

CRYPTORCHIDISM AND MALE FERTILITY:
A STUDY OF THE DETERMINANTS OF INFERTILITY
AMONG FORMERLY CRYPTORCHID AND CONTROL MEN

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Background: Cryptorchidism is a failure of the testis to descend into the scrotum from its initial site of development in the abdomen. The failure of the testis to descend results in significant histologic changes to the testicular tissues and increases risk for infertility and testicular cancer. Incidence of cryptorchidism is approximately 3% at birth declining to 1% at one year of age because of spontaneous descent. Little to no spontaneous descent occurs after six months of age and surgical correction by orchiopexy is recommended by age one to two.

Study Cohort: The Children's Hospital of Pittsburgh Male Fertility Study has been looking at the impact of cryptorchidism on male fertility since 1992. The study cohort comprises 1405 former cryptorchids and control men. Each of the subjects completed an extensive questionnaire that included questions on marriage and cohabitation, paternity, health problems, and environmental/occupational exposures. A subset of the full cohort returned in adulthood for evaluation of hormone levels and semen analysis (n=167).

Results: The articles presented here represent a selection of the study results looking at time to conception among formerly cryptorchid men, the impact of testicular suture on fertility, and the influence of age at orchiopexy on hormone levels and sperm count. Time to conception is

significantly increased among formerly bilateral (33.9 months), but not unilateral cryptorchid men (11.1 months) as compared to control men (8.8 months). Placement of a transparenchymal suture during orchiopexy greatly increases the risk of infertility (RR 7.56) among formerly cryptorchid men. Age at orchiopexy is significantly negatively correlated ($r = -0.274$) with inhibin B and positively correlated ($r = 0.229$) with FSH.

Conclusions: Cryptorchidism negatively impacts fertility in the human male. It can increase time to conception and reduce sperm counts, especially among formerly bilateral cryptorchid men. Surgical technique utilizing placement of a suture through the testis can greatly increase risk for future infertility and should be avoided. With an incidence of 3% at birth and a prevalence of 1% at one year, it represents the single most common birth defect among human males. Cryptorchidism is of public health importance because it leads to significantly increased risks for both testicular cancer and infertility. Future research should focus on identifying environmental and behavioral causes of cryptorchidism and on optimizing treatment.

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1. Cryptorchidism and Male Fertility

1.1. Introduction

Cryptorchidism is the condition of non-descent of the testis, and the diagnosis is defined as deviation or arrest of the testis at any point along the normal path of its descent from an origin below the kidneys down into the scrotal sac (Rezvani, 1987). Undescended testis is the most common genital anomaly identified at birth among human males. Cryptorchidism occurs in approximately 3% of human males at birth and decreasing to 1% during the neonatal period because of spontaneous descent (Berkowitz, 1993). The most common treatment for this condition over the last 50 years has been surgical correction by orchiopexy. The undescended testis represents the single most common disorder of male sexual differentiation. There are two potential negative outcomes of cryptorchidism, infertility and testicular cancer. Despite the widespread frequency of undescended testis, there is a persistent lack of knowledge about many aspects of its etiology, natural history, treatment, and outcomes. The overall goal of this work will be to better understand the impact of cryptorchidism on fertility.

Undescended testes are one of the most common causes of infertility among males. Despite the knowledge that male specific fertility problems account for 30-50% of the infertility among couples (Bhasin, 1994; Thompson, 1994), there are very few data addressing specific potential causes of male infertility when compared to the abundance of research on causes of infertility in women. The Children's Hospital of Pittsburgh Male Fertility Study has been investigating potential for fertility after orchiopexy among a large group of formerly cryptorchid men and a matched set of controls. This study of formerly cryptorchid men and controls who underwent surgery at Children's Hospital between the years of 1955 and 1975 was designed to address the

following primary hypotheses; (1) Men who have a history of bilateral or unilateral cryptorchidism will have a higher prevalence of infertility as measured by paternity than matched controls; (2) Formerly cryptorchid males who are infertile will have a later age at surgery, higher pre-treatment location of their undescended testis, and will be more likely to have had placement of a testicular suture as compared with fertile formerly cryptorchid males. The study also collected data on other potential causes of infertility unrelated to cryptorchidism, including varicocele, sexually transmitted disease, partner conception problems, lifestyle factors (smoking, alcohol use, drug use), and potential chemical exposures. The study has established a large (n=1405) cohort of formerly cryptorchid and control men who had surgery at the Children's Hospital of Pittsburgh. Published data from the study indicate that fertility is markedly reduced among married or cohabiting men with a history of bilateral cryptorchidism, with only 59.4% success in fathering children, as compared to 93.8% of control men (Lee et al., 1997). Fertility is also reduced among the formerly unilaterally cryptorchid group with only 88.4% of those attempting having children (Lee et al., 1996). The results of the CHP Male Fertility Study published to date show conclusively that cryptorchidism is a strong risk factor for male infertility.

1.2. Cryptorchidism and Orchiopexy

Cryptorchidism was first described in detail by John Hunter in 1786, and the first attempts at surgery (orchiopexy) to correct maldescent were made by Rosenmerkal in 1820 (Hinman, 1991). The significance of having both testes descended was recognized centuries ago when *duo testes bene pendulum* (two well descended testes) was a requirement for priesthood candidates in the early Middle Ages. Pathologic changes have been noted in the undescended testis in studies as

far back as the 1930's. It was also recognized at this time that the testis of very young children with cryptorchidism could be histologically normal (Cooper, 1929). More recent histopathologic studies of cryptorchid testes have shown a progressive deterioration of germ and Leydig cells the longer the testis remains in the cryptorchid state (Huff, 1991, 1993).

Significant negative outcomes related to cryptorchidism are testicular carcinoma and infertility. Studies have shown an increased relative risk for testicular cancer of from 4 to 10 times for cryptorchid men (Campbell, 1959; Giwercman, 1988; Haughey, 1989). While orchiopexy does not appear to protect against subsequent carcinoma (Chilvers, 1986), a testis in a scrotal position facilitates more rapid diagnosis of any malignant change. However, good data on children who underwent orchiopexy before age five years have yet to be accumulated. Excess risk of carcinoma associated with cryptorchidism has been shown to be positively associated with age at orchiopexy (Pottern, 1985). The prevailing beliefs are that significant damage occurs to the testis beginning as early as six months of age, few testes descend spontaneously after one year of age, and that advances in the field of pediatric anesthesia allow for earlier intervention to correct cryptorchidism by surgical orchiopexy (American Academy of Pediatrics, 1996).

The second negative outcome of cryptorchidism is reduction in fertility among affected men. This reduction has been observed in both unilateral and bilateral maldescent, but is more profound in men with bilateral cryptorchidism. Previous investigations by Chilvers et al. (1986) found rates of oligospermia (low sperm count) and azoospermia (no sperm) of 31% and 14% among formerly unilateral cryptorchids and 31% and 42%, respectively, among formerly bilaterally cryptorchid men. Fertility assessed by paternity shows similar patterns of reduced fertility in formerly cryptorchid men. The "normal" paternity rate is believed to be about 85%,

while paternity among formerly unilaterally cryptorchid men is 60% to 80%, and among formerly bilateral cryptorchids is 50% to 60% (Elder, 1988). Most studies of fertility in formerly cryptorchid men to date have occurred in populations of men where orchiopexy was performed without the realization that testicular damage occurs by two years of age. The recent recognition of the early age of testicular damage has led to the recommendation for correction of maldescended testis before the age of two in order to maximize fertility potential and to reduce the potential for cancer mortality.

1.3. The Children's Hospital Male Fertility Study

The Children's Hospital of Pittsburgh Male Fertility Study has been investigating risk factors for male infertility related to cryptorchidism since 1992. The current database includes detailed records for 1405 formerly cryptorchid men and matched controls. This large well-defined cohort was identified by medical record review of patients who were surgically treated between 1955 and 1975. The case group comprises men who were surgically treated for cryptorchidism by orchiopexy at Children's Hospital of Pittsburgh. The control group was ascertained by medical record review and underwent a surgical procedure thought to be unlikely to affect future fertility (appendectomy, tonsillectomy, adenoidectomy, circumcision, or orthopedic procedure). The controls are matched to cases on date of surgery (± 2 months), age at surgery (± 3 years), and race. Paternity outcomes published to date have agreed with data from earlier studies of cryptorchidism. Formerly bilaterally cryptorchid men have shown markedly reduced paternity rates as compared to controls, with only 59.4% of those attempting paternity having success in comparison to 93.8% of control men. Among the unilateral group 88.4% who attempted achieved paternity. Both of these comparisons are statistically significant ($p < 0.05$). Relative

risks computed from these figures show formerly bilateral males to be 6.6 times, and formerly unilateral males 1.6 times more likely than controls to be infertile (Lee 1996, 1997).

1.4. Subfertility and Time to Conception

Previous studies have shown comparable paternity reductions in formerly cryptorchid men, and others have shown impaired semen characteristics among men with undescended testes. While these gross comparisons of paternity and sperm characteristics are vital to our understanding of the impact of cryptorchidism on fertility, they do little to increase our understanding of the complex interaction of factors which characterize infertility. Cryptorchidism is not the only cause of infertility among males. Other causes include infectious agents (sexually transmitted diseases, mumps), chemical exposures (lead, chemotherapeutic agents), radiation, and varicocele. There are also numerous potential causes of infertility in the man's female partner (ovulatory disorders, tubal infertility). Infertility can also result from a combination of subtle male and female factors, which when combined lead to infertility in the couple. The measurement of paternity or semen characteristics also tells us nothing about the determinants of infertility, or the effect of subfertility. Subfertility is defined as a delay in conception. In the present study it is defined as conception occurring after longer than one year of intercourse without the use of contraception. The overall goal of this work is to better understand the determinants of infertility (and subfertility) among formerly cryptorchid and control men.

