ENDOCRINE FACTORS AND RISK OF TESTICULAR GERM CELL CANCER

by

Hannah Kate Weir

A thesis submitted in conformity with the requirements for the degree of Doctor of Philosophy
Graduate Department of Community Health
University of Toronto

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ABSTRACT

A population-based case-control study was conducted in the province of Ontario to examine the relationship between prenatal and pubertal endocrine exposure on the risk of testicular germ cell cancer. Cases included males aged 16 to 59 with a diagnosis of testicular germ cell cancer between 1987 and 1989. Controls were a population-based random sample frequency matched by 5-year age groups. Data were collected on 502 cases and 346 case mothers and 975 controls and 522 control mothers using a self-administered questionnaire to subjects and a phone interview to mothers.

A number of prenatal and perinatal exposures were found to be statistically significantly associated with risk of testicular germ cell cancer. Risk was elevated for in-utero exposure to exogenous hormones (i.e. prescription hormones including diethylstilbestrol (DES), injections and pills to determine pregnancy and birth control pills); mothers' cessation of birth control pill use within 3 months of conception; and preterm birth (delivery more than two weeks earlier than expected). Risk was decreased for heavy (12+ cigarettes per day) maternal smoking; young (<20 years) maternal age; and bleeding and threatened miscarriage. In addition, this study reports a protective effect from later puberty. These risk factors are discussed with respect to the hypothesis that endocrine exposures are associated with risk of testicular germ cell cancer.
Acknowledgments

I would like to thank a number of individuals and organizations for their contribution to my career development and to this project. First, I would like to thank my supervisor, Dr. Nancy Kreiger, for her patience, and continued support and guidance over the past six years. I would also like to thank the members of my thesis committee, Dr. Loraine D. Marrett and Dr. Gerarda Darlington, for their constructive comments and advice.

I would like to thank my colleagues at the Ontario Cancer Treatment and Research Foundation for their support and help on this project; and the faculty and students at the University of Toronto for making this a great and challenging learning experience.

I am especially grateful to the National Health Research and Development Program of Health and Welfare Canada for providing me with a National Health Fellowship.

And last, I would like to thank my family and friends for their support and encouragement. I am especially grateful to Kyle and Sydney for their unwavering belief in their mom. I couldn’t have done this without you!
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Data for this dissertation come from a case-control study of malignant germ cell tumours in Ontario residents (funded by the Ontario Ministry of Health). The Ph.D. candidate was co-investigator, along with Dr. Loraine D. Marrett, on this study. As co-investigator, the incumbent was involved in all aspects of the study including: preparing drafts of the grant proposal and all interim and the final reports submitted to the funding agency; the day-to-day running of the study including the hiring and training of staff; questionnaire development and pretesting; drafting letters to physicians, subjects and mothers; and development of study procedures (including contact and follow-up schedules and scripts, coding and abstracting forms, data entry manuals and data quality checks). To enlist the co-operation of oncologists who would be involved in this study, the incumbent made a presentation of the proposed study at the Oncology Grand Rounds at the Princess Margaret Hospital.

Data on testicular cancer cases and controls were used for this dissertation.
CHAPTER 1
INTRODUCTION

Testicular cancer is uncommon, usually occurring in boys, and young and middle aged men. The five-year relative survival rate for men diagnosed with testicular cancer is over 90%. Despite the rarity of the tumour, and its good prognosis, testicular cancer does have an impact on morbidity in men. Treatment for testicular cancer usually results in removal of the affected testicle which can lead to impaired fertility. Men with a history of testicular cancer are at greater risk of developing cancer in the contralateral testicle, and thus require continued medical attention and follow-up (Osterlind et al., 1991). Since treatment results in residual impairment, primary prevention is important.

The majority of testicular cancers are of germ cell origin. The age-specific incidence rates for these cancers closely parallel endocrine activity in children and young adults. Increasing incidence rates have been observed both in Ontario and throughout the world (Tomatis et al., 1990; Holowaty et al., 1995).

Numerous investigations have been conducted to examine the etiology of this cancer. Apart from the association of testicular germ cell cancer with a history of undescended testicle (Morrison, 1976; Mostofi, 1973), little consistent information regarding risk factors has emerged, and the reason for the increasing incidence remains unknown.

The descriptive epidemiology of testicular germ cell cancer suggests that exposures operating in the prenatal period or at puberty may be important in the etiology of this cancer. To examine the etiologic relevance of such exposures, particularly as they relate to hormone exposures, a case-control study was conducted in Ontario. Both subjects and their mothers completed questionnaires covering a wide range of medical and demographic conditions. Included in the study was histologic review of eligible cases for the purpose of classifying tumours according to the major histologic subgroups of testicular germ cell cancer: seminoma and nonseminoma.

1.1. Objectives

The main objective of this dissertation was to test the hypothesis that hormone exposures,
in either the prenatal or pubertal period, are associated with risk of testicular germ cell cancer. To accomplish this objective, specific conditions of pregnancy thought to indicate relative exposure to pregnancy hormones, or factors influencing pregnancy hormone serum levels, were identified from the literature and tested for their association with cancer risk. Risk associated with maternal exogenous hormone use and relative age of subject's puberty were also considered as risk factors.

In addition, this analysis examined whether risk factors differed according to the two major histologic subgroups of testicular germ cell cancer (seminoma and nonseminoma), and assessed the effect of agreement between subject and mother responses, on odds ratio estimates for age at puberty.

1.2. Format of the dissertation

Discussion of the biology of testicular development, maturation and function, and testicular abnormalities, diagnosis and histologic classification are presented in chapters 2 and 3. Sources of prenatal hormone exposure are presented in chapter 4. The descriptive epidemiology of testicular cancer is reviewed in chapter 5, while literature regarding risk factors for testicular cancer is reviewed in chapter 6. Materials, methods and methodological issues related to this study are discussed in chapter 7. Since the objectives of the proposed dissertation comprise separate but related issues of epidemiologic interest, the format of this dissertation includes three separate manuscripts suitable for publication in an epidemiological journal (chapter 8). Chapter 9 comprises the summary and discussion.

1.3. Expected Contribution

Both exogenous and endogenous hormone exposures are implicated as risk factors for undescended testicle. Based on the observation that more case mothers than control mothers reported receiving hormones during pregnancy, or reported nausea (an indicator of rapidly rising, and possibly excessive, estrogen levels in early pregnancy) as a complication of pregnancy, Henderson et al. (1979) hypothesized a role for prenatal estrogen exposure and risk of testicular
cancer. A number of subsequent studies have interpreted their results as supporting this hormone hypothesis. More recently, the hormone hypothesis has been extended to include factors operating around the time of subject's puberty. Since testosterone and estrogen share a number of similar physiologic properties (promotion of cell growth and differentiation) such a connection may have biological relevance. Yet the results of these studies are not entirely consistent, making it hard to support the hypothesis that hormone exposures are associated with risk of testicular germ cell cancer.

To date, no study has undertaken a comprehensive examination of risk factors as they relate to both exogenous and endogenous sources of maternal hormone exposure and age at puberty. This study was undertaken to provide such a comprehensive review and evaluation of the hypothesis.

Furthermore, this study will identify strategies for future etiologic investigation of testicular germ cell cancer. In particular, the need for reporting odds ratio estimates according to histologic subgroups will be examined. While some studies have reported odds ratio estimates for seminoma and nonseminoma, separately, none has made a quantitative comparison of odds ratio estimates for the purpose of identifying whether risk factors differ by histologic subgroup. Such a strategy could prove useful in elucidating associations if risk factors differ. In addition, the effectiveness of two analytic strategies, both designed to characterize the true effect of poorly measured exposures, will be assessed with regard to age at puberty.
CHAPTER 2
TESTICULAR DEVELOPMENT, MATURATION AND FUNCTION

The underlying changes that predispose an individual to develop testicular cancer as an adult may happen during prenatal development (Skakkebaek et al., 1987). Endocrine activity during this period or at puberty may be of particular importance. A brief review of testicular development, maturation and function, and testicular abnormalities will be presented (Goodman, 1994; Oth, 1993) with emphasis on how endocrine factors may predispose toward testicular cancer in adult life.

2.1. Development:

Much of the human reproductive tract develops during the first several months of embryonic life in close association with the development of the urological tract (Hoar, 1982). In the upper abdomen of the developing fetus, the genital ridge forms. In the genital ridge are two sets of ducts: the Mullerian ducts, which give rise to female genitalia; and the Wolffian ducts, which give rise to male genitalia. The primitive male gonad develops as an outgrowth of the genital ridge. Contained within the primitive gonad are seminiferous chords which contain Sertoli cells, Leydig cells and gonadocytes. The gonadocytes are derived from the primordial germ cells which have migrated to this position from the yolk sac some time between the fifth and seventh week of gestation. The Leydig cells produce testosterone which stimulates the Wolffian ducts to undergo cell differentiation and growth, and the Sertoli cells produce Mullerian inhibiting substance (MIS) which causes the Mullerian ducts to regress and disappear.

During fetal life, the gonad descends internally from the upper abdomen until it appears in the inguinal region just above the pelvis. Final descent of the gonad (testicle) through the inguinal canal into the exterior scrotal sac occurs around the seventh or eighth month of gestation. The testicle is held in place within the scrotal sac by the spermatic cord. Failure of the testicle to be present in the scrotal sac at birth is a common condition among premature births, and spontaneous descent often occurs following birth.

While hormone regulation appears critical for proper descent of the male gonad, the specific hormones involved, and their modes of action, remains unclear (Wensing, 1988).
Estrogen may play a role in both testicular development and descent (Sharpe, 1993; Sharpe and Skakkebaek, 1993). Estrogen is produced in the neonatal gonad by conversion of testosterone by the Sertoli cells. It is hypothesized that estrogen may be involved in a complex regulatory feedback loop with FSH (follicle stimulating hormone): interference with regulation may disrupt Sertoli cell division and MIS production. In this manner, altered production of maternal endogenous estrogen, or exposure to exogenous sources of estrogen, could interfere with regression of the Mullerian ducts and this, in turn, could interfere with internal descent of the primitive male gonad. Altered MIS production may also be involved in suppressing or interfering with germ cell multiplication during fetal development (Hirobe et al., 1992).

During fetal development, the population of gonadocytes, Sertoli cells and Leydig cells increases. However, within six months following birth, Leydig cells disappear, testosterone levels decline and germ cell division ceases until the onset of puberty.

2.2. Maturation and Function

In prepubertal boys, the testicle appears as a small dense mass. At the onset of puberty, the testicle enlarges as tubules develop within the seminiferous chords, and the gonadocytes mature into spermatogonia. Spermatogenesis (sperm production) takes place throughout the entire thickness of the seminiferous tubule, and can be characterized by three distinct phases. The first phase (mitosis) begins at the basement membrane of the epithelium with the resumption of mitosis in the gonadocyte or germ cell (dormant since around the time of birth). Mitotic division results in the formation of two daughter cells, one of which will remain as a stem cell while the other continues on to phase two. In phase two (meiosis), cell division results in the production of two haploid spermatids. Phase three involves the transformation of the spermatid into the mature sperm. During this time, the chromosomes become tightly packaged as cytoplasm is lost and flagella form. Spermatogenesis takes approximately 64 days to complete in the adult male. Mature sperm are then deposited into the lumen of the seminiferous tubules. Sertoli cells produce a watery substance that helps in the transport of the sperm within the seminiferous tubules.
Adult male gonads contain approximately 250 meters of seminiferous tubules. While spermatogenesis continues throughout adult life, a study of 833 healthy, fertile men showed that sperm count and semen volume steadily increased to about age 25 years, followed by a plateau until the mid-30's, and then gradually declined with advancing age (Schwartz et al., 1985).

At puberty, Leydig cells reappear with resumption of testosterone production. The Leydig cells are present in the interstitial lining separating the seminiferous tubules. Sertoli cells are found throughout the thickness of the epithelium lining, alongside the maturing spermatogonia, and are believed to assist in the process of spermatogenesis. Their numbers may ultimately determine the number of mature sperm that are produced in adulthood (Orth, 1993). A tight conjunction, referred to as the blood-testis barrier, forms among the tightly packed Sertoli cells. Spermatogonia are present on the blood side of the barrier, while spermiogenesis takes place on the luminal side of the membrane. The blood-testis barrier is selectively permeable, allowing testosterone to cross freely.

In boys, the initiation of gonadal maturation, and the underlying endocrine changes that signify puberty, occur several years prior to the actual appearance of secondary sex characteristics (i.e. growth spurt, appearance of pubic and facial hair, and voice change). The usual course of events marking puberty is accelerated growth of the testicle and penis, followed by the adolescent growth spurt, appearance of hair and voice change. Based on observations on boys who reached puberty in the 1970's, the acceleration of penile growth takes place between age 10.5-14.5 years (Tanner, 1978). There is, however, considerable variability both in the onset of puberty, as well as the time it takes for a boy to progress through the pubertal changes and attain maturation. A boy may begin puberty before his peers, but not attain full maturation until after his peers.

The exact mechanism by which puberty is initiated or completed is not known; however, the onset of puberty appears to be influenced by both environmental and genetic factors (Tanner, 1978). Over the century, successive cohorts of children (boys and girls) have been growing to maturity more quickly, presumably due to their entering puberty at an earlier age. However, this secular trend of accelerated maturation may be slowing in some industrialized countries (Tanner, 1978). Child and adult height has also increased among these cohorts.
2.3. Undescended testicle

Undescended testicle, also called cryptorchidism, arises when one or both testicles fail to descend properly into the scrotal sac by one year of age. Confusion in definitively diagnosing this condition in newborns may arise because spontaneous descent can occur following birth, especially among preterm births. Likewise, difficulty in diagnosing this condition in children may arise because the testicle can remain retractile (capable of being withdrawn from the scrotal sac back up into the inguinal canal) in prepubertal boys. Failure of the testicle to descend properly may result from failure to descend internally (into the inguinal region), or externally (into the scrotal sac) due to structural problems, such as a short spermatic cord or a narrow inguinal canal. If the testicle can be located in the inguinal region, hormonal treatment or surgical correction may successfully position the testicle within the scrotal sac. It is advised to treat prior to the second year of life (Chilvers et al., 1986) although there is no evidence that earlier rather than later correction improves subsequent fertility in these boys. The evidence that earlier correction lessens the risk of testicular cancer is inconclusive (Pike et al., 1986; Potten et al., 1985; Prener et al., 1996; UK Testicular Cancer Study Group, 1994).

Measuring the prevalence of cryptorchidism is beset by problems of definition, especially if measured at birth, for reasons already mentioned. Despite the inherent problems with diagnosing cryptorchidism, there is evidence to suggest that the prevalence of this condition is increasing (Campbell et al., 1987; Chilvers et al., 1984). Over a twenty year period, the cumulative rate for surgical correction of an undescended testicle, among boys 0 to 14 years of age, doubled in England and Wales (Chilvers et al., 1984): the estimated rates being 1.4% among boys born in 1952 vs. 2.9% in boys born in 1977. Discharge rates increased most steeply for boys in the youngest age group (0-4 years) reflecting a trend to operate at an earlier age. The authors acknowledged that this doubling in discharge rates does not necessarily reflect a doubling in true prevalence but could, instead, result from an increase in unnecessary operations being performed on retractile testes in prepubertal boys. This trend probably reflects a growing awareness among pediatricians that cryptorchid boys may experience reduced fertility (Chilvers et al., 1986) and
possibly increased cancer risk (Mostofi, 1973).

More persuasive evidence for there being a true increase in the incidence of undescended testicle comes from two other sources. First, notification rates of malformations of external genitalia (cryptorchidism, hypospadias and hydrocele) reported at birth among males have increased in the past several decades in England and Wales, while rates for malformation of vaginal and external genitalia in females have decreased (Matlai and Beral, 1985). Second, in a study of 3,599 newborns, followed from birth to age 2, the prevalence of undescended testicle was 5.9% at birth and 1.6% at 3 months of age (Jackson, 1988). Compared to results from a similar study conducted in the same institution several decades earlier (Scorer, 1964), these data represent an increase of 40% for diagnoses made at birth, and 68% for diagnoses made at 3 months of age.
CHAPTER 3
DIAGNOSIS AND HISTOLOGIC CLASSIFICATION OF TESTICULAR GERM CELL CANCER

3.1. Diagnosis

Testicular cancer can be confused with testicular torsion (twisting of the spermatic cord), epididymitis (inflammation of the epididymis) and hydrocele (accumulation of fluid in the scrotal sac). Testicular cancer patients usually present with complaints of a painless enlargement due to a lump, swelling or hardness of one gonad (Horwich, 1991). If pain is reported, it is usually due to concomitant epididymitis or bleeding within the scrotal sac which may become acute following trauma to the scrotum. The majority of patients presenting with signs and symptoms of a testicular tumour have a primary cancer while the remaining few have metastatic disease due to an occult cancer. Delays in treatment occur because patients delay seeking medical attention and because physicians make erroneous diagnoses. In a retrospective review of adult testicular cancer patients, an initial incorrect diagnosis resulted in delays in treatment by as much as 10 months in some few cases (Nilsson et al., 1981).

Definitive diagnosis follows from radiological examination and serum assays for the two tumour markers, alpha fetoprotein (AFP) and human chorionic gonadotropin (HCG). Pathologic diagnosis requires either needle biopsy or orchiectomy, removal of affected testicle, thus allowing for histologic classification.

3.2. Histologic Classification

Testicular germ cell cancer comprise a heterogeneous group of tumours whose common origin is generally accepted as being that of the germ cell (Talerman, 1986). They are an unusual group of tumours in that they are capable of undergoing both embryonic (somatic) and extra embryonic (i.e. tumour elements that resemble the extra embryonic membranes of the developing embryo) differentiation (Grigor, 1981). Other testicular cancers do arise, including lymphoma, sarcoma, and tumours of Leydig and Sertoli cells. These latter two tumours are very rare (Mostofi, 1973) while lymphomas and sarcomas more commonly occur in men over the age of 65 years (Schottenfeld and Warshauer, 1982).
Two systems for classifying germ cell tumours are currently in use, one proposed by the British Testicular Tumour Panel (Pugh and Parkinson, 1981), the other by the World Health Organization (WHO), based on a proposal made by Mostofi and Sobin (1977). The latter is the most comprehensive and widely used system in North America, although revisions to this classification system have recently been proposed (Mostofi et al., 1990) as knowledge and understanding of the histogenesis of these tumours evolves.

The WHO classification system is based on the presumption that there is a precursor germ cell which can give rise to either seminoma (except spermatocytic seminoma) or nonseminoma. The latter group can then undergo further differentiation, beginning from embryonal carcinoma, and giving rise to teratomas with embryonic elements of endoderm, mesoderm and ectoderm, or tumors of extra embryonic elements, such as yolk sac tumours and choriocarcinoma. The WHO system includes the following histologic types: seminoma, spermatocytic seminoma, embryonal carcinoma, yolk sac tumours (also called embryonal carcinoma infantile type or endodermal sinus tumour), polyembryona, choriocarcinoma and teratoma (mature, immature and with malignant transformation). Seminoma tumours are highly differentiated and closely resemble the gonadocyte while spermatocytic seminoma, a rare tumour, more closely resembles the spermatogonia, and should not be included with germ cell tumours (Skakkebaek et al., 1987). Nonseminoma tumours are the least differentiated of all germ cell tumours because they least resemble the gonadocyte. However, these tumours are capable of differentiating (analogous to parthenogenesis) into somatic structures (mature and immature teratoma) or extra embryonic structures. Differentiation into extra embryonic structures occurs along one of two pathways: the viteline pathway (yolk sac tumours) or the trophoblastic pathway (choriocarcinoma). Germ cell tumours can occur in pure histologic form or as combinations of histologies.

The traditional view holds that seminoma and nonseminoma have evolved from different pathways. However, there is controversy over the histogenesis of these tumours. A new model of histogenesis is being considered based on the discovery of atypical germ cells. Atypical germ cells have long been observed in areas adjacent to invasive tumour. Biopsies have revealed these
atypical cells in the contralateral testes of men with unilateral germ cell cancer (Skakkebaek et al., 1982; van der Masse et al., 1986) and in the testes of men with undescended testicles but no tumour (Koide et al., 1987; Skakkebaek et al., 1981). Skakkebaek (1987) has postulated that these cells, which closely resemble both gonadocytes and seminoma, are a premalignant phase. The term carcinoma-in-situ (CIS) has been used to describe them (although strictly speaking, CIS is not a correct term since the origin of these cells is germ cell and not epithelium).

The malignant potential of the CIS cells was clearly demonstrated by the finding that out of 27 unilateral testicular germ cell patients found to have CIS in the contralateral testicle, 50% of patients went on to develop cancer within 5 years (von der Masse et al., 1986). There are anecdotal reports of CIS found in the testes of neonates and prepubertal boys with androgen insensitivity syndrome and gonadal dysgenesis (Muller, 1984; Muller et al., 1985). CIS was not observed in yolk sac tumours or differentiated teratomas in children whose genetic constitution was assumed normal (Soosay et al., 1991) or in a series of men who had undergone testicular biopsy as children but developed germ cell testicular cancer in adulthood (Cortes et al., 1994), or in a group of prepubescent boys operated on for undescended testicle (Parkinson et al., 1994). Studying CIS in children may be difficult because reliable diagnoses may best be performed on the post-pubertal testes.

The histologic relevance of CIS has been summarized by Skakkebaek et al. (1987) as follows: CIS is a malignant gonadocyte (possibly forming in early fetal life); CIS can either regress into embryonal carcinoma elements, or progress into seminoma (but not spermatocytic seminoma); and the ability of these cells to regress is lost as a function of age whereas the potential to progress to seminoma is retained with age. A modification of this hypothesis is put forward by Oliver (1987) who argues that clonal expansion, not regression, leads to tumour formation: CIS progresses to seminoma, and seminoma progresses to nonseminoma. Furthermore, Oliver believes that excessive mitogenic stimulation leads to clonal expansion of the tumour. Suppression of Mullerian inhibiting substance (MIS) (section 2.1), which is also thought to regulate mitotic activity in the fetal germ cell, could lead to formation of these atypical germ cells.
Clinicians have grouped testicular germ cell cancers into seminoma and nonseminoma according to their prognosis and treatment (Brawn, 1983; Einhorn and Donohue, 1977; Horwich, 1991). Seminomas comprise slightly more than half of all testicular germ cell cancers and tend to be diagnosed in older men (early 30's). These tumours are less aggressive and are generally treated by surgery and radiation therapy. Nonseminoma, which comprise approximately one quarter of all testicular germ cell cancers, tend to be diagnosed in slightly younger men (mid 20's). These tumours are more aggressive, requiring chemotherapy to supplement surgical treatment and have a poorer prognosis. The remaining one fifth of testicular germ cell cancers are tumours of mixed histology containing both seminoma and nonseminoma. They have an average age at diagnosis between that of seminoma and nonseminoma and are grouped with nonseminoma for descriptive epidemiology purposes, treatment and prognosis.
CHAPTER 4
SOURCES OF PRENATAL HORMONE EXPOSURE AND ASSOCIATED RISK WITH UNDESCENDED TESTICLE

As much as 30% of human cancers may be hormone-related (Henderson et al., 1982), including breast, ovary, endometrium, prostate, and possibly testis. The carcinogenic potential of estrogen and testosterone appears related to their physiologic properties, and their ability to stimulate cell proliferation and growth (IARC, 1987).

A brief review of maternal hormones will be presented (Goodman, 1994) along with factors thought to influence relative concentrations of circulating hormones in pregnant women.

4.1. Endogenous hormones

Both the sources and the relative concentration of circulating estrogen vary throughout the life span of the human female. In prepubescent girls and post menopausal women, relatively small amounts of estrogen, primarily estrone, are produced as a result of the indirect conversion of cholesterol to estrogen in adipose tissue. At puberty, the ovaries begin producing relatively large amounts of estrogen, primarily estradiol, as well as testosterone and estrone.

Menstruation is a complex system of hormone interactions influenced by luteinizing hormone (LH) and follicle stimulating hormone (FSH). During the first half of the menstrual cycle (the follicular stage), estrogen levels rise until mid-cycle when levels peak. Progesterone levels then begin to rise immediately preceding ovulation and the release of a mature egg. Following ovulation (the luteal phase), the corpus luteum begins to produce large quantities of steroid hormones, including progesterone, which help to prepare the uterine lining for the implantation of a fertilized egg. If pregnancy does not follow, the corpus luteum involutes approximately 12 days later; progesterone secretion ceases, and menstruation occurs.

In the event pregnancy does occur, the integrity of the corpus luteum is extended and steroid hormone production continues. In particular, the corpus luteum produces human chorionic gonadotropin (HCG), whose detection is widely used as an indicator of pregnancy. Starting in the second month of pregnancy, the placenta takes over production of steroid hormones including estrogen and progesterone. The levels of the latter two hormones continue
to rise rapidly throughout pregnancy. In addition to estradiol and estrone production, the placenta also produces relatively large quantities of estriol, an estrogen of weak estrogenic activity. During pregnancy, the placenta acts as a bridge allowing nutrients and hormones to circulate between the maternal and fetal circulatory systems.

Estrogen circulates in the blood plasma both loosely bound to albumin and more tightly bound to sex hormone binding globulin (SHBG). Metabolic inactivation of estrogen takes place in the liver by a process of 2-hydroxylation and conjugation. Estrogen metabolites are excreted by the kidneys into the urine in the form of estriol. Estradiol is the most biologically active of all the estrogens, followed by estrone and estriol.

