TESTICULAR CANCER – NEW ETIOLOGICAL UNDERSTANDING OF THE EPIDEMIC

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SUMMARY

The incidence of testicular cancer has increased dramatically for decades, but what causes this increase is not known. The aim of this thesis was to find some explanation for the increasing rates, and to understand more about the mechanisms that can cause testicular cancer. We have conducted a series of epidemiological studies to try to reach this goal.

First, we investigated the relevance of Clemmesen’s hypothesis that smoking during pregnancy can cause testicular cancer in the offspring. Using data from the Nordic Cancer Registries and from previous smoking surveys, we could investigate if there is a correlation between smoking habits among women at fertile ages and testicular cancer incidence among their presumed offspring. In support of the Clemmesen hypothesis, we found, with the exception of Finland, a strong both geographical and temporal correlation between female smoking prevalence and testicular cancer incidence in the Nordic countries.

Second, we tested the same hypothesis on the individual level. Using data from the Swedish Cancer Registry and from the Swedish Medical Birth Register, we conducted a population-based case-control study on 192 cases of testicular cancer, and 494 matched controls, born in Sweden in 1973 onwards. Data on smoking during pregnancy, recorded already when the mothers were pregnant, was manually retrieved from the delivery archives throughout Sweden. We found no association between pregnancy smoking and risk of testicular cancer, and concluded that the epidemic rise in testicular cancer is not due to the surge in smoking among women.

Third, using data from different Swedish registries, we could construct a cohort of almost 17,000 men surgically treated for undescended testis between 1964 and 1999. The men in the cohort were followed up for the occurrence and of testicular cancer, and we investigated if age at surgical correction of undescended testis affect the risk of developing testicular cancer in adulthood. We found that the risk among men who were treated after 13 years of age was twice that among men treated before 13 years of age. These results indicate that treatment before puberty decreases the risk of developing cancer, and that the misplacement of the testis is a factor in the development of testicular cancer.

Fourth, we used the same design and study subjects as in the second study, but with an additional 101 cases and 367 controls. We aimed at investigating if signs of placental malfunction, such as pregnancy induced hypertension and preeclampsia, are related to the risk of testicular cancer. We found a more than threefold decrease in risk among sons exposed to severe gestational hypertension, but a 60 percent increased risk among the sons of women with mild gestational hypertension. And the results for preeclampsia were similar. We speculate that the hormonal changes that seem to occur in severe hypertensive conditions during pregnancy are associated with a decreased risk of testicular cancer.
CONTENTS

LIST OF PAPERS .................................................................................................................. 3
INTRODUCTION .................................................................................................................. 5
BACKGROUND .................................................................................................................. 6
   Male reproductive development .................................................................................. 6
   Tumor classifications and definitions ....................................................................... 7
   Testicular carcinogenesis ......................................................................................... 8
   Descriptive epidemiology ......................................................................................... 10
   Recognized risk factors for testicular cancer ......................................................... 12
   Some clues on the etiology of testicular cancer ..................................................... 15
   Hypotheses on mechanisms and factors causing testicular cancer ....................... 16
   Potential risk factors for testicular cancer ............................................................. 19
OUTLINE .......................................................................................................................... 26
METHODS .......................................................................................................................... 27
   Sources of data .......................................................................................................... 27
   Study designs .............................................................................................................. 29
RESULTS .......................................................................................................................... 33
   Pregnancy smoking and risk of testicular cancer (study 1 & 2) ......................... 33
   Age at surgery for undescended testis and risk of testicular cancer (study 3) .... 34
   Gestational hypertensive disorders and risk of testicular cancer (study 4) ........ 37
DISCUSSION ..................................................................................................................... 39
   Methodological considerations .............................................................................. 39
   Findings and implications ......................................................................................... 41
   Suggestions on future research .............................................................................. 43
CONCLUSIONS .................................................................................................................. 44
ACKNOWLEDGEMENTS ................................................................................................. 45
REFERENCES ................................................................................................................... 47
LIST OF PAPERS
This thesis is based on the following papers:


INTRODUCTION

Germ cell testicular cancer is a disease with exceptional features\textsuperscript{1}: It is the commonest cancer among young men in many parts of the world. It shows striking ethnic and geographic differences in occurrence. It has increased dramatically in incidence in many populations during the past century. The increasing trend in testicular cancer risk follows a birth cohort pattern. There is a strong familial component of testicular cancer risk. The histopathology of the tumor is unique with cancer cells that can develop into virtually any cell type of the body. The tumor is remarkably sensitive to chemotherapy and is a model for a highly curable, advanced cancer.

Still, the etiology of testicular cancer is poorly understood and there is no satisfactory explanation to the epidemic increase in incidence.

The aim of the present thesis is to shed some new light on the enigmatic etiology of germ cell testicular cancer. Or, put in other words, to find some explanation for the figure below (Figure 1).

Figure 1. Richiardi et al. 2004\textsuperscript{2}. 
BACKGROUND

Male reproductive development

The principal idea is that testicular carcinogenesis starts in utero. Therefore, a brief section on important aspects and defects of male development follows3,4.

The gonad and its cells

Early in the fetal development (week 4 to 6) the primordial germ cells migrate from the yolk sac to invade the genital ridges, forming the indifferent gonads, and mature into gonocytes. Influenced by an activated male determining gene from the Y-chromosome, SRY, the indifferent gonads start their transformation into testes. Perhaps most importantly, SRY induce some cells of the genital ridge to differentiate into Sertoli cells, the cells that seem to orchestrate the coming differentiation of the germ cells and the other testicular cell lineages. The Sertoli cells aggregate around the germ cells to form the testis cords around week 7. Soon thereafter, mesenchyme cells that surround the testis cords give rise to the interstitial cells, most importantly the androgen producing Leydig cells.

By about week 8, the Sertoli cells start to secrete anti-Mullerian hormone which causes regression of the Mullerian ducts. Under the influence of human chorionic gonadotropin (hCG) secreted by the placenta, the Leydig cells in turn start to secrete testosterone that salvage the Wolffian ducts from degeneration, favors downstream masculinization, as well as testicular descent. The gonocytes, which may replicate mitotically but are prevented from entering meiosis by the Sertoli cells, will differentiate into spermatogonia during the second and third trimester.

During the first 3 to 6 months of life the hypothalamic-pituitary-gonadal axis of the newborn baby is transiently activated; there is a temporary surge in gonadotropins and sex hormones, and the number of Sertoli cells and Leydig cells increase. Hence, this period is sometimes referred to as ‘mini puberty’. The reason for this hormonal period is not known, but it may be to complete the transformation of gonocytes into spermatogonia, an event important for future fertility5. At puberty, the levels of gonadotropins and sex hormones increase again, and the spermatogonia, supported by the mature Sertoli cells, undergo meiosis and further maturation into sperm.

Male external development

After the start of testosterone production by the Leydig cells, a complex and not fully understood androgen-driven masculinization process starts3. Testosterone is converted into the more potent metabolite 5α-dihydrotestosterone (DHT), which together with testosterone binds to the androgen receptor present in the cells of the developing external genitalia. This leads to morphological changes, such as development of the prostate gland, formation of the scrotum, and the elongation of the genital tubercle and the fusion of the urethral folds to form the penile urethra.

A disorder, hypospadias, is a condition in which the urethra has not been properly fused and the urethral orifice is located somewhere along the underside of the penis. Given that as many as one in every 125 boys reportedly have this condition, the processes of the urethral fusion appears to be delicate6. Still, faults in the androgen receptor and
other genetic defects can explain only all small proportion of all cases of hypospadias, and the etiology of this condition remains poorly characterized.

**Descent of the testis**

The testes descend in two distinct phases. First, the transabdominal phase (week 8 to 15), which is basically the result of the growth and elongation of the fetus while the testes are in a fixed position; the cranial suspensory ligament regresses while the testis is anchored caudally to the internal inguinal ring by the swollen gubernaculum. Even if much of the knowledge derives from rodent models and therefore may not be applicable to humans, there is evidence that the regression of the cranial ligament is androgen dependant while the swelling of the gubernaculum is dependant on Insulin-like hormone 3 (INSL3) secreted by the Leydig cells, possibly augmented by anti-Mullerian hormone and/or androgens. The second, inguinoscrotal phase, occurs in the third trimester and is supposed to be completed by birth. This step is perhaps even less well understood, but in contrast to the transabdominal phase it appears to be truly androgen dependant, as it is not completed in men with androgen insensitivity syndrome. It has been proposed that shrinkage and shortening of the gubernaculum, together with increased intra-abdominal pressure, forces the testis through the inguinal canal, or that the migration of the testis into the scrotum is under neurotransmitter control by the genitofemoral nerve, which in turn is under influence of androgens.

Undescended testis, or cryptorchidism, occur in up to 9% of newborn boys with the highest rates among boys born preterm, with signs of impaired fetal growth, or with other congenital abnormalities such as hypospadias. Unilateral undescended testis is overall slightly more common than bilateral, whereas among full term boys about one third have bilateral undescended testis. The majority of undescended testes are found in a high scrotal or inguinal position, and only a small proportion of boys who undergo surgical correction (i.e., orchiopexy) for cryptorchidism have intra-abdominally placed testes. It thus appears as if the second stage of testicular descent goes awry in most instances. Testes that are undescended at birth often descend during the first year of life (up to 80%) . Some authors have speculated that this is an effect of the surge in sex hormones during mini puberty. Conversely, testes that are in a scrotal position at birth can ascend later in life (i.e., acquired cryptorchidism). In fact, as many as 1 to 2% of boys aged 6 to 13 years, and the majority of boys referred to outpatient clinics for cryptorchidism, may have acquired cryptorchidism. It has been proposed that failure of the postnatal elongation of the spermatic cord causes acquired undescended testis, but this remains to be verified.

**Tumor classifications and definitions**

Germ cell testicular cancer can be divided into two main histological subtypes, seminomas and non-seminomas. Seminomas account for approximately 50% of the tumors, differentiate along the gonadal cell lines, and resemble primordial germ cells and gonocytes. Non-seminomas, in turn, have retained pluripotency and include a number of different subgroups. These tumors display various stages of embryonic differentiation; ranging from highly undifferentiated cells resembling embryonic stem cells in the
embryonal carcinomas, to the highly differentiated cells of somatic tissue in the teratomas. Non-seminomas may also present as extraembryonic tissue, that is, as yolk sac tumors or as choriocarcinomas. In about 10% of the tumors, a mixture of cells from the different subtypes (with or without a seminoma component) is present. Germ cell tumors occasionally (<10%) arises in extra-gonadal sites along the midline of the body, possibly reflecting the migration route of the primordial germ cells during embryogenesis.

Clinically, non-seminomas have a more aggressive behavior, are less susceptible to radiation, and manifest at an earlier age. Moreover, mature teratomas, in contrast to other tumors, are less sensitive to platinum-based chemotherapy. These differences indicate etiological heterogeneity between the two groups. Most likely, however, key risk factors in terms of etiological fractions are shared (see below). Therefore, if not stated otherwise, seminomas and non-seminomas are treated as a single entity in this thesis.

In addition, testicular germ cell cancer is frequently divided into 3 groups depending on age of occurrence. First, the rare tumors of the newborns and infants that predominantly manifest as teratomas or yolk sac tumors. Second, the seminomas and non-seminomas of the adults. Third, the spermatocytic seminomas of the elderly. It is debated if the childhood tumors share etiology and pathogenesis with the tumors of the adults, and the spermatocytic seminomas are considered not to, as they develop from more mature germ cells21,22. For that reason, testicular cancer will hereon be synonymous only to testicular germ cell tumors of the adults. Correspondingly, testicular tumors originating from cells other than germ cells (<10% of all testicular tumors), for instance Sertoli cells or Leydig cells, are not considered further.

**Testicular carcinogenesis**

Germ cell testicular cancer is suspected to derive from development arrest and disturbed differentiation of primordial germ cells or gonocytes during fetal life. This idea is based on epidemiological findings, coupled with findings from histopathological studies.