Article #1 presents the results of an evaluation designed to measure subfertility by analysis of time to conception among the formerly cryptorchid and control men in the cohort. Time to conception is defined as the number of months required for a couple to conceive a child (Baird et

al., 1985; 1986). Measurement of time to conception allows us to compute fecundability, the monthly probability of conception. Fecundability is a measure of fertility which allows us to consider factors which can reduce conception probability, but which may not cause frank infertility. Infertility represents only one of the potential fertility reducing effects of cryptorchidism. Cryptorchidism is also known to lower sperm counts (Puri, 1988; Yavetz, 1992; Mandat, 1994), and in this way has the potential to reduce fecundability and thereby cause subfertility. A substantial body of research on subfertility in women already exists (Rachootin, 1982; Baird, 1985; Bolumar, 1996; Correa, 1996). Research on causes of male subfertility is confined to results from studies of women which include a few questions about their male partners (Bolumar, 1996), and one study other than article #1 which measured time to pregnancy using data from men (Joffe and Li, 1994). In the current study, subfertility was measured by univariate Kaplan Meier survival analysis, and multiple regression using a proportional hazards model (Cox, 1972). These statistical methods allow comparison of survival curves between different groups of subjects (bilateral, unilateral, and control), and allowed for the inclusion of a number of potential covariates which may be associated with time to conception (major illness, smoking, varicocele, partner conception problem).

1.5. Testicular Suture and Fertility

Article #2 presents the results of an analysis of the impact of placement of a transparenchymal testicular suture during orchiopexy. These sutures are placed through the testis and were utilized to secure the testis in the scrotal sac and to prevent re-ascent of the testis. It has been hypothesized that disruption of the blood-testis barrier that may be caused by testicular biopsy or the placement of a suture through the testis to anchor the testis in the scrotum could have a negative

impact on spermatogenesis, such as the development of anti-sperm antibodies. Laboratory animal studies suggest that placement of a testicular suture generates a potent inflammatory response that can cause local necrosis, vascular damage, and a significant immunologic response (Bellinger et al, 1989). A study searching for anti-sperm antibodies in semen of men who had had testicular biopsy at puberty failed to find antibodies (Cortes et al, 1990). An assessment of the Children's Hospital of Pittsburgh cohort of formerly cryptorchid men, also failed to find an increased incidence of antibody titers among boys operated upon during childhood (unpublished data). The analysis of the Children's Hospital cohort presented as article #2 found evidence of a significantly increased risk of infertility associated with the placement of a testicular suture (RR=7.56, 95% CI 1.66-34.39) (Coughlin et al, 1998). Bilateral cryptorchidism, varicocele, hormone treatment before orchiopexy, and partner conception problems also increased risk of infertility in men with a history of cryptorchidism.

1.6. Age at Orchiopexy and Potential Fertility

The analysis presented in Article #3 evaluates the impact of age at orchiopexy on fertility potential in adulthood. It has been hypothesized that earlier treatment will be followed by a decreased risk of infertility, but this has not previously been conclusively demonstrated. A 1975 report of 36 patients found that fertility was greater when patients were younger at the time of orchiopexy (Ludwig and Potempa, 1975). Another study of men with bilateral cryptorchidism, also reported an inverse correlation between age of orchiopexy and sperm concentration (Engeler et al, 2000). However, a meta-analysis by Chilvers et al (1986) analyzed the age of treatment and found no difference when comparing both unilateral and bilateral cryptorchidism treated before and after 9 years of age. Unfortunately, no patient in the Chilvers' analysis was treated during

the first few years of life. Efficacy of early therapy has been questioned even more for unilateral cryptorchidism. A study comparing patients having had surgery between 2 and 7 years of age with those having surgery between 10 and 12 years of age found no difference in fertility potential as an adult based upon testicular volume, LH, FSH and testosterone levels, and semen analyses (Cortes et al, 1996). Among another group of men who had had unilateral and bilateral cryptorchidism, again, sperm density was not found to correlate with age at the time of surgery (Gracia et al, 2000).

The analysis presented as article #3 (Coughlin et al, 1999) suggested a relationship between age at orchiopexy and hormone and semen measures of fertility potential, but subsequent analyses have failed to confirm this (Lee and Coughlin, 2001; Miller, Coughlin and Lee, 2002). However, the story remains incomplete as only a portion of these men have yet had attempted or demonstrated paternity and even fewer have been available for physical examination, hormone and semen studies. However, a third study in the Pittsburgh cohort did suggest an association between age of orchiopexy and testosterone levels among the unilateral group (Lee and Coughlin, 2002). Although all men evaluated had circulating testosterone levels within the adult male range, there was an inverse relationship with age of orchiopexy. This subtle finding is surprising and implies a risk of diminished Leydig cell secretion potential with the retention of the testis after infancy. The finding is also consistent with the finding of atrophy of Leydig cells in the cryptorchid testis (Hadziselimovic and Herzog, 1976). It may well be that there are subtle differences in hormone levels related to age at orchiopexy, but that these levels have little to do with the ability of formerly cryptorchid men to father children. However, additional studies will be necessary to confirm this hypothesis.

2. **Article #1: Time to conception after orchidopexy: evidence for subfertility?***

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2.1. Abstract

Objective: To determine whether time to conception is increased among men who were formerly bilaterally or unilaterally cryptorchid in comparison to a group of matched control men.

Design: Retrospective Cohort Study.

Setting: Human volunteers in an academic research environment.

Patients: Men who underwent orchidopexy between 1955 and 1971 at the Children's Hospital of Pittsburgh (n=547) and a group of matched control men (n=463) were surveyed by questionnaire.

Results: Of the men who attempted paternity the median time to conception for formerly bilateral men was 14 months, and 2 months for formerly unilateral and control men. Kaplan-Meier survival analysis showed a significantly longer time to conception among bilateral cases when compared to unilateral cases and controls, but not between unilateral cases and control men. Adjustment for confounders and covariates using a Cox Proportional Hazards model showed that formerly bilaterally cryptorchid men were 68% (95% C.I.=55% to 81%) less likely than formerly unilateral cryptorchid men or controls to conceive per month of unprotected intercourse.

Conclusions: Time to conception was increased among formerly bilaterally cryptorchid men when compared to both formerly unilaterally cryptorchid and control men. However, there were no significant differences in time to conception between the unilateral cryptorchid men and the control men. *Fertil Steril* 1997; 67:742-6.

Key Words: Cryptorchidism, Time to Conception, Orchidopexy, Epidemiology.

2.2. Introduction

Cryptorchidism appears to be a primary cause of male infertility. Previous research investigating cryptorchidism and orchidopexy is inconclusive as to the degree to which infertility occurs among men who have had either bilateral or unilateral cryptorchidism during childhood. Previous reports have indicated widely varying rates of paternity after orchidopexy (1-6). It is difficult to make conclusions concerning the previous research as different endpoints (semen and paternity), potentially biased samples (e.g. infertility clinics), and small sample sizes (e.g. 28-78), have weakened these earlier findings (1-6). The Children's Hospital of Pittsburgh Male Fertility Study is determining if male fertility is impaired by maldescent of one or both testes, and examining the effects of subsequent surgical correction by orchidopexy. The current cohort is much larger than that of other studies (>1000 individuals), is a generalizable sample of the Allegheny County Pennsylvania population, and utilizes paternity information which is likely a better measure of fertility than semen as men with impaired sperm counts still may father children (4,6).

An important potential adverse outcome of cryptorchidism and orchidopexy is the possibility of subfertility; that is, the ability to father children, but with longer waiting times to conception. In the present analysis, time to conception was compared between men who underwent orchidopexy and control men to determine if time to conception is significantly increased among formerly cryptorchid men in comparison to control men. Previous reports have not attempted to do this, but have rather reported on either semen parameters or absolute paternity, i.e. fathered a child or not. This approach utilizing time to conception has been well developed in the fertility and occupational hazards literature, but has not been used when examining male infertility

related to undescended testes (7-12). It has been shown by several authors that collection of retrospective data on time to pregnancy is feasible and generates sufficient statistical power to detect moderately reduced fertility utilizing relatively few pregnancies (8,10).