A number of factors, including smoking, age, maternal weight, alcohol and parity appear to influence endogenous levels of hormones in both nonpregnant and pregnant women. In a clinical study of 147 pregnant women (Bernstein et al., 1989), smokers had lower levels of estradiol (-17.6%), SHBG (-12.4%) and HCG (-21.5%) compared to nonsmokers and an inverse gradient was observed based on the number of cigarettes smoked per day. A second clinical study, of comparable size and which measured circulating hormones at a comparable gestational age, found a modest reduction in total serum estrogen in smokers (9%) (Petridou et al., 1990); however, smoking intensity was not reported. Of interest in the latter study, was the observation that birth weight was positively correlated with estrogen levels independent of smoking status. In the same study population, total estrogen and estradiol levels were reported to be lowest in pregnant women under age 20 (Panagiotopoulou et al., 1990), although results correlating age and estrogen levels in older women were equivocal. Maternal weight gain up through the 31st week of pregnancy was positively associated with total estrogen and estradiol levels among these women (Petridou et al., 1992).

In a clinical study of premenopausal nonpregnant women, using a cross-over design in which women alternated between consuming 2 and 0 alcoholic drinks per day for two weeks, alcohol consumption was found to elevate serum estradiol levels (27.5%) and serum estrone levels (21.2%) (Reichman et al., 1993). But when the association between estrogen levels and alcohol consumption was examined using data from a drinking pattern questionnaire, no association was
Chapter 4

reported (Dorgan et al., 1994). There are no studies to show the effect of alcohol consumption on maternal estrogen levels among pregnant women.

A number of studies have demonstrated that the hormone profile of women changes following first full term pregnancy (Bernstein et al., 1986; Mussey et al., 1987; Trichopolus et al., 1980). Serum samples taken from 34 women, and drawn at comparable gestational ages in both their first and second pregnancies, showed that total and unbound levels of estradiol were 7% and 17% higher respectively, in first compared to second full term pregnancies (Bernstein et al., 1986). Similar results have been obtained in other clinical studies of pregnant women (Panagiotopoulou et al., 1990). This alteration in hormone profile is thought to be due to an alteration in metabolism following first full term pregnancy (Bernstein et al., 1985; Bernstein et al., 1986).

Though the causes of pregnancy-related nausea and vomiting are not well understood, a seroepidemiologic study of first trimester pregnancies found that women who experienced this condition had higher estradiol levels (including nonprotein-bound estradiol) and sex hormone binding globulin levels than women who did not report nausea and vomiting: 26% and 37% higher for estradiol and SHBG, respectively (Depue et al., 1987). Hyperemesis was reported more often among younger, nulliparous women of high body weight (Depue et al., 1987).

4.2. Exogenous hormones

It has long been recognized that optimum levels of hormones are required to maintain pregnancy. Both natural and synthetic estrogens and progesterones have been given to pregnant and nonpregnant women for therapeutic benefit. Conjugation, or modification, of the molecular structure of natural estrogens improves absorption and slows metabolism thus allowing these preparations to remain active and circulate for longer periods of time. Synthetic estrogens may be more potent than natural ones because they circulate unbound to sex-hormone binding globulin (Arai et al., 1983). Examples of these preparations are detailed below:

* The synthetic nonsteroidal estrogen, diethylstilbestrol (DES), was widely prescribed in the United States (US) between 1941 and 1971, and in Canada
between 1944 and 1971. According to DES Action Canada (Nevin, 1988), over 400,000 pregnant women in Canada were exposed to DES within this period. DES was given to pregnant women for threatened miscarriage, history of miscarriage or threatened miscarriage, hypertension or diabetes in the belief that it would stimulate production of natural progesterone levels and, thereby, help maintain the pregnancy. DES was shown to be ineffective in preventing miscarriage in the early 1950’s (Dieckmann et al., 1953; Ferguson, 1953). However, it remained in use until 1971 when it was reported that DES may have caused vaginal clear cell adenocarcinoma in the daughters of women who had taken the medication while pregnant (Herbst et al., 1971).

* During the 1950’s and 1960’s, a common form of pregnancy testing included the administration of relatively large amounts of progesterone, often in combination with estrogen. If withdrawal bleeding did not occur following cessation of hormone exposure, this was thought to confirm pregnancy. (No association between these tests and congenital abnormalities reported at birth have been reported (Oakley et al., 1973; Torfs et al., 1981)).

* Oral contraceptives (OC) are a combination of hormones that when ingested, inhibit ovulation, fertilization and implantation. The first birth control pill was approved for use in the United States in 1960. Shortly thereafter, US drug companies began marketing birth control pills in other countries including Canada. Sequential birth control pills first in use contained higher doses of estrogen than the combination pills (pills containing estrogen and progesterone) now in use. OC are not 100% effective in preventing pregnancy, and women have conceived while taking birth control pills.
4.3. **Endocrine factors and risk of undescended testicle**

Administration of exogenous hormones, in the form of diethylstilbestrol (DES), to pregnant women has been reported to increase the incidence of cryptorchidism (Beral and Colwell, 1981; Cosgrove et al., 1977; Mills and Bongiovanni, 1977; Stillman, 1982; Whitehead and Leiter, 1981). A study by Rothman and Louik (1978) found that male children born to mothers who conceived shortly after terminating birth control pill use had a modest increase in the incidence of cryptorchidism.

Several case-control studies have attempted to evaluate the hypothesis that exposure to maternal estrogens is associated with increased risk of cryptorchidism (Beard et al., 1984; Berkowitz et al., 1995; Davies et al., 1986; Depue et al., 1984; McBride et al., 1991; Swerdlow et al., 1983). The results of these studies, which looked at exposure to exogenous hormones and endogenous hormones, are equivocal. However, a nested case-control study, using participants from the US Collaborative Perinatal Study, a prospective study designed to investigate etiologic factors thought to be associated with adverse pregnancy outcome, has provided more conclusive evidence that maternal estrogens increase risk of cryptorchidism. Case mothers compared to control mothers had statistically significantly elevated levels of both bound and unbound estradiol in the first trimester of their pregnancy (Bernstein et al., 1988).
CHAPTER 5
DESCRIPTIVE EPIDEMIOLOGY OF TESTICULAR CANCER

Since the majority of testicular tumours are of germ cell origin, and the vast majority of these tumours are diagnosed in men under 60 years of age, the descriptive epidemiology of testicular cancer, at least among boys and young and middle-aged men, reflects the epidemiology of testicular germ cell cancer. Tumours diagnosed in older men are usually of nongerm cell origin (Pike et al., 1987; Schottenfeld and Warshauer, 1982).

5.1. Burden of disease

Testicular cancer is rare, accounting for 1% of all cancers diagnosed in Ontario males. It is, however, the most commonly diagnosed cancer in young men in the age group 25-34 years (Cancer in Ontario, 1993). In 1991, 239 cases of testicular cancer were diagnosed in Ontario residents while in the same year, there were 20 deaths due to testicular cancer. The lifetime probability of developing testicular cancer is 0.3% (unpublished OCR data).

5.2. Age distribution

As can be observed in figure-5.1, there is a small peak in incidence in boys under age 5, followed by a much larger peak in men in their late 20’s to early 30’s. Following this peak, incidence declines with advancing age. Nonseminoma incidence peaks in men in their late 20’s while seminoma peaks somewhat later. For purposes of cancer registration, and according to tumour classification (World Health Organization 1976), tumours of mixed histologies, even those containing seminoma, are included with nonseminoma.

5.3. Incidence and mortality rates

Between 1964 and 1991, there was a statistically significant increase in testicular cancer incidence in Ontario: rates rose 79%, from 2.6 per 100,000 to 4.7 per 100,000 (Weir et al., manuscript in preparation). The increase in incidence appears to be due to a steady rise in the incidence of germ cell tumours, particularly among young men between 15-44 years (figure - 5.2). The incidence of nongerm cell tumours is low compared to germ cell tumours and has not
increased. Similar patterns of increase have been noted worldwide (Adami et al., 1994; Boyle et al., 1987; Brown et al., 1986; Heindal et al., 1990; Moller, 1993; Nethersell et al., 1984; Pearce et al., 1987; Stone et al., 1991). Incidence rates in prepubertal boys (<15 years) and older men (45-59 years) have not increased (figure-5.2). Similar patterns of increase in specific age groups has been noted in Denmark (Moller et al., 1995). In Ontario, the increase in incidence of germ cell tumours has been similar for both seminoma and nonseminoma (figure -5.3). A similar histologic pattern of increase has been noted in Denmark (Moller, 1993). In the mid 1970's, mortality rates declined, particularly for nonseminoma, both in Ontario (Marrett et al., 1986) and elsewhere (Li et al., 1982), coincident with the introduction of new chemotherapeutic agents used to treat nonseminoma (Einhorn and Donohue, 1977). This divergence in incidence and mortality trends has been noted elsewhere (Brown et al, 1986; Osterlind, 1986) and is due to improved survival for germ cell tumours, particularly nonseminoma (see section 5.4).

An examination of US and European data has shown that the increase in testicular cancer incidence is due to a birth cohort effect operating in men born at least since the early part of this century (Bergstrom et al., 1996; Hoff Wanderas et al., 1995; Moller, 1993; Roush et al., 1987; Zheng et al., 1995). This birth cohort effect is evident in Ontario data as well (Weir et al., manuscript in preparation); however, because the Ontario Cancer Registry only began to register cancer patients beginning in the mid 1960's, the birth cohort effect is only evident among Ontario males born in the mid-1940's onward. Of anecdotal interest is the finding that incidence, as measured by cumulative risk, temporarily declined among Danish men born in the early 1940's, when Denmark was occupied by German forces (Moller, 1989). Moller speculated that malnutrition, experienced by pregnant women, may have somehow altered risk in the son. A similar pattern of lower incidence among men born during wartime occupation was noted in Norway data (Hoff Wanderas et al., 1995).

The increase in incidence must be viewed as real. No organized, population-based, screening programs have been conducted, and there has been no major change in diagnostic procedures or histologic classification of these cancers. The early age at diagnosis, as well as the accessibility of the testicle for physical examination, suggest that occult cancers are not likely.
FIGURE-5.1. Frequency of testicular cancer cases by histologic type and age at diagnosis (Ontario incidence data 1964-1991)

Figure - 5.2. Age-adjusted* incidence rates (AAR) for testicular germ cell cancer in Ontario

* age-adjusted to the World Standard Population (Waterhouse et al., 1962)
Figure - 5.3  Age adjusted* incidence rates (AAR) for testicular cancer in Ontario by histologic type

* age-adjusted to the World Standard Population (Waterhouse et al. 1962)
As far as Ontario data are concerned, no changes in cancer registration over the past several decades could account for the magnitude of increase in incidence. 5.4. **Survival**

In Ontario, the 10-year relative survival rate for men diagnosed between 1979 and 1988 with seminoma and nonseminoma is approximately 94% and 86%, respectively (McLaughlin et al., 1995).

5.5. **Geographic variation and differences by race**

There are pronounced racial and ethnic differences in age-adjusted incidence rates between countries (table-5.1). Black and Asian populations having low incidence rates compared to Caucasian populations of northern and western Europe, North America and Australia (Parkin, 1992; Tomatis, 1990). Within populations, there are also pronounced racial and ethnic differences: US Blacks and Hispanics have much lower rates than whites (Brown et al., 1986; Newell et al., 1987; Spitz et al., 1986). Incidence rates among US Blacks do not appear to be increasing while rates among US whites are increasing (van der Eeden and Weiss 1989). Canadian and US rates appear somewhere in the middle in a comparison of international rates.

**TABLE-5.1.** Age-adjusted† incidence rates (AAR) and standard errors (SE) for testicular cancer per 100,000 in various populations

<table>
<thead>
<tr>
<th>Country</th>
<th>AAR (SE)</th>
<th>Country</th>
<th>AAR (SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Australia</td>
<td>3.4-4.2†</td>
<td>Poland</td>
<td>1.2-3.3‡</td>
</tr>
<tr>
<td>Canada</td>
<td>3.6 (0.1)</td>
<td>Romania</td>
<td>1.4 (0.3)</td>
</tr>
<tr>
<td>China</td>
<td>0.4-0.8‡</td>
<td>Switzerland</td>
<td>6.2-8.8‡</td>
</tr>
<tr>
<td>Denmark</td>
<td>8.4 (0.2)</td>
<td>UK</td>
<td></td>
</tr>
<tr>
<td>Estonia</td>
<td>1.6 (0.2)</td>
<td>England and</td>
<td>3.7 (0.1)</td>
</tr>
<tr>
<td>Finland</td>
<td>1.8 (0.1)</td>
<td>Scotland</td>
<td>5.0 (0.2)</td>
</tr>
<tr>
<td>Hong Kong</td>
<td>1.1 (0.1)</td>
<td>US</td>
<td></td>
</tr>
<tr>
<td>Japan</td>
<td>0.8-1.9‡</td>
<td>- white</td>
<td>4.9 (0.1)</td>
</tr>
<tr>
<td>India</td>
<td>0.6-1.0‡</td>
<td>- black</td>
<td>0.7 (0.1)</td>
</tr>
</tbody>
</table>

Adapted from: Cancer incidence in five continents, vol. VI (Parkin, 1992)
† Age-adjusted to the World Standard Population (Waterhouse et al, 1962)
‡ Range of rates given when more than one geographic area was reported

The within and between variations among incidence rates suggests that there is a genetic component to this cancer; and that this component may be a strong determinant, at least in nonwhite populations. Migrant studies (i.e. incidence rates reported by place of birth) could help
elucidate this relationship. Owing to the rarity of this tumour, these studies would be difficult to conduct.

An analysis of incidence data from Northern European countries (Adami et al., 1994) shows that incidence rates vary even for countries in close proximity. Denmark, which is reported to have one of the highest incidence rates in the world, has a rate that is nearly three-fold higher than that of Finland. In general, East European countries have much lower rates than those of West European countries, although all countries report a steady increase in incidence over the past several decades.

5.6. **Summary of descriptive epidemiology**

The descriptive epidemiology of testicular germ cell cancer suggests several intriguing clues to its etiology:

* the early age at incidence suggests that in-utero exposures (Clemmesen, 1981), and/or exposures of early childhood, are probably important, while the increasing incidence, and the birth cohort effect, suggest that exposure opportunities have changed over time: exposures may have increased in intensity; new risk factors may have been introduced; or protective factors may have decreased over time;

* the age distribution of testicular germ cell cancer closely parallels endocrine activity in children and young adults suggesting that endocrine exposure may be important; and

* among predominantly white populations, the descriptive epidemiology suggests that environmental determinants are strong, and have changed over time.
CHAPTER 6
STUDIES OF RISK FACTORS FOR TESTICULAR CANCER

The descriptive epidemiology of testicular germ cell tumours has prompted a number of investigators to focus on endocrine factors operating in-utero, or at puberty. Characteristics of these studies are detailed in table-6.1. These studies will be reviewed and information on odds ratio estimates according to histologic type, while limited, will be presented.

6.1. Prenatal and perinatal risk factors
6.1.1. In-utero hormone exposure

Henderson et al. (1979) were the first to conduct a case-control study designed to look at risk factors operating in the prenatal and perinatal period. The study included cases of testicular cancer diagnosed between 1972 through 1974 in Los Angeles county, California. In a matched analysis (79 pairs), using neighborhood controls, a nonsignificant but elevated risk was reported for exposure to hormone treatment around the time of conception or during pregnancy. The prevalence of exposure among control mothers was one-percent. When asked to report complications of pregnancy, 11 (10.3%) case mothers, compared to 3 (2.6%) control mothers, reported excessive nausea. From the investigators' knowledge that undescended testicle was a risk factor for testicular cancer, and that exogenous hormone exposure appeared to elevate risk for undescended testicle, Henderson and his colleagues postulated a unifying hypothesis: a major risk factor for both undescended testicle, and testicular cancer, is a relative excess of certain hormones (estrogen, and perhaps progesterone) at the time of testicular differentiation during the first trimester of pregnancy.

This report prompted a number of studies to investigate further the role of exogenous hormones and risk of testicular cancer. Schottenfeld et al. (1980) reported a nonsignificant, but slightly elevated risk of cancer among sons exposed to DES and other hormones in-utero. Eligible subjects had to have been born between 1950 and 1965. In order to control for the potential confounding effect of social class, 'peer' controls from childhood were selected in addition to hospital controls. Results from both analyses were similar. The prevalence of exposure among 'peer' and hospital control groups was 2.1% and 2.5%, respectively, and
TABLE-6.1. Characteristics of studies of prenatal, perinatal and pubertal exposures and risk of testicular cancer.

<table>
<thead>
<tr>
<th>Study</th>
<th>Location</th>
<th>Case definition</th>
<th>Control definition</th>
<th>Source of data / analysis</th>
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</thead>
<tbody>
<tr>
<td>Henderson et al., 1979</td>
<td>Cancer Surveillance Program (Los Angeles county)</td>
<td>testicular cancer age 15 - 40 years diagnosed 1972-74 N=79</td>
<td>neighborhood individually matched on sex and age</td>
<td>questionnaires to subjects and mothers</td>
</tr>
<tr>
<td>Schottenfeld et al., 1980</td>
<td>Memorial Sloan-Kettering Cancer Center, New York</td>
<td>testicular cancer diagnosed 1965-77 white males born 1950-65 N=193</td>
<td>#1 hospitalized for Hodgkin's disease or non-Hodgkin's lymphoma N=171</td>
<td>questionnaires to subjects and mothers</td>
</tr>
<tr>
<td>Depue et al., 1983</td>
<td>Cancer Surveillance Program (Los Angeles)</td>
<td>germ cell testicular cancer diagnosed 1973-79</td>
<td>neighborhood individually matched on sex and age living mother</td>
<td>questionnaires to subjects and mothers</td>
</tr>
<tr>
<td>Brown et al., 1986</td>
<td>3 Washington, DC area hospitals</td>
<td>testicular cancer diagnosed 1976-81 age 18-42 years,</td>
<td>hospital controls stratum matched on age and hospital, N=213</td>
<td>questionnaires to subjects and telephone interviews of mothers</td>
</tr>
<tr>
<td>Moss et al., 1986</td>
<td>northern California (California Tumor Registry) and north western Nevada</td>
<td>germ cell testicular cancer diagnosed 1976-81 alive and age 17-40</td>
<td>'peer' controls individually matched on race and age</td>
<td>questionnaires to subjects and mothers</td>
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<td></td>
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<td>analysis by histologic</td>
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<td>Study</td>
<td>Location</td>
<td>Case definition</td>
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<tr>
<td>Gershman and Stolley, 1988</td>
<td>Connecticut Tumor Registry, Connecticut</td>
<td>testicular cancer diagnosed 1945-80</td>
<td>Connecticut birth records individually matched on subject age, race, maternal age and attending</td>
<td>questionnaires to subjects and mothers</td>
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<td></td>
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<td>Connecticut resident born 1945-72</td>
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<td>patients not diagnosed with testicular, genital or lung cancer #1 radiotherapy centers</td>
<td>questionnaires to subjects</td>
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<td>aged &gt;19 years</td>
<td>patients not diagnosed with testicular, genital or lung cancer #1 radiotherapy centers</td>
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<td>N=259</td>
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<tr>
<td>Prener et al., 1992</td>
<td>Eastern Denmark (cities of Copenhagen and Gentofte)</td>
<td>germ cell testicular cancer cases within cohort of</td>
<td>subjects within cohort matched on age N=366</td>
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<tr>
<td>UK Testicular Cancer</td>
<td>nine health regions within England and Wales</td>
<td>testicular germ cell cancer diagnosed 1984-87</td>
<td>age matched by general practitioner N=794</td>
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<td></td>
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<td>questionnaire administered by interviewer to subjects supplemented from general</td>
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<tr>
<td>Gallagher et al., 1995</td>
<td>British Columbia and Alberta, Canada</td>
<td>germ cell testicular cancer diagnosed 1980-85</td>
<td>randomly age matched within province N=996</td>
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<td>mailed questionnaire</td>
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<tr>
<td>Moller and Skakkebaek, 1996</td>
<td>Danish Cancer Registry</td>
<td>testicular cancer diagnosed 1986-88 age 16-74</td>
<td>population-based frequency matched on age N=720</td>
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<td>telephone interview of subjects self-administered mailed</td>
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</table>
5.8% among cases.

A small ‘pilot’ study (Loughlin et al., 1980) was conducted in a Boston area hospital and included subjects born between 1948 and 1955, when DES was most widely prescribed in the Boston area. Of twenty-two case mothers and twenty-eight control mothers interviewed, two (5%) case mothers and 0 (0%) control mothers reported exposure.

To specifically test the hypothesis of elevated risk due to exogenous hormone exposure, Henderson and his colleagues conducted a second study (Depue et al., 1983) of similar design to the previously published study (Henderson et al., 1979). However, eligible men, diagnosed between 1973 and 1979, had to be between the ages of 16 and 30 years (i.e. born between 1943 and 1963), diagnosed specifically with a germ cell tumour, and had to have a living mother willing to participate in the study. Included in the exposure category were 5 case mothers and 1 control mother who had had an estrogen-progestin preparation administered as a pregnancy test. (It is not clear from the published report whether some of the original subjects were included in the follow-up study as cases were ascertained from the same population, and during some of the same time period, as the first study). Matched analysis was performed on 108 pairs. Compared to mothers who reported no exogenous hormone exposure, a statistically significant elevated risk was found, with more case mothers (9.3%) than control mothers (1.9%) reporting drug use.

All subsequent case-control studies have failed to confirm an association between exogenous hormone use and testicular cancer (Brown et al., 1986; Gershman and Stolley, 1988; Moss et al., 1986). The prevalence of hormone exposure was higher in these studies than previous reports probably reflecting the fact that the 1940+ birth cohort was now entering a period of increased risk for testicular cancer. However, prevalence of exposure was similar among cases and controls. Matching on obstetrician (Gershman and Stolley, 1988), and on childhood friends (Moss et al., 1986), in two of these studies, may have resulted in over matching on maternal socio-economic status and obstetric care thus making it difficult to find a true association. In the third study (Brown et al., 1986), subjects with a history of undescended testicle were excluded from analysis. If undescended testicle is an intermediate condition in the causal pathway, leading from hormone exposure to cancer (as might be expected since these
hormones are hypothesized to increase risk of undescended testicle and testicular cancer) then excluding these subjects from the analysis might selectively lead to the exclusion of subjects with hormone exposure.

6.1.2. Conditions associated with pregnancy

Women who experience excessive nausea and vomiting during pregnancy are reported to have higher levels of circulating estradiol in their first trimester of pregnancy (Depue et al., 1987). Sons of women who experience nausea and vomiting might therefore be at increased risk of testicular cancer. An association between nausea, voluntarily reported as a complication of pregnancy, and testicular cancer was first reported by Henderson et al. (1979). The association may have been confounded by the fact that medication for nausea was taken by 12 of the 14 mothers. A modestly elevated risk (OR=1.4) was reported for nausea requiring medical treatment during the index pregnancy, but excess risk was only found among first born sons (OR=4.4) (Depue et al., 1983).

The results of two other case-control studies remain inconclusive. Compared to mothers who reported no nausea during pregnancy, a slight, but statistically nonsignificant, elevated risk was reported among mothers who took medication to treat nausea (Moss et al., 1986). In the same study, risk was nonsignificantly elevated for reported nausea (regardless of treatment) among first born sons. A second hospital based case-control study found a statistically nonsignificant elevated risk for nausea (Brown et al., 1986), but risk was not further elevated for women who took medication for this condition. No attempt was made in the latter study to explore the relationship between nausea and medication use among first born sons. Other conditions of pregnancy that have been investigated include bleeding and spotting, possible indicators of threatened miscarriage, and toxemia. Brown et al. (1986) reported a nonsignificant but slightly elevated risk for toxemia, but risk was not elevated for mothers who reported taking medication for this condition. In the same study, elevated risk was found for bleeding and spotting during the index pregnancy, but risk was not further elevated for mothers who took medication for this condition.
Risk due to pregnancy related nausea and threatened miscarriage may be due to an altered hormonal milieu in the mother. In the case of threatened miscarriage, it is not clear whether this condition indicates an excess or insufficiency of pregnancy hormones. A recently completed prospective study of women who experienced first trimester bleeding reported that, compared to women who did not experience bleeding, women who bled and who eventually spontaneously aborted, had lower mean estrogen levels than women whose pregnancy went to term (Azogui et al., 1996). Women with low second trimester serum estrogen also appear to be at increased risk for pregnancy loss (Santolaya-Forgas et al., 1996).

6.1.3. Maternal age

Data from a clinical study on pregnant women have shown that levels of circulating estrogen were lowest in women under 20 years of age (Panagiotopoulou et al., 1990). These data suggest that risk for testicular germ cell cancer may be associated with maternal age. Several case-control studies report little difference between cases and controls with respect to maternal age at subject’s birth (Henderson et al. 1979; Swerdlow et al., 1986). Swerdlow et al. (1986) reported that among first born sons, there was a positive association between maternal age and risk of testicular cancer, and that the gradient in risk was more pronounced for seminoma than nonseminoma.

6.1.4. Birth order and sibship size

The results of several clinical studies (Trichopoulos et al., 1980; Bernstein et al., 1986; Mussey et al., 1987; Panagiotopoulou et al., 1990) suggest that nulliparous women have higher concentrations of circulating estrogens than parous women. This would suggest that first birth sons are at increased risk of testicular germ cell cancer due to exposure to relatively higher endogenous hormone levels than subsequent born sons.

Depue et al. (1983) first reported a modestly elevated risk for testicular cancer among first born sons. Two other case-control studies, one conducted in England, the other in Denmark, confirm an elevated risk for first born sons, and also showed a gradient in risk: increasing risk for
testicular cancer with decreasing birth order and decreasing sibship size (Prener et al., 1992; Swerdlow et al., 1986). This relationship held when the number of live born children, or full term births, was used as the unit of measure (Swerdlow et al., 1986). Both investigators analyzed their data according to histologic subgroup, and reported that the gradient in risk was more pronounced for nonseminoma than seminoma. Two other studies report no association between birth order and risk of testicular cancer (Brown et al., 1986; Moss et al., 1986) though the use of ‘peer’ controls in the latter study may have resulted in overmatching on birth order.

