain or child anthropometrics modulate the relationship between pregnancies and breast cancer? (Study V)

SUBJECTS AND METHODS

SETTING

The studies were all based on Swedish data. Sweden provides excellent opportunities for epidemiological research. The most important advantage is the long tradition of good population statistics, and most importantly, the national registration numbers (NRN). Further, Sweden has a fairly homogenous population in terms of social standards and access to medical care (117).

National registration numbers

Since 1947, all residents in Sweden are assigned a unique ten-digit number. The first six digits consists of the birth date (yymmdd), the following two is (for NRNs assigned before 1990) a code of birth hospital, the ninth a code which denotes sex and birth order (0 for the first girl at that hospital and day, 1 for the first boy, 2 for the second girl and so on) and the final digit is a check digit which can be calculated from the previous ones. This registration number is used in all Swedish registries and institutions, like schools and health care. It is also noted on all drivers licenses, passports and other identity cards. This system makes it possible to link all records for everyone who has been resident in Sweden at some point after the beginning of 1947.

The Swedish cancer registry

The Swedish Cancer Registry was founded in 1958, and is maintained by the National Board of Health and Welfare. It has from the beginning been mandatory to report all malignant diseases to the registry at diagnosis. This obligation concerns both clinician and pathologist, making the completeness of the registry very high. In the to date only published evaluation, the completeness was 96%, based on comparisons with other registries in 1978 (118). However, the majority of the missing 4% were diseased cases where the diagnosis was found on death certificates. Today, cancers found incidentally at autopsy are entered with a special notation of their origin. Cancers only denoted at death certificates are not entered, as the validity of such records are considered too low. The total deficit is probably under 1% today. Nation-wide rates of the diseases covered by the registry are yearly published in "Cancer Incidence in Sweden" (2). The cancer diagnoses are recorded according to ICD-7 (and in later years also newer versions of ICD). To the cancer registry, data from the death and emigration registries are linked, making it possible to directly exclude subjects who have died or emigrated during follow-up.

The cancer registry also include a few non-malignant conditions, including hydatidiform mole (investigated in study I and III). The completeness for such lesions are lower, probably due to less awareness of the obligation to report such diagnoses. An evaluation has estimated the deficit to 25% (119).

The Swedish in-patient registry

The Swedish in-patient registry should now more appropriately be called the Swedish patient registry. It was founded in 1964 but covered by then only a minor proportion of Sweden, 4 out of 24 counties. In 1987 it had expanded to cover all Sweden. The idea of the registry is to record all admissions to Swedish in-patient care. The admission is reported with patient NRN, diagnosis according to ICD, and dates of admission and exit of hospital. In later years, a gradual expansion has started into out-patient care as well, resulting in the name change. The completeness for in-patient care has been estimated to between 98 and 99% for later years (120). Some specific diagnoses have lower completeness, among them "normal childbirth".

The registry of the total population (RTP) and the registry of births

Statistics Sweden is the governmental agency responsible for population statistics in Sweden. They keep a register known as "The national person and address registry" (SPAR) or the registry of the total population (RTP). This forms the basis of Swedish population statistics and includes all Swedish citizens and all permanent residents of Sweden.

"The registry of births" at Statistics Sweden has not been scientifically described to my knowledge. It was formed in 1961, and it is simply a part of the Swedish population statistics. It records all newborns in order to give them their first official address. The registry includes the NRNs of the mother and infant and address details.

The Swedish medical birth registry

This is a totally different registry than the former one. It is a research registry kept by the National Board of Health and Welfare. The medical birth registry was founded in 1973, covers all Sweden, and has according to the agency almost no deficit. It is based on the standardized antenatal care and birth records for all children born 1st January 1973 or later. The included information has varied over the years. In the seventies, a few medical and reproductive background variables and variables on the childbirth were recorded along with personal identification details. In the eighties and onwards, more and more social background variables have been introduced, as smoking, employment and family situation. Previous abortions are not recorded for integrity reasons. The validity of the registry data is high (121).

Standardized antenatal care and birth records

By 1st January 1973, the antenatal care records and birth records were standardized throughout Sweden. The result was a six page form, in two sections. The first section is filled out by the midwife during maternal care at the first visit (social, medical and reproductive background information) and at the following visits

(changes in weight and health). The second part is filled out at the hospital and includes information on the birth and the newborn child. The record also has non-standardized pages, which were not used in the studies.

STUDY DESIGN

Study I

Study I is a cohort study of women with hydatidiform mole and their breast cancer risk. All women with a diagnosis of hydatidiform mole (ICD-7: 173, histo-pathological code 801) in the cancer registry from the registry's start in 1958 until the end of 1993 were identified. These 3371 women were followed-up for a diagnosis of breast cancer (ICD-7: 170), death or emigration in the cancer registry until the end of 1993. 81 women died or emigrated before end of follow-up. Rates of breast cancer specific to age, sex and calendar period were accessible through published data from the cancer registry. Using these rates, an expected number of breast cancer cases was calculated based on the time of follow-up of the cohort, and compared to the observed number.