2.3. Materials and Methods

2.3.1. Subjects

Men who had undergone an orchidopexy for either bilateral or unilateral cryptorchidism at Children's Hospital of Pittsburgh (CHP) between 1955 and 1971 were identified by a review of medical records. During this time period greater than 85% of all orchidopexies performed in Allegheny County, which includes the City of Pittsburgh, were performed at CHP. Therefore this sample is likely representative of the Allegheny County general population. Institutional Review Board (IRB) approval was secured from the Children's Hospital of Pittsburgh Human Subjects Committee. Selection criteria excluded all subjects with retractile testes. Control subjects, matched for age (± 3 years), date of surgery (± 2 months) for a minor problem (e.g. adenoidectomy (n=4), tonsillectomy and adenoidectomy (n=258), circumcision (n=54), appendectomy (n=139), or orthopedic procedure (n=8), and race were selected by medical record review. Controls were selected and their medical records screened for any conditions that could potentially affect fertility later in life. Conditions that were criteria for exclusion among controls included a history of cryptorchidism or hernia, while exclusion criteria for both cases and controls included hypospadias, retractile testis, severe central nervous system disorder, multiple congenital abnormalities, debilitating chronic disease, or other genitalia abnormalities. The identified case and control men were then located and asked to complete a 70 item questionnaire concerning general health, marriages, co-habitation, use of birth control, length of unprotected

intercourse before conception, miscarriages and elective abortions, lifestyle factors (e.g. smoking, alcohol and drug use), and potential exposure to chemical and other occupational hazards. Detailed data were not collected on the man's spouse other than self-reported physician diagnoses of a conception problem. Surveys have been received from over 70% of the individuals identified for recruitment. Differential response rates between the bilateral, unilateral, and control groups were 74%, 70%, and 67% respectively. The major reason for non-response was that it was not possible to locate the individuals 20-30 years after their surgery. The refusal rate for individuals located was less than 10% for both the cases and controls. Individuals less likely to be located and to complete a survey included men with an earlier date of surgery, and non-white men. The present analysis includes information from 1010 formerly cryptorchid (bilateral n=72, unilateral n=475), and control men (n=463). Of these 1010 men, 471 (224 controls, 247 cases) attempted to conceive a child and had estimated time to conception for the Kaplan-Meier analysis, while 467 men (221 controls, 246 cases) contributed complete data to the Cox Proportional Hazards analysis. All men who attempted to conceive a child without the use of birth control were included in the analyses, whether or not they successfully conceived. Men who failed to conceive after actively attempting are included as censored intervals in both the Kaplan-Meier and Cox analyses.

2.3.2. Statistical Methods

In order to test the null hypotheses that formerly cryptorchid men have similar times to conception as control men, we compared time to conception data from unilateral and bilateral cases, and control men, using Kaplan-Meier Lifetable (13) methods and Cox Proportional Hazards Analysis (14). Time to conception data are self-reports of the time period from the

beginning of unprotected sexual intercourse to conception of a child. Data were analyzed on a Vax-VMS mainframe computer system using the Biomedical Computer Program (BMDP Statistical Software, Inc., Los Angeles, CA) (15). The BMDP 1L and 2L programs were utilized for the Kaplan-Meier Survival and Cox Proportional Hazards analyses respectively. The Kaplan-Meier analysis allows for comparison of time to conception distributions between orchidopexy cases and control men using the log rank test while the Cox analysis allows inclusion of other potential independent contributors to subfertility such as varicocele, smoking, alcohol, frequency of intercourse, contraceptive method used, age at orchidopexy, and occupational exposure(s). It also allowed control for potential confounders such as age and race. The Cox Proportional Hazards model was used to estimate a set of regression coefficients for prognostic factors for time to conception among formerly cryptorchid and control men.

2.4. Results

The demographic characteristics of the entire cohort of 547 case and 463 control men are shown in Table 2-1. There were no significant differences in the racial distributions (94.1% and 96.1% white), mean age (38.2 vs. 38.3 yrs.), mean year of surgery (both 1964), or mean age at surgery (7.2 vs. 7.1 yrs.). These data reflect that the two groups were accurately matched in the initial phase of the study. In addition the cases and controls were similar with respect to mean age at conception (27.8 vs. 27.3), proportion who ever smoked (49.4% vs. 48.3%), proportion reporting a previous sexually transmitted disease (7.5% vs. 7.6%), or reported ever being diagnosed with a varicocele (4.6% vs. 4.1%).

Table 2-1: Comparison of Demographic and Confounding Variables

Variable*	Cases (n=547)	Controls (n=463)
Age (yrs.)	38.2	38.3
Age at Surgery (months)	86.2	85.3
Year of Surgery	1964	1964
Race (% White)	94.1	96.1
BMI(Wt. kg./Ht m ²)	26.7	26.3
Ever Married (%)	69.5	77.1
Ever Smoked (%)	49.4	48.3
Ever Drink (%)	87.2	84.8
Varicocele (%)	4.6	4.1
STD (%)	7.5	7.6
Vasectomy (%)	6.9	8.4
Unplanned Pregnancies (%)†	10.0 (n=211)	15.3 (n=210)
Age at Conception (years)†	27.8 (n=255)	27.3 (n=261)

*Entire cohort: n=1,010. P > 0.05 for all variables.

†Includes only men who achieved a conception and had complete data.

Figure 2-1 shows the univariate Kaplan-Meier survival distributions for the bilateral cases, unilateral cases, and control men. Median time to conception was 14 months for the formerly bilateral group and 2 months for both the formerly unilateral and control groups. Figure 2-1 also presents the statistical comparisons by log rank test of the three distributions, and shows no statistically significant differences between the unilateral and control groups (P>0.05). There is however a significant difference between the survival distributions of the bilateral cases, and the unilateral cases and control men (P<0.001). This indicates longer times to conception for those men who were formerly bilaterally cryptorchid as compared to both the formerly unilaterally cryptorchid and control men. Only 45% of the bilateral men were able to conceive within 6 months, as compared to 69% of the unilateral cases and 70% of the controls. By 1 year 85% of

the controls and 80% of the unilateral cases had conceived, while only 48% of the bilateral cases had successful conceptions.

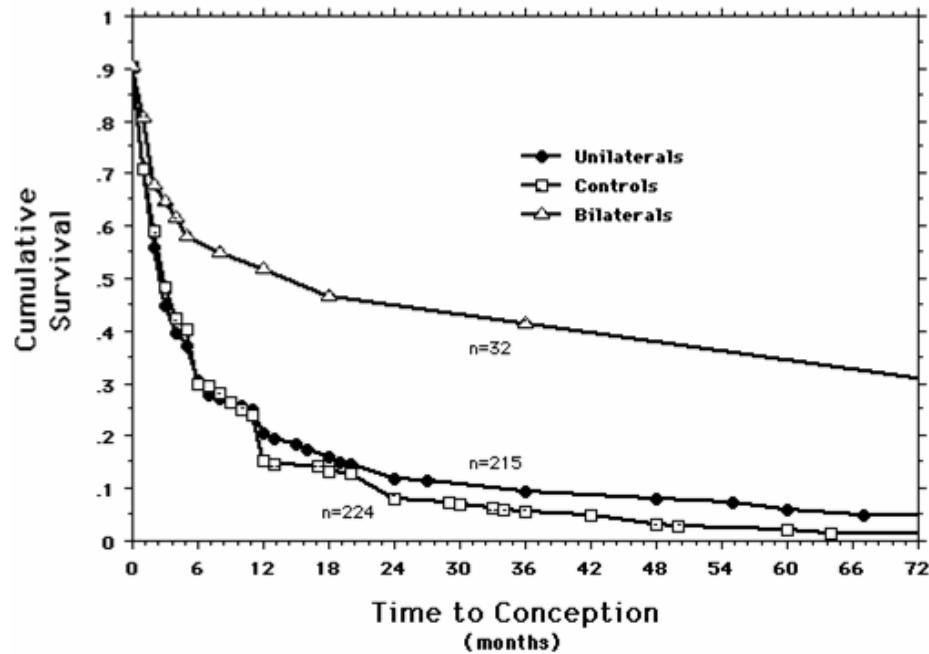


Figure 2-1: Kaplan-Meier Survival time to conception for formerly bilateral and unilateral cryptorchid and control men. *P* values for: bilateral group versus control group = 0.003, bilateral versus unilateral = 0.0007, and unilateral versus control = 0.8023, respectively.

Cox proportional hazards analysis was used to determine if time to conception differed significantly among the three groups after controlling for potential confounders. Variables included in the initial backwards stepwise model were age at study enrollment (continuous), age at surgery (continuous), year of surgery (5-year groups), varicocele (yes/no), mumps (yes/no), sexually transmitted disease (yes/no), epididymitis (yes/no), use of alcohol (yes/no, ever > 30 drinks/month), cigarettes (yes/no, ever regular smoker), other drugs (yes/no, ever regular user), occupational exposure to chemicals (yes/no, ever regular exposure), and conception problems in the female partner (yes/no, told by doctor). These covariates were included because of their

known potential to affect conception rates. The results for the final model are shown in table 2-2. The variables that contributed significantly to the model were conception problems in the female partner, varicocele, and bilateral cryptorchidism. The hazard ratio $\exp(\beta_i)$ can be interpreted as the relative risk of an event for an individual with the risk factor (i.e. cryptorchidism) present compared with an individual with the risk factor absent. Men who were formerly bilaterally cryptorchid were only 32% as likely to conceive a child per month of unprotected intercourse as formerly unilaterally cryptorchid and control men. Men whose wives had conception problems were 28% as likely to conceive as men whose wives did not. Men who had a varicocele were 55% as likely to conceive. Therefore after adjusting for potential confounders, female conception problems and varicocele, formerly bilateral men still have longer times to conception than formerly unilateral men and controls.

Table 2-2: Cox Model Results*

Variable	Relative risk of conception†	P
Bilateral cryptorchidism	0.3204 (0.1874, 0.5478)	<0.001
Partner conception problem	0.2765 (0.1897, 0.4003)	<0.001
Varicocele	0.5525 (0.3389, 0.9008)	0.009

* Number attempting conception: n=467.

† Values in parentheses are confidence interval.