The widely accepted model for testicular carcinogenesis postulates that testicular tumor cells develop from precursor lesions called intratubular germ cell neoplasia unclassified (ITGCNU), also known as carcinoma in situ (CIS)23. The CIS cells, which are found in the seminiferous tubules, are pluripotent cells that may evolve into either seminomas or non-seminomas; although the exact pathway through which the CIS cells differentiate into their final morphological state is not known (e.g., non-seminomatous tumors perhaps always evolve through a stage of seminoma)21,22. Numerous investigators have found that CIS cells are morphologically similar to primordial germ cells and gonocytes, and that there are similarities in gene expression, telomerase activity, genomic imprinting, and immunohistochemical characteristics between these cell types21,22. Hence the conclusion that primordial germ cells or gonocytes transform into pre-CIS or CIS during embryogenesis and progress into invasive cancer in adulthood. It should be noted, however, that two models of origin of CIS cells have been put forward. CIS cells has been hypothesized to be initiated at a spermatocyt-stage during pubertal meiosis, and, in addition, pluripotent spermatogonial stem cell-like cells, that perhaps could give rise to testicular cancer, have been identified in adult mouse testis24-26.
Irrespective of how CIS develop into overt cancer, it is certainly a precursor lesion in a substantial proportion of the cases; if not in all. First, CIS is a frequent finding in testicular tissue adjacent to invasive tumors. Second, the risk of developing invasive cancer after a diagnosis of CIS seems to be markedly increased (an estimated 50% risk within 5 years after a diagnosis of CIS). Third, CIS is more common among men with a high risk of developing testicular cancer (and in some studies reflect the expected lifetime risk of developing testicular cancer in that given group), for instance among men with contralateral testicular cancer, extra-gonadal testicular cancer, and likely among men with fertility problems, or a history of undescended testis. Fourth, the prevalence of CIS in the general population appears to be low, seemingly mirroring the life time risk of developing testicular cancer among healthy males. Furthermore, and in line with the prevailing theory of an embryonic origin, CIS cells have been described in aborted trisomy 21 fetuses. Also, in a case report, a 10 year old boy diagnosed with atypical germ cells closely resembling CIS cells was followed up until he developed invasive cancer in adulthood. Additionally, it is often stated in the literature that in effect all mature CIS lesions will eventually progress into frank cancer.

Some of these findings and conclusions are however based on scarce data, and are somewhat contradictory to other findings. For instance, if CIS develop during fetal life (or if pre-CIS develop into CIS at puberty) and 50% of all CIS will progress into invasive cancer within 5 years; this would imply that 50% of all testicular cancers would develop, at the latest, within 5 years after puberty, which is not the case (see below). Moreover, according to one study from Denmark, 5 men were diagnosed with CIS and 1 man with invasive testicular cancer (<0.5%) among 1,335 cryptorchid men who underwent surgical correction between the ages of 0 to 19 years (median 11.7 years). This figure is considerably lower than would be expected in a high risk group such as Danish cryptorchid men, and less than one third compared to the prevalence of CIS previously reported among adult Danish men with a history of cryptorchidism. Another research group from Switzerland looked for pre-CIS/CIS in 440 men who underwent routine testicular biopsies at prepubertal orchiopexies in 1970 to 1979. Twenty two patients (5%) had signs of CIS at biopsy, but no overt tumors had been diagnosed among those (14 men) followed-up in 1997.

One possible explanation for these findings is that CIS or pre-CIS lesions behave dynamically, and that the occurrence of testicular cancer in adulthood is not simply a reflection of the prevalence of CIS at birth. The initiating step in the pathogenesis of testicular cancer may very well occur during embryogenesis, and CIS may be the precursor of all germ cell testicular cancers; but if CIS can be regarded as a not-yet-invasive testicular tumor and can be included in the definition of testicular cancer remains to be confirmed. Nonetheless, with the lack of good animal models, CIS has become an invaluable tool in better understanding the histopathology and tumor biology of testicular cancer.
Descriptive epidemiology

Given that testicular cancer affects young men, that treatment is needed, and that a diagnosis is generally confirmed by histopathological examination, there is little reason to suspect that the fascinating findings of the descriptive studies are artifacts.

Age-incidence patterns
The incidence of testicular cancer is low in childhood, steeply increases after puberty, peak at 30 to 35 years of age, and then declines to almost nil by the age of 60. This age-incidence curve is largely the same in different populations, suggesting that the window of susceptibility (or age at exposure) to harmful exposures is rather narrow, invariate, and should occur before adulthood. Furthermore, the sudden and steep increase in incidence starting at around puberty implicates sex hormones in the progression of invasive cancer. It can also be speculated that the decrease in occurrence among the elderly reflect the fact that there are no precursor lesions left to advance into overt cancer.

The age-incidence curves of seminomas and non-seminomas are different. Non-seminomas manifest clinically at 25 to 29 years of age, whereas seminomas peak in incidence some 10 years later. This observation is in favor of a differential etiology between seminomas and non-seminomas, but may also be explained by, for instance, the more rapid growth and higher capacity to metastasize of non-seminomas.

Occurrence
Although rare in absolute terms, testicular cancer is the most common cancer among young men in many parts of the world; with life time risks approaching 1% in high-incidence populations. There are moreover considerable geographical and ethnical variations in occurrence, with on average 6 times higher incidences in developed areas compared to developing areas. The highest rates, around 8-10 per 100,000 world standard population, are found in Denmark, Norway and parts of Switzerland and France. In Africa and Asia, the incidence is typically less than 2 per 100,000.

Notably, the incidence vary several folds between neighboring countries, and these differences do not necessarily reflect ethnicity. Concurrently, the incidence is rather homogenous within countries, suggesting that few country specific exposures or habits rather than a multitude of air pollutants or alike are etiologically important factors. Though there are exceptions, there are for instance about 2-fold variations in incidence between regions in France, and an about 5-fold ethnic higher incidence in European-Americans compared to African-Americans in the US.

Temporal trends
Globally, the rate of testicular cancer has continued to increase during much of the 20th century. Increasing trends have been reported in most European countries (with the exception of historically high and stable rates in Switzerland), the US white population (while not to the same extent in the black population), Canada, and Oceania. In Europe, several countries have experienced annual increases of 2 to 6% for several years, doubling the rate every 20 years or so. Fortunately, in more recent times (men born after around 1960), there has been reports of a leveling off in incidence. This change of trend is indicated primarily in high-risk countries, suggesting that the epidemic has
reached a more mature phase in these countries but will continue to increase in coming years in low risk countries\textsuperscript{1,2,50,52,53}.

Although period effects have been reported, age-period-cohort modeling has generally shown that the risk of testicular cancer follows a cohort effect, implying that important risk factors are related to year of birth\textsuperscript{2,49,50,52,54}. Indeed, in some populations the risk is tenfold higher among men born in the 1960s compared to men born in the early 1900s\textsuperscript{54}, further suggesting that newly introduced environmental exposures are strong risk factors for testicular cancer. Interestingly, temporal trend analyses have uncovered that the increasing trend in testicular cancer incidence was temporally halted among men born right before or during the Second World War in Sweden, Norway, Denmark, and some other European countries\textsuperscript{2,47,50,52,54,55}. One straightforward explanation to this finding is that testicular cancer risk is rapidly affected by every-day changes in consumption (in early childhood or during pregnancy) of products that were scarce, or possibly restricted, in these countries during the war. However, the ‘war-cohort-effect’ has not been demonstrated in countries such as Finland and Poland, which were arguably more affected by the war.

Seminomas and non-seminomas
Temporal trends for seminomas and non-seminomas seem to be generally similar, supporting the inference that seminoma and non-seminoma are etiologically similar\textsuperscript{1,2,49,52,56}. This idea is further supported by the fact that the distribution of seminomas and non-seminomas is similar in populations with highly differing overall occurrence, and that the ratio of seminomas to non-seminomas over time has been fairly stable in most populations\textsuperscript{1,47}.

Prognosis
The great turning point in the treatment of testicular cancer came in the 1970s with the introduction of platinum-based chemotherapy\textsuperscript{1}. Today, more than 90\% survive there cancer for more than 5 years, and most countries have experiences decreasing mortality rates\textsuperscript{50}.

Migration studies
Migration studies may effectively disentangle between the effects of genetics and environment on risk of disease. For testicular cancer, the risk in the first generation of immigrants is similar to the risk in their country of origin\textsuperscript{57-60}, and this risk appears to be similar irrespective of age at immigration and duration of stay\textsuperscript{57,59}. Several studies have also reported that the risk of testicular cancer in the second generation approaches that of the country of immigration\textsuperscript{59-62}, lending strong evidence to the theory that environmental factors operating in utero or early in life are critical in the causation of testicular cancer. Interestingly, these results do not seem to apply to all countries. For example, men born abroad to Danish parents appear to maintain unchanged high risks. A recent investigation showed that the risk among men born outside of Denmark (and who later immigrated to Denmark), and who had at least one Danish parent, was the same or higher compared to men born and raised in Denmark by Danish parents\textsuperscript{59}. Another study have reported that the second generation of Danish immigrants to Sweden are at high risk of
developing testicular cancer if both parents are Danish, but not if only one of the parents is Danish.63.

Recognized risk factors for testicular cancer

Inherited susceptibility
Some 1 to 3% of all testicular cancers cluster in families.64,65 Brothers of men with testicular cancer have an 8 to 10-fold increased risk of developing testicular cancer, whereas the relative risk among father-son-pairs is about 4.65-70. Indeed, these familial risks are among the highest for any cancer. Albeit difficult to distinguish between genes and environment, it has been estimated that for an environmental factor to give rise to this kind of risk estimates, the risk factor need to be extraordinarily potent.65 Since no such risk factor is known, genetics arguably play an important role in familial testicular cancer. And there are additional findings supporting the role for a genetic component. Even if results are conflicting, it appears as if familial testicular cancer more often is bilateral, and have an earlier age at onset (which implicates a hereditary component).64,66-69 Testicular cancer is moreover associated with various rare genetic disorders, for instance Klinefelter’s syndrome (47, XXY) and XY-gonadal dysgenesis.65 Geographic clustering and ethnic differences in incidence, such as the highly differing rates between whites and blacks in the US, furthermore indicate a genetic pathway. Given the rarity of testicular cancer, no twin study with an adequate sample size have been conducted so far (although results are in line with a greater concordance among monozygotic twins than dizygotic twins).71

A segregation analysis has indicated a single major gene with an estimated frequency of 3.8% and a lifetime risk of developing testicular cancer of 43% among affected homozygous.72 Linkage studies and association studies have, however, thus far not robustly identified any certain genomic region that can account for a substantial part of the cases, suggesting that multiple susceptibility loci with feeble effects are involved.65,73

Undescended testis
The risk of testicular cancer is increased approximately 5-fold among patients with a history of undescended testis (reviewed by Dieckmann and colleague74), and 5 to 10% of all testicular cancer patients have had undescended testis.75 Two mechanisms may be in action: cryptorchidism itself causes testicular cancer, or both conditions share etiology.

Mechanistic investigations on the association between undescended testis and testicular cancer are unfortunately complex. First, the risk of testicular cancer is perhaps not increased among cryptorchid men whose testes naturally descend in childhood.76 Second, the risk of testicular cancer is most likely higher in bilateral relative to unilateral cryptorchidism,76 and the risk may depend on the specific position of the ectopic testis (e.g., higher risk in intra-abdominally placed testes). Third, acquired cryptorchidism is a recently acknowledged phenomenon, and its potential effects on testicular cancer risk have not been thoroughly investigated.

To disentangle between the two mechanisms outlined above, several investigators have assessed the risk of testicular cancer in the normally descended testis (contralateral),
compared to the risk in the undescended testis (ipsilateral), in men with unilateral undescended testis. In a recent meta-analysis, we found that the pooled relative risks for testicular cancer in the ipsilateral and contralateral testis were 6.33 (95% CI, 4.30 to 9.31) and 1.74 (95% CI, 1.01 to 2.98), respectively. This finding suggests that cryptorchidism and testicular cancer share etiology, but in particular support the theory that the ectopic position of the testis is a factor in the development of testicular cancer.

Another approach is to investigate if the risk of testicular cancer is affected by age at surgery for undescended testis, as the risk should increase with age at correction if the ectopic position is damaging. Most previous studies on this matter are retrospective case-control studies with small samples. Several, but not all, suggest that the risk of testicular cancer is partly affected by age at treatment, with men treated after around 10 years of age at greatest risk. Hence, these results are compatible with both mechanisms.