Study II

For the second study we used a matched case-control design. The idea was to identify as many breast cancer cases as possible and compare them to controls on histories of hyperemesis. However, to reduce dilution cases and controls had to have had at least one pregnancy within a region covered by the in-patient registry at the time of birth. If a woman did not meet this criteria, she had by definition no chance to turn out positive for hyperemesis. The cancer registry was searched for all women with a diagnosis of breast cancer from 1964 (when the in-patient registry started) to 1997. Among these women, all who had a registered birth in the Swedish Registry of Births after 1st January 1964 but before cancer diagnosis were identified. These 30,419 women were considered as potential cases and matched to four controls based on year of birth. Controls had to be alive, resident in Sweden and free from breast cancer by the time of diagnosis of the case. Among all those who met the criteria, the controls were randomly selected from those registered in the Swedish Registry of Births. The Swedish Medical Birth Registry and the Swedish In-patient Registry were then searched for all diagnoses of hyperemesis for all subjects, and for some potential confounding variables. Since the in-patient registry did not cover all Sweden initially, many of the 30,419 cases and their matched controls had not had a birth covered by the in-patient registry. All subjects who did not at any birth live in a county covered by the registry were accordingly excluded. If a risk-set (a case and its matched controls), had no case or no controls after this, the whole risk-set was excluded. This left us with data on 13,079 cases and 34,348 matched controls.

Study III

Study III used a nested case-control design within the cohort of women with hydatidiform mole reported to the Swedish Cancer Registry. The cohort was not identical to the one in Study I, as it covered a few additional years, but more or less the same. All women with a diagnosis of hydatidiform mole (ICD-7: 173, histo-pathological code 801) in the cancer registry from 1958 until the end of 1997 were identified (3797 women) and followed up for breast cancer diagnoses (ICD-7: 170). 73 women had such diagnoses. They were, if plausible, matched to four controls each on date of birth and date of diagnosis of hydatidiform mole. 285 controls were selected this way. All women who were still alive and resident in Sweden were contacted by letter and asked for permission to use data from their medical records. 86% responded and all but one of those gave permission. Additionally, permission was assumed from deceased and emigrated subjects. We were able to retrieve medical records for 49 cases and 142 of their individually matched controls. Like in study II, due to the matched design, all controls in risk-sets where information on the case was not available also had to be excluded, a major reason for exclusion of control subjects. From the medical records, we abstracted information on duration from last menstrual period until evacuation of the mole, and information on parity of the woman.

Study IV

The fourth study investigated the possible relationship between abortions and breast cancer. As described in the background section, some previous studies have been questioned due to the potential of recall bias when using retrospective data in this field. As there is no abortion registry in Sweden with identifiable data (this is to my knowledge only available in Denmark), we did not have resources to gather a large cohort. Probably, such a cohort would not have been feasible due to ethical constraints anyway, since recording abortions is a highly sensitive issue in Sweden.

Instead we used a case-control design with prospectively collected data from medical records, a method also used in other recent studies on the subject. As previously described, any woman who attends maternal care answers questions on reproductive history. Through linkage of the Medical Birth Registry with the Cancer Registry, we identified 4518 women who had given birth after 1st January 1973 and had a subsequent diagnosis of breast cancer. These were considered as eligible cases.

To each case, we aimed to match two controls on year of birth (within three years from the case), year of giving birth (within one year from the case) and hospital where the birth took place. The controls also had to be alive and resident in Sweden by the time of diagnosis of the case. This control selection was possible for all but a few cases, who were only matched to one control. The controls were randomly selected from those who met the criteria in the Medical Birth Registry.

As the cases were spread at practically all Swedish hospitals, we had no means of collecting information on all of them. Therefore, 23 hospitals were chosen. Most of these were situated in Mid-Sweden, but they varied in size and location in order to

reflect the background population to some extent. The hospitals covered 1,988 cases, or 44% of the original sample.

For exposure information, we tried to locate the antenatal care and birth record for the subject's first birth after 1973 for all subjects. The choice of the first birth after 1973 as index birth is not self-evident. The woman's last birth before the date of diagnosis of cancer would have given information on the reproductive history for a larger proportion of life. As we chose the first birth after 1973, the subjects were to a higher degree young and nulliparous, which we hoped would increase the validity of the response regarding the first, and assumedly most important, abortions. Figure 2 illustrates an example of a subject and the exposure information we could retrieve.