6.1.5. Birth weight and gestational age

In the first case-control study to report on prenatal exposure, prematurity was reported twice as often among cases as controls (Henderson et al., 1979). In a subsequent study, Depue et al. (1983) reported that a birth weight of less than 6 lb. significantly increased risk. Another study reported no association between low birth weight and risk of cancer (Moss et al., 1986). Parturition in humans appears to be proceeded by a surge in pregnancy related estrogen (Mazor et al., 1994). In a prospective study of pregnant women (N=241), women (N=23) who delivered preterm had higher estrogen levels, as measured in midtrimester salivary samples, than women whose pregnancy went to term (McGregor et al., 1995). No measurements were taken in the first trimester of pregnancy.

Since prematurity and low birth weight are highly correlated, Brown et al. (1986) classified cases and controls according to prematurity (gestational age of 7 or 8 months) and low birth weight (weight \( \leq 5 \) lb.). Compared to births of normal weight and normal gestational age, risk was elevated for low birth weight regardless of gestational age. Risk was not elevated for premature births in the absence of low birth weight. Moss reported that gestational age of 10 months doubled risk for testicular cancer (Moss et al., 1986).

6.1.6. Maternal prepregnancy weight and pregnancy weight gain

Obese women have been shown to have lower levels of sex hormone binding globulin (SHBG) (de Moor and Joosens, 1970; Dorgan et al, 1995) and, therefore, higher levels of
unbound estrogens. Further, there is evidence that maternal weight gain in pregnancy is weakly, but positively, associated with total circulating estrogens (Peridou et al., 1992). This suggests that maternal weight at, or weight gain during, pregnancy may be associated with risk of cancer.

A significantly positive gradient between prepregnancy weight (adjusted for height) and risk of testicular cancer was reported by Depue et al. (1983), although heavy body mass or obesity was not specifically examined. No other study has reported on prepregnancy weight and risk of cancer. Maternal weight gain during pregnancy has not been examined as a risk factor for testicular cancer.

6.1.7. Cigarette smoking and alcohol consumption

Data from clinical studies on pregnant women found that cigarette smoking during pregnancy reduced estrogen levels (Bernstein et al., 1989; Petridou et al., 1990), while alcohol consumption among nonpregnant premenopausal women elevated levels (Reichman et al., 1993). These data suggest that cigarette smoking during pregnancy may reduce risk, while alcohol consumption during pregnancy may elevate risk of testicular cancer.

Three case-control studies have reported that maternal cigarette smoking was not associated with risk of testicular cancer in the son (Henderson et al., 1979; Moller and Skakkebaek, 1996; Swerdlow et al., 1986). None quantified risk by duration or intensity of smoking. A third study (Brown et al., 1986) reported that compared to nonsmokers, mothers who smoked more than one pack per day had a statistically nonsignificant reduction in risk (OR=0.8, 95% CI 0.4-1.2) while those who smoked one or less packs per day had a statistically nonsignificant elevated risk (OR=1.5, 95% CI 0.8-2.9). The same study reported a greater than two-fold increased risk for mothers who consumed 2 or more alcoholic drinks per week compared to nondrinkers.

6.1.8. Underlying hormonal disorders in the mother

Two studies have looked at conditions which might indicate underlying hormonal disorders. Depue et al. (1983) reported that case mothers were less likely than control mothers
to report surgical treatment for menorrhagia: in most instances the treatment was after the birth of the index subject. Moss et al. (1986) reported an elevated risk for nonseminoma and mothers report of breast cancer.

To look at whether an estrogen disorder in the mother is responsible for an increased risk of testicular cancer in the son and increased risk of breast, endometrial and ovarian cancer in the mother, mothers of 2,204 Danish testicular cancer patients were followed for the occurrence of cancer (Kroman et al., 1996): risk of developing one of the three estrogen related female cancers was not elevated among these women.

6.2. Age at puberty

The incidence of testicular cancer increases rapidly in boys in their mid teens, suggesting that events around the time of puberty may be of importance. Since a birth cohort effect has been evident since the early part of this century, better nutrition leading to earlier puberty has been postulated as playing a role (Roush et al., 1987). Moss et al., (1986) were the first to report an association between age at puberty and risk of testicular germ cell cancer. Early puberty, defined as puberty before the age of 14 years, and measured by the age at which pubic hair first appeared, was reported to increase risk for nonseminoma in men under the age of 30 years (OR=2.4; p<0.01). Risk was not elevated for men with seminoma, or for men over the age of 30 years. Approximately 68% of all controls reported puberty before age 14. Swerdlow et al. (1989) reported reduced risk of testicular cancer among men who reported a late relative age at puberty as compared to classmates (OR= 0.59, 95% CI 0.35-0.98). However, compared to subjects who reported the same age at puberty as their classmates, early age at puberty did not elevate risk. Similar results were obtained in a Danish case-control study (Moller and Skakkebaek, 1996).

In a population-based case-control study conducted in England (UK Testicular Cancer Study Group, 1994), subjects were asked to report an actual age of events marking puberty (voice change, starting to shave, first nocturnal emission and first masturbation to orgasm). An inverse gradient was reported: decreased risk for testicular germ cell tumours with increasing age of event. Compared to men whose voice change occurred <13 years of age, those who recorded
an age of 16+ years were at significantly less risk (OR=0.45, 95% CI 0.25-0.80). Controls in this study were individually matched to cases by age and general practitioner. Gallagher et al. (1995) in Canada reported no association between age at voice breaking and age at shaving and cancer risk.

6.3. **Miscellaneous exposures**

Other risk factors that should be considered as potential confounders in an analysis of prenatal and pubertal exposures include genetic factors, undescended testicle and socio-economic status of the mothers.

6.3.1. **Genetic factors**

There are anecdotal reports of testicular germ cell cancer occurring among family members (Shreyaskumar et al, 1990; Goss and Bulbul, 1990; Heindal et al., 1996). Based on cases registered in the UK Registry for Familial Testicular Cancer, it is estimated that approximately 1.5% of all testicular germ cell cancer cases have a first degree relative diagnosed with testicular germ cell cancer (UK Testicular Cancer Study Group, 1992). These familial cases tended to be diagnosed at younger ages than nonfamilial cases. A large population-based cancer incidence study, using data in the Danish Cancer Registry, reported a statistically significantly elevated familial testicular cancer risk, which was more pronounced among brothers than between fathers and sons (Westergaard et al., 1996). Using data from the population-based Swedish Twins Registry, Braun et al., (1995) reported excess risk among dizygotic twins compared to monozygotic twins or the general population.

While these findings support a genetic component in the etiology of testicular cancer, they also could be interpreted as supporting the hypothesis that in-utero exposures to maternal hormones may be a factor: brothers share a similar prenatal environment, and maternal hormone levels are thought to be higher among multiple compared to singleton births, and higher yet among dizygotic twin pregnancies compared to monozygotic twin pregnancies.
6.3.2. Undescended testicle

Several anomalies of the genito-urinary tract have been associated with increased risk of developing testicular cancer, including undescended testicle (Batata et al., 1982; Giwercman et al., 1987; Morrison, 1976; Prener et al., 1996; Pinczowski et al., 1991); inguinal hernia (Morrison, 1976; Pinczowski et al., 1991; Prener et al., 1996) and possibly testicular torsion (Chilvers et al., 1987). Of particular interest is undescended testicle because of its strong and consistent association with cancer risk. Morrison (1976) was the first to report elevated risk (OR=8.8, 95% CI 2.3-56.3) for testicular cancer among army recruits born with an undescended testicle. Since then, numerous other case-control studies have confirmed this association (Depue et al., 1983; Gallagher et al., 1995; Henderson et al., 1979; Moss et al., 1986; Prener et al., 1996; Strader et al., 1988; Swerdlow et al., 1987; UK Testicular Cancer Study Group, 1994) with 1-3% prevalence of cryptorchidism reported among controls and 9-12% among cases.

Strader et al. (1988) examined risk by laterality of cancer using polychotomous logistic regression. While risk was significantly elevated (OR=8.0, 95% CI 4.2-15.3) for cancer occurring on the same side (ipsilateral) as the undescended testicle, there was a nonsignificant but elevated risk (OR = 1.6; 95% CI 0.6-4.1) of cancer occurring in the contralateral testicle. This finding is consistent with others, but not all, who report that risk appears to be preferentially, but not exclusively, higher in the involved testicle (Henderson et al., 1979; Pike et al., 1986; Pottern et al., 1985; Prener et al., 1996). Such a finding supports the view that there is an underlying condition that gives rise to both undescended testicle and testicular cancer.

On the other hand, an undescended testicle presents a unique micro-environment where heat (Swerdlow et al., 1988) or altered hormonal regulation (Giwercman et al., 1987) may play a role in increasing risk. If the micro-environment is the responsible factor then restoration of the testicle to a normal position outside the body should result in a reduction in risk. Supporting this view are several studies that have shown that risk of testicular cancer decreases with decreasing age of orchiopexy (surgical correction) (Pottern et al., 1985; UK Testicular Cancer Study Group, 1994): orchiopexy performed before 10 years of age eliminated excess risk of testicular cancer (UK Testicular Cancer Study Group, 1994). However, not all studies report decreased risk with
early age at correction (Pike et al., 1986; Prener et al., 1996).

6.3.3. Socio-economic status

Several case-control studies (Graham and Gibson, 1972; Depue et al., 1983; McDowall and Balarajan, 1986; Swerdlow et al., 1991) have reported increased risk of testicular cancer among men of high socio-economic status. Several studies have reported on the relationship between parental socio-economic status and risk of testicular cancer in the son (Kardaum et al., 1991; Swerdlow et al., 1991; Prener et al., 1992; Moller and Skakkebaek, 1996). A case-control study, conducted in England to investigate the relationship between cancer risk and lifetime history of socio-economic status, as measured by father’s occupation at birth and during childhood, and subject’s occupation at diagnosis (Swerdlow et al., 1991), reported a positive gradient between increasing socio-economic status and risk of testicular cancer in all three time periods, but the strongest association was reported with subject’s occupation at diagnosis. Two case-control studies conducted in Denmark reported a statistically nonsignificantly positive association between social class, as measured by parental occupation in childhood (as listed on birth certificates and school records) (Prener et al., 1992) or highest maternal education level (Moller and Skakkebaek, 1996) and risk of testicular cancer in the son. A case-control study conducted in the US found an association between broad occupational groups of parents and risk of cancer in the son (Kardaum et al., 1991).

6.4. Summary

Several issues arising from the literature need to be investigated:

Exogenous hormones exposure: DES was prescribed in Canada from 1944 to 1971. Men with potential for exposure entered a period of risk (age 20 through 40) beginning in the mid 1960’s, and will remain at risk throughout this century. Several studies restricted eligibility to cases born within the relevant exposure period (Loughlin et al, 1980; Gershman and Stolley, 1986; Moss et al., 1986), but their results remain equivocal, in part because the cohort of men with potential for DES exposure only began to enter a period of maximum cancer risk in the mid to late 1980s
(either at or after these studies were conducted). The present study should add to knowledge regarding risk from DES exposure because this study included a greater number of cohort members who were at, or had past, the age of maximum cancer risk. In addition, this study also has the advantage of including other forms of exogenous hormone exposure, namely birth control pills, which were not included in earlier studies.

**Endogenous hormone exposure:** Issues not fully explored regarding the role of maternal endogenous estrogens and testicular cancer include: cigarette smoking and alcohol use; maternal age; maternal weight and pregnancy weight gain; and underlying hormonal differences. In the absence of strong associations, consistency with other studies and the presence of a dose response relationship may provide some insight into the role these risk factors play in the etiology of testicular cancer.

**Age at puberty:** The relationship between puberty and testicular cancer is intriguing. Age at incidence patterns for testicular cancer appear to closely follow endocrine activity, and measures of fertility, both in young boys and young and middle aged men. Since incidence rates start to rise around the time of puberty, it follows that an early onset of puberty might be expected to increase cancer risk: the germ cell would be exposed to testosterone earlier and perhaps for longer periods of time. In the absence of a clearly defining event in a boy to mark puberty, the validity of subject responses and the use of surrogate measures become a concern. Validity of responses may be examined by assessing the effect of mother-subject agreement on odds ratio estimates.

**Histologic subgroup analysis:** The descriptive epidemiology suggests that putative exposure opportunities are increasing the risk of both seminoma and nonseminoma. Issues not fully explored include whether seminoma and nonseminoma have different risk factors. Tumours of mixed histologies containing seminoma should be excluded because it can not be known to which histologic group they should grouped.
CHAPTER 7
MATERIALS, METHODS AND METHODOLOGICAL ISSUES

7.1. Source of data

Data used for this dissertation come from a population-based case-control study of malignant germ cell tumours. The focus of this study was to identify factors that place an individual at increased risk of developing a malignant germ cell tumour. These tumours occur primarily in the testes in males, and the ovaries in females, but can occur at extra gonadal sites in both males and females. Since the primary focus of this dissertation was on endocrine factors and risk of testicular germ cell cancer, mothers' questionnaire data was the primary source of exposure information. Methods used to collect these data are presented below.

7.2. Case ascertainment

Eligible for study were all histologically confirmed cases of primary malignant germ cell cancers of the testes (ICD-9 site 186 and ICDO-M histology code 906-910: World Health Organization 1976, 1977) diagnosed in Ontario residents between 16 and 59 years of age, and diagnosed January 1, 1987 through December 31, 1989. Cases were identified from pathology reports received by the Ontario Cancer Registry. Consent forms for eligible cases were sent to a physician named on the pathology report. The consent form asked for permission and the necessary information (address and telephone number) for sending a questionnaire to the case.

7.3. Control ascertainment

Controls comprised a population-based random sample of men between the ages of 16 and 59 years and resident in Ontario and were identified from the Enumeration Composite Records of the Ontario Ministry of Revenue. These records are property tax assessment rolls that list all members of a household (owners, tenants and occupants) including children. As mandated by provincial law, these records are partially updated the latter part of each year, and completely updated every 3 years by means of a provincial census. This method of control selection was chosen because these records comprised a representative list of the general population of Ontario and because the file contained age.
Controls were selected to have the same age distribution as the cases, based on recent Ontario incidence data, and were selected in November, 1988, following a provincial census. Because the Ministry of Revenue considered age to be confidential information, their staff sampled controls (within five-year age groups and according to specified frequencies), and provided information on the age group used to select the control as well as the necessary information for contacting the subject by mail. A pseudo date of diagnosis was calculated for all participating controls based on subject's birth date (information provided on the subject's questionnaire) and his age as of the mid-point of the year in which his record was selected (July 1, 1988).

7.4. Data collection

Data were collected through the use of a self-administered questionnaire mailed to all subjects. This method of data collection was deemed the least costly in a study covering a large geographic area such as Ontario. Subjects were sent an introductory letter and asked to complete an accompanying self-administered questionnaire and return the questionnaire by means of a self-addressed business reply envelope. All subjects whose completed questionnaire was not returned within two weeks of mailing, were contacted by telephone, or sent a postcard reminder. If there was no response, a questionnaire and reminder letter were sent as a third and final invitation to participate in the study. Questionnaires not returned were considered 'lost to follow-up'. Questionnaires returned as 'undeliverable' were excluded from response rate calculations.

Subjects were requested to give permission, and to provide the necessary information, for sending their mother a questionnaire. In order to expedite data collection, and to improve data quality and response rates, a questionnaire was mailed to the mother and the mother was interviewed by telephone.

7.4.1. Pretesting of questionnaires

Pretesting the questionnaire(s) was used to assess the clarity of the question wording,
the efficiency of the layout of the questionnaire, the effectiveness of the questionnaire to elicit a full range of responses, and the adequacy of the responses. Questionnaires were pretested on three groups of people: colleagues; patients seen at a hospital outpatient clinic; and a random sample of Toronto residents. Of particular note, subjects reported difficulty in recalling an exact age at events marking puberty (appearance of pubic hair, starting to shave, growth spurt and voice change). As a result, this question was reworded to include questions of time (age) of pubertal events relative to that of their peers (later than, earlier than, same time as).

7.4.2. Questionnaire content

Sample copies of the mother’s questionnaire and selected questions from the subject’s questionnaire are attached (Appendix 1 and 2). Information obtained in the subjects’ questionnaire covered medical history, including diagnosis and treatment (hormonal or surgical) for undescended testicle, and relative time (age) at events marking puberty (appearance of pubic hair, starting to shave, growth spurt and voice change). Mothers provided information on their level of education, medical and menstrual histories, and detailed information on the index and all other, pregnancies. Information was obtained on maternal age; prepregnancy weight and pregnancy weight gain; birth control pill use; in-utero exposures to medication, alcohol and cigarettes; pregnancy-related toxemia, nausea and vomiting, and bleeding and threatened miscarriage; and perinatal factors, including birth outcome (vital status), birth weight, length of pregnancy (relative to expected date of delivery), type of delivery, birth defects and length (months) of breast feeding. Mothers also provided information on relative time (age) at events marking puberty for their sons.

7.5. Response rates

Of 621 eligible cases, 502 (80.8%) subjects participated in the study by returning a completed questionnaire. Reasons for nonparticipation by the cases included 13 physician refusals, 67 lost to follow-up, 25 case refusals and 14 deaths. One-thousand, four-hundred and
thirty-eight controls were sent questionnaires; 975 (67.8%) control subjects participated in the study by returning a completed questionnaire. Reasons for nonparticipation by controls included 117 refusals and 346 lost to follow-up.

Since permission to contact the mothers was obtained from subjects before study personnel could contact the mother directly, response rates among mothers could not be accurately determined. However, it was possible to calculate a participation rate based on the number of subjects and mothers participating in the study. Among 502 case participants, 346 (68.9%) case mothers participated. Among 975 control participants, 522 (53.5%) control mothers participated. When subjects provided a reason for not granting consent to contact his mother, the most frequently cited reason was 'mother deceased'. Among subjects who had a living mother, consent rates to contact mothers were higher among cases (82.4%) than controls (73.6%). And among mothers who were contacted, response rates among case mothers were higher (96.2%) than control mothers (88.6%).

7.6. Validation of exposure information

This study relied on questionnaire data for the reporting of relevant exposure information during a woman's reproductive years, and included her pregnancy history, past behavior (cigarette and alcohol consumption), and prescription medication use. These data were collected from women who ranged in age from 30 years to 89 years (mean age 57 years) at time of interview and who were asked to report on events that occurred 15 to 54 years ago (mean 30 years). Recall capacity among these mothers, particularly the older mothers in the study, and the reliability of questionnaire data to assess such information, was a concern.

In general, older respondents are able to give reproducible answers to a wide range of exposures (Cumming and Klineberg, 1994; Ridley et al., 1979), although the accuracy and reliability of self-reported data may vary according to the saliency and nature of the event. In particular, older respondents appear to have difficulty recalling accurately a chronological age of an event (Herzog and Dielman, 1985). Harlow and Linet (1989) reviewed 6 studies which assessed agreement between questionnaire data regarding reproductive histories and medical and
obstetric records. They concluded that birth weight and pregnancy history, including the number and outcome of pregnancies, were recalled with good accuracy, while length of menstrual periods, gestational age and unfavorable events occurring during pregnancy, such as threatened miscarriage and hospitalization, were recalled less accurately.

The experience of two previously conducted case-control studies of testicular cancer (Depue et al., 1983; Schottenfeld et al., 1980) suggested that validation of medication use (particularly hormone exposure) by means of medical records was not feasible (physicians, hospitals and pharmacies do not routinely keep records on subjects whose medical use occurred years in the past). This is particularly unfortunate since a large number of US women who were identified through their obstetric records as having been given DES while pregnant, either could not remember, or were unaware of, their exposure to hormones while pregnant (Tilley et al., 1985).

To summarize, the accuracy of self-reported reproductive history data will vary according to risk factor, and estimates of hormone exposure may be underestimates of true exposure prevalence. Further compounding problems of measuring hormone exposure is the use of surrogate measures, such as birth order, maternal age, pregnancy-related nausea, and smoking, to infer exposure since these measurements merely indicate the potential for exposure, and do not indicate the actual exposure of interest. Taken together, odds ratio estimates obtained for these exposures will be subject to an indeterminate amount of under reporting and misclassification.

7.7. Pathology review

Testicular germ cell cancer is routinely classified into two histologic groups, seminoma and nonseminoma, the latter group including tumours of mixed histology, even those containing seminoma. This classification scheme is routinely used by cancer registries for coding and reporting cancer data. Since the aim of this study was to categorize testicular cancer cases into three groups, seminoma, nonseminoma and tumours of mixed histology containing seminoma, the feasibility of using OCR pathology reports to classify cases was assessed as follows. Four-hundred and fifty-three incident cases of testicular germ cell cancer, diagnosed between 1982 and
1984, were identified, and pathology reports supporting the diagnoses was reviewed by a trained medical nosologist. The results of review were as follows: 237 (53.3%) seminoma; 196 (43.3%) nonseminoma; and 20 (4.4%) tumors of mixed histology. Because the percentage of mixed histology tumors was lower than that of a histologically reviewed series of incident cases (Brawn, 1982), the present study undertook pathology review of all eligible cases.

From information contained in the pathology report used to ascertain the case, the hospital pathology laboratory was identified and asked to provide tissue blocks supporting the diagnosis of testicular cancer. Pathology review was performed by Dr. Linda Sugar at the Princess Margaret Hospital in Toronto. Tissue review results were sent to the original pathology laboratory, and to the treating physician when requested. Pathology review was performed on 590 (95.0%) of 621 eligible cases and 481 (95.8%) of 502 participating cases.

While all laboratories sent material, some provided unstained slides in lieu of blocks, while others selected blocks for review. Therefore, it was not known if these blocks were representative of the tumour, which would be necessary for the recording of all specific histologic elements (seminoma, embryonal carcinoma, choriocarcinoma, teratoma and yolk sac tumour), and classifying tumours into histologic subgroups (seminoma, nonseminoma and tumours of mixed histology containing seminoma and nonseminoma). In fact, some blocks contained insufficient or no tumour. Therefore, it was decided to supplement the results of tissue review with information abstracted from the original pathology report. Dr. Hugh Richmond reviewed all relevant pathology information for this purpose.

7.8. Data analysis

Exposure categories were created based on the exposure distributions among control mothers or on relevant exposure categories reported in the literature.

7.8.1. Statistical analysis

Cases and controls were compared for all investigated exposures using unconditional logistic regression (Breslow and Day, 1980). Associations between testicular germ cell cancer
and known and unknown risk factors were described by means of maximum likelihood estimates of odds ratios and corresponding confidence intervals (CI). All odds ratio estimates were adjusted for subject’s age (5-year age groups) because age was used as a design variable during control selection.

In the first phase of data analysis, continuous variables were grouped with the objective of maintaining the full range of risk associated with the variable, and summary variables were created by combining variables. The likelihood ratio statistic was used to assess the contribution of individual and combined variables in a model and was calculated based on the reduction in residual deviance after the addition of the variable to a prior fit model. Two-way interaction was assessed by fitting a hierarchical model containing the two variables of interest, and then extending the model to include the cross product term. Potential confounding was identified by examining the confounder’s relationship to disease status, and exposure status in the control group. Confounding was assessed by comparing the age-adjusted odds ratio estimate with the odds ratio estimate adjusted for age and the potential confounder (Kleinbaum et al., 1982). Confounding was considered to be present if the age and confounder adjusted odds ratio estimate differed more than +/-15% from the age-adjusted odds ratio estimate. Statistical analysis was performed using SAS (1990) and EGRET (Statistics and Epidemiology Research Corporation, 1993).

7.8.2. Model building strategies

Variables selected for inclusion in the model-building phase of the analysis included known risk factors (age and undescended testicle), suspected risk factors (p<0.50), and potential confounders (as identified in the literature). Multivariate modeling, using backward elimination, was performed by fitting a full model (all variables included), and then assessing the contribution of each individual risk factor separately, while simultaneously adjusting for all other specified variables. Variables were sequentially eliminated until the final model contained only those variables with an adjusted odds ratio estimate > 2.0 and/or a p-value ≤ 0.10. Confounders were retained in the final model as well. The goodness-of-fit of the model was assessed using
Chapter 7

the Hosmer-Lemeshow test (Hosmer and Lemeshow, 1989).

7.8.3. Combining exposure information

Since the usual effect of nondifferential measurement error is to attenuate odds ratio estimates (Schlesselman, 1982), the true effect of an exposure can be obscured if the exposure is measured in such a way that nondifferential error is introduced. Measuring age at puberty was particularly problematic in this study because surrogates of exposure (appearance of hair, starting to shave, growth spurt and voice change) were used to assess the true exposure of interest (initiation of puberty), and because subjects were asked to report a relative age at puberty (earlier than, later than, same time as their peers) rather than a chronological age. In fact, the majority of subjects and mothers reported 'same time as' to all four questions regarding relative age of pubertal events. When 'earlier' or 'later' was reported, the overall level of agreement, as measured by the kappa statistic, was low (Appendix 3) suggesting agreement between respondents was likely due to chance alone. Poor measurement of exposure may introduce an indeterminate amount of measurement error and make it more difficult to detect an true association between age at puberty and risk of testicular germ cell cancer.

In the absence of a more precise way to measure puberty, two analytic strategies were used to minimize measurement error. The first method involved creating a summary variable (puberty score) based on the four separate exposure variables for subject and mother responses separately. The second method involved creating a composite variable, for each of the four puberty variables, based on subject-mother paired responses. The latter strategy was adapted from a method suggested by Marshall (1989) in which dichotomous exposure reports are combined, based on agreement between reports, provided the reports came from independent reporting sources. In this analysis, agreement was assigned to subject and mother pairs who both reported the same relative age at puberty (in the subject) while disagreement was assigned to those where one reported 'same time as' while the other reported 'earlier than' or 'later than'. Subject and mother pairs, where one reported 'earlier than' and the other reported 'later than', were eliminated from the analysis. The odds of disease was then compared between subject and
mother pairs where there was agreement of exposure against those where there was agreement of no exposure. According to Marshall (1989), subject and mother pairs with disagreement should be of intermediate risk.