Any existing geographical and temporal ecological correlations between undescended testis and testicular cancer would be in favor of a shared etiology. Interestingly, some geographical similarities clearly exist. Temporal trend analyses are, however, unfortunately complicated by the changing classification of cryptorchidism, differences in treatment regimes, and the lack of high quality registries. Besides, to correlate the occurrence of undescended testis and testicular cancer is challenging since testicular cancer manifest some 30 years after cryptorchidism, increasing the likelihood of spurious findings created over time. Previous studies have used data from either birth defect registries, medical examination of consecutive newborns in different time periods, or rates of cryptorchidism or orchiopexy from hospital discharge registries. Some studies have reported increasing trends, while some have not. Taken together, it appears as if cryptorchidism increased in occurrence between the 1950s and 1980s in several populations, but that rates thereafter have been rather stable.

Interestingly, the clear-cut ethnic differences in testicular cancer rates are for some reason not mirrored in cryptorchidism prevalence. In the US for instance, the incidence of testicular cancer among white men is 5 times greater than the incidence among black men, while the difference in cryptorchidism prevalence is negligible.

Shared risk factors between cryptorchidism and testicular cancer are also in favor of the idea of shared etiology. Frequently reported risk factors for undescended testis include low birth weight, preterm delivery, being born small for gestational age (SGA), and congenital malformations, including hypospadias. With the probable exception of low birth weight, none of these factors are however established risk factors for testicular cancer; and the association between impaired fetal growth and testicular cancer is still a hypothesis with limited support (see below). Furthermore, the few studies that have investigated the association between hypospadias and testicular cancer are inconclusive.

Several rare genetic disorders are associated with both testicular cancer and cryptorchidism. Notably, men with Kallman syndrome, a condition of congenital hypogonadotropic hypogonadism, enter puberty late, if at all, and these men have a high prevalence of cryptorchidism but a low risk of developing testicular cancer (suggesting
that proliferation stimuli from pituitary-gonadotropin-mediated signals are important in the progression of testicular cancer)\textsuperscript{105}.

In short, it is known that cryptorchidism is a risk factor for testicular cancer. But if this association is explained by shared etiology, adverse effects of cryptorchidism itself, or a combination of these mechanisms, is not known.

**Impaired fertility**

A record of fertility problems is a frequently reported risk factor for testicular cancer, and decreased fathering, even years before the cancer diagnosis, has been reported among testicular cancer patients\textsuperscript{106-110}. Shared genetic or environmental risk factors, rather than local or systemic tumor-effects, are presumably the chief explanation for this association. First, sibship size has been found to be inversely associated with testicular cancer risk in a study encompassing around 3,000 cases\textsuperscript{111}, and a study from Norway including almost 4,000 testicular cancer patients reported both a reduced number of children, and a reduced ratio of unlike-sex twins (an often used proxy for fertility), among their parents\textsuperscript{112}; indicating that parents to testicular cancer patients have impaired fertility. In accordance with the idea that testicular cancer is associated with ‘familial subfertility’, findings from another study indicate that brothers, but not sisters, to testicular cancer patients tend to father fewer children, and to have a lower proportion of unlike-sex twins\textsuperscript{113}. Second, Jacobsen and colleagues investigated the risk of testicular cancer in a cohort of men with fertility problems\textsuperscript{114}. Overall, the risk was increased 60\% among men in the cohort compared to the general Danish population, and the risk increased with increasing number of ‘subfertility-measures’ present (defined by semen analysis). If secondary effects of a tumor were to explain the association between abnormal semen analysis and testicular cancer, a trend with decreasing risk of testicular with increasing time since semen analysis should be expected, but no clear-cut trend was evident. Third, in men with unilateral testicular cancer, semen quality appears to be worse than it ought to be among men with only one well-functioning testis, suggesting that there is a pre-existing impairment of the spermatogenesis also in the contralateral testis\textsuperscript{115}. This notion is supported by findings of impaired spermatogenesis in 50\%, and signs of testicular dysgenesis (e.g., undifferentiated tubules, Sertoli cells only pattern, micro calcifications) in up to 25\%, of testicular biopsies from the contralateral testis in men with unilateral testicular cancer\textsuperscript{34}. If this association between testicular cancer and familial subfertility is caused by shared genetic factors, or by shared environmental factors, remains to be revealed.

There are worries that the semen quality has deteriorated during the past fifty years\textsuperscript{116}. The issue took wing in 1992, when Carlsen and colleagues published a comprehensive meta-analysis reporting a decreasing trend in sperm density between 1940 and 1990\textsuperscript{117}. Given the many methodological difficulties (e.g., differing study populations, geographical variations, differences in abstinence time) in investigating and interpreting temporal trends in semen characteristics, it is disputed whether or not a true declining trend exists\textsuperscript{118,119}. A later investigation, including additional studies and analyses, supported the findings by Carlsen and colleagues; although the authors concluded that this would not end the debate\textsuperscript{120}. Albeit, at least the remarkable geographical differences
in testicular cancer incidence in some Nordic countries seem to be paralleled by similar patterns in semen characteristics. Hence, as for cryptorchidism, there are some findings suggesting parallel geographical and temporal trends between testicular cancer and impaired fertility. It should be kept in mind, however, that any such existing trends do not necessarily reflect a true relationship between these conditions.

In short, there is plenty of evidence in favor for an association between impaired fertility and testicular cancer, and this association appears to be explained by shared environmental or genetic causes, rather than secondary tumor effects.

**Previous testicular cancer**
The strongest risk factor for testicular cancer is testicular cancer. Among men with a history of testicular cancer the risk is increased up to 20-fold, and the risk seem to decrease with age at diagnosis of the primary tumor. The second tumor is moreover not necessarily of the same histological type as the primary cancer, supporting the idea that seminomas and non-seminomas share etiology.

**Some clues on the etiology of testicular cancer**

Given the findings from histopathological studies, the descriptive epidemiology of testicular cancer, and the knowledge emerging regarding established risk factors; some conclusions on the etiology of testicular cancer should be possible to draw:

- Testicular cancer can be initiated in utero.
- Puberty is a critical period in testicular cancer progression.
- Genetic factors are important in testicular cancer etiology.
- The increasing trend is caused by (few) environmental or life-style factors.
- These factors have become more prevalent with time.
- These factors vary much and distinct in prevalence between populations.
- These factors operate before adulthood.
- These factors operate during a rather short period(s) of time.
- These factors are likely to operate in utero or early in life.
- These factors (if they operate in utero or early in life) perhaps reached their peak in prevalence in the 1960s in high-incidence countries.
- The supply/usage of these factors (if they operate in utero or early in life) was likely restricted during World War 2 in some countries.
However, important questions remain:

- Can testicular cancer be initiated after birth?
- To what extent is the life time risk of testicular cancer determined by prenatal cancer initiation compared to postnatal promotion (or initiation)?
- Which are these environmental factors causing the temporal trends?

Hypotheses on mechanisms and factors causing testicular cancer

The intriguing features of testicular cancer have encouraged a number of etiological hypotheses. It is beyond the scope of this thesis to discuss them all. Here follows a brief review on a selection of etiological hypotheses relevant for this thesis.

The estrogen hypothesis and the Testicular Dysgenesis Syndrome
Almost 30 years ago, Henderson and colleagues published a study reporting that excessive nausea and hormone use during pregnancy increases the risk of testicular cancer in the offspring. The authors hypothesized that a relative excess of certain hormones (in particular estrogens) during testicular differentiation is a major risk factor for testicular cancer. Over time, this hypothesis has been revisited and refined many times.

In 1993, Sharpe and Skakkebaek restated the hypothesis in the so called ‘estrogen hypothesis’ paper. Mechanistically, they proposed that estrogens directly or indirectly (by suppression of gonadotropin secretion via enhanced negative feedback by estrogens) interfere with Leydig and Sertoli cell function and number, resulting in decreased testosterone and perhaps anti-Mullerian hormone production, in turn leading to impaired development of the germ cells and the male genital tract. Moreover, they proposed several routes of exposure to endogenous or exogenous estrogens that may have changed over time, and that could have lead to increased in utero exposure to estrogenic compounds accommodating the temporal pattern of testicular cancer incidence.

The estrogen hypothesis has been further expanded to include other harmful substances and other mechanisms of action. In 2001, Skakkebaek and colleagues presented the idea of a Testicular Dysgenesis Syndrome (TDS), suggesting that testicular cancer, hypospadias, poor semen quality and undescended testis are all symptoms of one underlying entity. In short, according to the TDS hypothesis, a disturbed estrogen/androgen balance in fetal life impair normal Sertoli and Leydig cell function, interfere with the differentiation of fetal germ cells, as well as the production of testosterone, INSL3 and other factors; leading to the different symptoms of the syndrome. The factors proposed to disturb the fetal hormonal milieu are the so called endocrine disruptors. That is, chemicals that can alter hormone levels or actions within the body, such as compounds with estogenic effects and antiandrogens (e.g., androgen receptor-antagonists or compounds such as phthalates that can alter the Leydig cell function and thereby affect the testosterone production).
The TDS-hypothesis is based on several different findings and presumptions:\textsuperscript{128-134}: 1) parallel geographical and temporal trends of all conditions, 2) all conditions are present in different rare genetic abnormalities, 3) all conditions but testicular cancer can be induced in some animals exposed to endocrine disruptors, 4) wild-life natural experiments of exposure to certain endocrine disruptors has resulted in animals that are incapable of reproduction and that are ‘demasculinized’, 5) all conditions are initiated in utero, 6) all conditions share prenatal risk factors, 7) all conditions are risk factors for each other, and 8) frequent findings of testicular dysgenesis in undescended testes, and in the contralateral testis in unilateral testicular cancer.

The estrogen hypothesis and its enlargement to include the TDS is a biologically plausible, and mechanistically appealing, theory. Not least since the masculinization process in male development is hormone dependant and thus intuitively susceptible to endocrine disruption. In addition, even if it can be debated if the biological effects of most endocrine disruptors are strong enough to interfere with the fetal hormonal milieu in a significant way, the possible sources of estrogens and other endocrine disruptors (such as different pesticides and industrial chemicals) are plentiful and have increased over time, perhaps paralleling the temporal increase in testicular cancer incidence\textsuperscript{135}.

Given the immense impact of the estrogen hypothesis, a myriad of studies have investigated prenatal, perinatal, and maternal factors in relation to risk of testicular cancer, trying primarily to find mechanistic support for the hypothesis (see below).

\textit{Impaired fetal growth}

An association between impaired fetal growth and testicular cancer has been suspected for many years. Some of the early case-control studies reported an increased risk of testicular cancer among preterm or low birth weight babies (see below), and there is an apparent association between IUGR and cryptorchidism. Also, there has been an increased interest in the in utero period and in impaired fetal growth with regards so several other adult diseases in recent times.

This hypothesis is, from a mechanistic point of view, not as elaborately formulated as the estrogen hypothesis. In short, it has been proposed that impaired fetal growth somehow affect the maturation and functioning of Leydig cells and Sertoli cells, and that this leads to testicular cancer (as well as the other symptoms of the TDS)\textsuperscript{136}.

\textit{Smoking during pregnancy}

In 1994, Clemmesen hypothesized that smoking during pregnancy cause testicular cancer in the offspring\textsuperscript{137,138}. Clemmesen based his hypothesis mainly on observed parallel trends in female smoking related cancers and testicular cancer, while the hypothesis is compelling for other reasons as well:

1) Pregnancy smoking affects the in utero milieu, causing impaired fetal growth and interfering with pregnancy hormones (e.g., decreasing estrogen levels)\textsuperscript{139}. Moreover, tobacco contains several carcinogenic compounds that could pass the placenta and directly affect the developing fetus.
2) There has been an increase in usage of tobacco over time, smoking habits differ between countries, and the supply of cigarettes was probably scarce during war time.

3) Several\textsuperscript{140-144}, but not all\textsuperscript{145}, studies report a positive association between testicular cancer risk and maternal or familial lung cancer risk. Notably, one study found that the risk increased with decreasing time interval between birth and maternal lung cancer diagnosis, suggesting that environmental causes, rather than shared genetic pathways, are involved\textsuperscript{146}. On the other hand, increased risk of testicular cancer has also been reported among relatives of self-reported non-smoking lung cancer cases, suggesting a role for shared genetic pathways\textsuperscript{146}.

4) Pregnancy smoking is reportedly associated with both low sperm count and other indicators of impaired fertility\textsuperscript{147-150}, as well as undescended testis\textsuperscript{151,152}.