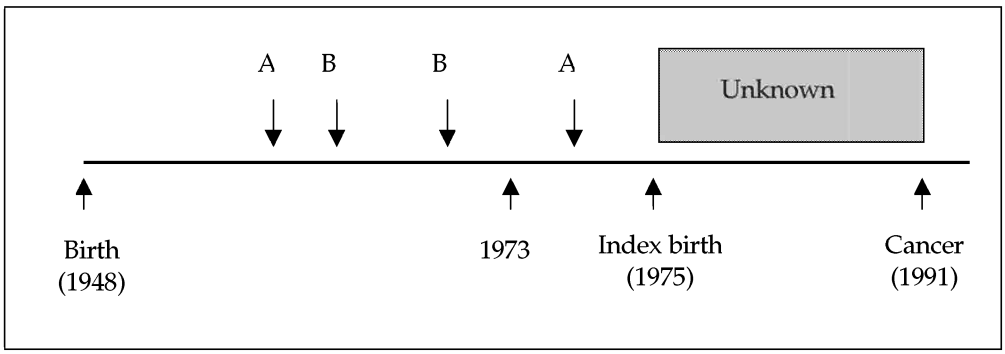

Figure 2. Schematic example of a subject with reproductive events over the line (A=abortion, B=birth) and registry data under the line.

We were able to retrieve records with full exposure information for 1,813 cases. We only used one control for each case. If the first control could not be retrieved, we tried to retrieve information on the second one. This resulted in complete exposure data on 1,759 case-control pairs or 88% of the original sample (and not 84% as I erroneously state in the article). The major reason for exclusion was that the child was born in the first half of 1973, which meant that the chart was not in use when the mother first visited her maternal care clinic.

For abortions, information was in most cases available on which year and in which gestational month the abortion took place, and whether the abortion was induced, spontaneous or other. From the notes on the records, all abortions in the latter category could be considered as spontaneous.

A number of potential confounding variables were also abstracted from the antenatal and birth charts. These included reproductive history, smoking, previous use of oral contraceptives, height, pre-pregnancy weight, occupation and a very crude variable on social conditions (single, cohabiting with the father of the child or other). The two latter ones were of too poor quality to be accounted for in the analysis. Smoking was noted as smoker or not at the time of interview. Use of oral contraceptives was only noted as a date when this use stopped.

A challenge in analysis was how to take into account the age differences at index birth. As the cases had to have a pregnancy after 1st January 1973 and a diagnosis of cancer before 31st December 1991, the cases were on average older than the population mean at the index birth. The three-year matching interval decreased this difference compared to controls, but the remaining age differences still threatened to bias the estimates. A higher age at index birth would mean that we had information on reproductive events for a longer period of life (Figure 2). To correct this, we excluded all reproductive events from analysis for the older in each pair, which took place after the age at interview of the youngest. We had no information on what date this interview took place for each subject, so the date was estimated to 32 weeks before the index birth.

Study V

The final study was based on the same material and methods as study IV. The major difference in the study population was that anyone who did not have complete data on anthropometric measures also had to be excluded. The major reason for this additional exclusion was that no maternal weight at delivery had been recorded, and weight increase during pregnancy accordingly could not be calculated. This left us with data on 1,548 case-control pairs.

As Study V only took into account data from one birth, and not historical events, the age differences at interview could be accounted for simply by adding age into the regression model.

STATISTICAL METHODS

Studies II-V were all matched case-control studies and analyzed with conditional logistic regression (122). The analyses were made with PROC PHREG in SAS statistical software (123).

ETHICAL APPROVAL

All studies were approved by the research ethical committees at Karolinska Institutet (Studies I, II, III and V) and Uppsala university (Study IV).

RESULTS

Study I

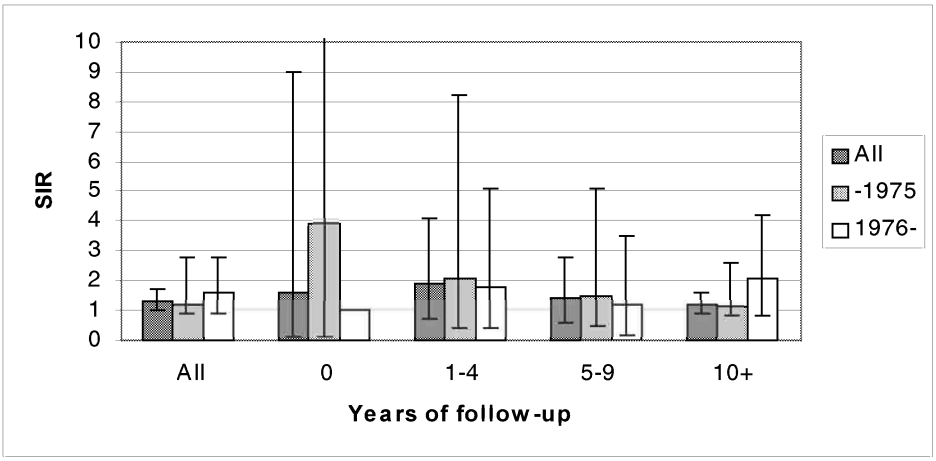

Table 1. Standardized incidence ratios of breast cancer following hydatidiform mole by years of follow-up and calendar period

Among 3371 women and 57,075 person-years of follow-up 59 breast cancer cases were identified. Compared to the 43 expected, this resulted in an overall odds ratio of 1.3 (95% CI 1.0-1.7).