2.5. Discussion

Subfertility is an important potential outcome of cryptorchidism in childhood which has to date been ignored by previous studies of fertility outcome after orchidopexy (1-6). These studies either did not collect time to conception data, had no appropriate control group, or were too small in size to allow for statistical comparison of time to conception data. The Children's Hospital of Pittsburgh Male Fertility Study is the first investigation of the fertility outcome of formerly

cryptorchid men with enough detailed data on time to conception to allow for the analysis of subfertility. The results of this analysis show longer times to conception for formerly bilaterally cryptorchid men as compared to both formerly unilaterally cryptorchid men and controls.

Time to conception is data which can be reliably collected and which do not require a large sample size to detect moderately reduced fertility (8). We attempted to validate the self-report data by comparing men's reported time to conception with a randomly selected sub-sample of their spouses (n=58). The Pearson correlation for these two groups was 0.8402 and was highly significant ($P < 0.0001$). This indicates that in this sample, men as a group were recalling time to conception proportionally to their female partners. Time to conception is not a measure without potential biases and problems. Selection bias associated with contraceptive practice results because of the exclusion of unplanned pregnancies from the analysis. Time to conception data for unplanned pregnancies is not meaningful because couples cannot report time to conception for a pregnancy that was not planned. Because of this, among those couples who do not use birth control carefully, the couples with the highest fecundity will tend to conceive and be excluded from the analysis, while those with the lowest fecundity will remain in the data set (9,16). If present, the bias will be toward increased time to conception among controls who have higher fertility and will tend to have more unplanned pregnancies which are excluded from analysis. The need to collect information on a large number of potential confounders and covariates is necessitated by the complexity of the biologic function of reproduction. Many factors have the potential to influence time to conception and must be included in the analysis. A potential limitation of the present study is the failure to collect detailed data on the subject's female partner. We were therefore limited to including covariates concerning the male, except for

previously stated partner conception problems. The potential for missing data for some factors can lead to the exclusion of the record from analysis when utilizing multivariate methods like the Cox Proportional Hazards Model.

While paternity is an all-or-none phenomenon, it may be accomplished over time even if the sperm count is low (17-19). It is reasonable to hypothesize that increased time to conception is related to diminished sperm quality and number. Potential biologic mechanisms responsible for the diminished sperm production include diminished numbers of germ cells (sperm precursors) and greater germ cell damage after cryptorchidism (20-22). Men who have bilateral cryptorchidism would sustain such damage to the germ cells of both testes and this may result in reduced capacity to produce a sufficient number of quality sperm in order to achieve conception. Unilaterally cryptorchid men also have some damage. However, since the contralateral testis is descended, this testis may compensate and be the primary contributor of sperm (23,24). This compensation, based upon the results from this study, is sufficient so that formerly unilaterally cryptorchid men have the ability to father children at the same rate as control men.

2.6. Errata (changes made to original manuscript)

Page 11- Changed from-“...mean time to conception for bilateral men was 33.90 months, 11.11, and 8.78 month for unilateral and control men, respectively.” to more appropriate measure of median time to conception ” ...median time to conception for formerly bilateral men was 14 months, and 2 months for formerly unilateral and control men.”

Page 13- Added- “Conditions that were criteria for exclusion among controls included a history of cryptorchidism or hernia, while exclusion criteria for both cases and controls included...” to clarify which exclusion criteria were for only for controls and which were for both cases and controls.

Page 14- Corrected error- Unilateral n=475, not 445.

Page 15- Changed from- “...was used to estimate a set of regression coefficients that are prognostic factors for time to conception...” to “...was used to estimate a set of regression coefficients for prognostic factors for time to conception...”

Page 15- Clarified that demographic characteristics are for “the entire cohort of 547 case and 463 control men.”

Page 16- Changed from mean to median values for time to conception. See Page 11 change above.

Page 17- Added variable types to the following- “Variables included in the initial backwards stepwise model were age at study enrollment (continuous), age at surgery (continuous), year of surgery (5-year groups), varicocele (yes/no), mumps (yes/no), sexually transmitted disease (yes/no), epididymitis (yes/no), use of alcohol (yes/no, ever > 30 drinks/month), cigarettes (yes/no, ever regular smoker), other drugs (yes/no, ever regular user), occupational exposure to

chemicals (yes/no, ever regular exposure), and conception problems in the female partner (yes/no, told by doctor).

Page 19- Deleted- “Selection bias can be associated with contraceptive practice. Also, the need to collect data on many potential confounders and covariates is concerning.”

Page 19- Added for clarification- “...because couples cannot report time to conception for a pregnancy that was not planned.”

Page 19- Added- “If present, the bias will be toward increased time to conception among controls who have higher fertility and will tend to have more unplanned pregnancies which are excluded from analysis.”

Page 20- Deleted- “Again the effect of this problem is reduced by having a large study population like that in the present study.”

2.6.1. General comments on revisions made to the original manuscript

Table 2-1 should show the time to conception cohort rather than the full study cohort. Unfortunately the original paper data are not available for re-analysis and cannot be included here. Median time to conception has replaced the mean previously reported. Mean is not appropriate here because of censoring in the data. Formerly bilateral men have a disproportionately large amount of censoring due to longer time to conception in this group and their mean time to conception will be underestimated. In the Cox analysis the proportionality assumption was tested, and met, by examination of the log-log plot for the bilateral, unilateral, and control men. Variable-variable interactions were not tested in the Cox analysis, though subsequent analyses of time to conception from the completed CHP study data found no

significant interactions between variables. The Pearson correlation is not a measure of validity between men and their partners, a more appropriate measure would be the intra-class correlation coefficient to test agreement between pairs of individuals. In addition, large sample size does nothing to reduce bias, systematic bias in a large sample simply results in a large biased sample. Having a large sample does moderate the random effects of sampling and reduces the effect that any one individual outlier or group of outliers may have on the overall sample.

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3. Article #2: Testicular suture: a significant risk factor for infertility among formerly cryptorchid men

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3.1. Abstract

Background/Purpose: Although fertility is decreased after cryptorchidism, the importance of risk factors, including parenchymal testicular suture is unknown. The aim of this study was to examine the relationship between parenchymal testicular suture and failure to conceive a child for 1 year or longer among formerly cryptorchid men.

Methods: Men who underwent orchidopexy between 1955 and 1972 at the Children's Hospital of Pittsburgh (n=619) were surveyed by questionnaire and their medical records reviewed. Only the men who attempted to conceive a child (n=387) are included.

Results: Logistic regression analysis determined significant risk factors for infertility. Testicular suture was significantly related to infertility (OR=7.56, 95% CI=1.66, 34.39), as were bilateral cryptorchidism (OR=5.51, 95% C.I.=1.58, 19.24), varicocele (OR=4.72, 95% CI=1.42, 15.75), hormone treatment before surgery (OR=3.68, 95% CI=1.22, 11.11), and partner conception problem (OR=3.32, 95% CI=1.11, 9.90).

Conclusions: Transparenchymal testicular suture was independently related to infertility among formerly cryptorchid men who underwent orchidopexy. Bilateral cryptorchidism, hormone treatment, varicocele, and partner conception problems were also associated with increased infertility.

Index Words: Cryptorchidism, Infertility, Orchidopexy, Epidemiology.

3.2. Introduction

The major risks for infertility after cryptorchidism have not been studied completely. In this study, we have analyzed a cohort of 387 formerly cryptorchid men who have fathered a child or attempted paternity.

Cryptorchidism is one of the primary causes of male infertility. At birth, 3.0% of boys have unilateral and 1.9% have bilateral cryptorchidism. After birth spontaneous descent does occur reducing the overall prevalence to 1 to 1.5% by three months of age [1-3]. Fertility after cryptorchidism has been assessed in a number of studies by both semen analysis and paternity [4-10]. Accurate paternity information may be the better measure of fertility as men with reduced sperm counts can still father children [9,10]. The Children's Hospital of Pittsburgh Male Fertility Study is attempting to determine if male fertility is compromised by maldescent of one or both testes. Results to date have indicated that formerly bilateral and unilateral cryptorchid men have reduced fertility in comparison to control men, and that formerly bilaterally cryptorchid men have longer times to conception than unilateral and control men [11-13]. The current cohort is much larger (>1200 individuals) than that of the other studies; is a generalizable sample of the Allegheny County, Pennsylvania population; and has an appropriate control group for comparisons. Many of the previous studies have had small samples (<100 individuals), biased ascertainment (infertility clinic populations), or have used varying outcome measures (paternity or sperm counts).

In our previously published works, we have identified that cryptorchid men have reduced fertility as compared with control men [11-13]. We now wish to investigate which treatment and lifestyle factors contribute to the risk of infertility among formerly cryptorchid men. The

hypothesis to be tested is that there are specific treatment modalities such as placing a suture through the testis, treatment with human chorionic gonadotropin (hCG) hormone, and unrelated disorders such as varicocele and female conception problems, which contribute significantly to infertility among formerly cryptorchid men. Laboratory research in animals [14] suggests that testicular sutures similar to those frequently utilized as part of orchidopexy surgery generate a potent inflammatory response which may lead to local necrosis, vascular damage, or immunologic response which has the potential to compromise fertility. The Children's Hospital of Pittsburgh Male Fertility Study provides the first opportunity for consideration of the effect of trans-parenchymal testicular suture on fertility in formerly cryptorchid men.