The childhood nutrition theory
In essence, the childhood nutrition theory postulate that high-caloric intake during early childhood promotes or conserves testicular cancer precursor lesions that have evolved during embryogenesis\textsuperscript{74}. This hypothesis stems largely from findings of an association between adult height and testicular cancer (see below), but there are other observations in accordance with this theory: many countries have experienced a increase in food supply during the past century, food was partly restricted during World War 2, developed countries experience more testicular cancer compared to underdeveloped countries, testicular cancer has historically been suspected to be associated with high social class (although this association seems to have diminished over time, possibly reflecting decreases in social class variations) that may have had better access to food\textsuperscript{153}, and twins with longer arms and legs (a proxy for childhood nutrition) than their co-twin, have a higher risk of developing testicular cancer\textsuperscript{154}.

Briefly on other hypotheses
The descriptive epidemiology of testicular cancer alone suggests that infectious agents are important in the etiology of testicular cancer. Indeed, Newell and colleagues have suggested that in parallel with Hodgkin’s disease and in accordance with the paralytic polio model, occurrence of childhood infections at later ages can result in more a severe disease and in the end in testicular cancer development\textsuperscript{155}.

Some investigators have reported a high ecological correlation between per capita consumption of cheese, milk, and animal fats, and testicular cancer\textsuperscript{156}. The authors suggest that the increased incidence in testicular cancer during the past 50 years is associated with the increased consumption (perhaps during pregnancy or before puberty) of milk and dairy products. The authors speculate that in addition to fats, proteins and calcium, milk and dairy products contain high levels of female sex hormones (including estrogens) that could explain the association.

Testicular atrophy, which has been found in up to 75\% of testicular cancer patients, has been proposed to be the final common pathway in the induction of testicular cancer\textsuperscript{157}. In short, it has been postulated that atrophy causes mitogenesis which can lead to mutagenesis and cancer development, and furthermore that germ cell proliferation is promoted by increased levels of gonadotropins due to decreased negative feed back from the atrophic testes.
Ochratoxin A, a mycotoxin and carcinogen in animals, and a naturally occurring contaminant of cereals, pig meat, and other foods, has been hypothesized to cause testicular cancer\textsuperscript{158}. The mechanism proposed is that consumption of Ochratoxin-A-contaminated foods during pregnancy or childhood induces lesions in testicular DNA, and that puberty promotes these lesions.

Increased mitotic activity in the testis after testicular trauma, and increased levels of testosterone among men with high physical activity has also been implicated in testicular cancer etiology.

Potential risk factors for testicular cancer

A great challenge with testing these hypotheses is the difficulties in obtaining pertinent and reliable exposure data; and in many instances to simply understand what mechanisms the exposures under study are reflecting. Moreover, many of the hypotheses are tested in retrospective questionnaire-based studies with several sources of possible bias, or in register-based studies that are constrained by the limited information available in the registries. Nevertheless, a plethora of factors have been investigated with respect to risk of testicular cancer, and some interesting and fairly robust associations seem to exist. Here follows a brief review on selection of these factors, primarily discussed in the context of the hypotheses outlined above.

Diethylstilbestrol and other exogenous estrogens

During pregnancy, maternal estrogen levels are significantly elevated. The endogenous estrogens are, however, to a large extent bound to circulating sex hormone binding globulin (SHBG), which in effect may protect the fetus from the high levels of estrogens. Diethylstilbestrol (DES), on the other hand, is a synthetic estrogenic drug that does not bind well to SHBG\textsuperscript{159}. The drug was prescribed to some 5 million pregnant women from the late 1930s to the 1970s to prevent miscarriages and pregnancy related disorders\textsuperscript{160}. But the prescription was stopped after DES was revealed to cause clear-cell cancer of the vagina in female offspring\textsuperscript{161}. Later it was discovered that DES exposure during pregnancy can cause a broad range of other adverse pregnancy outcomes and reproductive tract problems, such as ectopic pregnancy, spontaneous abortion, preterm birth, cryptorchidism, hypospadias, and perhaps low sperm count\textsuperscript{159,160,162}.

The association between DES exposure, or exposure to other hormones, during pregnancy and risk of testicular cancer has been investigated many times\textsuperscript{78,83,162-166}. A recent comprehensive systematic review reports a doubling in risk of testicular cancer (as for cryptorchidism and hypospadias) for DES exposure during pregnancy\textsuperscript{159}. The authors conclude, however, that the results do no constitute conclusive evidence of an estrogenic mode of action, since they found little evidence in favor of an association between exposures to other estrogenic compounds and risk of testicular cancer. Rather, the association can be related to the condition for which DES was prescribed.

Taken together, DES exposure appears to be associated with all four symptoms of TDS, lending support to the theory of a shared etiology. It should be noted, though, that DES exposure can hardly explain the secular trends in testicular cancer incidence\textsuperscript{167}.  

19
**Birth order and sibship size**

Higher levels of estrogens have been reported among nulliparous compared to parous women\textsuperscript{168-170}. Furthermore, primipara may experience elevated levels of serum free estrogens, as the levels of SHBG perhaps are lower in first pregnancies\textsuperscript{164,168}. In addition, firstborns are in general exposed to infectious agents at later ages.

In total, being first born seem to be associated with a marginally increased risk of testicular cancer\textsuperscript{78,111,142,163-166,171-182}. If this is explained by a high risk among firstborns per se, or a lower risk among later born, is unclear. The interpretation is further confused by the fact that birth order is associated with sibship size, which probably is related to parental fertility (and socioeconomic status) and therefore likely with testicular cancer risk (see above). Sibship size has been reported to be inversely associated with testicular cancer risk in several\textsuperscript{111,142,183}, but not in all\textsuperscript{78,171,176,178,181}, studies. In the end, sibship size, and not only birth order, is probably associated with risk of testicular cancer\textsuperscript{111}.

**Severe nausea**

Nausea during the first trimester of pregnancy is considered normal, but in some 0.5% of live births the mother experiences severe nausea and vomiting in early pregnancy, that is *hyperemesis gravidarum*. The pathophysiology of severe nausea is not known. Altered levels of pregnancy hormones, especially elevated levels of hCG, but also increased levels of estrogens, may be involved in the etiology\textsuperscript{184,185}.

The estrogen hypothesis was formulated partly on the basis of Henderson and colleagues finding of an increased risk of testicular cancer among sons of women who experienced severe nausea during pregnancy\textsuperscript{78}. Indeed, several, but not all, other studies also report an association above unity\textsuperscript{163-166,172-174,179,181,186}.

**Bleeding or spotting during pregnancy**

Bleeding or spotting during pregnancy has a multitude of causes, and it is somewhat unclear what it stands for with respect to risk of testicular cancer. To some extent it may be an indicator of hormonal treatment during pregnancy. Several of the studies that have investigated the association between bleeding during pregnancy (sometimes including threatened miscarriage) and risk of testicular cancer report a risk above unity\textsuperscript{78,83,163,166,172-174,181,186,187}.

**Maternal age at pregnancy**

Studies on maternal age and estrogen levels are equivocal. Some studies report that young mothers (<20) have lower estrogen levels, whereas the highest estrogen levels are found in mothers between either 20 and 24 years\textsuperscript{169}, or 20 and 34 years\textsuperscript{188}. Other investigators report no apparent association between age and estrogen levels\textsuperscript{189}. Moreover, androgen levels appear to be higher in younger mothers\textsuperscript{170}. In addition, low maternal age is likely associated with a higher risk of severe nausea during pregnancy (as is being pregnant for the first time), and it has been proposed that the continuous decline in maternal age at first full-term pregnancy is a partial explanation to the increasing trends in testicular cancer\textsuperscript{190}.

Results from studies on maternal age and risk of testicular cancer are conflicting\textsuperscript{78,142,166,172-182}. A few recent studies have reported higher risks among sons of mothers aged less than 20 at delivery\textsuperscript{173,174}. 

20
Twin pregnancy

Twin pregnancies are characterized by increased levels of several hormones, including estrogens, hCG, and testosterone\(^{191,192}\), and dizygosity may be associated with even higher levels of estrogens\(^{71}\). Twinning is additionally associated with several adverse pregnancy outcomes, such as preterm delivery and low birth weight.

With some exception\(^ {171}\), most studies, including several cohort studies, report risks above unity\(^ {142,175,174,178,193-196}\). A recent meta-analysis estimated that twins have an 30% increased risk of testicular cancer, but no difference with respect to zygosity was reported\(^ {197}\). This meta-analysis did not, however, include the study by Swerdlow and colleagues that reported a significantly higher risk of testicular cancer in dizygotic compared to monozygotic twins\(^ {71}\).

Birth weight

Maternal estrogen levels during pregnancy are positively associated with birth weight\(^ {139}\), although perhaps not during the first trimester\(^ {170}\). In fact, birth weight is a widely used proxy for prenatal estrogen exposure in breast cancer research\(^ {198}\). Low birth weight, preferably adjusted for gestational duration, is often used as a proxy for impaired fetal growth, and low birth weight is furthermore associated with maternal smoking during pregnancy.

Birth weight and risk of testicular cancer has been extensively studied\(^ {163-166,172-175,177,179-182,186,199-201}\). The topic is reviewed in two recent meta-analysis\(^ {202,203}\), one showing no apparent association and the other an 18% increase in risk among those weighing less than 2500 grams at birth, compared to normal weight babies. Conversely, and in line with the estrogen hypothesis, a 12% increase in risk was found among those weighing more than 4000 grams compared to normal weight babies in one meta-analysis\(^ {202}\). Following the publication of these meta-analyses, at least 3 additional studies have been published on this topic\(^ {172,181,199}\); all indicating an increased risk for low birth weight babies, and one study reported a statistically significant inverse association with birth weight\(^ {199}\).

Gestational duration

Pregnancies that end preterm differ from term pregnancies in several aspects. Some underlying condition or exposure triggers the preterm delivery, high levels of hCG during the second trimester increase the risk of preterm birth\(^ {204,205}\), estrogen levels appear to be higher at the time of delivery\(^ {206}\), hormones may accumulate in the preterm infant, and cumulative hormonal exposure during pregnancy is lower.

The effect of gestational duration on testicular cancer risk has been studied several times. Most studies indicate increased risks of being born preterm (and some indicate a decreasing risks with increasing gestational age)\(^ {163,166,172,174,177,181,187}\), while others show little sign of an association\(^ {173,175,180,186}\). In general, the risks of being born preterm is reported to be low (<2).

Maternal obesity

A forthright way for the fetus to be exposed to relatively higher levels of estrogens is through increased bioavailability of endogenous estrogens. As previously discussed, fetal
bioavailability of estrogens is primarily regulated by the levels of SHBG, whose most important endogenous suppressor is insulin.\textsuperscript{127} Hence the reasoning that obesity increases insulin resistance, and thus the insulin levels, leading to low levels of SHBG and ultimately to high levels of bioavailable estrogens that can pass the placenta and affect the fetus. This theory is of particular interest since low levels of SHBG is a potential way through which the fetus can be exposed to high levels of estrogens during early pregnancy (that is before the surge in estrogens secreted by the placenta). Moreover, since more people have become overweight, this could be a partial explanation for the increasing testicular cancer rates\textsuperscript{207}. In addition, maternal weight may have an effect on the insulin/insulin-like growth factor (IGF) pathway, which has been implicated in other cancer forms\textsuperscript{208}.

Interestingly, an ecological correlation between maternal weight at delivery and testicular cancer occurrence in Norway has been reported\textsuperscript{208}. However, individual-level studies have, with few exceptions\textsuperscript{164}, so far given little support\textsuperscript{166,172,181,186}.

\textit{Gestational hypertension and preeclampsia}

The placenta is instrumental for fetal growth as well as for the production of pregnancy hormones, and placental malfunction has long been suspected to be involved in the etiology of testicular cancer. The pathogenesis of gestational hypertension and preeclampsia (i.e., pregnancy induced hypertension with proteinuria) is poorly understood. The disorders appear to be related to a diverse placental pathology, and to impaired placental perfusion. Moreover, different forms of hypertensive disorders during pregnancy may have a different etiology. For instance, mild late-onset preeclampsia could be a physiological response to minimally impaired placental perfusion in predisposed women, whereas the severe early-onset form is a physiological reaction of any woman to a profoundly reduced placental perfusion\textsuperscript{209}. Several studies have reported decreased levels of pregnancy estrogens in preeclamptic women\textsuperscript{210,211}, and even lower levels in early-onset severe preeclampsia have been reported\textsuperscript{212}. Some studies have also reported elevated levels of androgens in women with preeclampsia\textsuperscript{213,214}. And mothers who develop preeclampsia, especially severe preeclampsia\textsuperscript{215}, have been found to have higher levels of hCG\textsuperscript{204,211}. Furthermore, hypertensive disorders during pregnancy are associated with preterm birth and impaired fetal growth.