Subset analysis of women with hydatidiform moles before or after 1975 did not detect any distinct differences. All relative risks were above unity.

The same could be said for subset analysis on time from hydatidiform mole until breast cancer. A higher risk was noted the first years following hydatidiform mole than later, but the data were too small to exclude the possibility of chance.

Study II

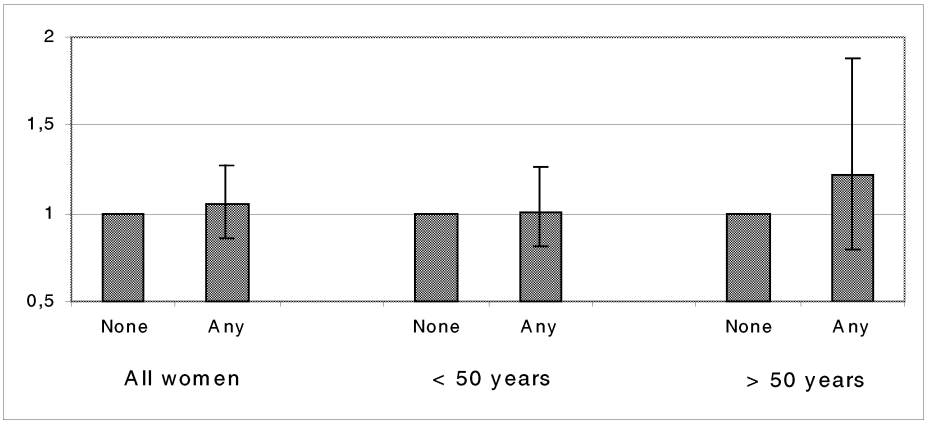

Table 2. Odds ratios of breast cancer following diagnosis of hyperemesis by age at breast cancer diagnosis.

148 cases (1.13 %) and 405 controls (1.18 %) had at least one record of hyperemesis. This resulted in a crude odds ratio of 1.04 (95% CI 0.85-1.26), for any versus no history of hyperemesis.

Adjustment did not alter this result. After adjustments for parity, age at first birth, and the number of pregnancies for which we had exposure information, the odds ratio remained 1.05 (95% CI 0.86-1.27).

Analysis of number of diagnoses did not reveal any trend towards lower breast cancer risk. The crude odds ratios decreased to 0.91 for three or more diagnoses of hyperemesis compared to none, but this risk decrease was due to the effect of increasing parity. After adjustment the odds ratio was 0.98.

Neither did the subset analysis on time between first diagnosis of hyperemesis and diagnosis of breast cancer reveal any clear pattern. A decreased risk was noted between 10 and 15 years following first diagnosis of hyperemesis, but as this risk decrease was not part of a trend, chance is a likely explanation for the result.

Study III

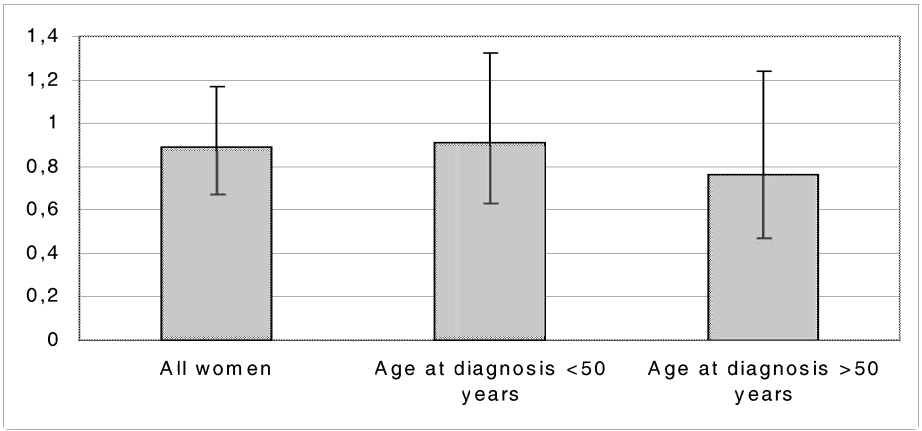

Table 3. Odds ratios of breast cancer before and after age 50, for each additional month of molar pregnancy.

A longer duration of molar pregnancy was associated with a decreased risk of breast cancer (Table 3). For each additional month, breast cancer risk decreased by 11% (95% CI 0.68-1.16). The decrease was more pronounced for post-menopausal breast cancer (after age 50). In this group, each additional month was associated with a 20% decrease in risk, whereas only a 4% risk decrease was noted for breast cancer before menopause. The strongest association was found when women with a duration of less than two months were compared to women with longer duration. In this comparison, women with longer duration had a relative risk of 0.44 (95% CI 0.29-0.96) compared to those with a shorter duration.

Adjustment for parity did not substantially alter the results. After adjustment, the risk was decreased by 24% and 9% for breast cancer after and before menopause, respectively. In the separate comparison of duration more than two months, compared to less than two months, the relative risk after adjustment was 0.41 (0.18-0.92).