3.3. Materials and Methods

Men who underwent either unilateral or bilateral orchidopexy at the Children's Hospital of Pittsburgh (CHP) between 1955 and 1972 were identified by medical record review. During this 17 year period, greater than 85% of all of the orchidopexies performed in Allegheny County, which includes the City of Pittsburgh, were performed at CHP. Because of this, this sample is likely representative of the Allegheny County general population. Institutional Review Board (IRB) approval was secured from the Children's Hospital of Pittsburgh Human Rights Committee in order to contact these former CHP patients for the purpose of data collection. Exclusionary criteria included retractile testis, genital abnormality, multiple congenital anomalies, major central nervous system disorder, and debilitating chronic disease. Eligible subjects were contacted by telephone and asked to complete a 70 item questionnaire which was then mailed to them if they consented. The questionnaire included items concerning general health, marriages, cohabitation, birth control methods, frequency of intercourse, miscarriages,

abortions, partner conception problems, military service, exposure to chemicals, and lifestyle factors (smoking, alcohol, and drug use). Questionnaires have been returned by 71% (619 of 872 total cases) of all formerly cryptorchid men. Response rates were 74% for bilateral and 70% for formerly unilateral men. The primary reason for non-response was a failure to locate an individual 20 to 30 years after their surgery at CHP. Individuals less likely to be located and to complete a survey included men with an earlier date of surgery, and non-white men. The present analysis includes data on 486 men who were either married or in a long-term (>1 year) cohabiting relationship with a woman. Infertility is defined as attempted conception with regular intercourse for greater than one year without success.

3.3.1. Statistical Methods

We utilized logistic regression analysis in order to determine the contribution of testicular suture and other covariates to infertility among formerly cryptorchid men, after performing univariate chi square tests to identify potential risk factors for infertility. All analyses were performed on a desktop computer utilizing the Statistical Package for the Social Sciences (SPSS) for MS Windows Release 6.1 (SPSS Inc., Chicago, IL.). Logistic regression analysis allows us to evaluate the association of testicular suture with infertility among formerly cryptorchid men independent of the other covariates. Forward stepwise selection was utilized for the regression analyses with an entry criteria of $p < 0.05$ and a removal level of $p > 0.10$. Potential covariates included in the initial model were transparenchymal testicular suture to position testis at surgery, age at surgery, partner conception problems, testicular biopsy at surgery, cryptorchidism (bilateral or unilateral), varicocele, hormone treatment, pre-operative testicular position, sexually transmitted disease, mumps, epididymitis, miscarriage or abortion, smoking, alcohol

consumption, illicit drug use, and family history of cryptorchidism. Each of these variables was entered into the model in the following form and was ascertained by either participant self-report or medical record review. Testicular suture is either yes or no, based on the operative record of the orchidopexy surgery. Cryptorchidism was entered as either unilateral or bilateral and was based on review of subject medical records. Partner conception problem was self-report for the female partner ever having been diagnosed with a fertility problem and was entered as yes or no. Miscarriage or abortion was self-reported (yes or no) of a partner ever having had either a miscarriage (spontaneous abortion) or induced abortion. Hormone treatment was also yes or no and based on medical record review. Varicocele was yes or no and based on self-report of diagnosis of varicocele. Testicular biopsy was yes or no and based on review of the operative record. Pre-operative testis location was divided into low (upper scrotum or at external ring), medium (within canal or at internal ring), or high (ectopic or intra- abdominal), and entered as a block of two categorical variables with low pre-operative location as the baseline category. No was considered the baseline category for each of the yes or no variables, while unilateral cryptorchidism was the baseline for the cryptorchidism variable.

3.4. Results

The general demographic characteristics of the formerly cryptorchid men are shown in table 3-1. The number of formerly cryptorchid men with complete data for this analysis was 387. As can be seen in the table, the cohort is mostly white (94.1%), has an average body mass index of 26.7, and nearly 70% have been or are presently married. The results from the univariate analyses comparing infertile and fertile men are shown in table 3-2. These analyses were used to screen variables before inclusion into the regression model. Significantly different percentages

Table 3-1: Selected Demographic Characteristics

Characteristic	n=387
Age (mean years)	38.2
Age at surgery (mean months)	94.7
Year of Surgery (mean)	1964
Race (% white)	92.0
BMI (wt kg/ht m2)	26.7
Ever Married (%)	94.5
Ever Smoked (%)	52.2
Ever Drank Alcohol (%)	79.9
Formerly Unilaterally Cryptorchid (%)	94.0
Testicular Suture Used (%)	71.8
Varicocele (%)	5.3
Hormone Treatment (%)	9.0
Partner Conception Problem (%)	9.2

Table 3-2: Infertile vs. Fertile Men

Variable	Infertile	Fertile	X² P-value
Testicular Suture (%)	90.5	68.6	0.004
Bilaterally Cryptorchid (%)	14.3	4.6	0.004
Partner Conception Problem (%)	25.0	6.5	<0.001
Marijuana Use (%)	42.9	35.2	0.269
Use Other Drugs (%)	10.7	13.2	0.614
Military Service (%)	26.4	23.5	0.641
Ever Married (%)	96.4	94.3	0.508
Miscarriage or Abortion (%)	11.3	29.2	0.005
Race (% white)	96.4	91.2	0.451
Ever Smoke (%)	58.9	51.1	0.276
Biopsy of Testis (%)	35.7	21.1	0.016
Hormone Treatment (%)	21.4	7.0	<0.001
Varicocele (%)	15.1	3.6	<0.001

between the infertile and fertile groups suggests a causal or confounding relationship between infertility and the individual variable. Individual variables significant in this univariate analysis should be eligible for inclusion in the multiple logistic regression as their significance indicates a

potential relationship with infertility. Variables were included in the initial multivariate model if significant at the $p < 0.10$ level in the univariate analysis. Those variables included in the initial model were cryptorchidism, testicular suture, partner conception problem, miscarriage or abortion, hormone treatment, varicocele, and testicular biopsy.

Table 3-3 shows the results of the final logistic regression model. Testicular suture (OR=7.56, 95% C.I. 1.66, 34.39) was significantly associated with infertility independent of cryptorchidism, hormone treatment, varicocele, and partner conception problems. Overall, factors significantly associated with infertility were having a testicular suture, hormone treatment before surgery, bilateral cryptorchidism, a conception problem in the female partner, and having had a varicocele. Odds ratios derived from the final model show men who had a transparenchymal testicular suture have 7.6 times the odds of infertility as those who did not have a fixation suture placed at the time of orchidopexy surgery. Formerly bilateral cryptorchids had 5.5 times the odds, men diagnosed with a varicocele 4.7 times the odds, men who received hormone treatment before surgery 3.7 times the odds, and those whose partners have conception problems have 3.3 times the odds as men without these conditions. It is also important to remember that formerly cryptorchid individuals can be affected by more than one of these risk factors and be at greatly increased risk for infertility. Miscarriage or abortion and testicular biopsy did not contribute significantly and were dropped from the final model.

Table 3-3: Final Logistic Regression Model Results

Variable	OR Estimate	Significance	95% C.I.
Testicular Suture	7.56	0.009	1.66, 34.39
Bilateral Cryptorchidism	5.51	0.007	1.58, 19.24
Varicocele	4.72	0.012	1.42, 15.75
Hormone Treatment	3.69	0.021	1.22, 11.11
Partner Conception Problem	3.32	0.032	1.11, 9.90
Preoperative Location		0.020	
Medium	0.7697	0.621	0.273, 2.17
High	2.84	0.060	0.962, 8.37

NOTE: Overall Model $\chi^2 = 43.96$ ($p < 0.0001$ with 7 d.f.)

3.5. Discussion

Reduced fertility and increased subfertility among cryptorchid men has been demonstrated in our study cohort and presented in several publications [11-13]. These data indicate that paternity failure is twice as common in formerly unilaterally cryptorchid (10.5%) men as controls (5.4%) when attempted paternity is considered [11] and about 7 times (38%) as common among formerly bilaterally cryptorchid men [12]. Other previously published works have reported paternity rates of 67% to 92% among married formerly unilateral cryptorchid men [4-8, 15-17] and 13% to 62% among formerly bilateral cryptorchids [4-8, 17,18]. What is not known is what other risk factors are significant for infertility among formerly cryptorchid men. Evaluation of this question requires a large cohort and detailed information on surgical and lifestyle factors which may influence fertility. The cohort comprising the Children’s Hospital of Pittsburgh Male Fertility Study can provide just this type of information. To date we have received detailed surveys, and compiled medical and surgical records for over 600 formerly cryptorchid men.

This is the first report that would suggest that a testicular suture has a detrimental effect upon fertility among formerly cryptorchid men who undergo orchidopexy. Further evaluation will be

necessary to verify this effect and to determine the mechanism of action. The mechanism may be vascular, immunologic, local necrosis or any combination of these and other factors. Violation of the blood-testis barrier could result in the development of antisperm antibodies which have the potential to effect male fertility [19, 20]. It has been shown in an animal model that histological evidence of tissue necrosis, inflammation, and diminished spermatogenesis can be produce in testes subject to transparenchymal suture [14]. Although some of the changes can be attributed to tissue necrosis from direct tissue trauma, suture material, which is absorbable, was found to create more inflammatory change than non-absorbable suture. In our patient cohort, silk suture (non-absorbable) was used for testicular fixation. Since testicular fixation has been shown to be superior with dartos pouch placement of testes, transparenchymal sutures should be avoidable in most instances. When suture fixation is necessary (for the short spermatic cord which necessitates extra precaution to prevent post-operative ascent due to tissue tension) paratesticular tissue can usually be successfully sutured with non-absorbable sutures.