7.8.4. **Comparative analysis of risk factors by histologic type**

Testicular germ cell cancer is comprised of two major histologic subgroups, seminoma and nonseminoma. The different age at incidence distributions of these two histologic types (appendix-4) suggests the possibility that they have different risk factors. Dubin and Pasternack (1986) have proposed the use of polychotomous logistic regression as an analytical strategy for assessing whether risk factors differ according to case groups. The advantage of this method over that of constructing two separate dichotomous logistic regression models is that it allows for the simultaneous estimation of case-specific odds ratios and direct hypothesis testing between case groups.

Because the age distributions of the two histologic groups differed, and because it was proposed to use a single comparison groups (comprised on all controls) whose age distribution more closely resembled that of all cases combined, the concern was raised that problems might be encountered in modeling age as a risk factor. Age was assessed in three models: all controls and all cases; all controls and seminoma cases; and all controls and nonseminoma cases. Each model was fit with age as a categorical variable (5 year age group) and then extended to include age as a continuous variable. Age as a continuous variable did not statistically significantly improve the fit in either of the three models. In addition, interaction between the two age variables was assessed by fitting each model with the two age variables and then extending the model to include their cross-product term. Interaction in all three models was not statistically significant. The results of these analyses suggested that age as a 5-year categorical variable adequately controlled for this variable in the comparative analysis.

Cases diagnosed with seminoma or nonseminoma were identified for analysis, while cases with mixed histologies (e.g. the tumour contained both seminoma and nonseminoma) were excluded from further analysis. To identify relevant risk factors, two multivariate logistic
regression models, one for seminoma and one for nonseminoma, were fit and risk factors were retained according to the criteria used to construct a multivariate model (see 7.8.2). Risk factors identified for either seminoma or nonseminoma were included in the comparative analysis. To assess whether these risk factors differed for seminoma and nonseminoma, polychotomous logistic regression was used to simultaneously calculate histologic specific log odds ratio estimates and standard errors using the CATMOD procedure in SAS (1990). All estimates were adjusted for subjects' age. The null hypothesis was tested that the difference between the two histologic specific odds ratio estimates was equal to zero.
CHAPTER 8

RESULTS

8.1. Introduction

The objectives of this dissertation comprise three separate but related issues of epidemiologic interest. Chapter 8 contains three manuscripts in publication format, addressing these objectives.

The first paper, titled "Prenatal and perinatal exposures and risk of testicular germ cell cancer", examines the association between germ cell cancer risk and specific conditions of pregnancy thought to indicate relative exposure to maternal hormones, and factors thought to influence pregnancy hormone serum levels.

In the second paper, titled "Age at puberty and risk of testicular cancer", cancer risk is examined according to the relative age at events marking puberty in the subject. Both mothers and subjects provided information on pubertal events, and their responses are combined to assess the effect of agreement on odds ratio estimates for age at puberty.

The third paper, titled "Comparison of risk factors by histologic subgroup of testicular germ cell cancer", examines whether risk factors differ according to the two major histologic subgroups of testicular germ cell cancer (seminoma and nonseminoma).
8.2. PRENATAL AND PERINATAL EXPOSURES AND RISK OF TESTICULAR GERM CELL CANCER

INTRODUCTION

The early age at incidence of testicular germ cell cancer suggests that childhood exposures, possibly occurring in-utero, may be important (Clemmesen, 1981). Exposure to maternal hormones, from exogenous and endogenous sources, may be such an exposure (Henderson et al., 1979). A number of case-control studies have attempted to interpret their results based on this mechanism (Brown et al., 1986; Depue et al., 1983; Gershman and Stolley, 1988; Moller and Shakkebaek, 1996; Moss et al., 1986; Prener et al., 1992; Schottenfeld et al., 1980; Swerdlow et al., 1987); however, these results are not entirely consistent, making it difficult to support the association. The present study was undertaken to test specific associations, and by extension, to include other pregnancy-related factors where there is clinical evidence supporting a mitigating influence on maternal hormone levels, and to examine how these exposures, and other prenatal and perinatal exposures, are associated with risk of testicular germ cell cancer.

MATERIAL AND METHODS

Subject ascertainment

A population-based case-control study was conducted in the province of Ontario, and included all histologically confirmed cases of primary malignant germ cell cancers of the testes (ICD-9 site 186 and ICDO-M histology code 906-910: WHO 1976; WHO 1977) in Ontario residents between 16 and 59 years of age, and diagnosed January 1, 1987 through December 31, 1989. Cases were identified from pathology reports received by the Ontario Cancer Registry. Consent forms for eligible cases were sent to a physician named on the pathology report. The consent form asked for permission and the necessary information (address and telephone number) for sending a questionnaire to the case.

Controls comprised a population-based random sample of men between the ages of 16
and 59 years and resident in Ontario. The sample was drawn from the Ontario Ministry of Revenue’s Enumeration Composite Records, which are property tax assessment rolls that list all members of a household, and included age. Controls were selected to have the same age distribution as cases (within 5-year age groups), based on recent Ontario incidence data.

**Subject response**

Of 621 eligible cases, 502 (80.8%) subjects participated in the study by returning a completed questionnaire. Reasons for nonparticipation by the cases included 13 physician refusals, 67 lost to follow-up, 25 case refusals and 14 deaths. One-thousand, four-hundred and thirty-eight controls were sent questionnaires; 975 control subjects (67.8%) participated in the study by returning a completed questionnaire. Reasons for nonparticipation by controls included 117 refusals; and 346 lost to follow-up.

**Data collection**

Data were collected through the use of a self-administered questionnaire mailed to all subjects. An explanatory letter detailing the purpose of the study, and including a telephone number for purposes of study validation, was enclosed with the questionnaire. Subjects were requested to give permission, and to provide the necessary information, for sending their mother a questionnaire. For expediency of data collection, and to improve response rates, mothers were interviewed by telephone.

Subjects provided information on socio-demographic factors and occupational and medical histories, including history and treatment for undescended testicle. Mothers provided information on their level of education, medical and menstrual histories, and detailed information on all pregnancies. Information was obtained on maternal age; prepregnancy weight and pregnancy weight gain; birth control pill use; in-utero exposures to medication, alcohol and cigarettes; pregnancy related toxemia, nausea and vomiting, and bleeding and threatened miscarriage; and perinatal factors, including birth outcome (vital status), birth weight, length of pregnancy, type of delivery, birth defects and duration (months) of breast feeding. Three-
hundred and forty-six case mothers and 522 control mothers participated in the study. Mothers’ data form the focus of this paper.

Several previously conducted case-control studies attempted to validate maternal hormone exposure through medical records (Depue et al., 1986; Schottenfeld et al., 1980) but were unsuccessful as physicians, hospitals and pharmacies do not routinely keep records on subjects whose medical use occurred many years in the past. For this reason, the present study did not attempt to validate medication use.

Data analysis

Cases and controls were compared for all investigated exposures using unconditional logistic regression (Breslow and Day, 1980). Associations between testicular germ cell cancer and known and unknown risk factors were described by means of maximum likelihood estimates of odds ratios and corresponding confidence intervals (CI). All odds ratio estimates were adjusted for subject’s age (5-year age groups) because age was used as a design variable during control selection.

In the first phase of data analysis, continuous variables were grouped with the objective of maintaining the full range of risk associated with the variable, and summary variables were created by combining variables. The likelihood ratio statistic was used to assess the contribution of individual and combined variables in a model and was calculated based on the reduction in residual deviance after the addition of the variable to a prior fit model. Two-way interaction was assessed by fitting a hierarchical model containing the two variables of interest, and then extending the model to include the cross product term. Potential confounding was identified by examining the confounder’s relationship to disease status, and exposure status in the control group. Confounding was assessed by comparing the age-adjusted odds ratio estimate with the odds ratio estimate adjusted for age and the potential confounder (Kleinbaum et al., 1982). Confounding was considered to be present if the age and confounder adjusted odds ratio estimate differed more than +/-15% from the age-adjusted odds ratio estimate. Statistical analysis was performed using SAS (1990) and EGRET (Statistics and Epidemiology Research
Variables selected for inclusion in the model-building phase of the analysis included known risk factors (age and undescended testicle), suspected risk factors (p<0.50), and potential confounders (as identified in the literature). Multivariate modeling, using backward elimination, was performed by fitting a full model (all variables included), and then assessing the contribution of each individual risk factor separately, while simultaneously adjusting for all other specified variables. Variables were sequentially eliminated until the final model contained only those variables with an adjusted odds ratio estimate > 2.0 and/or a p-value ≤ 0.10. Confounders were retained in the final model as well. The goodness-of-fit of the model was assessed using the Hosmer-Lemeshow test (Hosmer and Lemeshow, 1989).

Exposure categories were created based on exposure distributions among control mothers or on relevant exposure categories reported in the literature. Unconditional multiple logistic regression modeling was used to calculate age-adjusted odds ratio estimates (AOR), ninety-five percent confidence intervals (95% CI) and likelihood ratio statistics and accompanying probabilities (p-value) using EGRET (Statistics and Epidemiology Research Corporation, 1993). All odds ratio estimates were adjusted for subject age by 5-year age groups. Multivariate modeling was performed using backward elimination retaining factors with p≤0.10.

Exogenous hormone exposure was determined from use of prescription hormones (e.g. diethylstilbestrol (DES) or premarin); prescription medication for conditions associated with threatened miscarriage; injections or pills to determine pregnancy; and use of birth control pills at the time of conception. Because reported exposures to exogenous hormones were uncommon, these exposures were combined into a dichotomous exogenous hormone exposure variable, coded ‘yes’ if the mother reported exposure to any of the sources of exogenous hormones, and ‘no’ if she reported no exposure to all sources of exogenous hormones.

Maternal prepregnancy body weight and pregnancy weight gain were adjusted for maternal height by using weight/height$^2$ as the weight variable. Congenital anomalies and maternal cancer diagnoses were coded to ICD-9 (World Health Organization, 1977). There is
difficulty in distinguishing at birth between an undescended testicle that will require treatment, and an undescended testicle that will spontaneously descend within the first year of life or remain retractile during childhood. To distinguish between these conditions, undescended testicle was defined to have occurred among subjects who reported surgical or hormonal treatment for the condition. All other subjects, even those who reported an undescended testicle but who did not report treatment, were considered unexposed.

RESULTS

There was no statistically significant difference in the mean age of cases (30.6 yr.) and controls (30.1 yr.). Mothers ranged in age between 30 and 89 years (mean 57 years). There was no statistically significant difference in the proportion of case mothers (26.1%) and control mothers (27.8%) who completed some or all post-secondary education.

As shown in table-1, more case mothers (4.7%) than control mothers (1.4%) reported exposure to exogenous hormones (p<0.01) (AOR=4.58, 95% CI 1.79-11.72). Among case mothers, 6 (1.8%) reported taking prescription hormones (5 during the first three months of pregnancy) compared to 1 (0.2%) control mother (AOR=7.33, 95% CI 0.85-63.25); 4 (1.2%) case mothers reported being given pills or injections to determine pregnancy compared to 2 (0.4%) control mothers (AOR=4.57, 95% CI 0.47-44.46); 3 (0.9%) case mothers continued taking birth control pills after becoming pregnant compared to 0 control mothers (AOR=undefined); and 4 (1.2%) case mothers and 4 (0.8%) control mothers took prescription medication for threatened miscarriage (AOR=1.05, 95% CI 0.50-2.20). Among mothers, 1.8% of case mothers and 1.3% of control mothers could not or did not report prescription hormone exposure. Similar results were obtained for other sources of exogenous hormones exposure and for the summary exogenous hormones exposure variable.

Selected pregnancy-related conditions are also shown in table-1. There was no statistically significant difference among case and control mothers with respect to reported vomiting during pregnancy (p=0.36), nausea and vomiting requiring physician treatment.
Chapter 8

(p=0.62), toxemia (p=0.28), or bleeding and threatened miscarriage (p=0.26). These odds ratio estimates did not change when the analysis was restricted to first born children. The protective effect of bleeding and threatened miscarriage became more pronounced (but not statistically significant) following adjustment for prescription hormone or unspecified medication for threatened miscarriage use, and for bleeding and threatened miscarriages that occurred within the first four months of pregnancy.

Prepregnancy weight, pregnancy weight gain, and maternal cigarette smoking and alcohol consumption are shown in table-2. There was no difference in the distribution of prepregnancy weight (p=0.44) and weight gain (p=0.46) between case and control mothers. More control mothers (15.6%) than case mothers (9.4%) reported smoking 12 or more cigarettes per day (p=0.02). Among smokers, the addition of duration (smoked entire pregnancy versus smoked less than 9 months) to a model containing number of cigarettes smoked per day did not significantly improve the model fit (p=0.51). There was no difference in the distribution of alcohol consumption between case and control mothers (p=0.45). Among drinkers, the addition of duration (consumed alcohol entire pregnancy versus consumed alcohol less than nine months) to a model containing number of drinks consumed per week did not significantly improve the model fit (p=0.47). Smoking and drinking were statistically significantly associated among case (p<0.01) and control mothers (p<0.01). There was no evidence of interaction between the two variables.

Table-3 shows selected menstrual and medical conditions among mothers. There was no difference between case and control mothers with respect to physician treatment for menstrual problems prior to the index pregnancy (p=0.72), prior pregnancy loss (p=0.86), birth defects reported in prior pregnancies (p=0.36), regularity of menstrual cycles reported during their 20’s and 30’s (p=0.59), or diagnosis of cancer in the mother (p=0.50), which included cancer preceding or following the birth of the index subject. Compared to women who never used birth control pills prior to the index pregnancy or who discontinued use more than three months prior to pregnancy, discontinuing birth control pill use within 3 months prior to becoming pregnant
was associated with a statistically nonsignificant (p=0.19) elevated risk.

Table-4 shows selected prenatal and perinatal factors. More cases (12.7%) than controls (8.7%) were born preterm, defined as births occurring more than two weeks earlier than expected. There was no difference in the distribution of birth weights (p=0.54), type of delivery (p=0.80), number of births (single vs. multiple) (p=0.58) or birth order (first vs. subsequent) (p=0.76) between cases and controls. Among subjects with a mother participating in the study, thirty (8.8%) cases and 6 (1.1%) controls reported treatment for undescended testicle (p< 0.01). More case mothers (4.5%) than control mothers (1.2%) reported a congenital anomaly of the genital organ in the subject. However, there was no difference in the proportion of nongenital birth defects reported among cases (2.3%) and controls (2.1%).

The distribution of mother's age at subject's birth differed (p=0.03) according to case status: fewer case mothers (7.4%) than control mothers (12.7%) were less than 20 years of age. Because of the obvious correlation between birth order and maternal age, and because of the estimated profiles associated with birth order and maternal age being different, interaction between birth order and maternal age was assessed as follows: maternal age and birth order were included in a model containing subject age and the model was extended to include the cross-product term. Interaction was of borderline statistical significance (p=0.07). Among young mothers (<20 years) and mothers between the ages of 20 and 24 years, first births were associated with statistically nonsignificant elevated risk (AOR=1.54, 95% CI 0.91-2.61 and AOR=1.75, 95% CI 0.48-6.35, respectively). Among older mothers (25+ years), first births were associated with a statistically nonsignificant reduced risk (AOR=0.66, 95% CI 0.39-1.11).

Slightly more control mothers (52.5%) than case mothers (48.2%) breast fed the subject. Among breast-fed subjects, the addition of duration (<4 months vs. 4+ months) did not improve the model fit (p=0.78).

Results of multivariate modeling, including frequencies, adjusted odds ratio estimates (AOR) and 90% confidence intervals (90% CI) are shown in table-5. Compared to nonsmoking mothers, there was decreased risk (AOR=0.62, 90% CI 0.43-0.92) among mothers who smoked 12 or more cigarettes per day. Compared to mothers who did not report exposure to any form
of exogenous hormones during pregnancy, exogenous hormone use during pregnancy elevated risk for testicular germ cell cancer (AOR=4.28, 90% CI 1.71-10.57). Compared to mothers who did not use birth control pills, or who stopped use more than 3 months prior to conception, birth control pill cessation within 3 months of conception elevated risk for testicular germ cell cancer (AOR= 2.16, 90% CI 1.00-4.64 ). Undescended testicle elevated risk for testicular germ cell cancer (AOR=7.88, 90% CI 3.65-17.04) as did preterm birth (AOR= 1.63, 90% CI 1.08-2.46 ). Following adjustment for the confounding effect of exogenous hormone use during pregnancy, a statistically significant protective effect was found for bleeding and threatened miscarriage (AOR=0.55, 90% CI 0.33-0.92). Young maternal age (< 20 years) was protective (AOR=0.54, 90% CI 0.34-0.87). Similar results were obtained (data not shown) when subjects treated for an undescended testicle were excluded from the analysis.

**DISCUSSION**

Administration of exogenous hormones to pregnant women is associated with adverse effects on the male reproductive tract, including undescended testicle (Stillman, 1982). Given this association, and the strong association reported between undescended testicle and testicular cancer (Morrison, 1978; Mostofi, 1973), Henderson et al. (1979) proposed the hypothesis that exposure to excess maternal hormones (particularly estrogen) early in pregnancy elevated risk for testicular cancer. Based on the observation that more case mothers than control mothers reported nausea as a complication of pregnancy (nausea is thought to be an indicator of rapidly rising, and possibly excessive, hormone levels in early pregnancy), their hypothesis included endogenous sources of hormones as well. A number of etiologic investigations of testicular cancer have extended this hypothesis to include other sources of exogenous hormone (e.g. pregnancy tests (Depue et al., 1983)); other indicators of relative endogenous hormone levels (e.g. threatened miscarriage (Schottenfeld et al., 1980)); factors that might influence endogenous hormone levels (e.g. birth order and maternal age (Prener et al., 1994; Swerdlow et al., 1986) and maternal weight (Depue et al., 1983); and underlying menstrual differences between case and control mothers (e.g. menstrual problems (Depue et al., 1983)).
Exogenous hormone exposure

Several early case-control studies reported that exposure to exogenous hormones, in the form of hormone use for threatened miscarriage and pregnancy tests, elevated risk for testicular cancer (Depue et al., 1983; Henderson et al., 1979; Loughlin et al., 1980; Schottenfeld et al., 1980). More recently conducted studies, however, have failed to confirm the association, although matching and exclusion criteria used in these studies may account for attenuated risk. Two studies (Gershman and Stolley, 1988; Moss et al., 1986) may have overmatched on obstetric care by using controls drawn from obstetric records and childhood friends. The third study used age-matched, hospital cancer controls and excluded subjects with history of undescended testicle (Brown et al., 1986).

The present study reports a statistically significant elevated risk for testicular germ cell cancer among mothers who reported exogenous hormone use. This was due to exposure to DES, pills and injections to determine pregnancy and to birth control pill use at the time of, and immediately following, conception. Unspecified prescription medication taken for threatened miscarriage did not elevate risk.

Caution should be used in interpreting these results since these findings are based on very small number and this study did not include validation of exposure status. Because DES has been associated with adverse health effects in both male and female offspring (Herbst et al., 1971; Stillman, 1982), and physicians may selectively query testicular cancer patients about maternal hormone exposure, or case mothers may be more likely to recall details of medication use than control mothers. Problems with self-reported prescription hormone exposure have been noted in a follow-up study of DES exposed women (Tilley et al., 1985). Many women, whose obstetric records indicated exposure to DES, could not remember, or where unaware of their exposure to prescription hormones. Recall bias in retrospective studies may be more likely to occur where recall is poor (Coughlin, 1990). In the DES follow-up study, self-initiated enrollment and referrals were better able to recall details of their DES exposure than were women who were recruited for study (Tilley et al., 1985). Since participation rates among case
mothers were higher than control mothers, this also raises concerns about potential selection bias in the present study.

**Indicators of relative endogenous hormones levels**

Given the retrospective nature of this study, it was not possible to include direct measurement of maternal hormone levels. However, several indicators of relative hormone levels were examined, including nausea and vomiting, as an indicator of excess levels of circulating maternal hormones (Depue et al., 1987) and bleeding and threatened miscarriage.

Four previous studies reported elevated risk for nausea (Brown et al., 1986; Depue et al., 1983; Henderson et al., 1979; Moss et al., 1986), particularly among first born sons. While the present study found no evidence of an association between vomiting and risk of testicular germ cell cancer, or between physician treatment for nausea and vomiting and cancer risk (even when the analysis was restricted to first born sons) the direction of odds ratio estimates is consistent with these previous reports. Several case-control studies have reported an excess of risk among mothers who reported bleeding and threatened miscarriage (Gershman and Stolley, 1988; Schottenfeld et al., 1980) even among mothers who did not report taking medication for the condition (Brown et al, 1986). Such observations, along with that of excess nausea, could be interpreted as supporting the hypothesis of 'modified hormonal milieu in the mother' (Depue et al., 1983); although, a priori, it is not clear in which direction risk due to bleeding and threatened miscarriage should operate: bleeding and threatened miscarriage may indicate a relative excess of endogenous hormones; or less than optimum levels thereby requiring treatment by exogenous hormones. The present study found a modest protective effect associated with bleeding and threatened miscarriage that became more pronounced following adjustment for exogenous hormone use. The protective effect was most pronounced for events that occurred in the first four months of pregnancy. Since women who have lower levels of pregnancy-related estrogen appear to be at increased risk of pregnancy loss (Azogui et al., 1996; Santolaya-Forgas et al., 1996), this may explain the protective effect of bleeding and threatened miscarriage, especially when it occurs in early pregnancy. The fact that these results differ from previous
reports may be explained by the differences in the gestational age at which the bleeding and threatened miscarriage took place, and the manner in which the confounding influence of exogenous hormone use, in response to bleeding and threatened miscarriage, was analyzed.

Factors influencing maternal hormone levels

Factors known to influence maternal hormone levels include maternal age, birth order, maternal weight and pregnancy-related weight gain, and cigarette smoking (Bernstein et al., 1986; Panagiotopoulou et al., 1990; Petridou et al., 1990; Reichman et al., 1993; Trichopoulos et al., 1980).

A clinical study of pregnant women suggests that estradiol levels change with increasing maternal age, being lowest in women under the age of 20 years (Panagiotopoulou et al., 1990), highest in women between 20 and 24 years, and of intermediate values in older women (25+ years). Results of the present study are consistent with this observation; risk was lowest in the youngest age group of mothers. Conversely, a number of clinical studies on pregnant women have found reduced estradiol levels in second compared to first pregnancies (Bernstein et al., 1986; Panagiotopoulou et al., 1990; Trichopoulos et al., 1980), leading to speculation that estrogen metabolism is altered following first births (Bernstein et al., 1986).

Apart from a positive association reported among first born sons (Swerdlow et al. 1986), little difference between case and control mothers with respect to maternal age has been reported (Brown et al., 1986; Henderson et al., 1979). On the other hand, 4 case-control studies report a higher proportion of first births among cases compared to controls (Depue et al., 1985; Prener et al., 1992; Moller and Skakkebaek, 1996; Swerdlow et al., 1986), and two of these studies report decreasing risk of testicular cancer with increasing birth order (Prener et al., 1992; Swerdlow et al., 1986), although the clinical significance of the latter finding, as it relates to hormonal metabolism, is not stated. As with two other studies (Brown et al., 1986; Moss et al., 1986), the present study found no difference in the proportion of first compared to subsequent births among cases and controls. Since maternal age and birth order are correlated, and the clinical data suggest they have opposing effects on maternal hormone levels, a birth order effect
may depend on maternal age. The present study found some evidence for such an interaction. Modestly elevated risk for first compared to subsequent births was found among younger mothers (<25 years), but not among older mothers (25+ years). This suggests that at lower levels of maternal hormones, as indicated by maternal age, the effect on estrogen metabolism due to birth order may be present; however, at higher levels of maternal hormones, as indicated by maternal age, this birth order effect is not evident. Future studies looking at birth order and risk of testicular germ cell cancer should consider potential interaction between maternal age and birth order.

Obese women have been shown to have lower levels of sex hormone binding globulin (de Moor and Joosens, 1970; Dorgan et al., 1995) and higher levels of unbound estrogens. A weak, but positive, association has been reported between maternal weight gain during pregnancy and circulating estradiol levels (Petridou et al., 1992). This suggests that maternal weight at pregnancy, or weight gain during pregnancy, may be associated with risk of cancer. One study, which looked at prepregnancy weight (Depue et al., 1983), found a statistically significant positive gradient with body mass and cancer risk; however, the association was not tested specifically among mothers with a heavy body mass. The present study found no association between risk of testicular germ cell cancer and heavy maternal body mass. Furthermore, no association was found between cancer risk and weight gain during pregnancy.

The present study found decreased risk for testicular germ cell cancer for men whose mothers smoked 12 or more cigarettes per day while pregnant, but no reduction in risk when mothers smoked less than 12 cigarettes per day. Similar results were reported by Brown et al. (1986) who found a slightly reduced risk among women who smoked more than one ‘pack’ of cigarettes per day. Clinical data suggest a mitigating influence for cigarette smoking on cancer risk: levels of circulating estradiol are inversely associated with the number of cigarettes smoked per day (Bernstein et al., 1989; Petridou et al., 1990). In support of an ‘antiestrogenic’ effect of cigarette smoking, it is worth noting that among post menopausal women, cigarette smoking has been associated with decreased risk for endometrial cancer, an estrogen-sensitive cancer (Baron et al., 1990).
Alcohol consumption was not associated with risk of testicular germ cell cancer despite the evidence that alcohol consumption elevates circulating estradiol level in premenopausal, nonpregnant women (Reichman et al., 1993), and the fact that one other study reported a statistically significant elevated risk among men whose mothers reported consuming two or more alcoholic drinks per week (Brown et al., 1986). The fact that the present study could not document an alcohol effect on risk of testicular cancer may have to do with problems eliciting this information using self-reports.