Study IV

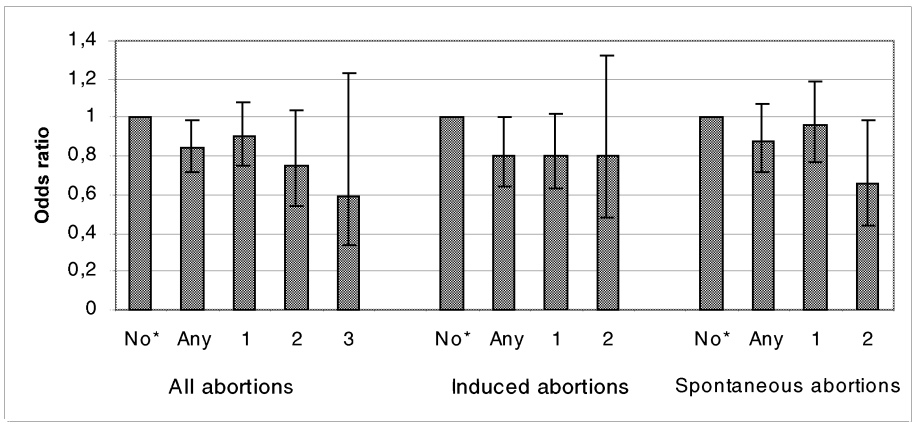

Table 4. Adjusted odds ratios of breast cancer by number of abortions, induced abortions and spontaneous abortions respectively.

383 cases (22%) and 473 controls (27%) reported a history of at least one abortion. This yielded an unadjusted odds ratio of 0.75 for ever having an abortion versus never. 172 cases (10%) and 228 controls (13%) reported at least one induced abortion yielding an unadjusted odds ratio of 0.73. A history of at least one spontaneous abortion was reported by 228 cases (13%) and 282 controls (16%), yielding an unadjusted odds ratio of 0.78.

Adjustment for potential confounders did not substantially alter the results. After adjustment for calendar period of birth, number of births, age at first birth and height the odds ratios were 0.84 for any abortion, 0.80 for any induced abortion, and 0.88 for any spontaneous abortion, respectively.

Statistically significant trends toward lower breast cancer with increasing number of abortions were found for spontaneous abortions and for abortions overall. Three or more abortions compared to none resulted in an odds ratio of 0.59 (95% CI 0.34-1.03).

Study V

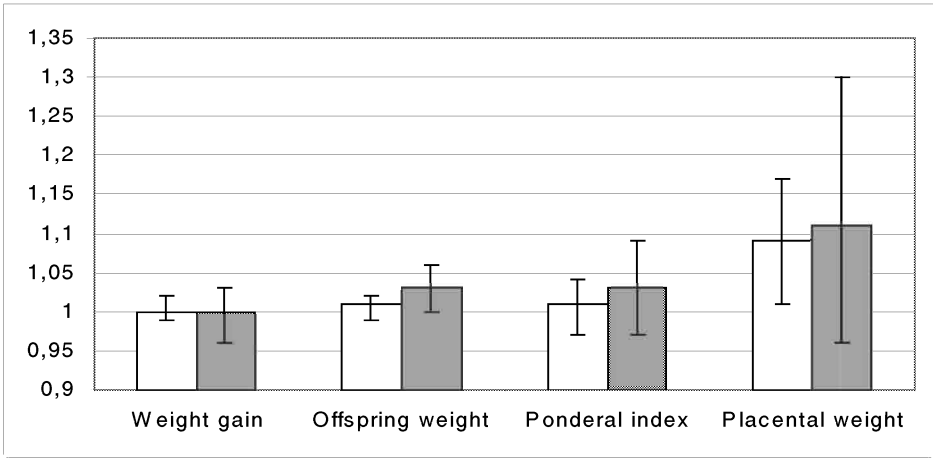

Table 5. Odds ratios of breast cancer for each increment in weight gain, offspring weight, offspring ponderal index and placental weight for all women (white) and nulliparous women (gray). An increment equals for weight gain 1 kg, for offspring weight 100 grams, and for placental weight 100 grams.

Weight gain during pregnancy was not associated with breast cancer risk. Both for the group as a whole and for nulliparous women, the relative risks were 1.00.

Offspring birth weight was associated with a slight increase in subsequent maternal breast cancer risk. Each additional 100 grams was associated with a 1% increase in risk in the group as a whole, and a 4% risk increase in nulliparous women. Both reached a marginal statistical significance (95% CI 1.00-1.03 and 1.01-1.06, respectively). Ponderal index did not have a stronger association to subsequent maternal breast cancer risk than birth weight alone. Among nulliparous women, a slight increase in breast cancer risk was noted with increasing ponderal index, but it did not reach statistical significance.