Testicular suture also increases odds of infertility in a model including only unilateral cryptorchid men (data not shown). Therefore, the concept of sympathetic orchidopexy as a consequence of immunologic phenomenon may have some validity. It will be important to evaluate testicular volume, sperm parameters, and titers of antisperm antibodies among formerly cryptorchid men who did or did not have a scrotal suture in order to identify potential mechanisms for the association. We have recently begun collecting such data from our cohort of formerly cryptorchid men.

The findings in the present analyses concerning bilateral cryptorchidism, varicocele, partner conception problems, and hormone therapy are not unexpected and agree with previously

published studies of male infertility. Varicocele has often been found to be associated with infertility. A conception problem in the man's female partner is also an expected association. The significance of having had hormone treatment is likely the result of selection bias. Only those men whose testes failed to descend after hormone treatment would have been referred for orchidopexy. Further analysis also revealed that hormone treatment was significantly correlated with position ($p=0.038$) and inclusion of pre-operative position in the model reduced the independent effect of hormone treatment on infertility (data not shown). These men may well have worse outcomes than those who were only treated by orchidopexy since treatment failure may be indicative of higher testicular position as well as compromised testicular potential.

The results of studies such as the one presented here may allow us to develop a predictive profile for those patients who have undergone orchidopexy as to potential success in reproduction. Identification of risk factors for infertility among formerly cryptorchid men will allow us to work toward the primary prevention of infertility in this group. The identification of the testicular suture as a risk factor for infertility among formerly cryptorchid men may mandate a reassessment of fertility after cryptorchidism excluding this risk factor. It may also indicate that use of a transparenchymal testicular suture during orchidopexy should be avoided if at all possible. Similar recommendations would also apply to surgery for testicular torsion.

3.6. Errata (changes made to original manuscript)

Page 27- Changed- “RR” (relative risk) to “OR” (odds ratio) since RR is incorrect. Logistic regression models produce odds ratios, not relative risks.

Page 33- Again, changed- “relative risk” and “RR” to “odds ratio”, “odds”, or “OR” to correct error of reporting relative risk where odds ratio should be.

Page 33- Added for clarity- “Miscarriage or abortion and testicular biopsy did not contribute significantly and were dropped from the final model.”

Page 34-5-Deleted- “Testicular suture was strongly associated with infertility in the present study. Placing a suture through the testis to move it into the scrotum and hold it in position was a common practice in orchidopexy throughout the years of our study, and about 75% of the men included in the present analysis had such a suture. Since one could hypothesize that testes which had higher pre-treatment locations would be more likely to be sutured in order to fix them in a scrotal position, further evaluation was done to determine whether the suture risk was the result of testicular position. This possibility was evaluated by correlating suture placement and pre-operative position. No significant correlation was found between pre-operative location and testicular suture ($p=0.832$, data not shown). Inclusion of pre-operative location in the regression model actually increased the strength of the association between suture and infertility.”

Paragraph deleted because correlation is not an appropriate measure of association between testicular position and placement of a testicular suture.

Page 36- Deleted- “Since this relationship is present among the unilateral as well as the bilateral group.” and Added- “Testicular suture also increases odds of infertility in a model including only unilateral cryptorchid men (data not shown).” for clarity.

3.6.1. General comments on revisions made to the original manuscript

Interaction terms were not included in the logistic regression model in an effort to keep the analysis as simple as possible. The present study was exploratory in nature, as prior studies had not considered the potential impact of testicular suture on fertility. Any subsequent analyses should include potential interactions between terms. A cutoff p-value of <0.10 was arbitrarily chosen for inclusion of variables in the initial logistic model. This value is potentially too conservative and variables which could be significant in the presence of others included in the model might be missed. Alternatively we should increase this cutoff or include all potential covariates in the initial model.

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4. Article #3: Age at unilateral orchiopexy: effect on hormone levels and sperm count in adulthood

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4.1. Abstract

Purpose: We determined whether there are differences in hormone levels, such as inhibin B, follicle-stimulating hormone (FSH), luteinizing hormone (LH), and testosterone, and sperm density in men with a history of unilateral cryptorchidism as stratified by age at orchiopexy.

Materials and Methods: A total of 84 men with a history of unilateral cryptorchidism presented to our institution for serum hormone measurement and semen analyses. These parameters were compared using Pearson's correlations and analysis of variance among 4 groups stratified by age at orchiopexy (range 1 month to 11 years).

Results: Comparison by Pearson's correlation analysis showed that age at orchiopexy significantly correlated inversely with inhibin B ($r = -0.274$, $p=0.012$), and positively correlated with FSH ($r = 0.229$, $p=0.036$). Comparison of mean hormone levels and sperm densities by ANOVA test for a linear trend showed that there is a significant correlation between age of surgery and inhibin B ($p=0.032$), and testosterone ($p=0.029$), while sperm density, FSH, and luteinizing hormone were not significantly related. Post-hoc comparisons of the individual means at surgery and at the time of this study demonstrated a significantly higher inhibin B level in the youngest age group than in 2 of the 3 older groups.

Conclusions: Men who previously had unilateral cryptorchidism and who underwent orchiopexy by the age 2 years have higher inhibin B and lower FSH profiles than those who underwent surgery later in life. This finding suggests an overall beneficial effect of early orchiopexy in boys born with unilateral cryptorchidism.

Key Words: testis, cryptorchidism, fertility, inhibin, FSH.

4.2. Introduction

In cryptorchidism a testis fails to descend into the scrotum from its initial site of development in the abdominal cavity. Descent normally occurs late in the third trimester of gestation, at approximately week 38 of development. To date the literature suggests that failure of the testis to descend into the scrotum results in histological changes that are detectable by the second year of life, and become progressively worse with age [1-4]. Because of these early histological changes, the recommended age for orchiopexy has progressively declined, and surgery by age two years is now the standard of care [5]. Retrospective studies comparing sperm concentration with age of orchidopexy have shown variable results, with some studies showing higher sperm density among those who had early orchiopexy [6,7,8], and others showing no difference between men who had early or late orchiopexy [9]. Chilvers et al [9] reviewed data of 7 articles which compared age of treatment and sperm count in men with a history of cryptorchidism and noted no effect of age at treatment. A recent study by Taskinen et al observed that no patient treated before 4 years had severe sperm defects [8]. Data published to date comparing paternity with age of treatment show no improvement in the paternity rate in men who underwent orchiopexy at a younger age [10-13].

More recently, researchers have begun to investigate hormonal biomarkers of testicular function, such as follicle stimulating hormone (FSH) and inhibin B, as indicators of the integrity of testicular function. Inhibin B is the principal form of inhibin in men and is produced by Sertoli cells, functioning to regulate FSH secretion by the pituitary in a closed loop negative feedback system [14-16]. These studies indicate that FSH and inhibin B are indicators of spermatogenesis. Levels of inhibin B and FSH are lower and higher, respectively, in men with abnormal sperm counts than in those with a normal semen analyses [14-16]. Inhibin B levels in

subfertile men studied by Pierik et al. negatively correlated with FSH and positively correlated with total sperm count [17]. These published reports of inhibin B and FSH levels, and their relationship to spermatogenesis, indicate that these serum hormones may be useful markers of fertility and potential fertility.

Given that serum levels of inhibin B and FSH reflect the underlying integrity of the seminiferous tubule, measurements of these parameters may be used as predictors of fertility in men with a history of cryptorchidism. We hypothesize that men who underwent orchiopexy early in life have higher inhibin B and lower FSH levels than those who underwent surgery later in childhood. If the integrity of testicular function is preserved by early surgery, hormone levels and sperm density will be positively reflected among men operated on early in life.

4.3. Materials and Methods

Subjects in this study represent a subset of men in the male fertility study at our institution. This study of fertility comprises more than 1200 men with a history of cryptorchidism and matched controls that underwent surgery at our institution between 1955 and 1974. Phase 1 of the study, which began in 1992, involves analysis of a detailed paternity questionnaire that includes information on marriage and cohabitation, paternity, health problems, and potential chemical exposures. Participants in the paternity questionnaire who live in the region were then asked to participate in further clinical testing as part of the second phase of the study. Data presented in this report include laboratory hormone and semen analyses from the 84 men with a history of cryptorchidism who have been seen to date as part of phase 2. Two patients were excluded; one with hypergonadotropic eunuchoidism who was taking exogenous hormones, and one who was chronically ill and receiving multiple medications which altered the hormone profile.

Each participant in the second phase of the study was scheduled for a visit of less than 1 hour to the general clinical research center at our institution. The study protocol was approved by the Human Rights Committee (Institutional Review Board) of Children's Hospital of Pittsburgh and informed consent was obtained at the time of the clinic visit. During this visit a single sample of blood and semen was collected, and the participant was examined by the study principal investigator (P.A.L.). Semen analyses were performed following World Health Organization (WHO) guidelines on samples obtained by masturbation after 48 to 72 hours of sexual abstinence. Blood samples were analyzed to determine levels of luteinizing hormone (LH), FSH, testosterone and inhibin B. LH and FSH levels were determined by the Delfia fluoroimmunoassay method, testosterone was measured by a commercially available radioimmunoassay kit (Diagnostic Products Corp., Los Angeles) and inhibin B was assayed by a commercially available double-antibody enzyme-linked immunosorbent assay from Serotec Ltd. (Oxford, UK). The inhibin B assay was performed by the Reproductive Endocrine Laboratory at the Massachusetts General Hospital (Dr. P. Sluss), while all other assays were performed in the endocrine laboratories at our hospital.