Some studies have investigated the association between hypertensive disorders (including convulsions) during pregnancy and risk of testicular cancer\textsuperscript{78,163,173,174,177,181,182}. Almost all of these studies have reported point estimates between 1 and 2, but no study has reported a statistically significant association. Cook and colleagues found a 60\% increased risk for hypertension during pregnancy, but no association for preeclampsia\textsuperscript{173}, while Aschim and colleagues reported a significant 2-fold risk for seminomas among women with hypertension during pregnancy, but no association with the other subtypes of testicular cancer\textsuperscript{174}. Interestingly, Aschim and colleagues also reported a more than doubling in risk of testicular cancer in deliveries complicated by retained placenta, and speculated that uteroplacental hormonal disturbances and early malfunction of the placenta could be a causative factor for testicular cancer.

Unfortunately, none of the studies on hypertensive diseases during pregnancy and risk of testicular cancer have separated the mild forms from the severe forms.
Smoking during pregnancy
Several studies have investigated the possible association between pregnancy smoking and testicular cancer in the offspring \(^{78,142,163,166,172,176}\). These studies give virtually no support for an association. All of these studies are, however, questionnaire-based studies with several possible sources of biased measurement of smoking. First, data was collected decades after the exposure. Second, some participation rates are low (<50%). Third, smokers have a higher morbidity and mortality compared to non-smokers. Fourth, the hazards of pregnancy smoking were not well-known when most cases were born, but well-known when the studies were conducted.

Age at puberty
Since sex hormones are strongly implicated in the progression of testicular cancer, the pubertal period is intuitively of interest. Unfortunately, it can be difficult as an adult to remember when entering puberty, and there is no simple benchmark for when puberty begins. Therefore, studies investigating age at puberty with respect to testicular cancer have a high risk bias. Nevertheless, questionnaire-based studies on surrogates for age at puberty, such as signs of secondary sexual characteristics, have rather consistently shown that a late age at puberty has a protective effect (and in some studies that an earlier age at puberty is harmful) on the risk of developing testicular cancer \(^{77,165,176,216}\), although some studies disagree or are more inconclusive \(^{164,217-219}\). Moreover, overt baldness has been proposed as a biomarker for higher androgen exposure during adolescence and early adult life, and one study report an inverse association between baldness and risk of testicular cancer \(^{179}\).

It is debated what mechanism is responsible for this association. One possible explanation is that those who enter puberty early will develop their ‘predetermined’ testicular tumor in beforehand. Other proposed mechanisms include shared intrauterine events (including intrauterine growth), and increased childhood nutrition. Interestingly, a decreasing secular trend in age at puberty appears to have taken place \(^{220,221}\).

Adult height
Adult height, which is associated with caloric intake during early childhood, appears to be strongly associated with testicular cancer risk. The largest study yet, including more than 6,000 testicular cancer patients, showed striking evidence in favor of a positive association \(^{222}\). The risk among those tallest (>194 cm) was increased 3-fold, whereas there was a 20% reduction in risk of being short (<170 cm), compared to those 175 to 179 centimeters long; and there was an uninterrupted trend in between. This association is supported by several, but not all, previous studies \(^{103,179,201,218,219,223-228}\) (further discussed by Dieckmann and colleagues \(^{74,222}\)). Interestingly, adult height appears to be associated with testicular cancer independently of size at birth, suggesting that factors affecting postnatal growth may underlie this observation \(^{201,225}\).

Several mechanisms other than childhood nutrition may however operate. Testicular cancer risk and adult height can share intrauterine determinants (perhaps other than those affecting fetal growth), adult height can be affected by diseases in childhood, and adult height is affected by age at puberty. Furthermore, it has been hypothesized that the association between height and testicular cancer is explained by alterations in the IGF-pathway \(^{229}\); although there is yet little support from analytical studies \(^{230}\). Interestingly
adult height has increased with time, possibly accommodating the secular trends in testicular cancer incidence221.

**Dairy consumption and body size**
While height reflects dietary intake at early ages, weight and to a large extent body mass index (BMI) instead reflect dietary intake throughout life.

Overall, the bulk of evidence suggests that BMI is not associated with testicular cancer179,201,218,223-228,231. There are some evidence of an association between consumption of dairy products, milk, and fats, and risk of testicular cancer218,228,231-233. However, with respect to the childhood nutrition theory, the most interesting period to investigate would be early childhood (<2 years). So far, the only study who has investigated nutrition during childhood found no association, but they did not investigate the ages below 2 in specific218.

**Other risk factors**
Other frequently investigated risk factors include scrotal trauma, inguinal hernia, infectious diseases, occupation, physical activity, hydrocele, and vasectomy etcetera. In summary, few factors have shown a consistent association, especially not over time, interpretation is hampered by methodological difficulties in assessing the relationship, and few factors would have the capacity to explain any substantial part of the dramatic increase in testicular cancer incidence. Unfortunately, it is beyond the scope of this thesis to discuss all of these potential risk factors. Please see previous comprehensive reviews and textbooks discussing these factors74,167,234.

Regarding exposure to different endocrine disruptors, such as persistent organochlorine pollutants, including the previously commonly used pesticide DDT, still only few studies have been conducted and evidence is limited235. There are reports of positive associations, for instance between chlorinated biphenyls (PCBs) in maternal blood and testicular cancer risk in the offspring, but this remains to be verified in larger studies236. In short, there is little empirical evidence in favor of any endocrine disrupting chemical at present.

**Seminomas and non-seminomas**
Several of the studies discussed above investigate the risk of testicular cancer stratified by histological subtype, and there are some differences reported. Two large register-based studies have studied seminomas and non-seminomas (and even mixed tumors) separately174,177. Although both studies report some apparent heterogeneity between the subtypes, none of the studies could report a statistically significant heterogeneity between seminomas and non-seminomas. Given this, together with the similarities in the descriptive epidemiology of the two subtypes, and the other findings discussed before, there is at present rather little reason to suspect that seminomas and non-seminomas differ substantially in major determinants.

**In summary**
Despite the fact that much evidence indicates that the fetal period is instrumental in the development of testicular cancer, evidence from analytical studies is equivocal. Unfortunately, the estrogen hypothesis is difficult to investigate retrospectively; and so, if
these inconclusive findings reflect that there is no association between excess estrogen exposure during fetal life and testicular cancer risk, or that the surrogates thought to reflect estrogen exposure are invalid or does not reflect estrogen exposure during the right critical time window, is not known. Notably, fewer than expected results from analytical studies seem to oppose the estrogen hypothesis compared to what would be expected if only chance was in play. Studies on birth weight suggest that impaired fetal growth is a risk factor for testicular cancer, but not too strong. Moreover, a marginally increased risk of testicular cancer for low birth weight is expected given the well-known association between impaired fetal growth and cryptorchidism, and this is not always taken into account. The increased risk of testicular cancer recently reported by Dieckmann and colleagues is highly interesting. It is, however, quite difficult to see how adult height, or childhood nutrition in general, can explain the geographical differences in incidence seen in for instance the Nordic countries. Perhaps is therefore this association interesting from a more mechanistic point of view. Although there is no direct evidence in favor of Clemmesen’s theory that pregnancy smoking causes testicular cancer, the theory is appealing, not least since smoking has become more and more common with time.
The aims of the present thesis are:

1. to investigate the association between smoking during pregnancy and risk of testicular cancer in the offspring (Study 1 and 2).

2. to better understand the mechanisms behind association between undescended testis and testicular cancer (Study 3).

3. to investigate if age at surgery for undescended testis affects the risk of developing testicular cancer (Study 3).

3. to investigate the association between signs of placental malfunction and risk of testicular cancer (Study 4).
METHODS

The studies included in this thesis are based on data from different Swedish registries, from the Nordic Cancer Registries, from previous smoking surveys, and from antenatal charts stored at the delivery units throughout Sweden. The registries in the Scandinavian countries are population based, and are generally of a high standard. All linkage between the different Swedish registries was carried out using the national registration number (NRN), a unique personal identifier referred to in all hospital records and official registries in Sweden since 1947.

Sources of data

Previous smoking surveys
In study 1, data on smoking prevalences among women (and men) aged 25 to 29 years were collected from previously published smoking surveys conducted in the Nordic countries.

Sweden
In 1963, Statistics Sweden carried out a survey of smoking habits based on a sample of 55,930 randomly selected men and women (response rate 94.2%)\(^{237}\). By combining these data with other data sources, Nordlund estimated trends in prevalence of smoking, in five-year age groups, for successive five-year birth cohorts of Swedish men and women born from 1904 to 1948\(^{238}\).

Norway
By combining data from several surveys conducted in 1954 onwards in Norway, Ronneberg and colleagues estimated the prevalence of current smokers among Norwegian men and women by five-year birth cohorts, between 1890 and 1974, and five-year age groups\(^{239}\).

Denmark
In 1951 to 1954 the National Board of Health in Denmark carried out a nation-wide health and morbidity survey in which 34,018 men and women were asked about their smoking habits (response rate 83%)\(^{240,241}\). Current smokers in 1954 were presented in 10-year age groups, whereas the proportions starting to smoke, divided into five-year periods, were presented for five-year birth cohorts born 1905 through 1939. Due to the 10-year stratification of current smokers, no exact figure on the prevalence of current smokers at the age of 25 to 29 could be derived for different five-year birth cohorts. To estimate this prevalence, we assumed that the age of current smokers in 1954, in each five-year birth cohort born 1905 through 1939, was that of the corresponding 10-year age group. The fraction that stopped smoking, that is, the difference between the proportion that had ever started smoking up to 1954 and the proportion that were actual smokers in 1954, was then assumed to be distributed in accordance with the proportion that started to smoke in the different five-year periods.
Finland
Smoking habits in Finland, according to five-year birth cohorts born in 1896 to 1965, have been estimated by Martelin\textsuperscript{242}. The smoking prevalence among Finnish men and women, in five-year age groups, born in 1916 to 1940, was based primarily on data from a study concerning mainly sexual behaviour among Finns, performed in 1971, with 2,152 participants (response rate 91.4\%)\textsuperscript{243}.

The Nordic Cancer Registries
The Swedish Cancer Registry was established in 1958 and obtains mandatory reported data from both clinicians and pathologists on all newly diagnosed malignant neoplasms. Information on the site and histopathological features of the tumors is recorded.

In study 1, age- and calendar year-specific data on testicular cancer incidence was collected from the population-based cancer registries in the other Nordic countries, dating back to 1943, 1952 and 1952 in Denmark, Norway and Finland, respectively. All registries obtain mandatory reporting from both clinicians and pathologists.

For testicular cancer, all registries are estimated to be more than 95\% complete\textsuperscript{54}.

The Swedish Medical Birth Register
The Swedish Medical Birth Register was initiated in 1973 and includes prospectively collected information during pregnancy, delivery, and the neonatal period for 98\% of all pregnancies in Sweden\textsuperscript{244}. Information is collected on standardized records used all over Sweden. At the first visit to the antenatal care unit, which occurs before the 15\textsuperscript{th} week of gestation in 95\% of the pregnancies\textsuperscript{245}, information on prior pregnancies, height, and smoking habits (recorded either as number of cigarettes smoked per day or categorized into 3 groups: non-smokers, $<10$ cigarettes per day, and $\geq10$ cigarettes per day) of the mother is recorded. Also, a first measurement of weight, haemoglobin, blood pressure, and a dipstick urine sample for protein and glucose is recorded. Thereafter, a pregnant woman generally visits the antenatal care unit 13 times before delivery\textsuperscript{246}. During these visits, as well as at the delivery ward, the same measurements are repeated according to specific routines. After delivery, information on birth weight, placental weight etcetera are recorded. All records are stored at the archives of the delivery units, and part of the information is forwarded to and computerized by the Medical Birth Register.