Increasing placental weight was associated with a statistically significant 8% increase in maternal breast cancer risk, for each additional increment of 100 grams. This risk was more pronounced among nulliparous women (OR 1.19, 95% CI 1.04-1.34).

Adjustments did not substantially alter the results. All results remained at their original levels, except the association between placental weight and breast cancer risk in nulliparous women. The risk increase in this group was after adjustment 11% and the result was no longer statistically significant.

DISCUSSION

FINDINGS AND IMPLICATIONS

Hydatidiform moles

The results from study I indicate that exposure from hCG, as measured by proxy variable hydatidiform mole, does not decrease breast cancer risk. Instead, the data suggest a moderate risk increase. At least one more study has investigated the relationship between hydatidiform moles and subsequent breast cancer risk (124). This study was carried out based on the clinical interest in hydatidiform moles, and did not use the diagnosis as a proxy variable for hCG exposure. A similar result was noted as in Study I, with the same methodology, but with less precision. Future studies should use a different design, which allows investigators to take other potential confounding factors into account, especially reproductive history. Actually, one such study has been performed (as part of a study on reproductive factors), but did only identify one case and one control with molar pregnancy (78). Due to the relative rareness of this exposure, case-control studies are hard to perform.

The duration of molar pregnancies and its relationship to subsequent breast cancer risk has to my knowledge not been studied before. The results from Study III suggest that the increased risk of breast cancer following molar pregnancies (as reported in Study I) is confined to molar pregnancies with a short duration. Shorter pregnancies have been reported to increase breast cancer risk, as compared to pregnancies with a longer duration. This might seem as a paradox when compared to the results from study IV, but aborted pregnancies were in that study compared to not having a pregnancy, and not to having a longer pregnancy. In conclusion, the data suggest that the protection against pregnancies is dependent on duration of hormone exposure.

Hyperemesis

The presented data suggest that hyperemesis during pregnancy do not alter the effect on subsequent maternal breast cancer risk, as opposed to the two previous studies which reported increased and decreased risks, respectively. The study was the first based on prospectively recorded data. The prevalence of exposure is in accordance with previous reports (112). The fact that both previous studies reported results in favor of their (conflicting) hypotheses might reflect interviewer bias in their retrospective design. Chance is, however, the most probable explanation for the conflicting results.

On hCG and breast cancer protection

The presented data from studies I-III do, overall, not support the theory that pregnancies protect against breast cancer through hCG, in contrast to the results from animal experiments.

The experiments performed primarily on Sprague-Dawley rats, but in recent years also on other rodent strains, have certainly increased our knowledge of the pregnancy induced protection against breast cancer. Still, to infer the results from experimentally induced mammary cancer in rats on physiological conditions in women is dangerous. Rat, mouse and human mammary cancers are different in several aspects. As an example, rat mammary tumors are practically always hormone-dependent whereas mice tumors are hormone-independent. Human breast cancer can be either (125). It is also important to distinguish the role hCG have in breast cancer pathogenesis, from that of a potential tissue stabilizing effect long before tumor initiation. Many human cancers, among them breast cancer, express hCG and hCG/LH receptors for so far unknown reasons (101, 126). The specificity of the hCG protection on rat mammary cancer may also be questioned. In experiments on another rat model, administration of estrogens has had a similar protective effect as hCG had in the Russo experiments (127, 128).

In conclusion, epidemiological data from human populations are needed to support the promising experimental data. Such data are still largely missing.

As a highly speculative hypothesis, I have mentioned in study III that different isoforms of hCG, acting in different periods of pregnancy, could have different effects on the breast. The placental hormones are known to typically have multiple effects (129). The forms of hCG present in early pregnancy have been reported to differ from the ones present later (130). Early forms are more acidic than later forms. Different hCG isoforms have also been reported to have different effects (131, 132). If isoforms active early in pregnancy had a different effect on breast cancer risk than those acting later, it could theoretically explain the disagreement between the results from studies I and II and those from animal and *in vitro* experiments. This theory would also fit the results from study III. However, the data available on hCG isoforms is sparse, and such studies have mainly been performed to evaluate screening methods for Down's syndrome.

Although different hCG isoforms may not be the answer, other aspects of duration of hormone exposure may. The studies I and II are based on the assumption of a dose-dependent effect of hCG, as both hyperemesis and hydatidiform moles are conditions where the exposure doses are high. The duration of high exposure, on the other hand, is not longer than in normal pregnancies. If the relationship instead have a threshold dose, and thereafter is dependent on duration, completely different studies are needed.

Abortions

The presented data in Study IV adds to the growing body of evidence that having an abortion does not increase breast cancer risk, as compared to not having a pregnancy. Positive associations were absent both for induced and spontaneous abortions. Instead, the data suggest that also aborted pregnancies, at least if followed by a childbirth, contribute to the protection against breast cancer provided by pregnancies. This suggestion is strengthened by the dose-response relationship observed for abortions in general and subsequent breast cancer risk.