**Underlying menstrual differences between case and control mothers**

Depue et al. (1983) reported a protective effect of surgical treatment for menorrhagia, and speculated on an underlying menstrual difference between case and control mothers. However, the authors noted that surgical treatment mostly occurred after the birth of the index son. The present study, where possible, specifically examined conditions operating before the index pregnancy. No association was found to suggest underlying menstrual differences between case and control mothers as characterized by irregular menstrual cycles during the mothers' reproductive years, or by physician treatment for menstrual problems prior to the birth of the index subject. Other indicators of underlying differences between case and control mothers, as evidenced by prior pregnancy loss, congenital anomalies reported in prior pregnancies, and mothers' diagnosis of cancer did not differ between case and control mothers. It should be recognized that many of the mothers participating in this study were yet entering a period of risk for reproductive cancers. However, in a retrospective cohort study of mothers who gave birth to testicular cancer cases, there was no evidence that these women were at higher risk for developing estrogen-related cancers (i.e. breast, ovary and endometrium) (Kroman et al., 1996).

Discontinued use of birth control pills within three months of conception appears to elevate risk. Risk may be elevated due to exposure to an exogenous hormone or because endogenous maternal hormone levels are altered immediately following cessation of birth control pills use.
Other exposures

A number of other exposures were examined in this study, including birth weight, type of delivery, number of births and breast feeding, and found not to be associated with testicular cancer risk. The present study reported a statistically significant elevated risk for preterm births that was independent of birth weight. The one other study to examine the association between birth weight and gestational age (Brown et al., 1986), reported elevated risk among preterm births that were also low birth weight. The relevance of this finding to the estrogen hypothesis is not clear, although estrogen levels, as measured in third trimester amniotic and salivary fluids, of pregnant women who subsequently went on to preterm deliveries were higher than among women who delivered at term (Depue et al., 1983; Mazor et al., 1996; McGregor et al., 1995).

As with numerous other case-control studies (Depue et al., 1983; Henderson et al., 1979; Moss et al., 1986; Schottenfeld et al., 1980), the present study found a strong association between undescended testicle and testicular germ cell cancer whether reported by the mother as a birth defect in the son, or reported by the son as a medical condition requiring hormonal or surgical treatment. Because there is evidence of an association between maternal hormone exposure and undescended testicle, and between undescended testicle and testicular germ cell cancer, adjustment for the confounding effects of undescended testicle may be necessary in an analysis of maternal hormone exposure. On the other hand, an undescended testicle may be an intermediate condition on the causal pathway leading from maternal hormone exposure to testicular germ cell cancer, and adjustment would be inappropriate because it would result in attenuated odds ratio estimates (Thompson, 1994). Since the inclusion of undescended testicle in a multivariate model, or the exclusion of subjects with undescended testicle, did not appreciably change the odds ratio estimates for surrogate measures of maternal hormone exposure, the evidence from this study suggests that undescended testicle in neither a confounder nor an intermediate variable.

In summary, the over all findings of the present study add support to the hypothesis that maternal hormone exposures are associated with risk of testicular germ cell cancer. In addition
to evidence that prescription hormone use and pregnancy tests may elevate risk, birth control pill use while pregnant may constitute a new source of putative exogenous hormone exposure. Not all previous findings, such as nausea and birth order, which have been interpreted as supporting the hormone hypothesis, could be verified. However, problems with using surrogates to measure endogenous hormone exposure may account for the difference between studies. Several risk factors emerged in this study not previously interpreted as supporting the hormone hypothesis. They include a protective effect associated with early pregnancy bleeding and spotting, heavy maternal cigarette consumption and young maternal age; and elevated risk due to preterm birth and cessation of birth control pill use just prior to conception.
REFERENCES


Table-1. Frequency† distribution and percent (%) of testicular germ cell cancer cases and controls, age-adjusted odds ratio estimates (AOR) and 95% confidence intervals (95% CI) for exogenous hormone use and selected conditions of pregnancy.

<table>
<thead>
<tr>
<th>Exogenous hormone use</th>
<th>No. cases</th>
<th>No. controls</th>
<th>AOR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>no</td>
<td>324 (95.3)</td>
<td>508 (98.6)</td>
<td>1.00</td>
</tr>
<tr>
<td>yes</td>
<td>16 (4.7)</td>
<td>7 (1.4)</td>
<td>4.58 (1.79,11.72)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>p &lt;0.01</td>
</tr>
<tr>
<td>Vomiting</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>no</td>
<td>255 (75.2)</td>
<td>398 (77.4)</td>
<td>1.00</td>
</tr>
<tr>
<td>yes</td>
<td>84 (24.8)</td>
<td>116 (22.6)</td>
<td>1.17 (0.84,1.62)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>p=0.36</td>
</tr>
<tr>
<td>Physician-treated nausea and vomiting</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>no</td>
<td>308 (90.6)</td>
<td>473 (91.7)</td>
<td>1.00</td>
</tr>
<tr>
<td>yes</td>
<td>32 (9.3%)</td>
<td>43 (8.3%)</td>
<td>1.13 (0.70,1.84)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>p=0.85</td>
</tr>
<tr>
<td>Bleeding/threatened miscarriage</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>no</td>
<td>317 (93.5)</td>
<td>469 (91.1)</td>
<td>1.00</td>
</tr>
<tr>
<td>yes</td>
<td>22 (6.5%)</td>
<td>46 (8.9)</td>
<td>0.74 (0.43,1.26)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>p=0.26</td>
</tr>
<tr>
<td>Toxemia</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>no</td>
<td>321 (94.7)</td>
<td>495 (96.3)</td>
<td>1.00</td>
</tr>
<tr>
<td>yes</td>
<td>18 (5.3)</td>
<td>19 (3.7)</td>
<td>1.45 (0.74,2.84)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>p=0.28</td>
</tr>
</tbody>
</table>

† Number of cases and controls does not add up to total study subjects because of missing data
Table-2  Frequency† distribution and percent (%) of testicular germ cell cancer cases and controls, age-adjusted odds ratio estimates (AOR) and 95 % confidence intervals (95 % CI) for maternal prepregnancy weight and pregnancy weight gain adjusted for height, maternal cigarette smoking and alcohol consumption during pregnancy.

<table>
<thead>
<tr>
<th>Pre pregnancy weight*</th>
<th>No. cases</th>
<th>No. controls</th>
<th>AOR(95%CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 18.2</td>
<td>27 (9.2)</td>
<td>44 (9.2)</td>
<td>0.85 (0.51,1.43)</td>
</tr>
<tr>
<td>18.2 - 25.5</td>
<td>247 (83.7)</td>
<td>353 (80.4)</td>
<td>1.00</td>
</tr>
<tr>
<td>&gt; 25.5</td>
<td>21 (7.1)</td>
<td>42 (9.6)</td>
<td>0.72 (0.41,1.25)</td>
</tr>
</tbody>
</table>

Weight gain during pregnancy* ‡

<table>
<thead>
<tr>
<th></th>
<th>No. cases</th>
<th>No. controls</th>
<th>AOR(95%CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>lowest tertile</td>
<td>29 (10.7)</td>
<td>42 (10.3)</td>
<td>1.05 (0.63,1.75)</td>
</tr>
<tr>
<td>intermediate</td>
<td>224 (82.4)</td>
<td>324 (79.6)</td>
<td>1.00</td>
</tr>
<tr>
<td>highest tertile</td>
<td>19 (7.0)</td>
<td>41 (10.1)</td>
<td>0.70 (0.40,1.25)</td>
</tr>
</tbody>
</table>

Smoked cigarettes (no. cigarettes / day)

<table>
<thead>
<tr>
<th></th>
<th>No. cases</th>
<th>No. controls</th>
<th>AOR(95%CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>241 (71.1)</td>
<td>350 (68.1)</td>
<td>1.00</td>
</tr>
<tr>
<td>1-11</td>
<td>66 (19.5)</td>
<td>84 (16.3)</td>
<td>1.13 (0.78,1.64)</td>
</tr>
<tr>
<td>12 +</td>
<td>32 (9.4)</td>
<td>80 (15.6)</td>
<td>0.57 (0.37,0.90)</td>
</tr>
</tbody>
</table>

Drank alcohol (no. drinks / week)

<table>
<thead>
<tr>
<th></th>
<th>No. cases</th>
<th>No. controls</th>
<th>AOR(95%CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>232 (68.2)</td>
<td>360 (69.9)</td>
<td>1.00</td>
</tr>
<tr>
<td>&lt; 2</td>
<td>93 (27.4)</td>
<td>125 (24.3)</td>
<td>1.16 (0.84,1.60)</td>
</tr>
<tr>
<td>2 +</td>
<td>15 (4.4)</td>
<td>30 (5.8)</td>
<td>0.78 (0.41,1.49)</td>
</tr>
</tbody>
</table>

†  Number of cases and controls does not add up to total study subjects because of missing data.
*  Adjusted for maternal height.
‡  Categories based on tertiles among controls.
Table 3. Frequency† distribution and percent (%) of testicular germ cell cancer cases and controls, age-adjusted odds ratio estimates (AOR) and 95% confidence intervals (95% CI) for menstrual and pregnancy conditions prior to index pregnancy, and maternal cancer diagnosis.

<table>
<thead>
<tr>
<th></th>
<th>No. cases</th>
<th>No. controls</th>
<th>AOR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Physician treated menstrual problems prior to index pregnancy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>no</td>
<td>313 (92.3)</td>
<td>473 (92.4)</td>
<td>1.00</td>
</tr>
<tr>
<td>yes</td>
<td>26 (7.7)</td>
<td>39 (7.6)</td>
<td>1.10 (0.65, 1.87)</td>
</tr>
<tr>
<td>Prior pregnancy loss *</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>no</td>
<td>284 (83.6)</td>
<td>423 (82.8)</td>
<td>1.00</td>
</tr>
<tr>
<td>yes</td>
<td>55 (16.2)</td>
<td>88 (17.2)</td>
<td>0.97 (0.67, 1.41)</td>
</tr>
<tr>
<td>Birth defects reported in prior pregnancy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>none</td>
<td>326 (94.2)</td>
<td>498 (96.3)</td>
<td>1.00</td>
</tr>
<tr>
<td>nongenital</td>
<td>19 (5.5)</td>
<td>18 (3.5)</td>
<td>1.63 (0.83, 3.17)</td>
</tr>
<tr>
<td>genital</td>
<td>1 (0.3)</td>
<td>1 (0.2)</td>
<td>1.34 (0.08, 21.73)</td>
</tr>
<tr>
<td>Menstrual cycles 20's/30's</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>regular</td>
<td>290 (87.1)</td>
<td>436 (85.7)</td>
<td>1.00</td>
</tr>
<tr>
<td>irregular</td>
<td>43 (12.9)</td>
<td>73 (14.3)</td>
<td>0.89 (0.59, 1.35)</td>
</tr>
<tr>
<td>Mothers' cancer</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>none</td>
<td>318 (93.8)</td>
<td>472 (91.7)</td>
<td>1.00</td>
</tr>
<tr>
<td>site 174,182,183</td>
<td>8 (2.4)</td>
<td>14 (2.7)</td>
<td>0.75 (0.31, 1.80)</td>
</tr>
<tr>
<td>all other sites</td>
<td>13 (3.8)</td>
<td>29 (5.6)</td>
<td>0.71 (0.36, 1.40)</td>
</tr>
<tr>
<td>Discontinued birth control pill use within 3 months of conception</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>no‡</td>
<td>326 (96.7)</td>
<td>501 (97.1)</td>
<td>1.00</td>
</tr>
<tr>
<td>yes</td>
<td>11 (3.3)</td>
<td>15 (2.9)</td>
<td>1.79 (0.76, 4.24)</td>
</tr>
</tbody>
</table>

† Number of cases and controls does not add up to total study subjects because of missing data.
* Includes pregnancy loss due to miscarriage, therapeutic abortions and stillbirths, and counts multiple births as one pregnancy.
‡ Includes those who never used birth controls pills prior to conception and those who discontinued use more than 3 months prior to conception.
Table 4  Frequency† distribution and percent (%) of testicular germ cell cancer cases and controls, age-adjusted odds ratio estimates (AOR) and 95% confidence intervals (95% CI) for selected prenatal and perinatal factors.

<table>
<thead>
<tr>
<th>Pregnancy length</th>
<th>No. cases (%)</th>
<th>No. controls</th>
<th>AOR(95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt; 2 weeks early</td>
<td>42 (12.7)</td>
<td>43 (8.7)</td>
<td>1.55 (0.98,2.46)</td>
</tr>
<tr>
<td>within 2 weeks of expected</td>
<td>259 (78.2)</td>
<td>394 (79.6)</td>
<td>1.00</td>
</tr>
<tr>
<td>&gt; 2 weeks late</td>
<td>30 (9.1)</td>
<td>58 (11.7)</td>
<td>0.80 (0.50,1.28)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>p=0.09</td>
</tr>
<tr>
<td>Birth weight (grams)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;2500</td>
<td>35 (13.0)</td>
<td>50 (11.8)</td>
<td>1.09 (0.68,1.75)</td>
</tr>
<tr>
<td>2500-3900</td>
<td>206 (76.6)</td>
<td>317 (74.9)</td>
<td>1.00</td>
</tr>
<tr>
<td>&gt;3900</td>
<td>28 (10.4)</td>
<td>56 (13.2)</td>
<td>0.78 (0.48,1.28)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>p=0.54</td>
</tr>
<tr>
<td>Type of delivery</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>head first</td>
<td>309 (92.2)</td>
<td>470 (91.3)</td>
<td>1.00</td>
</tr>
<tr>
<td>breech</td>
<td>11 (3.3)</td>
<td>20 (3.9)</td>
<td>0.77 (0.36,1.65)</td>
</tr>
<tr>
<td>cesarean section</td>
<td>15 (4.5)</td>
<td>25 (4.9)</td>
<td>0.97 (0.50,1.89)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>p=0.80</td>
</tr>
<tr>
<td>Breast-fed</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>no</td>
<td>176 (51.9)</td>
<td>245 (47.8)</td>
<td>1.00</td>
</tr>
<tr>
<td>yes</td>
<td>164 (48.2)</td>
<td>271 (52.5)</td>
<td>0.82 (0.62,1.09)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>p=0.18</td>
</tr>
<tr>
<td>Birth order</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>subsequent birth</td>
<td>229 (67.6)</td>
<td>344 (67.3)</td>
<td>1.00</td>
</tr>
<tr>
<td>first birth</td>
<td>110 (32.4)</td>
<td>167 (32.7)</td>
<td>1.05 (0.78,1.41)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>p=0.76</td>
</tr>
<tr>
<td>Maternal age</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;20 years</td>
<td>25 (7.4)</td>
<td>64 (12.7)</td>
<td>0.50 (0.29,0.84)</td>
</tr>
<tr>
<td>20-24 years</td>
<td>114 (33.6)</td>
<td>142 (28.1)</td>
<td>1.00</td>
</tr>
<tr>
<td>25+ years</td>
<td>200 (59.0)</td>
<td>300 (59.3)</td>
<td>0.83 (0.61,1.13)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>p=0.03</td>
</tr>
<tr>
<td>Birth defects</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>none</td>
<td>322 (93.1)</td>
<td>500 (96.7)</td>
<td>1.00</td>
</tr>
<tr>
<td>nongenital</td>
<td>8 (2.3)</td>
<td>11 (2.1)</td>
<td>1.34 (0.52,3.42)</td>
</tr>
<tr>
<td>genital</td>
<td>16 (4.6)</td>
<td>6 (1.2)</td>
<td>4.01 (1.54,10.45)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>p=0.01</td>
</tr>
</tbody>
</table>

† Number of cases and controls does not add up to total study subjects because of missing data.
Table 5: Results of multivariate modeling of risk factors associated (p ≤ 0.10) with testicular germ cell cancer.

<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>No. cases</th>
<th>No. controls</th>
<th>AOR (90% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Smoked cigarettes (no. cigarettes / day)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>227</td>
<td>333</td>
<td>1.00</td>
</tr>
<tr>
<td>1-11</td>
<td>65</td>
<td>82</td>
<td>1.11 (0.80, 1.54)</td>
</tr>
<tr>
<td>12+</td>
<td>31</td>
<td>76</td>
<td>0.62 (0.43, 0.92)</td>
</tr>
<tr>
<td>Exogenous hormones use</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>no</td>
<td>311</td>
<td>484</td>
<td>1.00</td>
</tr>
<tr>
<td>yes</td>
<td>12</td>
<td>7</td>
<td>4.28 (1.71, 10.57)</td>
</tr>
<tr>
<td>Discontinued birth control pill use within 3 months of conception</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>no</td>
<td>312</td>
<td>477</td>
<td>1.00</td>
</tr>
<tr>
<td>yes</td>
<td>11</td>
<td>14</td>
<td>2.16 (1.00, 4.64)</td>
</tr>
<tr>
<td>Treatment for undescended testicle</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>no</td>
<td>294</td>
<td>485</td>
<td>1.00</td>
</tr>
<tr>
<td>yes</td>
<td>29</td>
<td>6</td>
<td>7.88 (3.65, 17.04)</td>
</tr>
<tr>
<td>Pregnancy length (actual to expected)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt; 2 weeks early</td>
<td>41</td>
<td>42</td>
<td>1.63 (1.08, 2.46)</td>
</tr>
<tr>
<td>within 2 weeks of expected</td>
<td>253</td>
<td>392</td>
<td>1.00</td>
</tr>
<tr>
<td>&gt; 2 weeks late</td>
<td>29</td>
<td>57</td>
<td>0.81 (0.53, 1.23)</td>
</tr>
<tr>
<td>Bleeding / threatened miscarriage</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>no</td>
<td>302</td>
<td>448</td>
<td>1.00</td>
</tr>
<tr>
<td>yes</td>
<td>21</td>
<td>43</td>
<td>0.55 (0.33, 0.92)</td>
</tr>
<tr>
<td>Maternal age</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 20 years</td>
<td>25</td>
<td>61</td>
<td>0.54 (0.34, 0.87)</td>
</tr>
<tr>
<td>20-24 years</td>
<td>110</td>
<td>139</td>
<td>1.00</td>
</tr>
<tr>
<td>25+ years</td>
<td>188</td>
<td>289</td>
<td>0.85 (0.65, 1.12)</td>
</tr>
</tbody>
</table>

Hosmer Lemeshow (1989) goodness-of-fit test: chi-square = 6.03, p = 0.64
8.3. AGE AT PUBERTY AND RISK OF TESTICULAR CANCER

Introduction

Testicular germ cell cancer is predominantly a disease of young and middle aged men. Age-specific incidence rates of testicular germ cell cancer, which closely parallel endocrine activity, appear to be increasing among post-pubescent men but not in prepubescent boys. This increase has been observed in Ontario (Holowaty et al., 1995) as well as elsewhere in the world (Adami et al., 1994; Brown et al., 1986; Moller, 1993; Stone et al., 1991), and is likely due to a birth cohort effect operating in men born at least since the 1940's (Weir et al., 1997), and possibly earlier (Bergstrom et al., 1996; Zheng et al., 1996).

Both the early age at onset and the increasing incidence of testicular germ cell cancer suggest that putative exposures operate early in childhood, or young adulthood, and may have changed over time. Age at puberty, and the acceleration of endocrine activity, may be this exposure (Roush et al., 1987) as it is consistent with the descriptive epidemiology of this cancer: a decline in age at puberty in both boys and girls (Tanner, 1978), coincident with the birth cohort phenomenon observed in population-based incidence data.

Several studies have been undertaken to examine the association between age at puberty and risk of testicular germ cell cancer: some of these report an association (UK Testicular Cancer Study Group, 1994; Moller and Skakkebaek, 1996; Moss et al., 1986), while others report either no association (Depue et al., 1983; Gallagher et al., 1995) or inconclusive results (Swerdlow et al., 1989). With no clear defining event, such as menarche, to mark the onset of puberty in boys, these studies have relied on a number of surrogates to indicate exposure status (e.g. age starting to shave, voice change, and appearance of facial and pubic hair). Such surrogates only estimate the exposure of interest and are subject to an indeterminate amount of measurement error.

The main objective of this study was to test the hypothesis that advanced or delayed relative age at puberty is associated with risk of developing testicular germ cell cancer. A secondary objective was to assess the effectiveness of two analytic strategies, both designed to characterize the true effect of poorly measured exposures: the first strategy was an
examination of the association between multiple measures of exposure and risk of testicular germ cell cancer; the second strategy used combined measures of exposure, reported from different sources (subjects' questionnaires and mothers' questionnaires), to assess the effect of relative age at puberty on odds ratio estimates.

Methods

A population-based case-control study was conducted in the province of Ontario, and included all histologically confirmed newly diagnosed cases of testicular germ cell cancer (ICD9 code 186, ICDO-M 906-910) (WHO, 1976; WHO, 1977) in Ontario residents diagnosed between 1987 and 1989, aged 16-59 years, and reported to the Ontario Cancer Registry by pathology reports. Controls were a random sample of the Ontario population, drawn from the Enumeration Composite Records of the Ontario Ministry of Revenue, and selected to have the same age distribution as that expected for cases based on Ontario incidence data.

Data were collected through the use of a self-administered standardized questionnaire mailed to subjects. Another questionnaire was mailed to subject mothers, when they were available, and completed by telephone interview. Subjects provided information on socio-demographic factors, and occupational and medical histories. Both subjects and mothers were asked about events marking puberty in the subject: appearance of body hair (pubic, chest and axillary hair), starting to shave, growth spurt and voice change. Ages at occurrence of these events were reported relative to peers: earlier than, later than or at the same time as other boys. (In a pretest of the questionnaire, subjects had difficulty reporting the actual age of occurrence of these events.)

When information on at least three of these four puberty variables was available, two summary variables were created, one from subject responses and the other from mother responses, as shown in table-1. In addition, a composite variable (subject-mother pair) was created, adapted from a method described by Marshall (1989), originally designed to combine dichotomous exposure variables according to agreement. For the purpose of this analysis,
subject and mother responses to relative time of puberty events, recorded as a trichotomous response variable (earlier, same, later) were jointly classified as shown in table-2 for each puberty variable. The following number of discordant pairs (i.e. one reported later and the other reported earlier) were excluded from the analysis: 23 growth spurt, 4 voice change, 14 starting to shave and 18 appearance of body hair.

Table-1. Puberty Score based on subjects’ responses and on mothers’ responses independently.

<table>
<thead>
<tr>
<th>Puberty score</th>
<th>Definition of puberty score</th>
</tr>
</thead>
<tbody>
<tr>
<td>3-4 early</td>
<td>3 or 4 variables reported as earlier</td>
</tr>
<tr>
<td>1-2 early</td>
<td>1 or 2 variables reported as earlier and a fewer number of variables reported as later</td>
</tr>
<tr>
<td>Same</td>
<td>3 or 4 variables reported as same or an equal number of variables reported as earlier and later</td>
</tr>
<tr>
<td>1-2 later</td>
<td>1 or 2 variables reported as later and a fewer number of variables reported as earlier</td>
</tr>
<tr>
<td>3-4 later</td>
<td>3 or 4 variables reported as later</td>
</tr>
</tbody>
</table>

Table-2. Classification of subject-mother pair responses.

<table>
<thead>
<tr>
<th>Subject-mother pair</th>
<th>Subject and mother responses</th>
</tr>
</thead>
<tbody>
<tr>
<td>Both earlier</td>
<td>subject and mother reported earlier</td>
</tr>
<tr>
<td>One earlier/ one same</td>
<td>subject or mother reported earlier/ the other reported same</td>
</tr>
<tr>
<td>Both same</td>
<td>subject and mother reported same</td>
</tr>
<tr>
<td>One later/ one same</td>
<td>subject or mother reported later/ the other reported same</td>
</tr>
<tr>
<td>Both later</td>
<td>subject and mother reported later</td>
</tr>
</tbody>
</table>
Chapter 8

Unconditional logistic regression modeling was used to calculate age-adjusted odds ratio estimates (AOR) and ninety-five percent confidence intervals (95% CI) using the statistical package EGRET (Statistics and Epidemiology Research Corporation, 1993). All reported odds ratio estimates were adjusted for subjects’ age by 5-year age groups.

Results

Of 621 eligible cases, 502 case subjects and 346 of their mothers completed questionnaires. One-thousand, four-hundred and thirty-eight controls were sent questionnaires; 975 control subjects and 522 of their mothers participated in the study.

Frequencies, age-adjusted odds ratio estimates (AOR) and ninety-five percent confidence intervals (95% CI) for the original four puberty variables and puberty score are shown in table-3 for all subjects, subjects with mothers, and mothers. AORs for the four puberty variables are shown in figure-1 and AORs for puberty score are shown in figure-2. For all four puberty variables, in all 3 comparisons, more controls reported later puberty than cases.

Among subjects, using as referent subjects who reported an event having occurred ‘at the same time’ as his peers, the protective effect of later puberty was statistically significant for the appearance of hair (AOR=0.49, 95% CI 0.34-0.69) and starting to shave (AOR=0.69, 95% CI 0.53-0.91). There was no association between earlier puberty and risk of testicular germ cell cancer according to subjects’ responses. Compared to subjects whose puberty score was defined as same, the protective effect of later puberty was somewhat greater among subjects who reported 3 or 4 later puberty events (AOR=0.47, 95% CI 0.30-0.74) than when 1 or 2 later puberty events were reported (AOR=0.67, 95% CI 0.49-0.91). Similar results were obtained for only subjects who had a mother participating in the study. In addition, a protective effect of later puberty was also statistically significant for voice change (AOR=0.48, 95% CI 0.28-0.84).