In study 2 and 4, data available in the Medical Birth Register was complemented with data manually collected from the delivery archives in Sweden.

The Hospital Discharge Register
In 1964, the Swedish National Board of Health and Welfare initiated the Hospital Discharge Register, which compiles data on individual discharges from Swedish public hospitals. Virtually all patients in Sweden are treated at public hospitals, making the Discharge Register representative of hospitalizations in the Swedish population. Each record contains medical and administrative data, including the dates of admission and discharge, surgical procedures performed, the main diagnosis at discharge, and up to five contributory diagnoses. The surgical procedures were coded from 1964 to 1996 according to the Swedish Classification of Operations and Major Procedures, first through sixth editions, and from 1997 onward according to the Classification of Surgical Procedures, 1997 edition. The diagnoses at discharge were coded according to the Swedish version of
the *International Classification of Diseases*, with the 7th revision (*ICD-7*) used for the years 1964 through 1968, the 8th revision (*ICD-8*) for 1969 through 1986, the 9th revision (*ICD-9*) for 1987 through 1996, and the 10th revision (*ICD-10*) thereafter.

In 1964, the Discharge Register covered 6 of the 26 health care regions in Sweden. Gradually, more hospitals were included, and by 1975, 16 health care regions were fully covered (including the three largest cities in Sweden: Stockholm, Gothenburg and Malmö). Since 1987, the registry has covered all hospitals in Sweden.

The validity of the registry was evaluated in 1987, when information in the registry was compared to what was recorded in the clinical charts (900 charts)\(^{247}\). In total, an erroneous diagnostic code was present in about 6% of the cases, and 11% of the procedures were either not reported or erroneously coded. Moreover, the number of dropouts in the reporting to the registry is estimated to between 1 and 2%.

*The Register of Population and Population Changes*

The Register of Population and Population Changes contains the official Swedish population data, including dates of death and migration; the data have been available since 1960.

**Study designs**

*Study 1*

In this study, we investigated the ecological correlation between smoking habits among women in fertile ages and the incidence of testicular cancer in the presumed offspring cohorts in Sweden, Norway, Denmark, and Finland.

By using information from the Nordic Cancer Registries, and from the previous smoking surveys, we created data sets describing, in each country, smoking habits at the age of 28 (approximate mean age of women at giving birth in the Nordic countries during the study period\(^{248}\)) in five different female birth cohorts and the incidence of testicular cancer in the generation of the presumed offspring. Prevalence of smoking at 25 to 29 years of age for female five-year birth cohorts 1910 to 1940, were assumed to correlate well with the prevalence of smoking during pregnancy. Incidences of testicular cancer were calculated for the male five-year birth cohorts 1938 to 1968, that is, the birth cohorts born 28 years after the female birth cohorts. To avoid effects of truncation of follow up and to include the largest number of cases, the incidence of testicular cancer between the ages of 20 to 34 in each male birth cohort was calculated. Furthermore, for comparison with the data on female smoking, information on smoking prevalence at 25 to 29 years of age was also collected for male five-year birth cohorts 1910 to 1940. The age at having children is generally higher among males than females, and similar analysis was therefore performed for male smoking prevalences at 30 to 34 years of age. In total, five male and female birth cohorts with data on smoking prevalence and five presumed offspring male birth cohorts with known testicular cancer incidence could be constructed for each country. Pearson’s correlation coefficient was calculated, and uni- and multivariate linear
regression analyses were performed on testicular cancer incidence, with female and male smoking as explanatory variables.

**Study 2**
This study is a population-based case-control study on maternal smoking during pregnancy and risk of testicular cancer. We used the Swedish Medical Birth Register to define a cohort of virtually all men born in Sweden in 1973 onwards, in which we conducted a nested case-control study through linkage to the Swedish Cancer Registry.

The study base in this study is defined by the person time experienced by the cohort of singleton males in the Swedish Medical Birth Register born in June 1973 (when maternal smoking habits started to be recorded) onwards. There were 266 cases of testicular germ-cell cancer (age $\geq 15$ at cancer diagnosis) in the study base before 2002, ascertained through the Cancer Registry. Information from the Cancer Registry allowed us to define cases as either seminomas or non-seminomas (including cancers of mixed histological pattern). Potential controls were the first 3 men born at the same hospital after a case, and who were alive and without testicular cancer at the time of diagnosis of the corresponding case.

For those born before 1983, data on smoking were collected manually from the birth records in the delivery archives. We excluded 32 cases (12%) and 79 controls (10%), for whom the chart was untraceable, and 41 (15%) cases and 117 (15%) controls, for whom there was no information on smoking (mainly because smoking was not registered in 1 Swedish county before 1983). After exclusion of another 2 controls with missing values in covariates, and 1 case and 108 controls in risk sets with no case or no controls, 192 cases and 494 control subjects remained for analysis. Other exposure variables included were maternal age at delivery, birth order, gestational duration and birth weight; all information on these study variables was retrieved from the Medical Birth Register.

Odds ratios (OR) with corresponding 95% confidence intervals (CI) were estimated through conditional logistic regression.

**Study 3**
In this study, we investigated the risk of testicular cancer according to the age at orchiopexy in a cohort of men who underwent surgical correction for undescended testis in Sweden between 1964 and 1999. We used data from the Swedish Hospital Discharge Register to assemble the study cohort, which was followed from 1965 to 2000 through linkage to the Swedish Cancer Registry and the Register of Population and Population Changes.

We confined our study cohort to all subjects in the Discharge Register who had received a diagnosis of cryptorchidism (main or contributory discharge diagnosis ICD-7 code 757.00, ICD-8 code 752.10, ICD-9 code 752F, or ICD-10 code Q53.0, Q53.1, Q53.2, or Q53.9) between January 1964 and December 1999 and who had been treated with orchiopexy (surgical procedure code 6790, KFH00, or KFH10) before 20 years of age. The restriction to those who underwent surgery before 20 years of age was done to minimize the influence of selection bias due to, for instance, referral of men seeking treatment for infertility to the urology department.
One subject in whom testicular cancer had already been diagnosed, 205 subjects who emigrated from Sweden after orchiopexy and did not immigrate back to Sweden before the beginning of follow-up, and 12 subjects with conflicting information on sex or date of orchiopexy were excluded from the study cohort, leaving 16,983 subjects for the analysis. Among 2,667 (15.7%) subjects who were surgically treated for cryptorchidism more than once, we used the date of the last admission as the index date of orchiopexy.

The members of the cohort were followed from 15 years of age or the age at orchiopexy plus 1 year, whichever occurred later, to the date of diagnosis of testicular cancer, the age of 55, emigration, death, or December 31, 2000, whichever occurred first. The first year after orchiopexy was excluded to reduce the risk of overdiagnosis of testicular cancer.

We estimated the relative risk of testicular cancer by calculating the standardized incidence ratio (SIR). Expected numbers of testicular cancer were based on the 5-year age- and period-specific rates in the Swedish general population. We estimated 95% CIs assuming a Poisson distribution. Analyses were also stratified according to age at orchiopexy and calendar period of orchiopexy. In categorizing age at orchiopexy, we focused on the ages around puberty. Categories are therefore smaller for the group between 10 and 15 years of age and larger for ages before and after. Thirteen years of age was set a priori as the cutoff for analyses of surgery before and after puberty.

We used Cox regression analysis, with age as the temporal axis, to estimate the hazard ratio for testicular cancer associated with age at orchiopexy in internal comparisons. Covariates included in the model were the calendar period of follow-up (1965 to 1990, 1991 to 1995, and 1996 to 2000), calendar period of orchiopexy, and region where the orchiopexy was performed (two categories: the three largest cities in Sweden and the rest of Sweden). There is colinearity among birth cohort, age at orchiopexy, and calendar period of orchiopexy (e.g., a child operated on at 5 years of age in 1970 must have been born in 1964 or 1965). To analyze the effect of age at orchiopexy adjusted by calendar period of orchiopexy, we therefore used only three categories for the latter variable (1964 to 1969, 1970 to 1974, and 1975 to 1999), assuming no period effect after 1974. Both visual inspection of a graph (with the log of the cumulative hazard on the y axis and the log of the survival time on the x axis) and a formal test based on Schoenfeld residuals (P=0.68) indicated that the proportional-hazard assumption was met. There was no evidence of interaction between the calendar period of follow-up and the effect of age at orchiopexy.

We also conducted a sensitivity analysis, restricting the cohort to men born between 1964 and 1980, among whom the estimate of the effect of age at orchiopexy could not be biased by the lack of information about orchiopexy before 1964 (when the Discharge Register was established) or by the termination of the recruitment 2000.

**Study 4**

In this study, we have used the same design and study subjects as in study 2 (see above), but with additional cases (because in this study we included all men born in January 1973 onwards, while in study 2 we included only men born only from June 1973 onwards due to no recording of smoking before that time). The aim of this study was primarily to
investigate the association between signs of placental malfunction/hypertensive disorders during pregnancy, and risk of testicular cancer in the offspring.

We identified 293 singleton cases of testicular cancer (aged ≥15 at diagnosis), born in January 1973 onwards and diagnosed before 2002. After excluding 18 (2%) twin controls, 293 cases and 861 controls remained for analysis.

The following variables were analyzed: maternal smoking during pregnancy, maternal age at delivery, birth order, gestational duration, birth weight, maternal height, BMI before pregnancy, weight gain during pregnancy, anemia during pregnancy (defined as at least one haemoglobin value of less than 100 g/l any time during pregnancy), mild and severe gestational hypertension (defined as at least two measurements of systolic blood pressure of 140 or more and/or diastolic blood pressure of 90 or more after gestational week 20, or at least one measurement of systolic blood pressure of 160 or more and/or diastolic blood pressure of 100 or more after gestational week 20, respectively), mild and severe preeclampsia (defined as mild or severe hypertension, respectively, with proteinuria during pregnancy -defined as at least one positive dipstick urine sample for protein after gestational week 20-), glucoseuria during pregnancy (defined as at least one positive dipstick urine sample for glucose any time during pregnancy), placental weight, maternal allo immunization (diagnosis ICD-8 codes: 634.91, 634.92 or 634.93), neonatal jaundice (diagnosis ICD-8 codes: 778.93, 778.94 or 778.96), and cryptorchidism (diagnosis ICD-8 code: 752.10).

Odds ratios with corresponding 95% CIs were estimated through conditional logistic regression. All multivariable models, adjusted for age and place of birth by design, included maternal age at delivery and birth order. Gestational duration and birth weight were not retained in the final models because they exerted limited confounding. Additionally, analyses were conducted separately for seminomas and non-seminomas.
RESULTS

Pregnancy smoking and risk of testicular cancer (study 1 & 2)

Ecological association (Study 1)
We found a strong correlation between smoking prevalence among women at fertile ages and testicular cancer incidence among presumed offspring cohorts. With the exception of Finland, this correlation was present within each country as well as in the combined analysis, i.e. a strong both temporal and geographical correlation (Figure 2). We found no correlation between male smoking prevalence and testicular cancer.

![Graph showing correlation between female smoking prevalence and testicular cancer incidence among men aged 20 to 34.](image)

Figure 2. The correlation between female smoking prevalence at 25 to 29 years of age and incidence of testicular cancer in the presumed offspring cohort.

The Pearson coefficient of correlation ($r$) between female smoking prevalence and testicular cancer incidence was 0.99 ($P=0.0008$) in Sweden, 0.85 ($P=0.07$) in Norway, 0.87 ($P=0.06$) in Denmark, 0.31 ($P = 0.6$) in Finland, and for all countries combined 0.93 ($P<0.0001$). The correlation between male smoking prevalence and testicular cancer was 0.47 ($P=0.04$) for all countries combined. In multivariate models including male and female smoking prevalence the correlation with female smoking remained virtually unchanged ($r = 0.91$ ($p < 0.0001$)), but the correlation with male smoking disappeared ($r = -0.2$ ($P=0.4$)). There were no substantial changes when analyzing male smoking prevalence at 30 to 34, instead of at 25 to 29, years of age.