The scientific material presented in this matter is vast, and sufficient to give reasonably safe advice from a clinical point of view. The question is, however, still interesting for epidemiologists and other cancer researchers, because of the differential results from different study designs. The question has been addressed with a variety of experimental and epidemiological designs, and given the consistency in results within each design, the differences between them must reflect methodological challenges.

Anthropometric factors

The results from study V are more of explorative nature. We found no evidence of an effect of maternal weight gain during pregnancy on subsequent breast cancer risk, in accordance with at least one previous study (38). Both placental weight and the child's birth weight were positively associated with subsequent maternal breast cancer risk, although both associations were very modest from a clinical point of view. The results confirm previous studies, which have indicated that pregnancy characteristics could act as proxy variables for factors which modulate the protection of pregnancies on future breast cancer risk.

SYSTEMATIC AND RANDOM ERRORS

Bias

Selection bias

In study I, the registration only covered approximately 75% of all women with hydatidiform mole during the period. It is, however, highly unlikely that the under-reporting (presumably due to lack of knowledge of the reporting obligation among Swedish doctors) would in any way be correlated to future risk of breast cancer for the women. In the case-control studies, all controls were randomly selected from the nationwide registers defining the study-base, making selection bias unlikely. The case selection was limited by a number of reasons, like the completeness of the cancer registry for hydatidiform moles in study III, or the prerequisite of being parous in study IV. These are, however, generalizability matters and not sources of bias.

Observation bias

All case-control studies (II-V) were based on prospectively collected exposure information from medical records (III-V) or registries (II). This reduced the risk of differential reporting based on case-control status to a minimum. There is a theoretical possibility of bias due to differential reporting based on factors correlated to risk of disease, as socio-economic index in study IV. Still, if confounding by socio-economic index in itself (discussed later) was probably small, this hypothetical bias need to be miniscule.

Non-differential misclassification could be a more serious problem. As induced abortions is a morally sensitive issue, underreporting has probably resulted in a non-differential misclassification of the exposure in study IV. This is the case in practically all studies on abortions and breast cancer risk. By using medical records, I believe that we have used answers from an interview where the women were optimally motivated. Still, the women only reported 18% as many previous induced abortions as previous live births. The quota between performed abortions and live births in Sweden based on population statistics is approximately 30% (133). Nevertheless, under-reporting could not explain the absence of a positive association between abortions and breast cancer, as we found consistent risk estimates below null, and non-differential misclassification only creates bias towards null.

In all studies the investigated exposures were only proxy variables of the underlying hypothesized risk factor, and accounted only for part of the exposure from this factor. This could be perceived as a non-differential misclassification. In studies I-III, the proxy variables only measured a minor part of the life-time hCG exposure. In study IV, only abortions before the index birth could be taken into account, and in study V, only variables from one pregnancy were analyzed. This might have made true relationships harder to detect, and it means that null results have less power to completely rule out a true effect.

Confounding

Reproductive factors

Adjustments for parity and age at first birth were made in all studies where such information was available. No further adjustments for reproductive history was made, since these are the most important factors (24).

In study I, no information on reproductive factors was available. This is a major limitation, since it is not unrealistic to believe that hydatidiform moles decrease the number of subsequent pregnancies, although a history of hydatidiform mole has been reported not to affect fertility or outcome of subsequent pregnancies (134).

In studies IV and V, we had no information on reproductive factors after the index birth. This could have resulted in confounding in study IV, given that subsequent pregnancies would be correlated to number of abortions. However, all women were parous, and the effect on breast cancer risk of additional pregnancies is modest (26).

Any relationship between such additional pregnancies and previous abortions is presumably even more modest.

Age at menarche

Age at menarche is inversely correlated to breast cancer risk, and could well be correlated to pregnancy related factors as the ones studied in this thesis. We had no information on age at menarche in any of the studies. However, the age at menarche varies little in the Swedish population (135), so any confounding by this factor was probably modest.

Treatment

Theoretically, the use of cytotoxic drugs in the management of molar pregnancies could have increased breast cancer risk in studies I and III. An increased risk has been reported following cytotoxic combination therapy, but not single-agent methotrexate therapy (136, 137). Methotrexate has been used in Sweden since the mid-seventies, but since stratification on date of diagnosis did not substantially alter the results of study I, and since none of the cases had a diagnosis of choriocarcinoma (indicating heavier treatment), the potential treatment has probably not influenced the results.

Socio-economic factors

We had no information on socio-economic index for the subjects in any of the studies. Breast cancer is positively associated to socio-economic index (138). As also abortions are negatively correlated to socio-economic index (139, 140), this could act as a confounding factor in study IV. As it has been shown that the relationship between socio-economic index and breast cancer to a large extent can be explained by final height and reproductive factors (141), I believe that we have been able to eliminate the major part of this confounding.

Smoking

Smoking has under normal circumstances little correlation to breast cancer (8). However, it has been suggested that smoking during pregnancy might increase subsequent maternal breast cancer risk (142). In the studies where adjustment for smoking status was possible (studies IV and V), this adjustment had no impact on the estimates as smoking was evenly distributed between cases and controls. The most recent data also argues against a relationship between smoking during pregnancy and an altered maternal breast cancer risk (143).