Based on mothers’ reports alone, the protective effect of later puberty was statistically significant for growth spurt (AOR=0.54, 95% CI 0.33-0.87) and voice change (AOR=0.39, 95% CI 0.18-0.87). Earlier puberty did not significantly elevate risk for testicular germ cell
cancer. Compared to sons whose puberty score was defined as 'same', a nonsignificant but somewhat greater protective effect was observed when mothers reported 3 or 4 later puberty events in their sons (AOR=0.54, 95% CI 0.26-1.11) than when 1 or 2 later puberty events (AOR=0.83, 95% CI 0.55-1.26) were reported. Odds ratio estimates for earlier puberty were elevated, but these estimates were not statistically significant, and there appeared to be no difference in odds ratio estimates when the mother reported 3 or 4 earlier events (AOR=1.32, 95% CI 0.65-2.66) or 1-2 earlier events (AOR=0.41, 95% CI 0.85-2.33)

Table-4 shows frequencies, age-adjusted odds ratio estimates and 95% confidence intervals for subject-mother pairs. AORs are also shown in figure-3. Compared to subject-mother pairs who both reported a relative age of same, protective effects of later puberty were observed for all four puberty variables when both the mother and the subject reported later puberty, and when one reported later puberty and one reported same age at puberty. The protective effect of later puberty was similar when the mother and son both reported later puberty, as when the mother or son, but not both, reported later puberty. Statistical significance was achieved only when either the son or mother, but not both, reported later puberty: this was presumably due to the larger number of responses in the former comparison. AORs were elevated when both subjects and mothers reported earlier puberty, but not when earlier puberty was reported by either the mother or subject, alone. Similar results were obtained when 44 (5%) mothers who reported having had help from their sons in completing their questionnaires were excluded from the analysis. Measures of association (kappa), between son and mother pairs, for the four measures of puberty (starting to shave, voice change, growth spurt and appearance of hair) were 0.25, 0.18, 0.15 and 0.17, respectively. Agreement on 'later' responses ranged between 0.18 and 0.30 while those for 'earlier' ranged between 0.12 and 0.29.

Discussion

Moss et al. (1986) first reported an association between age at puberty, as measured by the appearance of pubic hair, and risk of testicular germ cell cancer. Since then a number of other case-control studies (Moller and Skakkebaek, 1996; Swerdlow et al., 1989; UK Testicular
Cancer Study Group, 1994) have provided evidence to suggest a protective effect of later puberty on risk for testicular germ cell cancer using age of starting to shave and voice change as surrogates to estimate puberty status. Gallagher et al. (1995) did not report a statistically significant inverse association; however, there was evidence of decreased risk among boys reporting later age at puberty.

The results of the present study are generally consistent with these previous reports: a protective effect of later puberty was evident for four indices of puberty (appearance of body hair, starting to shave, growth spurt and voice change) as reported by either subjects themselves, or their mothers. The present study used two analytic strategies, each designed to characterize the true effect of poorly measured exposures, to examine the association between age at puberty and risk of testicular germ cell cancer. The first strategy examined the association between multiple surrogate measures of exposure and risk of testicular germ cell cancer through the creation of a composite variable (puberty score) based on the four original puberty variables. Greater protection was conferred on those men who reported the greatest number of events marking later puberty. Risk was not elevated for any measure of earlier puberty as reported by subjects. Risk appeared nonsignificantly elevated, however, based on the mothers' reports of earlier puberty, but risk was not further elevated among those with a greater number of earlier puberty events.

The second strategy, as proposed by Marshall (1989), used agreement between measures of exposure from different sources (mothers' and subjects' questionnaires) to examine the association. The odds of disease was then compared between subject and mother pairs where there was agreement regarding exposure (both reported earlier puberty or both reported later puberty) against those where there was agreement of no exposure (both reported puberty as same time as peers). According to Marshall (1989), subject and mother pairs with disagreement should be of intermediate risk. Risk was modestly elevated when subject-mother pairs both reported earlier puberty, and was modestly decreased when either reported later puberty for all four measures of puberty. But risk was not further decreased when both reported later puberty. This result is not surprising given the low measures of agreement...
between son and mother pairs in which chance agreement could not be ruled out.

The results of the present study confirm the impression drawn from preceding studies, that later puberty offers significant protection from testicular germ cell cancer. The inclusion of mothers’ data did provide some evidence to suggest that earlier puberty may modestly increase risk. Since age-specific incidence rates of testicular germ cell cancer closely parallel endocrine activity in children and young adults, it seems reasonable to speculate that earlier endocrine exposure (age at puberty), with proportionately prolonged exposure, would result in increased incidence.

The lack of a strong association between earlier age at puberty and elevated risk of testicular germ cell cancer may be due to unavoidable problems in recalling events marking the onset of puberty. The accelerated rate of growth of children in some industrialized countries, which has led to earlier puberty among successive cohorts of boys, may be slowing (Tanner, 1978). If this is the case, then the age distribution for onset of puberty may be skewed toward young ages. Children who experience earlier puberty may do so over a matter of months, while children who experience later puberty may do so over a matter of years. For this reason, early puberty may be more difficult for the subject and/or his mother to distinguish than later puberty.

The finding that risk of testicular germ cell cancer is decreased with later puberty, while perhaps elevated with earlier puberty, taken with the suggestion that there has been a shift in the distribution of age at puberty in the population over time, could account for an increase in incidence of testicular germ cell cancer. Over the past century, the age at puberty, as measured by menarche in girls, has decreased on average 0.3 years per decade (Tanner, 1978). Subtle shifts in the distribution of an exposure in the population can have a profound impact on disease incidence rates (Rose, 1985). Age at puberty may be a particularly important risk factor because exposure is complete in the population since nearly all subjects go through puberty at some point in adolescence.

As age at puberty has decreased in the population, the protective effect of later puberty has diminished, and successive cohorts of boys are now at increased risk. This phenomenon would help explain why, in Ontario, the incidence of testicular germ cell cancer has increased
75% from 1964 (4.0 per 100,000) to 1991 (7.0 per 100,000) (Weir et al., 1997). If the accelerated rate of growth is slowing in the population, the increase in incidence of testicular cancer should also start to slow within the next several decades.
REFERENCES


Table 3: Frequency distribution and percent (%) of testicular germ cell cancer cases and controls, age-adjusted odds ratio estimates (AOR) and 95% confidence intervals (95% CI) for events marking puberty and puberty score by source of data.

<table>
<thead>
<tr>
<th>Appearance of Hair</th>
<th>All Subjects</th>
<th>Subjects with mothers</th>
<th>Mothers $\dagger$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cases</td>
<td>Controls</td>
<td>AOR</td>
</tr>
<tr>
<td>Earlier</td>
<td>96 (20.4)</td>
<td>166 (18.2)</td>
<td>1.01</td>
</tr>
<tr>
<td>Same time</td>
<td>327 (69.6)</td>
<td>575 (63.2)</td>
<td>1.00</td>
</tr>
<tr>
<td>Later</td>
<td>47 (10.0)</td>
<td>169 (18.6)</td>
<td>0.49</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Starting to Shave</th>
<th>All Subjects</th>
<th>Subjects with mothers</th>
<th>Mothers $\dagger$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cases</td>
<td>Controls</td>
<td>AOR</td>
</tr>
<tr>
<td>Earlier</td>
<td>88 (18.4)</td>
<td>178 (19.1)</td>
<td>0.83</td>
</tr>
<tr>
<td>Same time</td>
<td>285 (59.6)</td>
<td>495 (53.2)</td>
<td>1.00</td>
</tr>
<tr>
<td>Later</td>
<td>105 (22.0)</td>
<td>258 (27.7)</td>
<td>0.69</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Growth Spurt</th>
<th>All Subjects</th>
<th>Subjects with mothers</th>
<th>Mothers $\dagger$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cases</td>
<td>Controls</td>
<td>AOR</td>
</tr>
<tr>
<td>Earlier</td>
<td>83 (17.4)</td>
<td>165 (17.8)</td>
<td>0.89</td>
</tr>
<tr>
<td>Same time</td>
<td>318 (66.8)</td>
<td>584 (62.9)</td>
<td>1.00</td>
</tr>
<tr>
<td>Later</td>
<td>75 (15.8)</td>
<td>179 (19.3)</td>
<td>0.76</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Voice Change</th>
<th>All Subjects</th>
<th>Subjects with mothers</th>
<th>Mothers $\dagger$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cases</td>
<td>Controls</td>
<td>AOR</td>
</tr>
<tr>
<td>Earlier</td>
<td>60 (12.7)</td>
<td>113 (12.3)</td>
<td>0.99</td>
</tr>
<tr>
<td>Same time</td>
<td>380 (80.5)</td>
<td>714 (77.9)</td>
<td>1.00</td>
</tr>
<tr>
<td>Later</td>
<td>32 (6.8)</td>
<td>89 (9.7)</td>
<td>0.69</td>
</tr>
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</table>

<table>
<thead>
<tr>
<th>Puberty Score</th>
<th>All Subjects</th>
<th>Subjects with mothers</th>
<th>Mothers $\dagger$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cases</td>
<td>Controls</td>
<td>AOR</td>
</tr>
<tr>
<td>3-4 early</td>
<td>49 (10.2)</td>
<td>88 (9.4)</td>
<td>0.88</td>
</tr>
<tr>
<td>1-2 early</td>
<td>78 (16.3)</td>
<td>161 (17.3)</td>
<td>0.77</td>
</tr>
<tr>
<td>Same</td>
<td>242 (50.4)</td>
<td>394 (42.2)</td>
<td>1.00</td>
</tr>
<tr>
<td>1-2 later</td>
<td>83 (17.3)</td>
<td>193 (20.7)</td>
<td>0.67</td>
</tr>
<tr>
<td>3-4 later</td>
<td>28 (5.8)</td>
<td>97 (10.4)</td>
<td>0.47</td>
</tr>
</tbody>
</table>

† Numbers of cases and controls do not sum to total number of study subjects because of missing data.
* Based on subjects' reports.
†† Based on mothers' reports.

*Based on subjects' reports.
†† Based on mothers' reports.
Table 4  Frequency † distribution and percent (%) of testicular germ cell cancer cases and controls, age-adjusted odds ratio estimates (AOR) and 95% confidence intervals (95% CI) for events marking puberty in the subject by subject-mother paired responses.

<table>
<thead>
<tr>
<th>Subject-mother pairs</th>
<th>Cases No.(%)</th>
<th>Controls No.(%)</th>
<th>AOR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Appearance of hair</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>both earlier</td>
<td>13 (4.4)</td>
<td>10 (2.3)</td>
<td>1.62</td>
<td>0.69 - 3.81</td>
</tr>
<tr>
<td>one earlier/ one same</td>
<td>60 (20.1)</td>
<td>84 (19.5)</td>
<td>0.91</td>
<td>0.61 - 1.35</td>
</tr>
<tr>
<td>both same</td>
<td>183 (61.4)</td>
<td>218 (50.6)</td>
<td>1.00</td>
<td>-</td>
</tr>
<tr>
<td>one later/ one same</td>
<td>33 (11.1)</td>
<td>95 (22.0)</td>
<td>0.43</td>
<td>0.28 - 0.68</td>
</tr>
<tr>
<td>both later</td>
<td>9 (3.0)</td>
<td>24 (5.6)</td>
<td>0.47</td>
<td>0.21 - 1.03</td>
</tr>
<tr>
<td><strong>Starting to shave</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>both earlier</td>
<td>20 (6.3)</td>
<td>23 (5.1)</td>
<td>1.18</td>
<td>0.62 - 2.25</td>
</tr>
<tr>
<td>one earlier/ one same</td>
<td>54 (17.0)</td>
<td>73 (16.1)</td>
<td>0.94</td>
<td>0.62 - 1.42</td>
</tr>
<tr>
<td>both same</td>
<td>160 (50.3)</td>
<td>196 (43.2)</td>
<td>1.00</td>
<td>-</td>
</tr>
<tr>
<td>one later/ one same</td>
<td>57 (17.9)</td>
<td>116 (25.6)</td>
<td>0.64</td>
<td>0.43 - 0.94</td>
</tr>
<tr>
<td>both later</td>
<td>27 (8.5)</td>
<td>46 (10.1)</td>
<td>0.77</td>
<td>0.46 - 1.31</td>
</tr>
<tr>
<td><strong>Growth spurt</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>both earlier</td>
<td>13 (4.3)</td>
<td>13 (2.8)</td>
<td>1.41</td>
<td>0.63 - 3.15</td>
</tr>
<tr>
<td>one earlier/ one same</td>
<td>58 (19.2)</td>
<td>86 (18.7)</td>
<td>0.94</td>
<td>0.64 - 1.40</td>
</tr>
<tr>
<td>both same</td>
<td>176 (58.3)</td>
<td>239 (52.1)</td>
<td>1.00</td>
<td>-</td>
</tr>
<tr>
<td>one later/ one same</td>
<td>46 (15.2)</td>
<td>95 (20.7)</td>
<td>0.67</td>
<td>0.45 - 1.00</td>
</tr>
<tr>
<td>both later</td>
<td>9 (3.0)</td>
<td>26 (5.7)</td>
<td>0.50</td>
<td>0.23 - 1.09</td>
</tr>
<tr>
<td><strong>Voice change</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>both earlier</td>
<td>10 (3.2)</td>
<td>9 (2.0)</td>
<td>1.62</td>
<td>0.64 - 4.09</td>
</tr>
<tr>
<td>one earlier/ one same</td>
<td>47 (15.0)</td>
<td>67 (14.6)</td>
<td>0.99</td>
<td>0.65 - 1.50</td>
</tr>
<tr>
<td>both same</td>
<td>236 (75.4)</td>
<td>317 (69.2)</td>
<td>1.00</td>
<td>-</td>
</tr>
<tr>
<td>one later/ one same</td>
<td>18 (5.8)</td>
<td>53 (11.6)</td>
<td>0.50</td>
<td>0.28 - 0.88</td>
</tr>
<tr>
<td>both later</td>
<td>2 (0.6)</td>
<td>12 (2.6)</td>
<td>0.23</td>
<td>0.05 - 1.06</td>
</tr>
</tbody>
</table>

* Numbers of cases and controls do not sum to total number of study subjects because of missing data.
Figure 1. Age-adjusted odds ratio estimates (AOR) by relative time of puberty for events marking puberty by source of data.

**Subjects**

![Graph showing AOR estimates for subjects.]

**Subjects with mothers**

![Graph showing AOR estimates for subjects with mothers.]

**Mothers**

![Graph showing AOR estimates for mothers.]
Figure-2. Age adjusted odds ratio estimates (AOR) for puberty score by source of data.

Figure-3. Adjusted odds ratio estimates (AOR) for events marking puberty for subject-mothers pairs.
8.4. COMPARISON OF RISK FACTORS BY HISTOLOGIC SUBGROUP OF TESTICULAR GERM CELL CANCER

INTRODUCTION

Testicular germ cell cancer is routinely classified into two histologic groups, seminoma and nonseminoma, the latter group including all other and mixed histology tumours. This classification scheme has been a useful prognostic indicator and is routinely used by cancer registries for coding and reporting cancer data. Because these two histologic groups have different age at incidence distributions, suggesting the possibility of different risk factors, or different age at exposure opportunities, etiologic studies have begun to include histologic specific analyses of risk factors. This could be an effective strategy if risk factors differed according to histologic type. However, the presence of mixed histology tumours containing seminoma and nonseminoma is problematic since it is not clear to which, if either, histologic group these tumours belong.

Data from a case-control study of testicular germ cell cancer, which included detailed histologic information on eligible cases and was designed to examine a number of prenatal, perinatal and pubertal factors, form the basis of this study. Dubin and Pasternack (1986) have proposed the use of polychotomous logistic regression as a way to evaluate evidence that risk factors differ among multiple case groups. Histologic-specific odds ratio estimates for seminoma and nonseminoma, excluding mixed histology tumours containing seminoma, were compared to a single control group. The advantage of this method over that of constructing two separate dichotomous logistic regression models is that it allows for the simultaneous estimation of case-specific odds ratios and direct hypothesis testing between case groups. Data from this study were used to evaluate the hypothesis that risk factors differ between testicular seminoma and nonseminoma relative to controls.

METHODS

Subject ascertainment

A population-based case-control study, described in detail elsewhere (Weir et al., manuscript in preparation) was conducted in the province of Ontario. Briefly, cases (N=621)
of malignant testicular germ cell cancer diagnosed in Ontario residents between 16-59 years of age, and diagnosed January 1, 1987 through December 31, 1989, were identified from pathology reports received by the Ontario Cancer Registry. Controls (N=1,438) comprised a population-based sample of Ontario residents drawn from the Enumeration Composite Records of the Ontario Ministry of Revenue and frequency matched to have the same age distribution of that expected for cases based on recent incidence data. Five-hundred and two (502) cases and 975 control subjects participated in the study. Three-hundred and forty-six (346) case mothers and 522 controls mothers participated in the study.

Data collected

Histologic review:

Pathology laboratories throughout the province of Ontario routinely forward copies of cancer reports to the Ontario Cancer Registry. In preparing for this study, a review of these pathology reports was undertaken to assess the feasibility of using them to classify tumours according to the histologic groups, seminoma, nonseminoma and mixed histology tumours containing both seminoma and nonseminoma. Four-hundred and fifty-three reports regarding incident cases diagnosed between 1982 and 1984 were reviewed by a trained medical nosologist. The results of review were as follows: 237 (53.3% seminoma); 196 nonseminoma (43.3%); and 20 tumors of mixed histology (4.4%). Since the percentage of mixed histology tumours was smaller than that reported in the literature (Brawn, 1982), the present study undertook histology review of all eligible cases.

Pathology laboratories were contacted and asked to provide tissue blocks supporting the diagnosis of testicular cancer. Pathology review was performed by Dr. Linda Sugar at the Princess Margaret Hospital in Toronto. While all of the laboratories sent material, some provided unstained slides in lieu of blocks, while others selected blocks for review. Therefore, it was not known if the tissue blocks provided for review were representative of the tumour, which would be necessary for definitive classification of tumours into histologic subgroups; and the recording all specific histologic elements included in the diagnosis of nonseminoma (embryonal carcinoma, choriocarcinoma, teratoma and yolk sac tumour). It was decided then,
to abstract information from the original pathology report and use it to supplement the results of tissue review data. Dr. Hugh Richmond, from St. Michael's Hospital in Toronto, reviewed all relevant pathology reports.

**Questionnaires**

Subjects and their mothers completed mailed questionnaires, described in detail elsewhere (manuscripts in preparation), covering a variety of socio-demographic, medical, occupational and reproductive risk factors. Included in the subjects’ and mothers’ questionnaire were questions on relative (to son’s peers) age at puberty, as measured by starting to shave, appearance of hair, growth spurt and voice change. A summary (puberty score) variable was created from the four individual puberty variables as follows: early (1-4 variables reported as earlier and a fewer number of variables reported as later); same (1-4 variables reported as same or an equal number reported as earlier and later); later (1-4 variables reported as later and a fewer number of variables reported as earlier).

**Data analysis**

Since the primary focus of this analysis was to compare histologic-specific odds ratio estimates, two case groups (seminoma and nonseminoma) were identified while cases with mixed histologies (containing both seminoma and nonseminoma) were excluded from analysis. The analysis was restricted to subjects between the ages of 16 and 44 years because there were no nonseminoma mothers participating in the study among older participants. A single comparison group (all controls) was used in all analyses. To improve stability of the odds ratio estimates, analysis was restricted to those variables where there were five or more exposed cases and controls within either histologic group. Analysis was performed in two stages: the first stage identified the relevant risk factors; and the second stage performed a comparative analysis between risk factors.

To identify risk factors that were associated with either all cases combined, or a histologic-specific case group, three separate logistic regression models were constructed using EGRET (Statistics and Epidemiology Research Corporation, 1993) software. All-cases,
seminoma cases and nonseminoma cases, respectively. The comparison group for all three analyses consisted of all-controls combined.

All previously investigated risk factors (manuscripts in preparation) were considered for inclusion in the individual models. Risk factors were retained if they were associated (p≤0.10) with the respective case group. All models included subject's age (5 year age groups). Log odds ratio estimates and their standard error estimates were used to construct adjusted odds ratio estimates (AOR) and 95% confidence intervals (95% CI) for all-cases combined.

To compare odds ratio estimates between histologic groups, polychotomous (two case group vs. one comparison group) logistic regression (PLR) was performed using the CATMOD procedure in SAS (1990). PLR was performed on a model which included all risk factors identified in the first stage analysis as being associated with one or both histologic-specific case groups or all-cases combined. The model included subjects' age (5 year age groups). Log odds ratio estimates and their standard error estimates were used to construct histologic-specific (seminoma and nonseminoma) age-adjusted odds ratio estimates (AOR) and 95% confidence intervals (95% CI). The null hypothesis, that the difference between the two histologic-specific log odds ratios was equal to zero, was tested according to the method of Dubin and Pasternack (1986) in which the difference between log odds ratio estimates was divided by an estimate of the standard error of the differences.

RESULTS
Histologic review

Figure-1 shows the percent of eligible cases by histologic group and age at diagnosis. The majority of cases, 332 (53.3%), were seminoma (mean diagnosis age 35.5 years), followed by nonseminoma, 180 (29.0%) (mean diagnosis age 28.0 years), and tumours of mixed histology, 109 (17.6%) (mean diagnosis age 31.0 years). The distribution by histology among participating subjects was: 275 (54.8%) seminoma; 141 (28.1%) nonseminoma; and 86 (17.1%) tumours of mixed histology. The distribution by histology among cases who had a mother participating in the study was: 181 (52.3%) seminoma; 107 (30.9%) nonseminoma; and 58 (16.8%) tumours of mixed histology.
Figure-2 shows the proportion of eligible cases expressing specific histologic elements by age at diagnosis. Among cases between the age of 16 and 19 years, the majority of tumours expressed embryonal carcinoma (82.4%), followed by teratoma (76.5%), yolk sac tumours (58.8%), choriocarcinoma (17.7%), and seminoma (11.8%). As age at diagnosis increased, there was a steady decline in the percent of tumours expressing nonseminoma histologic elements and a steady increase in the percent of tumours expressing seminoma. Among cases between the age of 55-59 years, 86.7% of tumours contained seminoma, 20.0% teratoma, 13.3% embryonal carcinoma and 6.7% yolk sac tumours.

Risk factors

As reported by mothers, the following pregnancy-related risk factors were found to be associated with seminoma and/or nonseminoma testicular germ cell cancer and to be of adequate sample size to permit comparison of odds ratios: in-utero exposures to exogenous hormones (including prescription hormones such as diethylstilbestrol or premarin, prescription medication for conditions associated with threatened miscarriage; injections or pills to determine pregnancy); daily consumption of cigarettes (0.1-11, 12+); bleeding and threatened miscarriage; and pregnancy length in relation to expected date of delivery (<2 weeks early, within 2 weeks, >2 weeks late) and breast feeding. As reported by the subject, the following variables were found to be statistically significantly associated with one or both histologic groups: treatment (hormonal or surgical) for an undescended testicle and later relative age at puberty.

Table-1 shows frequencies, adjusted odds ratio estimates and 95% confidence intervals for risk factors associated with all cases (from the EGRET analysis) and with seminoma and nonseminoma, separately (from the CATMOD analysis). In addition, the p-value associated with the test of difference (alpha=0.05) between the histologic specific odds ratios is shown. Hormones use during pregnancy was associated with a statistically significantly elevated risk for testicular germ cell cancer (AOR=3.48, 95% CI 1.07-11.31) that was evident for both seminoma and nonseminoma. Compared to mothers who did not smoke during pregnancy, heavy cigarette consumption (12+ cigarette per day) was associated with a statistically significant reduced risk for testicular germ cell cancer (AOR=0.59, 95% CI 0.36-0.96) that was evident for both
histologic subgroups. Moderate cigarette consumption (1-11 cigarettes per day) was not associated with risk in either histologic subgroups. Compared to subjects who were born within 2 weeks of their expected dates of delivery, early birth was associated with a statistically nonsignificant elevated risk for testicular germ cell cancer that was more pronounced for seminoma (AOR=2.33, 95% CI 0.97-5.61) than nonseminoma (AOR=1.49, 95% CI 0.55-5.61); however, histologic specific odds ratio estimates did not differ (p=0.46). Later births were not associated with either histologic type of testicular germ cell cancer. Bleeding and threatened miscarriage were associated with a statistically significant reduced risk for seminoma (AOR=0.41, 95% CI 0.17-0.99) but not nonseminoma (AOR=1.05, 95% CI 0.48-2.31). The difference between the odds ratio estimates was not statistical significant (p=0.09). Undescended testicle was associated with a statistically significantly elevated risk for testicular germ cell cancer (AOR=7.90, 90% CI 3.14-19.91) that was evident in both histologic groups. Compared to subjects who were not breast fed, a statistically significant protective effect for breast feeding was observed for seminoma (AOR=0.67, 95% CI 0.45-0.99) but not nonseminoma (AOR=1.12, 95% 0.69-1.80). Odds ratio estimates did not differ (p=0.07). Later relative age at puberty was associated with statistically significantly reduced risk for seminoma (AOR=0.49, 90% CI 0.30-0.81) but not nonseminoma (AOR=0.78, 90% CI 0.45-1.35). Odds ratios did not differ (p=0.18).