The geographical correlation between female smoking and testicular cancer across the four countries, was 0.98 ($P=0.02$) for the offspring cohort of 1942 to 1948, 0.94 ($P=0.06$) for 1947 to 1953, 0.98 ($P=0.02$) for 1952 to 1958, and 0.98 ($P=0.02$) for 1957 to 1963. Time periods/cohorts in this analysis include seven years, with two years overlap, since exactly contemporary cohorts could not be obtained.
Association on the individual level (Study 2)

There was no association with testicular cancer for maternal smoking during pregnancy (OR, 0.91; 95% CI, 0.64–1.30), and there was no evidence of a dose–response effect (Table 1). Older maternal age was weakly associated with risk of testicular cancer. No association was found with birth order, birth weight or gestational duration. Risk of seminoma was reduced among light smokers, but less so among heavy smokers.

Age at surgery for undescended testis and risk of testicular cancer (study 3)

The cohort consisted of 16,983 men who were surgically treated for cryptorchidism and followed for a mean (± standard deviation) period of 12.4±7.4 years, with a total of 209,984 person-years at risk. For 679 of the subjects, follow-up ended before December 31, 2000, because of a diagnosis of testicular cancer (56), emigration (436), reaching the age of 55 years (5), or death (182). The overall mean age at orchiopexy was 8.6±3.5 years, and the median age was 8.5 years. Mean age at end of follow-up was 27.5±7.6, and the median age was 26.9. See Table 1 in paper 3 for main characteristics of the cohort.

We identified 56 cases of testicular cancer during the follow-up period, as compared with 20 expected cases, resulting in a SIR of 2.75 (95% CI, 2.08 to 3.57). The SIR for testicular cancer among those operated on before the age of 13 years was 2.23 (95% CI, 1.58 to 3.06), whereas it was 5.40 (95% CI, 3.20 to 8.53) for those treated at age 13 or later (Table 2). There were no significant differences in risk between groups below (P=0.81) or above (P=0.68) the age of 13 years. The proportion of men who were 13 years of age or older at orchiopexy declined from 27.3% in the beginning of the study period to 5.4% during the late 1980s. Figure 3 shows the SIRs for testicular cancer according to the calendar period of orchiopexy among men who underwent the surgery before the age of 13 years and among those who were surgically treated when they were 13 years or older. The SIR for all men in the cohort decreased from 8.64 (95% CI, 4.47 to 15.10) among those operated on between 1964 and 1969 to 3.29 (95% CI, 1.95 to 5.21) for those operated on between 1970 and 1974, and it did not vary much after that. The relative difference between the SIRs for those who underwent surgery before the age of 13 years and those in whom surgery was performed at or after the age of 13 years was close to 2 in most of the calendar periods (Figure 3).

The within-cohort analysis of the effects of age at orchiopexy on the risk of testicular cancer were consistent with the findings obtained when the rates of testicular cancer in the study cohort were compared with Swedish national rates. The adjusted hazard ratio (HR) for testicular cancer among men who were 13 years of age or older when surgery was performed, as compared with those who were younger at the time of treatment was 1.99 (95% CI, 1.00 to 3.95). Analyses restricted to men born between 1964 and 1980 revealed a HR of 3.56 (95% CI, 1.34 to 9.47), based on 6 cases in which surgery was performed at 13 years of age or later and 23 cases in which surgery was performed at an earlier age.
Table 1. Characteristics of cases and individually matched controls and crude and adjusted odds ratios of germ-cell testicular cancer for pre- and perinatal characteristics.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Cases (n=192)</th>
<th>Controls (n=494)</th>
<th>Crude OR(^1,2) (95% CI(^1))</th>
<th>Adjusted OR(^1,2) (95% CI(^1))</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. (% of the total)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Maternal smoking (cigarettes/day)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>none</td>
<td>111 (58)</td>
<td>273 (55)</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>&lt;10</td>
<td>34 (18)</td>
<td>102 (21)</td>
<td>0.82 (0.53 - 1.29)</td>
<td>0.83 (0.52 - 1.30)</td>
</tr>
<tr>
<td>≥10</td>
<td>47 (25)</td>
<td>119 (24)</td>
<td>0.97 (0.64 - 1.47)</td>
<td>0.99 (0.65 - 1.52)</td>
</tr>
<tr>
<td>Maternal age (years)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;20</td>
<td>8 (4)</td>
<td>32 (6)</td>
<td>0.63 (0.28 - 1.43)</td>
<td>0.58 (0.24 - 1.37)</td>
</tr>
<tr>
<td>20 – 24</td>
<td>57 (30)</td>
<td>160 (32)</td>
<td>0.88 (0.59 – 1.31)</td>
<td>0.88 (0.58 - 1.33)</td>
</tr>
<tr>
<td>25 – 29</td>
<td>77 (40)</td>
<td>188 (38)</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>30 – 34</td>
<td>39 (20)</td>
<td>90 (18)</td>
<td>1.11 (0.70 - 1.75)</td>
<td>1.11 (0.70 - 1.76)</td>
</tr>
<tr>
<td>≥35</td>
<td>11 (6)</td>
<td>24 (5)</td>
<td>1.16 (0.52 – 2.58)</td>
<td>1.13 (0.50 - 2.54)</td>
</tr>
<tr>
<td>Each 5-year increase</td>
<td></td>
<td></td>
<td>1.14 (0.96 - 1.36)</td>
<td>1.14 (0.95 - 1.38)</td>
</tr>
<tr>
<td>Birth order</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Firstborn</td>
<td>83 (43)</td>
<td>216 (44)</td>
<td>0.98 (0.70 – 1.37)</td>
<td>1.14 (0.79 - 1.65)</td>
</tr>
<tr>
<td>Non-firstborn</td>
<td>109 (57)</td>
<td>278 (56)</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>Gestational duration (tertiles, days)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;277</td>
<td>67 (35)</td>
<td>155 (31)</td>
<td>1.27 (0.84 - 1.90)</td>
<td>1.34 (0.87 - 2.04)</td>
</tr>
<tr>
<td>277 – 285</td>
<td>59 (31)</td>
<td>171 (35)</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>≥286</td>
<td>66 (34)</td>
<td>168 (34)</td>
<td>1.12 (0.74 - 1.70)</td>
<td>1.11 (0.73 - 1.70)</td>
</tr>
<tr>
<td>Birth weight (tertiles, grams)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;3330</td>
<td>58 (30)</td>
<td>164 (33)</td>
<td>0.77 (0.51 - 1.15)</td>
<td>0.73 (0.48 - 1.11)</td>
</tr>
<tr>
<td>3330 – 3770</td>
<td>76 (40)</td>
<td>165 (33)</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>≥3780</td>
<td>58 (30)</td>
<td>165 (33)</td>
<td>0.77 (0.52 - 1.14)</td>
<td>0.78 (0.52 - 1.17)</td>
</tr>
</tbody>
</table>

1) OR, odds ratio; CI, confidence interval; SD, standard deviation.
2) Crude ORs are inherently adjusted for age and place of birth; Adjusted ORs are adjusted for all variables listed in the table, with the exception of maternal smoking that is not adjusted for birth weight.
Table 2. Standardized incidence ratio for testicular cancer according to the age at orchiopexy among men 15 to 55 years of age between 1965 and 2000.

<table>
<thead>
<tr>
<th>Age at orchiopexy</th>
<th>Observed</th>
<th>SIR(^1)</th>
<th>CI (95%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All ages</td>
<td>56</td>
<td>2.75</td>
<td>2.08 – 3.57</td>
</tr>
<tr>
<td>0 – 6</td>
<td>9</td>
<td>2.02</td>
<td>0.93 – 3.84</td>
</tr>
<tr>
<td>7 – 9</td>
<td>14</td>
<td>2.35</td>
<td>1.28 – 3.94</td>
</tr>
<tr>
<td>10 - 12</td>
<td>15</td>
<td>2.27</td>
<td>1.27 – 3.74</td>
</tr>
<tr>
<td>13 - 15</td>
<td>12</td>
<td>5.06</td>
<td>2.61 – 8.84</td>
</tr>
<tr>
<td>16 – 19</td>
<td>6</td>
<td>6.24</td>
<td>2.29 – 13.58</td>
</tr>
<tr>
<td>&lt;13</td>
<td>38</td>
<td>2.23</td>
<td>1.58 – 3.06</td>
</tr>
<tr>
<td>≥13</td>
<td>18</td>
<td>5.40</td>
<td>3.20 – 8.53</td>
</tr>
</tbody>
</table>

1) The Swedish general population was used as comparison group.

Figure 3. Standardized incidence ratio for testicular cancer according to calendar period of orchiopexy among men treated before the age of 13 years and those treated when they were 13 years or older. I bars denote the 95% confidence intervals.
Gestational hypertensive disorders and risk of testicular cancer (study 4)

We found a negative association between severe gestational hypertension and risk of testicular cancer (OR, 0.29; 95% CI, 0.12-0.74), whereas the risk was increased for mild gestational hypertension (OR, 1.62; 95% CI, 0.98-2.69). When we analyzed the effect of preeclampsia, the risk of testicular cancer was decreased for severe preeclampsia (OR, 0.27; 95% CI, 0.06-1.17), but slightly increased for mild preeclampsia (OR, 1.31; 95% CI, 0.56-3.03) (Table 3).

Low maternal BMI (<19) was associated with a decreased risk of testicular cancer (OR, 0.63; 95% CI 0.39-1.01), but there was no association with high BMI. Anemia during pregnancy was associated with an increased risk of testicular cancer (OR, 1.68; 95% CI, 0.99-2.86). We found no effect of maternal height, weight gain during pregnancy or glucoseuria during pregnancy. Cryptorchidism was associated with an almost threefold increased risk of testicular cancer. There was no effect of smoking, maternal age at delivery, birth order, gestational duration or birth weight on the risk of testicular cancer, and the risk estimates were similar to those reported in study 2 (data not shown).

Mild gestational hypertension was a risk factor for seminomas (OR, 5.55; 95% CI, 1.72-17.9), but was not associated with the risk of non-seminomas (OR, 1.17; 95% CI, 0.65-2.11). Severe gestational hypertension was associated with a decreased risk of both seminomas (OR, 0.24; 95% CI, 0.03-2.06) and non-seminomas (OR, 0.30; 95% CI, 0.10-0.84).
Table 3. Characteristics of cases and individually matched controls and crude and adjusted odds ratios of germ-cell testicular cancer for gestational hypertension, and for preeclampsia.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Cases (n=293)</th>
<th>Controls (n=861)</th>
<th>Crude OR(^{1,2}) (95% CI(^{1}))</th>
<th>Adjusted OR(^{1,2}) (95% CI(^{1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gestational hypertension</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>no</td>
<td>212 (72)(^{3})</td>
<td>632 (73)</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>mild</td>
<td>29 (10)</td>
<td>59 (7)</td>
<td>1.59 0.97 - 2.63</td>
<td>1.62 0.98 - 2.69</td>
</tr>
<tr>
<td>severe</td>
<td>5 (2)</td>
<td>54 (6)</td>
<td>0.29 0.12 - 0.74</td>
<td>0.29 0.12 – 0.74</td>
</tr>
<tr>
<td>missing</td>
<td>47 (16)</td>
<td>116 (14)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Proteinuria</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>no</td>
<td>191 (65)</td>
<td>571 (66)</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>yes</td>
<td>58 (20)</td>
<td>167 (19)</td>
<td>1.05 0.73 – 1.50</td>
<td>1.07 0.74 - 1.53</td>
</tr>
<tr>
<td>missing</td>
<td>44 (15)</td>
<td>123 (14)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Combinations of gestational hypertension and proteinuria</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No hypertension, no proteinuria</td>
<td>204 (70)</td>
<td>608 (71)</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>mild gestational hypertension without proteinuria</td>
<td>20 (7)</td>
<td>36 (4)</td>
<td>1.78 0.98 – 3.22</td>
<td>1.84 1.01 – 3.33</td>
</tr>
<tr>
<td>severe gestational hypertension without proteinuria</td>
<td>3 (1)</td>
<td>27 (3)</td>
<td>0.34 0.10 – 1.13</td>
<td>0.33 0.10 – 1.12</td>
</tr>
<tr>
<td>mild preeclampsia(^{4})</td>
<td>9 (3)</td>
<td>23 (3)</td>
<td>1.30 0.56 – 2.99</td>
<td>1.31 0.56 – 3.03</td>
</tr>
<tr>
<td>severe preeclampsia(^{4})</td>
<td>2 (1)</td>
<td>23 (3)</td>
<td>0.26 0.06 – 1.10</td>
<td>0.27 0.06 – 1.17</td>
</tr>
<tr>
<td>missing</td>
<td>55 (19)</td>
<td>144 (17)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1 OR, odds ratio; CI, confidence intervals.
2 Crude ORs are inherently adjusted for age and place of birth; Adjusted ORs are adjusted for maternal age at pregnancy and birth order.
3 Values in parentheses indicate percentages.
4 Mild and severe preeclampsia is defined as proteinuria in combination with mild and severe hypertension, respectively.
DISCUSSION

Methodological considerations

There are several methodological issues to consider regarding the papers of this thesis. Here follows a brief discussion on the most relevant concerns.