All statistical comparisons were done using a commercially available statistical software program on a personal computer. Using the Pearson correlation, initial comparisons were made of age at orchiopexy, hormone parameters and sperm density to determine whether parameters were significantly associated. All variables were considered to be independent continuous random variables associated by a linear relationship and to be approximately normally distributed. We then compared mean hormone levels and sperm density in 4 groups stratified by age at orchiopexy using 1-way analysis of variance to detect differences in mean hormone levels according to age at orchiopexy. A linear test for trend across the age groups was performed

along with post-hoc comparisons of individual mean hormone levels. The least significant difference and Games-Howell methods were utilized when there was homogeneous variance among the groups and when group variances differed significantly, respectively.

4.4. Results

Age at orchiopexy for 84 men with a history of unilateral cryptorchidism ranged from 1 month to 11.0 years (mean 6.3 years). In the whole cohort mean hormone levels plus or minus standard deviation were 115.70 ± 52.60 pg/ml inhibin B (normal 73-330), 6.49 ± 4.70 units per l. FSH (normal 1.10-7.90), 4.41 ± 3.26 units per l. LH (normal 1.70-9.20) and 595.26 ± 170.57 ng./dl testosterone (normal 285-980). Mean sperm density was $50.76 \pm 43.95 \times 10^6$ /ml. (normal greater than 20×10^6).

Table 4-1 shows the correlations of age at surgery, hormone levels, and sperm parameters. Age at surgery was significantly negatively correlated with inhibin B ($r = -0.274$, $p = 0.012$), and was positively correlated with FSH ($r=0.229$, $p=0.036$). Inhibin B also significantly negatively correlated with FSH and LH and positively correlated with sperm density (all $p<0.01$). In addition, FSH and LH correlated highly with each other as well as with inhibin B and sperm density. Sperm density correlated significantly with inhibin B, FSH and LH but not with age of surgery or testosterone. Direction of the correlations was predicted by the feedback loops involved. Inhibin B negatively correlated with both FSH and LH, and positively correlated with sperm density. Similarly sperm density also negatively correlated with FSH and LH, and positively correlated with inhibin B. The significant correlations of age at surgery with inhibin B and FSH suggests that there is a negative effect of late orchiopexy on hormone profiles in adulthood.

Table 4-1: Pearson's correlations in 101 patients

	Pt. Age at Surgery	Inhibin B	FSH	LH	Testosterone
Inhibin B	-0.274, p<0.05	---	---	---	---
FSH	0.229, p<0.05	-0.684, p<0.001	---	---	---
LH	0.006	-0.307, p<0.01	0.666, p<0.001	---	---
Testosterone	-0.205	0.161	-0.120	0.020	---
Sperm Density	-0.048	0.374, p<0.001	-0.414, p<0.001	-0.262, p<0.05	0.146, p<0.05

Table 4-2 shows the comparison of mean hormone levels and sperm density stratified by age at orchiopexy. The recommended age of surgery by 2 years was used as the youngest age group because we wished to test the hypothesis that surgery before age 2 is beneficial. The remaining subjects were divided into 3 year age groups. Analysis of variance test for linear trend of mean hormone levels by age at orchiopexy is significant for inhibin B ($p=0.032$) and testosterone ($p=0.029$). The general trend is for inhibin B and testosterone to fall with increasing age at orchiopexy, as is shown by lower values for inhibin B in each of the three older age groups and for testosterone in two (Older than age 5 to age 8 and Older than age 8 to age 11) of the three. In contrast, mean FSH increases with age at orchiopexy. Post-hoc tests of the differences among the individual age group means revealed significantly higher mean inhibin B (158.73 ± 59.99 pg./ml) in the youngest age group than in the older than 2 to 5-year (106.43 ± 53.54 pg./ml, $p=0.009$) and older than 8 to 11-year (103.77 ± 36.40 pg./ml, $p=0.004$). There was borderline significance in the youngest versus the older than 5 to 8-year (121.12 ± 59.42 pg./ml, $p=0.053$). These data support the idea that late age at orchiopexy can have a deleterious effect on hormone parameters and sperm density in adulthood.

Table 4-2: Analysis of variance comparison of means plus or minus standard deviation by patient age

	Age at Surgery (No. pts.)				p Value (test for linear trend)
	0 to 2 (10)	Older than 2 to 5	Older than 5 to 8	Older than 8 to 11	
Inhibin B	158.73 ± 59.99	106.43 ± 53.54* (20)	121.12 ± 59.42 (23)	103.77 ± 36.40* (31)	0.032
FSH	4.41 ± 3.21	6.12 ± 3.32 (20)	6.49 ± 6.40 (23)	7.40 ± 4.32 (31)	0.088
LH	4.80 ± 2.96	4.02 ± 1.40 (20)	4.91 ± 5.46 (23)	4.16 ± 1.80 (31)	0.806
Testosterone	652.50 ± 117.79	655.50 ± 171.46 (20)	569.52 ± 142.82 (23)	557.03 ± 192.94 (31)	0.029
Sperm Density	59.79 ± 42.03	48.04 ± 47.22 (16)	45.65 ± 33.74 (21)	53.28 ± 51.69 (24)	0.846

*Versus 0-2 age group mean p<0.01 by analysis of variance post hoc test.

4.5. Discussion

To our knowledge we report the first study to show an association between inhibin B with age at orchiopexy in men with a history of cryptorchidism. Mean inhibin B decreases (in 2 of 3 age groups) and FSH increases with increasing age at orchiopexy. Patients who underwent surgery by the age 2 years have higher inhibin B levels than the three groups that underwent surgery between ages 2 through 11 years. This difference may be evidence of a beneficial effect of early correction of cryptorchidism in the youngest age group. Data indicating that there are higher inhibin B levels among the youngest age group than in the other groups as well as the significant linear trends in mean inhibin B and testosterone levels suggest that early orchiopexy is beneficial.

The data presented suggest that early orchiopexy results in an increased potential for future fertility among patients born with cryptorchid testes. Our results suggest that inhibin B and FSH are useful as predictors of fertility potential among formerly cryptorchid men. There may be multiple causes for infertility in men with a history of cryptorchidism. There may be multiple causes of infertility, including the underlying cause of the testicular maldescent, anatomical position of the testis before surgery, surgical technique, testicular injury or acquired disease, in addition to age at orchiopexy. Normal hormone levels are not certain indicators of fertility among these men but they are indicative of the potential for normal sperm production.

Previously published research in fertile and infertile men indicates that inhibin B and FSH are biomarkers of the integrity of the seminiferous tubule [16-18]. Our observations among formerly cryptorchid men are consistent with these data. Inhibin B and FSH levels correlate strongly with sperm density measurements, with inhibin B positively correlated ($r=0.374$) and FSH negatively correlated ($r = -0.414$) with sperm density. These results are consistent with those published by Jensen et al. who indicated similar correlations of inhibin B ($r=0.38$), FSH ($r = -0.40$), and sperm density in 2 groups of normal men in Denmark [18]. Our results seem to show that inhibin B and FSH are indicators of the integrity of the seminiferous tubules in men with a history of unilateral cryptorchidism.

Published reports comparing age at orchiopexy with fertility have been inconsistent. It is now generally accepted that histological evidence of testicular deterioration is seen as age at orchiopexy increases [2-4], which is the reason for the current recommendation of surgery by the age 2 years [5]. To our knowledge the effect of early surgery on fertility in adulthood is much less clear at this time. Taskinen et al observed no semen abnormalities in men treated before age 4 years [8] . Others noted higher sperm parameters who underwent surgery at younger ages [6,7], while 6 of the 7 reports reviewed by Chilvers et al showed no relationship between age at orchiopexy and sperm count [9].

Measurements of inhibin B and FSH are indicators of the integrity of Sertoli cells and the seminiferous tubules, and their levels are closely associated with sperm density. Conversely, the collection and analysis of semen samples is notoriously problematic because of low participation rates, significant intra-individual and interindividual variation, the necessity for immediate processing of samples, and high variability between laboratories and individual technicians. Thus, the availability of serum biomarkers of spermatogenesis, such as inhibin B and FSH would

thus greatly simplify both studies of male infertility and the initial clinical evaluation of infertility patients.

4.6. Conclusions

Our study indicates that inhibin B and FSH are viable indicators of spermatogenesis among formerly unilateral cryptorchid men. Since inhibin B is an indicator of testicular integrity, to our knowledge the higher inhibin B levels found to be associated with younger age at orchiopexy is the first hormonal evidence of the benefit of early orchiopexy. These data support the findings of studies which have shown diminished numbers of germ cells in cryptorchid testes after two years of age in support of the current recommendation for orchiopexy before age 2.

Dr. P. Sluss, Reproductive Endocrine Laboratory, Massachusetts General Hospital, performed the inhibin B assay.

4.7. Errata (changes made to original manuscript)

Page 45- Added “All variables were considered to be independent continuous random variables associated by a linear relationship and to be approximately normally distributed.” Normality was tested by a visual check of a plot of the data points.