**DISCUSSION**

The current model of the histogenesis of testicular germ cell cancer suggests that all germ cell cancers arise from a common precursor germ cell (Skakkebaek et al., 1987). Testicular germ cell cancers are routinely classified into two histologic groups, seminoma and nonseminoma, the latter group including embryonal carcinoma, teratoma, choriocarcinoma, yolk sac tumours, and tumours of mixed histology, even those containing seminoma. This classification scheme has been a useful prognostic indicator, as evidence by differential survival (Li et al., 1982), and is routinely used by cancer registries for coding and reporting cancer data. Because these two histologic groups have different age at incidence distributions, suggesting the possibility of different risk factors (Brown et al., 1987; Stone et al., 1991) or
different age at exposure opportunities (Brown et al., 1987), etiologic studies have begun to include histologic-specific analyses of risk factors. This could be an effective strategy in elucidating associations if risk factors differed according to histologic type. However, the presence of mixed histology tumours, containing seminoma and nonseminoma, is problematic since it is not clear to which, if either, histologic group these tumours belong. Where histologic specific analysis has been conducted, these tumours have been included with the nonseminoma group (Moss et al., 1986; Prener et al., 1992) or excluded (Swerdlow et al., 1986; 1987) from the analysis. This later strategy seems preferable, at least for the purpose of assessing the evidence of different risk factors, since the inclusion of mixed tumours in the nonseminoma group may obscure differences between the two histologic groups. However, there are problems in using pathology reports to identify mixed histology tumors as demonstrated by the feasibility study undertaken as part of this study. It should be noted that in the Swerdlow et al. (1986; 1987) study, only 3% of cases were classified as mixed tumours leaving the possibility that not all mixed histology tumours were identified in their study.

As evidenced in this study (figure-1), and reported elsewhere (Brawn, 1983), the age at incidence distribution of histologic specific testicular germ cell cancer is multi-modal: nonseminoma peaks in men in their late 20's while seminoma peaks in men in their early 30's. The peak age at incidence of mixed histology tumours lies between the two groups. Detailed histologic information obtained on all eligible cases in this study (figure - 2) showed there was a steady decline in the proportion of tumours expressing nonseminomatous elements (apparent for all elements that comprise nonseminoma) which was inversely proportionate to the increase in the proportion of tumours expressing seminoma. This supports the view that the underlying potential of the germ cell to express different histologic elements is a function of the age at which the cancer is diagnosed (Brawn, 1983). The extent to which factors, other than age, are associated with specific histologic types of testicular germ cell cancers is a matter of speculation.

Of the case-control studies which included histologic specific analysis of risk factors, (Moss et al., 1986; Prener et al., 1992; Swerdlow et al, 1986; Swerdlow et al, 1987), one study (Moss et al, 1986) concluded that a cluster of ‘breast cancer like’ risk factors was preferentially associated with nonseminoma while another study concluded that hormone factors (Swerdlow
et al., 1986) and undescended testicle (Swerdlow et al., 1987) were preferentially associated with seminoma. In a third study (Prener et al., 1992), histologic specific results were presented, but no attempt was made to interpret results based on the findings. It is difficult to know how to interpret the findings from these studies since they included either mixed histology cancers with nonseminoma or they may not have excluded all mixed histology tumours.

This present study found some evidence to suggest that a number of risk factors appeared preferentially associated with one histologic group (seminoma) or both. The preferential association with seminoma may be a function of sample size since there were almost twice as many seminoma as nonseminoma cases in the study. However, there was little evidence to suggest that risk factors differed for seminoma and nonseminoma.

Further examination of histologic specific risk factors should be encouraged but future studies may need to consider including histologic review to properly classify cases. Evidence from this study suggests that prenatal, perinatal and pubertal risk factors are not likely to be as strong a determinant of histologic expression as the age of the subject at diagnosis.
REFERENCES


Table-1  Frequency† distribution and adjusted odds ratio estimate (AOR) and 95% confidence interval (95%CI) for selected exposures by histologic groups.

<table>
<thead>
<tr>
<th></th>
<th>Control No.</th>
<th>All No.</th>
<th>Sem No.</th>
<th>NS No.</th>
<th>Total (AOR, 95% CI)</th>
<th>Sem (AOR, 95% CI)</th>
<th>NS (AOR, 95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hormone use in pregnancy</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
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<td>403</td>
<td>282</td>
<td>155</td>
<td>81</td>
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<td>1.00</td>
<td>1.00</td>
<td>-</td>
</tr>
<tr>
<td>yes</td>
<td>5</td>
<td>10</td>
<td>5</td>
<td>5</td>
<td>3.48 (1.07, 11.31)</td>
<td>6.73 (1.81, 24.95)</td>
<td>4.09 (1.23, 13.63)</td>
<td>0.47</td>
</tr>
<tr>
<td>Mothers smoked in pregnancy</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 cigs. /day</td>
<td>272</td>
<td>208</td>
<td>120</td>
<td>58</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>-</td>
</tr>
<tr>
<td>1-11 cigs. /day</td>
<td>73</td>
<td>56</td>
<td>25</td>
<td>21</td>
<td>0.96 (0.64, 1.44)</td>
<td>0.80 (0.47, 1.37)</td>
<td>1.59 (0.85, 2.65)</td>
<td>0.08</td>
</tr>
<tr>
<td>12+ cigs. /day</td>
<td>63</td>
<td>28</td>
<td>15</td>
<td>7</td>
<td>0.59 (0.36, 0.96)</td>
<td>0.57 (0.31, 1.06)</td>
<td>0.56 (0.26, 1.21)</td>
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</tr>
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<td>Pregnancy length</td>
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<td></td>
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<td></td>
<td></td>
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<tr>
<td>&gt;2 wks early</td>
<td>34</td>
<td>37</td>
<td>22</td>
<td>11</td>
<td>1.52 (0.92, 2.53)</td>
<td>2.33 (0.97, 5.61)</td>
<td>1.49 (0.55, 4.03)</td>
<td>0.46</td>
</tr>
<tr>
<td>within 2 wks</td>
<td>325</td>
<td>228</td>
<td>125</td>
<td>66</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>-</td>
</tr>
<tr>
<td>&gt;2 wks late</td>
<td>49</td>
<td>27</td>
<td>13</td>
<td>9</td>
<td>0.84 (0.50, 1.41)</td>
<td>0.74 (0.37, 1.47)</td>
<td>0.98 (0.46, 2.08)</td>
<td>0.56</td>
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<td>Bleeding/ threat. miscarriage</td>
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<td>152</td>
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<td>1.00</td>
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<tr>
<td>yes</td>
<td>35</td>
<td>20</td>
<td>8</td>
<td>11</td>
<td>0.61 (0.32, 1.15)</td>
<td>0.41 (0.17, 0.99)</td>
<td>1.05 (0.48, 2.31)</td>
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</tr>
<tr>
<td>Undescended testicle</td>
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<td></td>
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<td>148</td>
<td>76</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>-</td>
</tr>
<tr>
<td>yes</td>
<td>5</td>
<td>26</td>
<td>12</td>
<td>10</td>
<td>7.90 (3.14, 19.91)</td>
<td>7.45 (2.50, 22.14)</td>
<td>10.41 (3.29, 32.90)</td>
<td>0.51</td>
</tr>
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<td>Subject breast fed</td>
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<td></td>
</tr>
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<td>157</td>
<td>88</td>
<td>42</td>
<td>1.00</td>
<td>1.00</td>
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<td>-</td>
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<tr>
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<td>135</td>
<td>72</td>
<td>44</td>
<td>0.83 (0.61, 1.14)</td>
<td>0.67 (0.45, 0.99)</td>
<td>1.12 (0.69, 1.80)</td>
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<tr>
<td>Puberty Score</td>
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<tr>
<td>1-4 early</td>
<td>112</td>
<td>83</td>
<td>44</td>
<td>22</td>
<td>0.90 (0.62, 1.31)</td>
<td>1.01 (0.63, 1.60)</td>
<td>0.71 (0.39, 1.28)</td>
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<tr>
<td>same</td>
<td>165</td>
<td>143</td>
<td>86</td>
<td>38</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>-</td>
</tr>
<tr>
<td>1-4 later</td>
<td>131</td>
<td>66</td>
<td>30</td>
<td>26</td>
<td>0.59 (0.40, 0.87)</td>
<td>0.49 (0.30, 0.81)</td>
<td>0.78 (0.45, 1.35)</td>
<td>0.18</td>
</tr>
</tbody>
</table>

† Frequencies do not sum to the total number of subjects due to missing data.

* P-value associated with null hypothesis that there is no difference between histologic-specific odds ratios.

Sem = seminoma, NS=nonseminoma
Chapter 8

Figure-1. Distribution of testicular germ cell cancer cases by histologic subgroup among eligible cases.

![Bar chart showing distribution of testicular germ cell cancer cases by histologic subgroup among eligible cases.]

Figure-2. Testicular germ cell cancer: expression of histologic cell types by age at diagnosis among eligible cases.

![Line chart showing percent of cases by age at diagnosis among eligible cases.]

- Seminoma
- Embryonal carcinoma
- Teratoma
- Choriocarcinoma
- Yolk sac tumour
CHAPTER 9
SUMMARY AND DISCUSSION

Exposure to prenatal and pubertal endocrine factors has been hypothesized to be associated with risk of testicular cancer (Henderson et al., 1979; Roush, 1987). The present study was undertaken as a comprehensive examination of evidence in support of this hypothesis. Results of this study have been compiled in three separate papers: 'Prenatal and perinatal exposures and risk of testicular germ cell cancer'; 'Age at puberty and risk of testicular germ cell cancer'; and 'Comparison of risk factors by histologic subgroup of testicular germ cell cancer' (chapter 8).

9.1. Prenatal endocrine factors
9.1.1. Exogenous sources of hormone exposure

DES was first prescribed in Canada in the early 1940's. It remained on the market until 1971 when it was withdrawn because published medical reports linked it to vaginal adenocarcinoma in young women exposed in-utero (Herbst et al., 1971). DES is suspected to cause a number of adverse reproductive tract abnormalities in males, including undescended testicle (Stillman, 1982), but the evidence linking DES exposure to testicular cancer is inconclusive (Henderson et al., 1979; Schottenfeld et al., 1980; Loughlin et al., 1980; Depue et al., 1983; Brown et al., 1986; Gershman and Stolley, 1988). In part, this may be due to the fact that these studies were limited to investigating cancers diagnosed in the youngest members of the cohort of men with potential DES exposure. Since testicular germ cell cancer peaks in men in their mid 20's to early 30's, this cohort of men only began entering a period of maximum cancer risk in the mid to late 1970's. These men will remain at risk throughout this century. This study sampled from this cohort of men in the ages of maximum cancer risk: eighty-nine percent of the cases participating in this study were diagnosed in men between the ages of 20 and 44 years and who were born between 1945 and 1969.

The present study reports a statistically significant elevated risk for testicular germ cell cancer among mothers who reported exogenous hormone use. This was due to exposure to DES and other exogenous hormones, pills and injections to determine
pregnancy, and birth control pill use at the time of, and immediately following, conception. Cessation of birth control pill use, just prior to conception, also elevated risk. Unspecified prescription medication taken for threatened miscarriage, however, did not elevate risk. Caution should be used in interpreting these results since these findings are based on very small numbers and this study did not include validation of exposure status. Given the retrospective design of this study, recall bias, resulting from differential recall between case and control mothers; and selection bias, resulting from selective participation my case mothers, may account for these findings (see sections 9.4.1 and 9.4.2).

9.1.2. Endogenous hormone exposure

Several published studies have interpreted their results with respect to the hypothesis that maternal endogenous hormone exposure is associated with testicular germ cell cancer (Henderson et al., 1979; Depue et al., 1983; Brown et al., 1986; Moss et al., 1986; Swerdlow et al., 1986; Prener et al., 1992; Moller and Skakkebaek, 1996). In the absence of direct maternal serum measurements, and where clinical evidence supports an association, these studies have used a number of surrogate measures to infer exposure to maternal hormones, including pregnancy related nausea and vomiting, threatened miscarriage, birth order and maternal age. Based on additional clinical evidence, the present study included additional measurements: maternal cigarette and alcohol consumption, heavy maternal weight, pregnancy related weight gain and gestational age.

The results of these studies and the present study are summarized in table-9.1 (page 100). It is difficult to compare results across studies because exposure variables were quantified differently, subset analyses were performed and biological interaction and confounding were not always assessed. Evidence in support of the hypothesis that in-utero exposure to maternal endocrine factors elevates risk for testicular germ cell cancer may come from consistency of results from various study populations, and the presence of a dose response relationships rather than the strength of the association as reported by any one
study.

Maternal estrogen levels are reported to be higher among first pregnancies compared to subsequent pregnancies (Bernstein et al., 1986) although this may be only true for young mothers. Estrogen levels are reported to be higher among women who experience pregnancy related nausea and vomiting (Depue et al., 1984). Maternal estrogen levels also appear to be associated with maternal age, being lowest in young mothers (<20 years), highest in mothers between the age of 20 and 24 years and of intermediate values in the oldest age group of mothers (25+ years) (Panagiotopoulou et al., 1990). This would suggest that first born sons, and sons born to women who experience pregnancy related nausea and vomiting, are at greater risk for testicular germ cell cancer while sons born to young mother are at lower risk. The evidence from the present study, when viewed in context of all the other studies, generally supports a weak association for endogenous hormone exposure and risk of testicular germ cell cancer. Because maternal age and birth order are strongly correlated (first births tend to occur in young mothers), it seems reasonable to speculate that there may be interaction between birth order and maternal age, and that risk due to first births may manifest itself in the absence of elevated maternal estrogen levels associated with older maternal age. This may also explain the association between pregnancy related nausea being strongest among first compared to subsequent births. Future studies should consider potential interaction between maternal age and birth order when assessing the hormone hypothesis. Additional clinical information on the effect of higher order pregnancies on maternal estrogen levels may help explain why several studies report a trend in reduced risk with increasing birth order.

Interpreting the data from threatened miscarriage in support of the maternal hormone theory is more difficult. While excess estrogen, due to an altered 'maternal hormonal milieu', may explain the finding that nausea elevates risk, it seems a less plausible explanation for why threatened miscarriage would elevate risk: clinical data suggests that hormonal insufficiencies are more likely to lead to bleeding and threatened miscarriage. In fact, the present study found a protective effect for bleeding and threatened miscarriage. Inabilities to properly adjust for the confounding effects of exogenous hormone use may explain the excess risk
reported by these other studies for bleeding and threatened miscarriage.

Preterm births or low birth weight appears to be consistently associated with elevated risk. In the present study risk associated with preterm birth was independent of risk associated with undescended testicle (a common condition found among premature births). While there is some clinical evidence that elevated estrogen levels precede preterm births (Mazor et al., 1996; McGregor et al., 1995), information on first trimester estrogen levels of pregnancies destined to be delivered preterm would help assess the plausibility of this association as it relates to the maternal hormone hypothesis.

Cigarette smoking has been shown to decrease maternal estrogen levels while alcohol consumption (2+ drinks per day) appears to elevate levels among premenopausal, nonpregnant women (Bernstein et al., 1989; Petridou et al., 1990; Reichman et al., 1993). Therefore sons born to mothers who smoked should be at decreased risk while sons born to mothers who drank should be at increased risk. The present study found that heavy maternal cigarette consumption decreased risk. This is consistent with one other study which reported modest decreased risk among mothers who smoked one or more packs of cigarettes per day. Unlike one other study, which reported elevated risk due to alcohol consumption of two or more drinks per week, the present study found no association. This may be due, in part, to problems in eliciting this information from questionnaires and because the range of exposures may not be sufficient to detect an association among pregnant women.

Obese women have been shown to have decreased levels of SHBG (Dorgan et al., 1995) which would, in turn, lead to higher concentrations of unbound estrogen. Therefore, sons born to women with heavy body mass should be at increased risk. The present study found no association between heavy body mass and cancer risk. This may be due to problems in identifying 'obese' women in the study and because SHBG levels may not depressed in moderately overweight women (Dorgan et al., 1995).

Taken together, the present study offers support for the hypothesis that endogenous maternal hormones are associated with risk of cancer. Limitations with using self-reported data, a retrospective design and surrogate measures to infer exposure may explain some of the inconsistencies of the present study with other published reports. The present
TABLE 9.1. Results of studies investigating the relationship between endogenous maternal hormone exposure and testicular germ cell cancer.

<table>
<thead>
<tr>
<th>Birth order</th>
<th>Maternal age</th>
<th>Nausea and vomiting</th>
<th>Threatened miscarriage</th>
<th>Gestational age</th>
<th>Cigarette smoking</th>
<th>Alcohol consumption</th>
<th>Weight and weight gain</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypothesized relationship</td>
<td>First birth increased risk</td>
<td>Increased risk with age</td>
<td>Increased risk</td>
<td>Not certain</td>
<td>Preterm increased risk</td>
<td>Decreased risk</td>
<td>Increased risk</td>
</tr>
<tr>
<td>Henderson et al., 1979</td>
<td>-</td>
<td>(+)</td>
<td>(+)</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Schottenfeld et al., 1980</td>
<td>(+)</td>
<td>+ first births</td>
<td>+ low birth</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Depue et al., 1983</td>
<td>-</td>
<td>(-) first births</td>
<td>+</td>
<td>+ low birth weight</td>
<td>(+) 1 pack per day</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Brown et al., 1986</td>
<td>-</td>
<td>(+) first births</td>
<td>- low birth weight</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Moss et al., 1986</td>
<td>-</td>
<td>(+) first births</td>
<td>- low birth weight</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Swerdlow et al., 1986</td>
<td>+ first vs. subsequent and trend</td>
<td>+ first births</td>
<td>+</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gershman and Stolley, 1988</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prener et al., 1992</td>
<td>(+) trend</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Moller and Skakkebaek, 1996</td>
<td>(+)</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Present study</td>
<td>(+) among younger mothers</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+ (1 pack per day)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

+ supports hypothesized relationship; - does not support hypothesized relationship; ( ) p > 0.05 (alpha = 0.05)
study does provide new evidence in support of the hypothesis that maternal hormone exposures are associated with risk of testicular germ cell cancer.

9.2. Age at puberty

The increasing incidence of testicular cancer appears due to a birth cohort effect operating in men at least since the early part of this century. Since age at puberty is decreasing in the general population coincident to the increase in population-based cancer incidence, initiation of endocrine activity, associated with puberty, may be important. Modest shifts in the distribution of a risk factors within the population can impact the incidence rate of a disease (Rose, 1985).

Later relative age at puberty, as measured by age starting to shave, voice change, growth spurt and appearance of hair, was found to be protective for testicular germ cell cancer. No association was found between earlier puberty and cancer risk. This statistically significant protective effect of later puberty is consistent with other studies (Moller and Skakkebaek, 1996; Swerdlow et al., 1989; UK Testicular Cancer Study Group, 1994).

On the other hand, unmeasured environmental determinants may be acting as confounders in the association between puberty and testicular cancer. For example, exposure to environmental and dietary estrogen-like chemicals (Colborn et al., 1996) has increased over the past several decades, and these chemicals may be responsible for the increase in male reproductive tract disorders, including testicular cancer.

9.3. Validity of Study Results

A major concern of any retrospective study, especially one that relies on self-reports to assess exposure status, is the validity of the information. This may be a particular concern with the present study since surrogate measures where used to infer exposure to endogenous hormones; self-reports were used to assess exposure to exogenous hormones; and because the reliability of this information, as it pertain to some exposures more than other, may be affected by the age of the subject, the age of the mother; the time since exposure and the saliency of the
event. All these concerns could lead to a considerable amount of measurement error. Since the usual effect of nondifferential misclassification, at least as it pertains to dichotomous exposure variables, is to attenuate odds ratio estimates (Schlesselman, 1982; Dosemeci et al., 1990), the results of this study may reflect conservative odds ratio estimates.

9.3.1. Recall bias

Recall bias occurs when there is differential recall of exposure status among the comparison groups. This bias can result in odds ratio estimates that are inflated or biased toward the null (Copeland et al., 1977). For most of the risk factors considered, especially those involving endogenous sources of hormones, it seems unlikely that there would be substantial differential recall between case subjects and controls subjects or between case mothers and control mothers since subjects and their mothers were unaware of the study hypotheses.

Recall bias may be more of a concern for odds ratio estimates involving exogenous hormone use because case mothers may be ruminating on past exposures more than control mothers, or physicians may be selectively querying testicular patients, and their mothers, about possible exposures. Less than one percent of control mothers reported hormone use or use of unspecified prescription medication for threatened miscarriage. Data on DES prevalence in the general population of Ontario is lacking, and it cannot be known whether mothers under reported exposure. Since validation studies of DES exposure have shown that many women either were unaware of exposure to DES or had difficulty recalling exposure (Tilley et al., 1985), it seems likely that these exposure percents are under-reports of the true prevalence of exposure. Studies may be more susceptible to recall bias when the exposure is rare (Coughlin et al., 1990), as in the case of DES.

Of particular interest is the fact that unspecified prescription medication use for threatened miscarriage did not elevate risk. Since DES was prescribed for threatened miscarriage, it seems reasonable to think that this particular exposure included DES. If DES did elevate risk for testicular germ cell cancer, then risk associated with unspecified prescription medication use for threatened miscarriage should also be elevated. The fact that risk was not
elevated supports the view that recall bias may be responsible for the association between exogenous hormone use and testicular germ cell cancer risk.

9.3.2. Selection bias

Selection bias occurs due to systematic differences in characteristics between those who are selected for study (or participate) and those who are (or do) not. Selection bias could be operating in this study due to the lower response rates of control subjects compared to case subjects, and from the lower participation rate among control mothers compared to case mothers. The most often cited reason by subjects for mothers nonparticipation was mother deceased. There could introduce selection bias if there were systematic differences between the mortality experiences of case and control mothers. As far as it was possible to look at maternal morbidity (mother diagnosed with cancer), there was no difference between the percent of case and control mothers who were diagnosed with cancer. A cohort study which looked at morbidity and mortality from hormonally related female cancers reported that mortality or morbidity rates among testicular germ cell cancer case mothers were no higher than among women in the general population (Kroman et al., 1996). If case mothers were more likely to be sick or deceased, then participation rates among case mothers would be expected to be lower than among control mothers.

Selection bias may be a concern in this study as it pertains to exogenous hormone use. The higher participation rate among case mothers appears due to a higher rate of consent from case subjects, and a higher response rate from case mothers. This raises the possibility that prior knowledge of the study hypothesis may have motivated cases and there mothers to participate in the study. As Tilley et al. (1985) have shown, in a validation study of DES use, women who initiated their own participation had more precise knowledge of their exposure to prenatal hormones. Not only may case mothers be more motivated than control mothers to participate in the study, but case mothers with prior knowledge of DES exposure may be the most motivated to participate.
9.3.3. Confounding

Confounding occurs when there is distortion in the apparent effect of one exposure brought about because the exposure is associated with that of another exposure. To act as a confounder, the two exposures must be associated independent of disease status, and the second exposure must be associated with the outcome of interest. This may be a particular problem with testicular germ cell cancer since few causal risk factors are known for this cancer, apart from age and undescended testicle. However, the threat of confounding was limited by frequency matching controls to cases by five-year age groups, and by using multivariate analysis. Model selection used backward elimination of potential risk factors to identify relevant exposures, and to identify confounders in the analysis. For example, the protective effect of bleeding and threatened miscarriage was clearly evident once adjustment was made for hormone use. The use of forward selection of risk factors, in the model building phase of this study, would have required prior knowledge of this association to properly characterize the association between bleeding and threatened miscarriage and testicular germ cell cancer.

The literature suggests that subject's socio-economic status, as reported by the subject at diagnosis, may be more strongly associated with risk for testicular germ cell cancer risk than socio-economic status as reported in childhood or by the parent. This may explain why there was no difference between the highest level of education as reported by case and control mothers. Maternal socio-economic status was not found to be a confounder in this analysis.

9.3.4. External validity of the study

The overall response rate among controls (67.8%) was lower than that of cases (80.8%) and this was primarily due to the fact that 24.1% (346/1,438) of controls, compared to 10.8% (67/671) of cases were lost to follow-up (the questionnaire was not returned within 12 weeks of being sent and the subject could not be contacted by telephone to verify a refusal). Since every attempt was made to contact non-respondents, it seems reasonable to infer that 'lost to follow-up' included many controls who never received a questionnaire.

Similar response rates have been observed in other case-control studies conducted on
young and middle-aged populations (Slattery et al., 1995). This finding may have implications for the generalizability of these results in that respondents may have been less mobile than the general population of men between the ages of 16 and 59 years. However, the lack of generalizability may not apply to risk factors related to prenatal, perinatal and pubertal endocrine exposures as much as they would to current risk factors, such as general state of health. The over-all findings of this study are in accordance with the maternal and pubertal hormone hypothesis stated in the literature, indicating that the results of this study may be valid.

9.4. Study strengths

This study has several strengths. Cases were identified from pathology reports routinely received by the Ontario Cancer Registry (OCR). Pathology reports constitute a major source for identifying cases in the OCR. Since case ascertainment by the OCR is nearly complete (Robles et al., 1988), it seems reasonable to suggest that nearly all eligible cases were identified for inclusion in this study. Controls comprised a random sample of the general male population of Ontario and were selected solely on the basis of age and thus free of selection bias that could be introduced as a result of matching on various socio-demographic variables, such as neighborhood and obstetric care or hospital.

Histologic review of all eligible cases allowed for detailed description of all incident cases and precise classification of testicular cancers into the histologic groups, seminoma, nonseminoma and mixed histology tumours.

9.5. Implication of study results to prevention strategies

Results of this study offer clues to the possible prevention of testicular germ cell cancer. There were several modifiable prenatal and perinatal risk factors identified in this study. Women are advised to cease birth control pill use and wait several months prior to attempting pregnancy; avoid any exposure to exogenous hormone sources while pregnant and breast feed. While heavy maternal cigarette smoking appeared to reduce testicular cancer risk in the son, it can not be advocated as a prevention strategy when it is associated with a number of adverse health
outcomes in both the mother and her offspring.