Study 1
By design, we lack individual-level information on exposure and disease in this study. In addition, we investigated a correlate of the true exposure of interest (i.e., pregnancy smoking), which introduces additional sources of bias. For instance, we miss information on the proportion of women that stopped smoking when they became pregnant, and we do not know if this proportion is different by country, or if it has changed over time. On the other hand, the assumption that female smoking prevalence among women in fertile ages correlates well with the prevalence of smoking during pregnancy is probably valid. Even so, given the underlying problems of ecological studies, it is not possible to make causal inferences, or to interpret the correlation as reflecting effects on the individual level. In our study, this is also indicated by the fact that although the correlation coefficient was close to 0.9 and highly significant, if we were to extrapolate the smoking prevalence to 0%, the estimated incidence of testicular cancer would be negative; which cannot be the case.

Nevertheless, the association between female smoking prevalence and testicular cancer was present both over time within countries, as well as in-between countries. This fact substantially strengthens the suspicion that the ecological correlation reflects a true individual-level association.

Study 2
The rational for conducting this study was the results of the ecological study, together with the other rather persuasive arguments in favour of the hypothesis. In addition, previous retrospective questionnaire-based studies on this topic are arguably hampered by methodological shortcomings that could have resulted in biased null risk estimates (see background section, and study 1 and 2). In that respect, the most important strength of this study is that we use prospectively collected exposure information. Moreover, the linkage between the registries should be of high quality, and the completeness of the registries is high. Thus, both selection bias and recall bias is virtually precluded.

The exposure information may, however, be uninformative or mis-informative with regards to risk of testicular cancer. The window of susceptibility to carcinogenic exposures is perhaps confined to some specific part of the fetal period, and different time windows are not satisfactorily taken into account in this study. On the other hand, the perhaps most likely period for carcinogenic exposures is the first trimester, and smoking exposure was measured at the end of the first trimester.

As discussed in the paper, the null findings of our study is unlikely explained by non-differential misclassification of smoking exposure, especially not given the high prevalence of smoking among pregnant women (~45%). Rather, a concern is the potential lack of power. However, any major increased risk of testicular cancer is unlikely given the rather narrow confidence intervals; the OR for smoking was 0.91 (95% CI, 0.64 –
Furthermore, we conducted analyses stratified by histological group, and in fact we found a statistically significant protective effect of pregnancy smoking for seminomas.

**Study 3**

This study is one of the largest studies ever investigating the association between age at surgery for undescended testis and risk of testicular cancer, and the cohort was constructed and followed-up using registries with high quality. In the light of the results, the perhaps most important question to consider is if some characteristic other than age at surgery can explain our findings (chance excluded). This question is also important since a Danish study, conducted shortly after our study and with a similar study design, did not find any association between age at orchiopexy and risk of testicular cancer.

There are at least two principally different ways by which our results could be biased. First, the risk could be ‘diluted’ among those treated before 13 years of age. Second, those treated at 13 years of age or older could be a selected group of cryptorchid men with a high risk of testicular cancer. This could happen if, for instance, many of those treated before 13 years of age had retractile testis, or acquired cryptorchidism, that would naturally have descended at puberty, and that might not be associated with testicular cancer. Unfortunately, we do not have detailed information on the type of cryptorchidism, and can not answer this question with certainty. On the other hand, as stated in the paper, the proportion of boys receiving surgical correction before 13 years of age for ‘harmless’ cryptorchidism would have to have been extremely high to dilute the risks we found. In addition, at least acquired cryptorchidism seems to be less common in young children. If so, and if this condition is not associated with testicular cancer, a higher risk of testicular cancer should be found in the youngest age-group. However, neither we, nor the Danish study, found evidence of an increased risk among those operated in the age group 0 to 6 years.

For some reason, calendar-period of orchiopexy had a rather strong effect on our risk estimates (more than 2-fold). Although this calendar-period effect does not seem to affect the effect of age at surgery on testicular cancer risk, it is an interesting finding because it indicates that there is some unidentified factor (perhaps some characteristic of cryptorchidism) with a rather strong effect on the risk of developing testicular cancer in play. It would be interesting to know what factor this is, but then we would need more detailed information of the study subjects.

It is conceivable that if the testis is in an ectopic position at or after puberty, this speed up the tumor progression. We miss sufficient data covering the full age-incidence curve of testicular cancer (the mean and median age at end of follow-up in our cohort was 27.5±7.6, and 26.9 years, respectively) to explore this topic. A substantial proportion of the study subjects were, however, followed-up ‘beyond’ the age-peak in incidence of testicular cancer (30 to 35 years of age), and we found no effect on the risk with attained age.

**Study 4**

As this study is of the same design and uses the same study subjects (with an additional study subjects, see above) as study 2, it shares some of the principal advantages and shortcomings. With regards to the main aim of the paper, this study has two strengths in particular. First, we use prospectively collected information from repeated antenatal care.
visits to assess the relationship between hypertensive disorders during pregnancy and risk of testicular cancer. Second, it is the first study that separates mild from severe forms of hypertensive disorders during pregnancy.

It is unclear to what extents our variables gestational hypertension and preeclampsia reflect impaired placental function and an altered hormonal milieu, and we had no opportunity to validate these variables against for instance blood samples. Hypertensive disorders are, however, widely used markers for hormonal changes during pregnancy in etiological research, and are well known to be associated with impaired fetal growth. From the paradoxical findings of a higher risk in mild gestational hypertension, compared to a substantially lower risk in severe gestational hypertension, it is tempting to suspect that some medical treatment is protective. Unfortunately, we miss information on medical treatment during pregnancy, and information on other potentially important factors.

The small sample size is a concern. Although this study was conducted with the aim of exploring the association between hypertensive disorders during pregnancy and risk of testicular cancer, chance can not be excluded as an explanation to the results. Hence, the results need to be verified in studies with larger samples.

**Findings and implications**

*Pregnancy smoking and risk of testicular cancer (Study 1 & 2)*

The findings of the individual-level study, together with the findings from previous questionnaire based studies, are strong evidence against the hypothesis that maternal smoking during pregnancy is responsible for the past century’s epidemic increase in testicular cancer incidence. In addition, two more case-control studies on this hypothesis has been published since we published our studies, an none of these studies found any evidence of an association\(^{181,250}\). The implications of the ecological study are rather few in the light of this conclusion. Nonetheless, few exposures suggested to cause testicular cancer would likely show this strong both temporal and geographical correlation with testicular cancer occurrence. Perhaps are the important, yet unidentified, risk factor(s) highly correlated with female smoking habits not only on the population-level, but also on the individual-level. Hopefully, the results of the ecological study will give some clue to which these factors are.

*Age at surgery for undescended testis and risk of testicular cancer (Study 3)*

Our results indicate that age at surgery for undescended testis has an effect on the risk of developing testicular cancer; the risk among those treated at 13 years of age or older was twice the risk among those who were treated at younger ages. Moreover, our results indicate that the risk of testicular cancer is increased among men with cryptorchidism irrespective of age at surgical correction. These results are in line with both mechanisms outlined above; that is, the ectopic position of the testis is a factor in the development of testicular cancer, and both conditions share etiology. Therefore, the results have both etiological and clinical implications.

Etiologically, the findings implicate that the life time risk of testicular cancer is not determined in utero. This is one of the first times this has been demonstrated in a larger study, since other postnatal risk factors for which there is substantial support, for instance
adult height, can still be explained by both prenatal and postnatal exposures and events. Thus, our results implicate that testicular cancer can be initiated and/or promoted after birth, and the results are also in line with the idea that CIS or pre-CIS behave dynamically.

The exact mechanisms responsible for our findings remain to be elucidated. That the ectopic position has some damaging effects on the testis is suspected, and, primarily for the sake of future fertility, age at orchiopexy has been successively lowered. One possibility is that increased temperature is harmful. One can also speculate that gonadotropins somehow are involved, for instance through increased proliferative drive due to inadequate negative feed-back from a mal-positioned and malfunctioning testis.

The results are also important with regards to the search for risk factors that can explain the past century’s dramatic increase in incidence. It is difficult to disregard the fact that despite 30 years of research with a focus on prenatal and perinatal factors, few, if any, etiologically important risk factors have been identified. Perhaps are strong risk factors operating after birth and are therefore missed, and perhaps are prenatal and postnatal risk factors interacting. It would be highly interesting to see what investigations on factors acting during puberty, or during other potentially critical postnatal time windows, could reveal.

In addition, the results add to the evidence base for treatment of undescended testis. Since the recommended age at surgery has been successively lowered for the sake of future fertility (it is presently down to 6 months), our findings have little impact on clinical practice. However, the strategy of not operating boys with undescended testis suspected to descend at puberty could be questioned.

**Gestational hypertensive disorders and risk of testicular cancer (study 4)**

Hypertensive disorders during pregnancy are associated with a malfunctioning placenta, and therefore indirectly with two of the main etiological hypotheses on testicular cancer: the estrogen hypothesis, and the idea that impaired fetal growth causes testicular cancer. Given this, our finding of a protective effect of testicular cancer for severe gestational hypertension/preeclampsia has two important implications. First, our results argue against the notion that impaired fetal growth is an important risk factor for testicular cancer. Second, the results are in line with the idea that hormones are mechanistically involved in the causation of testicular cancer. Preeclampsia is associated with lower estrogen levels during pregnancy, and therefore the results are in line with the estrogen hypothesis. Since other empirical evidences in favour of this hypothesis are rather weak, other potential mechanisms are important to consider. Interestingly, preeclampsia, and especially severe preeclampsia, appears to be associated with increased levels of hCG. This is of particular interest since hCG stimulates testosterone production during early fetal development.
Suggestions on future research

1) To thoroughly investigate the mechanisms behind the recognized risk factors for testicular cancer. In particular, to try to determine what lies behind the association between undescended testis and testicular cancer. Several important questions remain unanswered. It is for instance not known if the risk of testicular cancer is affected by age at surgery at young ages (<2 years), if the risk in the contralateral testis in men with unilateral cryptorchidism is affected by age at surgery of the undescended testis, or if the risk in the contralateral testis in men with unilateral testis is increased at all.

2) To a larger extent consider postnatal factors acting during puberty as potentially important etiological factors.

3) To better understand if and how hypertensive disorders during pregnancy is associated with testicular cancer. If hCG is important in the development of testicular cancer, this understanding would perhaps help us find important etiological factors.

4) Adult height is emerging as a strong risk factor for testicular cancer. It would be highly interesting to understand what lies behind this association.

5) To better understand if childhood testicular cancer is ‘the same disease’ as adult testicular cancer. If it is, to meticulously compare the descriptive epidemiology of these tumors over time and in-between countries could give valuable clues to what causes testicular cancer.

6) The possible association between diet and risk of testicular cancer is still poorly investigated. It would be particularly interesting to investigate diet during different periods, for instance during pregnancy, before 2 years of age, and during puberty.

7) To try to come up with some novel hypotheses. Many of the studies conducted today are exploring the same risk factors as 30 years ago, and little evidence has emerged.

8) To explore the TDS hypothesis in more depth. The hypothesis is certainly mechanistically appealing, but still there is little empirical evidence for the existence of TDS.
CONCLUSIONS

- Smoking during pregnancy does not explain the past century’s increase in testicular cancer incidence. But the factor responsible for the epidemic increase in testicular cancer incidence should share its geographical and temporal occurrence patterns with female smoking habits.
- Age at surgery for undescended testis appears to affect the risk of developing testicular cancer. This indicate that the ectopic position of the testis is a factor in the development if testicular cancer, and that the life time risk of testicular cancer is not determined solely in utero or early in life.
- All men with undescended testis should be treated before puberty.
- Signs of placental malfunction, such as gestational hypertension and preeclampsia, seem to be associated with the risk of developing testicular cancer. In particular, severe hypertensive disorders during pregnancy appear to be protective.
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