Chance

Chance can never be fully ruled out as an explanation for epidemiological results. The size of all the studies were limited by external factors, and not set based on power calculations. Studies I and III were limited by the number of reported molar pregnancies to the Swedish Cancer Registry, and the number of these who had developed breast cancer. Although a higher power could be wished for, especially in Study III, this constitutes to my knowledge the largest cohort of hydatidiform moles in the world. In Study III, a slightly higher precision could have been achieved by selecting a higher number of controls. While planning the study, we did not estimate the difficulties in obtaining informed consents and medical records to be as hard as it turned out. The improvement would, however, have been modest.

Study II was limited by the number of reported breast cancer cases following child births in the two registers. In spite of the high number of subjects in Study II, the power was reduced by the very low (but realistic) prevalence of exposure. The power could have been increased by using another definition of nausea than hyperemesis. However, as hCG levels have been shown to vary with the degree of nausea (92), the use of hyperemesis as proxy variable did probably refine the group to include a higher proportion of women with significantly increased hCG levels.

Studies IV and V were limited by resource constraints. As we both had more potential cases and potential controls than what we could get exposure information on, the highest possible power was achieved by a one-to-one matching design.

All studies were limited in scope, which is a strength as this reduces the risk of mass significance. For statistical significance, the alpha-level was set to 5% which is the custom in most epidemiological work today.

GENERALIZABILITY

Age and menopausal status

Breast cancer cases in all studies were younger than the population average. Given a restricted time of follow-up, the first breast cancer cases will always be the youngest, who will dominate the data. This might especially be a problem in studies IV and V. Regarding abortions (study IV), short follow-up periods result in early average ages at diagnosis in practically all prospective studies, both cohorts and case-control studies with prospective data. This should be kept in mind when comparing results from studies with different designs. In study IV the mean age at diagnosis was 40 years, an age at which only a minority of breast cancer cases have occurred. Furthermore, more than 90% were below 50 years of age, implicating that very few were post-menopausal. However, if aborted pregnancies would protect against breast cancer in a manner similar to that of term pregnancies, it seems reasonable to believe that any protection conferred by aborted pregnancies before menopause will only be stronger after menopause.

The results in study V could only be interpreted for pre-menopausal breast cancer. This is especially important when considering maternal weight gain, as obesity

and weight gain previously have been described to have different effects on pre- and postmenopausal breast cancer.

Potential effect modifiers

The histopathological type of breast cancer could not be investigated in any of the studies, as this information was not available in our data. Recent reports have suggested differences both in etiology and in mortality between ductal and lobular breast cancers. However, the role of reproductive factors in breast cancer etiology is, according to a recent report, little dependent on type of cancer (144).

Hereditary factors could also work as effect modifiers. Hormonal factors have been shown to have different effects on breast cancer risk among BRCA1/2 carriers than among non-carriers. Early age at first birth, for example, increases breast cancer risk in this group of women instead of decreasing (145). Since these factors, in spite of their relative rareness in the general population, are common among breast cancer cases, future studies focused on this group are needed.

CONCLUSIONS

I always feel reluctant to draw conclusions from my own studies alone, as epidemiological studies should be interpreted within the framework of all available evidence on the subject. Since this is my thesis, I will nevertheless give suggestions as to what conclusions could be drawn from my data, disregarding results from the rest of the scientific community.

From a clinical perspective

- Pregnancies resulting in induced or spontaneous abortions do not increase breast cancer risk, at least not if the woman at some point after this have a pregnancy which results in a child. This indicates that there is no need to take future breast cancer risk into account when considering induced abortions, and that a history of abortions is of little importance when assessing a patient's risk of breast cancer.

- Data suggest that hydatidiform moles increase future breast cancer risk, especially in women who have a short duration before evacuation, but the scientific evidence so far is too weak and the risk increase too small to warrant any changes in breast cancer prevention for this group of women.

- Neither hyperemesis, nor weight change during pregnancy has any impact on the protection against breast cancer conferred by pregnancies.

- Although both child weight and placental weight seem to modulate the protection against breast cancer conferred by pregnancies, the changes are too small to take into account in models predicting a woman's future breast cancer risk.

From an etiological perspective

- As measured by surrogate markers hyperemesis and hydatidiform moles, exposure to hCG does not seem to account for the protection against breast cancer conferred by pregnancies.

- If hCG does decrease breast cancer risk, the protection is confined to women with a longer duration of exposure.

- The finding of a weak protective effect by pregnancies resulting in an abortion suggests that the hormonal mechanism by which pregnancies protect against breast cancer is present even in the first trimester.

- The protection against breast cancer conferred by pregnancies can be modulated by weight of child and placenta. This indicates that hormones determining these measurements are also involved in the differentiation of the breast epithelium.

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