The prevented fraction is a measure of potential impact of a protective factor in the population (Kleinbaum et al., 1982) and is often used to estimate the public health importance of a risk factor. The prevented fraction due to later puberty is estimated to be 18.8% (Appendix 5). If all subjects who went through later puberty had, instead, gone through puberty at the same time as their peers, then 18.8% of all cases that would have occurred in the absence of exposure (later puberty) were actually prevented by the exposure. Another way of saying this is that 111 cases (between 1987 and 1989) may have been prevented as a result of having gone through puberty later than their peers.

Age at puberty may be an important risk factor in terms of explaining the increasing incidence of this cancer. The finding that risk of testicular germ cell cancer is decreased with later puberty, taken with the suggestion that there has been a shift in the distribution of age at puberty in the population over time, could account for an increase in incidence of testicular germ cell cancer. Exposure is complete in the population as nearly all boys go through puberty at some point during adolescence, and subtle shifts in the distribution of an exposure, especially one with a high prevalence rate, can have a profound impact on disease incidence rates (Rose, 1985). Secular trends in earlier onset of puberty suggest strong environmental determinants. If these determinants could be identified as they relate to age at puberty in boys, then it might be possible to design prevention strategies aimed at lowering the incidence of testicular germ cell cancer in the population by delaying the onset of puberty, or halting the continued advance in earlier onset of puberty. Since an inverse association with post pubertal recreational activity and cancer risk has been recently reported (UK Testicular Cancer Study Group, 1994; Gallagher et al., 1995), studies of physical activity in childhood may offer similar clues for prevention. Future direction.

9.6. **Future direction**

* The number of testicular germ cell cancer cases which can be attributed to any of the exposures identified in this study is small. Etiologic investigation of other risk factors should be encouraged.
Since a protective effect was shown for later puberty was shown in this study, and increasing incidence for this cancer may be related to the decline in the age at puberty in boys, studies on risk factors for earlier onset of puberty should be encouraged.

Future studies examining the association between endocrine exposure and risk of testicular germ cell cancer would benefit from a more direct assessment of prenatal and pubertal endocrine exposure. Etiologic investigations of men with undescended testicles may suggest strategies for investigation of testicular cancer. A number of case-control studies, conducted to evaluate the hypothesis that exposure to maternal estrogen was associated with increased risk of undescended testicle (Swerdlow et al., 1984; Beard et al., 1984; Depue et al., 1984; McBride et al., 1991; Berkowitz et al., 1995), used similar surrogate measures to infer exposure to maternal hormones. These results were equivocal with regards to the hypothesis. However, a nested case-control study, in which early pregnancy serum was drawn from study participants, found that case mothers had statistically significantly higher levels of bioavailable estrogen than did control mothers (Bernstein et al., 1988). Perhaps long term follow-up of men born to women enrolled in pregnancy-related cohort studies, as suggested by Senturia (1987), may provide more definitive results by which to assess the hypothesis that exposure to elevated levels of maternal hormones elevates risk of testicular germ cell cancer.

This study included two analytic strategies to help characterize one of its poorly measured exposures. The first strategy, which combined multiple indicators (age starting to have, voice change, growth spurt and appearance of hair), did provide evidence that multiple indicators were preferable to single indicators of exposure. This method should be used in future studies. In the second method, mothers data were combined with subjects data to assess the effects of agreement on odds ratio estimates for age at puberty. The restriction of results to subject-mother paired data indicated a more
pronounced effect on the odds ratio estimate (elevated for early puberty and decreased for late puberty) for several measures of exposure compared to when subjects or mothers individually reported exposure. But lack of agreement among subject-mother pairs resulted in small sample sizes and large confidence intervals, and made it difficult to interpret the results. This method should not be pursued since it is inefficient. Mothers do not appear better able to recall details of son’s puberty than the subject himself.

* This present study found little evidence to suggest that risk factors differ for seminoma and nonseminoma, and more studies are required to assess these findings. However, problems with using pathology reports to properly classify cases into histologic groups necessitated the inclusion of detailed histologic examination of cases in this study. Future studies will need to consider including similar histologic review, as well as sample size considerations to detect statistically significant differences between histologic-specific odds ratios. In addition, future research into biomarkers for testicular cancer may identify etiologically distinct types of cancer.

* In the absence of direct maternal serum measurements, more information is needed on factors that influence or are indicative of endogenous maternal hormone levels. For example, hormone levels by maternal age controlling for gravidity.
REFERENCES


Nevin MM. Forty-seven years after its first use as a wonder drug for pregnant women, DES's sad legacy is still with us. Can Nurse 1988(Mar);17-19.


Oliver RTD. Clues from natural history and results of treatment supporting the monoclonal origin of germ cell tumors. Cancer Surv 1990;9(2):333-68.


Sharpe RM and Skakkebaek NE. Are oestrogens involved in falling sperm counts and disorders of the male reproductive tract? Lancet 1993;341:1392-95.


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APPENDIX 1 Mothers questionnaire.
This questionnaire is part of a research study to improve our understanding of factors related to health and disease in Ontario.

Thank you very much for your help.
The following questions refer to your child, _____________, before age 16.

1. Did your child ever have any of the following childhood diseases? If so at what age?

<table>
<thead>
<tr>
<th>Disease</th>
<th>Age</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 MUMPS</td>
<td></td>
</tr>
<tr>
<td>2 MUMPS ORCHITIS (MUMPS WITH TESTICULAR INFECTION)</td>
<td></td>
</tr>
<tr>
<td>3 INFECTIOUS MONONUCLEOSIS</td>
<td></td>
</tr>
<tr>
<td>4 SCARLET FEVER</td>
<td></td>
</tr>
<tr>
<td>5 CHICKEN POX</td>
<td></td>
</tr>
<tr>
<td>6 MEASLES (RUBEOLA)</td>
<td></td>
</tr>
<tr>
<td>7 GERMAN MEASLES (RUBELLA)</td>
<td></td>
</tr>
<tr>
<td>8 MENINGITIS</td>
<td></td>
</tr>
<tr>
<td>9 WHOOPING COUGH (PERTUSSIS)</td>
<td></td>
</tr>
</tbody>
</table>

2. Was your child immunized against any of the following diseases?

<table>
<thead>
<tr>
<th>Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 DIPHTHERIA</td>
</tr>
<tr>
<td>2 PERTUSSIS (WHOOPING COUGH)</td>
</tr>
<tr>
<td>3 TETANUS</td>
</tr>
<tr>
<td>4 POLIO</td>
</tr>
<tr>
<td>5 MUMPS</td>
</tr>
<tr>
<td>6 MEASLES</td>
</tr>
<tr>
<td>7 GERMAN MEASLES</td>
</tr>
<tr>
<td>8 SMALLPOX</td>
</tr>
</tbody>
</table>

3. Did your child have any allergies (e.g. hay fever, asthma, eczema)?

<table>
<thead>
<tr>
<th>Response</th>
<th>Age</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 NO</td>
<td></td>
</tr>
<tr>
<td>2 YES</td>
<td>At what age did these begin? __________</td>
</tr>
</tbody>
</table>

4. Did your child have any immune disorder (e.g., rheumatoid arthritis, lupus erythematosus)?

<table>
<thead>
<tr>
<th>Response</th>
<th>Age</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 NO</td>
<td></td>
</tr>
<tr>
<td>2 YES</td>
<td>At what age was it diagnosed? __________</td>
</tr>
</tbody>
</table>

5. Did your child ever have x-rays of the abdomen below the waist?

<table>
<thead>
<tr>
<th>Response</th>
<th>Age</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 NO</td>
<td></td>
</tr>
<tr>
<td>2 YES</td>
<td>At what age(s)? __________</td>
</tr>
</tbody>
</table>
6. Before age 16, did your child take any growth hormone?
   1 NO
   2 YES  At what age(s) did this begin? _____
          At what age(s) did this stop? _____

7. Did your child take any cortisone either orally or by injection?
   1 NO
   2 YES  At what age(s) did this begin? _____
          At what age(s) did this stop? _____

8. Did your son have any of the following conditions of the groin or scrotum before age 16?
   If so, at what age? Which side had the abnormality? What was the treatment, if any?

<table>
<thead>
<tr>
<th>Condition</th>
<th>Age</th>
<th>Side</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>HERNIA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>UNDESCENDED TESTICLE (NOT PRESENT IN THE LOWER SAC AT BIRTH)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TESTICULAR INFECTION</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TESTICULAR INJURY</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>OTHER TESTICULAR ABNORMALITY (please specify)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

9. When did these events occur compared to other boys his own age?
   voice change   EARLIER LATER AT THE SAME TIME
   starting to shave EARLIER LATER AT THE SAME TIME
   body hair (chest, pubic, armpit) EARLIER LATER AT THE SAME TIME
   growth spurt   EARLIER LATER AT THE SAME TIME

If you are _____________'s natural (biological) mother, please continue.
If not, thank you for your help.
The following questions refer to your pregnancy with ____________.

10. Did your doctor use pills or injections to determine that you were pregnant?
   1. NO
   2. YES

11. Did you take birth control pills at any time during the year before you became pregnant?
   1. NO
   2. YES → To the best of your knowledge, had you stopped taking the pill before you became pregnant?
      1. NO → For how many months after you became pregnant did you take the pill? __________
      2. YES → How many months after you stopped taking the pill did you become pregnant? __________

12. Did you have any illnesses (e.g., German measles, flu, etc.) during this pregnancy?
   1. NO
   2. YES → (a) What did you have? ________________________________
       (b) During which month(s) of this pregnancy? ________________
       (c) Did you have a fever? → 1. NO
       2. YES

13. Did you take any hormones [e.g., DES (diethylstilbestrol), Premarin, etc.] during this pregnancy?
   1. NO
   2. YES → (a) What did you take? ________________________________
       (b) During which month(s) of this pregnancy did you take it? __________

14. Did you take any other prescription medication during this pregnancy?
   1. NO
   2. YES → (a) What did you take? ________________________________
       (b) During which month(s) of this pregnancy did you take it? __________
15. During this pregnancy, were you hospitalized or treated by a doctor for morning sickness (nausea and/or vomiting)?

1 NO
2 YES →
   (a) Were you hospitalized?
       1 NO
       2 YES
   (b) Did you take medication?
       1 NO
       2 YES
   (c) Did you lose weight because of this morning sickness?
       1 NO
       2 YES

16. Did you have x-rays of your abdomen below the waist during this pregnancy?

1 NO
2 YES → During which month(s) of this pregnancy? ________

17. Did you smoke cigarettes during this pregnancy?

1 NO
2 YES →
   (a) During which month(s) of this pregnancy? ________
   (b) About how many cigarettes per day? ________

18. Did you drink any alcoholic beverages (e.g., beer, wine, or liquor) during this pregnancy?

1 NO
2 YES →
   (a) During which months of this pregnancy? ________
   (b) About how many drinks per week? ________

19. Were you employed in the year before this pregnancy?

1 NO
2 YES →
   (a) What was your job title(s)? ________________________
   (b) What was the type of industry or business? ________________________

20. Were you employed during this pregnancy?

1 NO
2 YES →
   (a) For which months of this pregnancy did you work? ________
   (b) What was your job title(s)? ________________________
   (c) What was the type of industry or business? ________________________
21. Was the father of the child employed in the year before this pregnancy?
   1 NO
   2 YES — (a) What was his job title(s)? ________________________
               (b) What was the type of industry or business? __________

22. Was he employed during this pregnancy?
   1 NO
   2 YES — (a) During which months of your pregnancy did he work? _________
               (b) What was his job title(s)? ________________________
               (c) What was the type of industry or business? __________

23. Did the father of the child smoke cigarettes regularly at the time you became pregnant?
   1 NO
   2 YES — About how many cigarettes per day? _______

24. During this pregnancy, did you ever sleep on a heated waterbed or with an electric blanket turned on?
   1 NO
   2 YES — During what month(s) of this pregnancy? _______

25. Did you ever use a whirlpool or sauna during this pregnancy?
   1 NO
   2 YES — During what month(s) of this pregnancy? _______

26. Did the doctor give you hormones to start labour or to help labour progress?
   1 NO
   2 YES

27. What type of birth was it?
   1 HEAD FIRST
   2 BREECH
   3 CAESARIAN

28. Did you breastfeed?
   1 NO
   2 YES — For how many months? _______
29. Was there anything unusual about this pregnancy, birth or early childhood that you would like to mention?

Now we would like to ask some questions about you.

30. What is your date of birth? _____ / _____ / _____

31. What is the highest level of education that you have completed?

1. SOME OR ALL ELEMENTARY SCHOOL (GRADE SCHOOL)
2. SOME OR ALL SECONDARY SCHOOL (HIGH SCHOOL)
3. COMMUNITY COLLEGE OR SOME UNIVERSITY
4. COMPLETED UNIVERSITY

32. How tall are you? _____ FT _____ IN or _____ CM

33. At what age did you begin your menstrual periods? _____

34. Have you stopped having regular periods?

1. NO
2. YES ——> (a) At what age? _____
   (b) Did this occur naturally or surgically? _____

35. How would you describe your menstrual cycles during your 20's and 30's?

1. REGULAR, LESS THAN 21 DAYS
2. REGULAR, 21-35 DAYS
3. REGULAR, 36 DAYS OR MORE
4. IRREGULAR

36. During your 20's and 30's, how many days did your menstrual periods last?

1. LESS THAN 3 DAYS
2. 3 - 6 DAYS
3. MORE THAN 6 DAYS
4. VARIABLE
37. During your 20's and 30's, did you ever go without having a period for more than 3 months, except during or after a pregnancy?

1 NO
2 YES → (a) How many times did this happen? ______
   (b) When this happened, how long did you usually go without a period? ______

38. Have you ever been treated by a doctor for problems related to your menstrual periods?

1 NO
2 YES → (a) What was the problem(s)? ________________________________
   (b) What was the treatment? ________________________________
   (c) At what age(s) did you receive treatment? ______

39. Have you ever used birth control pills regularly for any reason?

1 NO
2 YES → please fill in the chart below for each period of time that you used birth control pills

<table>
<thead>
<tr>
<th>age started</th>
<th>age stopped</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

40. Have you ever had difficulty getting pregnant (i.e. tried for a year or more)?

1 NO
2 YES → Have you ever been told by a doctor that you had a fertility problem?

1 NO
2 YES → What was the cause? ________________________________

41. Have you ever had cancer?

1 NO
2 YES → (a) What type(s)? ________________________________
   (b) At what age(s)? ________________________________

42. Do you have diabetes?

1 NO
2 YES → (a) At what age was it diagnosed? ______
   (b) Is it treated by insulin?
      1 NO
      2 YES
43. Your age at pregnancy
44. Age of partner at pregnancy
45. Were you using any medication to help you become pregnant?
46. If you had any of the following conditions, during which months of pregnancy did they occur?
47. Your weight before pregnancy
48. What was the outcome of the pregnancy?
49. Were there any defects noted at birth or during the first year of life?

**FIRST PREGNANCY**

<table>
<thead>
<tr>
<th>Condition</th>
<th>Month(s) of Pregnancy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Threatened miscarriage</td>
<td></td>
</tr>
<tr>
<td>Bleeding/spotting</td>
<td></td>
</tr>
<tr>
<td>Nausea treated by a physician</td>
<td></td>
</tr>
<tr>
<td>Vomiting</td>
<td></td>
</tr>
<tr>
<td>Toxemia/eclampsia</td>
<td></td>
</tr>
</tbody>
</table>

- **LBs or KG**

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Year</th>
</tr>
</thead>
<tbody>
<tr>
<td>Live birth</td>
<td></td>
</tr>
<tr>
<td>Stillbirth</td>
<td></td>
</tr>
<tr>
<td>Miscarriage or spontaneous abortion</td>
<td></td>
</tr>
<tr>
<td>Therapeutic abortion</td>
<td></td>
</tr>
</tbody>
</table>

- **LBs or KG**

**SECOND PREGNANCY**

<table>
<thead>
<tr>
<th>Condition</th>
<th>Month(s) of Pregnancy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Threatened miscarriage</td>
<td></td>
</tr>
<tr>
<td>Bleeding/spotting</td>
<td></td>
</tr>
<tr>
<td>Nausea treated by a physician</td>
<td></td>
</tr>
<tr>
<td>Vomiting</td>
<td></td>
</tr>
<tr>
<td>Toxemia/eclampsia</td>
<td></td>
</tr>
</tbody>
</table>

- **LBs or KG**

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Year</th>
</tr>
</thead>
<tbody>
<tr>
<td>Live birth</td>
<td></td>
</tr>
<tr>
<td>Stillbirth</td>
<td></td>
</tr>
<tr>
<td>Miscarriage or spontaneous abortion</td>
<td></td>
</tr>
<tr>
<td>Therapeutic abortion</td>
<td></td>
</tr>
</tbody>
</table>

- **LBs or KG**

50. Your weight gain during pregnancy
51. Sex of child
52. Child's date of birth
53. Child's weight at birth
54. When was the child born in relation to the due date?
including any stillbirths, therapeutic abortions, and miscarriages.

<table>
<thead>
<tr>
<th>THIRD PREGNANCY</th>
<th>FOURTH PREGNANCY</th>
<th>FIFTH PREGNANCY</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 NO</td>
<td>1 NO</td>
<td>1 NO</td>
</tr>
<tr>
<td>2 YES</td>
<td>2 YES</td>
<td>2 YES</td>
</tr>
<tr>
<td>What medication(s)?</td>
<td></td>
<td>What medication(s)?</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>month(s) of pregnancy</td>
<td>month(s) of pregnancy</td>
<td>month(s) of pregnancy</td>
</tr>
<tr>
<td>1 THREATENED MISCARRIAGE</td>
<td>1 THREATENED MISCARRIAGE</td>
<td>1 THREATENED MISCARRIAGE</td>
</tr>
<tr>
<td>2 BLEEDING/SPOTTING</td>
<td>2 BLEEDING/SPOTTING</td>
<td>2 BLEEDING/SPOTTING</td>
</tr>
<tr>
<td>3 NAUSEA TREATED BY A PHYSICIAN</td>
<td>3 NAUSEA TREATED BY A PHYSICIAN</td>
<td>3 NAUSEA TREATED BY A PHYSICIAN</td>
</tr>
<tr>
<td>4 VOMITING</td>
<td>4 VOMITING</td>
<td>4 VOMITING</td>
</tr>
<tr>
<td>5 TOXEMIA/ ECLAMPSIA</td>
<td>5 TOXEMIA/ ECLAMPSIA</td>
<td>5 TOXEMIA/ ECLAMPSIA</td>
</tr>
<tr>
<td>1 DIPHTHERIA</td>
<td>LBS or KG</td>
<td>LBS or KG</td>
</tr>
<tr>
<td>1 LIVE BIRTH</td>
<td></td>
<td>1 LIVE BIRTH</td>
</tr>
<tr>
<td>2 STILLBIRTH</td>
<td></td>
<td>2 STILLBIRTH</td>
</tr>
<tr>
<td>3 MISCARRIAGE OR SPONTANEOUS ABORTION</td>
<td></td>
<td>3 MISCARRIAGE OR SPONTANEOUS ABORTION</td>
</tr>
<tr>
<td>4 THERAPEUTIC ABORTION</td>
<td></td>
<td>4 THERAPEUTIC ABORTION</td>
</tr>
<tr>
<td>1 NO</td>
<td></td>
<td>1 NO</td>
</tr>
<tr>
<td>2 YES</td>
<td></td>
<td>2 YES</td>
</tr>
<tr>
<td>What were they?</td>
<td></td>
<td>What were they?</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LBS or KG</td>
<td></td>
<td>LBS or KG</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>day  / month / year</td>
<td></td>
<td>day  / month / year</td>
</tr>
<tr>
<td>GMS or LBS OZ</td>
<td></td>
<td>GMS or LBS OZ</td>
</tr>
<tr>
<td>1 ON TIME (WITHIN 2 WEEKS)</td>
<td></td>
<td>1 ON TIME (WITHIN 2 WEEKS)</td>
</tr>
<tr>
<td>2 MORE THAN 2 WEEKS EARLY</td>
<td></td>
<td>2 MORE THAN 2 WEEKS EARLY</td>
</tr>
<tr>
<td>3 MORE THAN 2 WEEKS LATE</td>
<td></td>
<td>3 MORE THAN 2 WEEKS LATE</td>
</tr>
</tbody>
</table>
APPENDIX 2. Subjects questionnaire (selected questions).
37. For each of the conditions listed below, please indicate which side(s), your age at diagnosis, and the treatment (surgery and/or hormones).

*(if you had none of these, check the box beside NO PROBLEM)*

<table>
<thead>
<tr>
<th>Condition</th>
<th>Side (left, right)</th>
<th>Age at Diagnosis</th>
<th>Treatment (surgery, hormones)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hernia of the groin</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Undescended testicle</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Testicle not present in the lower sac at birth)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Testicular infection</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Testicular injury</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other testicular abnormality</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>(please specify)</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

38. During adolescence, when did these events occur compared to other boys? *(circle response)*

- Voice change
- Starting to shave
- Body hair (chest, pubic, armpit)
- Growth spurt

39. Have you and your partner ever conceived a child? *(circle number)*

1. No
2. Yes
APPENDIX 3. Agreement (Kappa statistic and 95% confidence interval) between subject and mother responses to relative age at events marking puberty in the subject.
APPENDIX 3. Agreement (Kappa statistic and 95% confidence interval) between subject and mother responses to relative age at events marking puberty in the subject.

<table>
<thead>
<tr>
<th>Pubertal event</th>
<th>Overall</th>
<th>Earlier</th>
<th>Later</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shave</td>
<td>0.25 (0.18, 0.31)</td>
<td>0.29 (0.21, 0.37)</td>
<td>0.30 (0.23, 0.38)</td>
</tr>
<tr>
<td>Voice</td>
<td>0.18 (0.10, 0.26)</td>
<td>0.17 (0.08, 0.26)</td>
<td>0.23 (0.11, 0.35)</td>
</tr>
<tr>
<td>Growth</td>
<td>0.15 (0.09, 0.22)</td>
<td>0.12 (0.04, 0.20)</td>
<td>0.18 (0.10, 0.27)</td>
</tr>
<tr>
<td>Hair</td>
<td>0.17 (0.10, 0.23)</td>
<td>0.14 (0.06, 0.21)</td>
<td>0.20 (0.12, 0.29)</td>
</tr>
</tbody>
</table>
APPENDIX-4.  Age distribution (%) of controls and cases (by histologic group).
APPENDIX-4.  

Age distribution (%) of controls and cases (by histologic group).

<table>
<thead>
<tr>
<th>Age</th>
<th>Controls</th>
<th>Cases</th>
<th>Nonseminoma†</th>
<th>Seminoma</th>
</tr>
</thead>
<tbody>
<tr>
<td>15-19</td>
<td>7.9%</td>
<td>3.2%</td>
<td>8.4%</td>
<td>1.1%</td>
</tr>
<tr>
<td>20-24</td>
<td>19.6%</td>
<td>17.6%</td>
<td>33.6%</td>
<td>5.0%</td>
</tr>
<tr>
<td>25-29</td>
<td>24.0%</td>
<td>26.9%</td>
<td>31.8%</td>
<td>23.7%</td>
</tr>
<tr>
<td>30-34</td>
<td>21.7%</td>
<td>24.9%</td>
<td>9.4%</td>
<td>35.7%</td>
</tr>
<tr>
<td>35-39</td>
<td>14.0%</td>
<td>15.6%</td>
<td>10.3%</td>
<td>19.9%</td>
</tr>
<tr>
<td>40-44</td>
<td>6.3%</td>
<td>9.0%</td>
<td>6.5%</td>
<td>9.8%</td>
</tr>
<tr>
<td>45-49</td>
<td>4.0%</td>
<td>1.7%</td>
<td>0.0%</td>
<td>2.7%</td>
</tr>
<tr>
<td>50-54</td>
<td>2.5%</td>
<td>1.2%</td>
<td>0.0%</td>
<td>2.2%</td>
</tr>
<tr>
<td>N=</td>
<td>521</td>
<td>346</td>
<td>107</td>
<td>181</td>
</tr>
</tbody>
</table>

† Excluding tumours of mixed histology containing seminoma.
APPENDIX 5. Number of prevented cases of testicular germ cell cancer due to later puberty.
APPENDIX-5. Number of prevented cases of testicular germ cell cancer due to later puberty (Kleinbaum et al., 1982).

<table>
<thead>
<tr>
<th>Puberty Score</th>
<th>Cases†</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Same time or earlier puberty</td>
<td>369</td>
<td>643</td>
</tr>
<tr>
<td>Later puberty‡</td>
<td>111</td>
<td>290</td>
</tr>
<tr>
<td>Total</td>
<td>480</td>
<td>933</td>
</tr>
</tbody>
</table>

† Restricted to participating cases who reported 3 or more puberty events.
‡ Case reported 1 or more later puberty events.

OR = 0.67

Prevented fraction (PF) of cases:

(a) \( PF = (I^*/I^* + I) \)

(b) \( PF = \frac{\text{Prev}(1-OR)}{\text{Prev}(1-OR) + OR} \)

Where:
- \( PF \) = Prevented fraction of cases.
- \( \text{Prev} \) = estimated prevalence of exposure among cases.
- \( I^* \) = Number of cases prevented.
- \( I \) = Number of cases that did occur.

(b) \( PF \) (due to later puberty) = \( \frac{[111/480](1-0.67)}{[111/480](1-0.67) + 0.67} = 18.8\% \)

Estimated number of cases prevented (\( I^* \)):

If \( PF = (I^*/I^* + I) \) then \( I^* = (PF \times I^*)/(1-PF) \)

\( I^* = (480(18.8\%))/(1-18.8\%) = 111 \) cases