Page 46- Replaced- “Age at surgery significantly correlated with...” with “Age at surgery was significantly negatively correlated with...” in order to state the direction of the correlation between the variables.

Page 46- Added- “negatively” and “positively correlated with” to “Inhibin B also significantly negatively correlated with FSH and LH and positively correlated with sperm density (all $p < 0.01$).” again to state the direction of the correlation.

Page 47- Added- “...because we wished to test the hypothesis that surgery before age 2 is beneficial.” to clarify reasoning behind the choice of age category cutpoints.

Page 47- Added- “...as is shown by lower values for inhibin B in each of the three oldest age groups and for testosterone in two (Older than age 5 to age 8 and Older than age 8 to age 11) of the three.” to better describe the trend in inhibin B and testosterone.

Page 48- Added- “(in 2 of 3 age groups)” to clarify the trend in inhibin B.

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5. General Discussion

The following section presents a discussion on the subjects of infertility and the etiology, pathophysiology, and epidemiology of cryptorchidism. It is provided as background material not represented as part of the three manuscripts presented above.

5.1. Infertility

Infertility is generally defined clinically as the inability to conceive a child after greater than one year of unprotected regular intercourse (Mosher and Pratt, 1991). Infertility is a defined state of childlessness, while *fecundity* is defined subjectively as the ability to conceive and carry a child to term. *Fecundability* is a statistician's term denoting the probability of pregnancy in an individual menstrual cycle (Joffe, 1989). Typical fecundability is approximately 0.2 per month (2 conceptions out of every 10 couples attempting) (Cramer et al., 1979). Long term studies show that more than 60% of couples achieve conception within the first 6 months of attempting (Joffe, 1989). *Primary infertility* is the inability to conceive a child by a man or woman who has never previously had a child, while *secondary infertility* is the inability to conceive by a man or woman who has previously had one or more children. *Subfertility* can be defined as low *fecundability* (monthly probability of conception) due to known or unknown causes. Individuals who are subfertile may conceive spontaneously or with medical assistance.

5.1.1. Female Infertility

Though this work is focused on infertility in males, it is important to briefly consider the potential causes of infertility in women. Infertility, after all, is not an individual, but rather is a couple based problem. Figure 5-1 shows the breakdown of female infertility causes from a study by Collins et al. (1983) which looked at infertility in 1145 Canadian women. Though numbers vary somewhat between studies, this example is quite typical with infertility due to ovulatory

disturbance in 43.5% of women, fallopian tube problems in 23.2%, cervical mucous defects in 7.3%, endometriosis in 5.8%, and unexplained infertility in 18.8%. Studies showing similar results have been published by Cates et al. (1985), Hull et al. (1985), Haxton and Black (1987), and Thonneau et al. (1991). Within each of these broad categories are numerous specific causes for infertility in an individual woman. Each case must be treated as unique since there are likely to be multiple contributing factors leading to infertility in each individual.

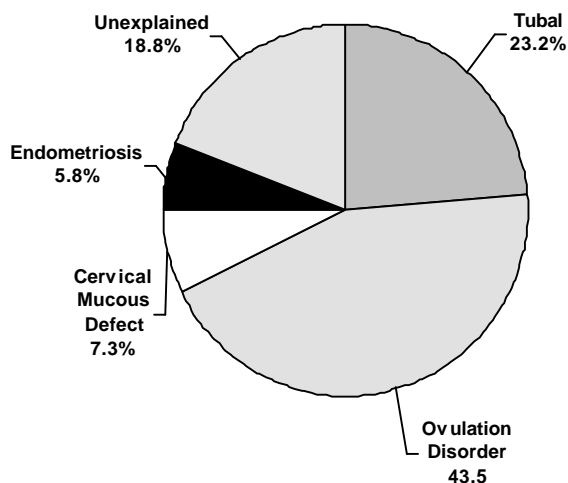


Figure 5-1: Causes of Female Infertility in Canada

5.1.1.1. Epidemiologic Factors and Female Infertility

Another considerations in looking at factors influencing female fertility are epidemiologic risk factors which can reduce fertility in women and thereby reduce the fertility of a couple. These include age, race, cigarette smoking, alcohol consumption, drug use, occupational exposures, and sexually transmitted disease (STD). Age is a primary factor in female fertility today since many women are delaying childbearing in order to compete in the workplace and to

provide a more stable financial base for future children. Fertility rates decline with age after age 30 and with duration of marriage (Menken et al, 1986; Howe et al., 1985; Van Noord-Zaadstra et al., 1991). This decline is likely the result of the aging of the reproductive organs and a decrease in sexual activity (Menken et al., 1986). While age has direct effects on the reproductive organs, the passage of time also increases the potential for exposure to diseases which can also reduce fertility including endometriosis and sexually transmitted disease (Jaffe and Jewelewicz, 1991). Race does not influence fertility directly, but can be a confounder in association with SES, and to other risk factors such as STDs, smoking, illegal drug use, and access to health care. Cigarette smoking has been associated with reduced fertility in a number of studies and has also been shown to reduce per cycle fecundability and increase time to conception (Howe et al., 1985; Olsen et al, 1983; Baird and Wilcox, 1985; Stillman et al., 1986; Joffe and Li, 1995). Chronic alcohol use can decrease fertility and cause disruption of normal menses (Smith and Asch, 1987). Illicit drugs such as marijuana and narcotics can affect hormone secretion thereby disrupting normal menstrual cycling (Smith and Asch, 1987). A number of occupational exposures have also been shown to have the potential to influence female fertility. These include noise, dry cleaning chemicals, mercury, cadmium, and textile dyes (Mueller and Daling, 1989). Finally, sexually transmitted disease has the previously discussed effect on fertility by causing such disorders as cervicitis, endometriosis, salpingitis, and PID. Each of these epidemiologic factors has the potential to reduce female fertility, and should be considered in any study designed to investigate infertility in women.

5.1.2. Male Infertility

Problems with fertility are common among young and middle-aged males, yet 60% of men visiting a doctor for infertility have allowed more than two years to go by before consulting a

doctor about the problem (Bruckert, 1991). Specialization in the field of andrology has been slow to be accepted as a legitimate field of practice in the medical community. Many men having reproductive problems are first seen not by an andrologist or urologist, but by a gynecologist as part of an infertility consult with their female partner. Research in male infertility has progressed greatly in the last twenty to thirty years, but there is still much that is unclear about problems of male reproduction. Figure 5-2 shows a breakdown of the causes of male infertility among 5061 consecutive patients seen at the Institute of Reproductive Medicine at the University of Münster as described by Nieschlag and Behre (1992). The major causes of male infertility include cryptorchidism, varicocele, endocrine hypogonadism, infections, and idiopathic infertility. Other less prevalent causes include coital disorders, sperm antibodies, testicular tumors, general diseases, and genital tract obstruction.

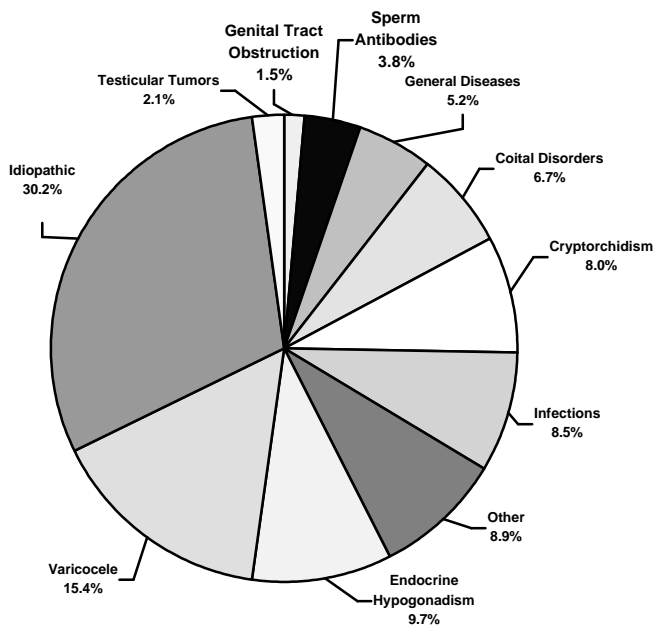


Figure 5-2: Causes of Male Infertility Among 5061 Infertility Patients

5.1.2.1. Epidemiologic Factors and Male Infertility

A number of other factors have the potential to influence male fertility and need to be considered by any study considering this subject. These include age, environmental exposures, smoking, and drug and alcohol use. Unlike women whose fertility declines with age, men do not seem to show a marked decline in fertility potential as they grow older. Schwartz et al. (1983) showed no significant decline in sperm density, semen volume, or total sperm count in their study of 833 fertile men aged 21 to 50 years. They did show a decline in sperm motility and percent normal cells, but the levels were still in the normal range and unlikely to effect fertility in these men. A number of other studies of sperm parameters have reached similar conclusions (Gallardo et al., 1996; Nieschlag et al., 1982). While semen parameters are only a proxy measure of fertility, others have attempted to look at factors influencing paternity. Joffe and Li (1994) considered time to pregnancy in 2,576 male cohort members, and concluded that age had no affect on male fertility in their sample. Research to date indicates that male age has little if any effect on fertility. While older men may have the capability to father children when we consider only semen parameters, other factors such as erectile dysfunction and declining libido may limit potential for paternity among older men. Older men also tend to have older female partners and this may reduce fertility potential as well.

Environmental exposures have the potential to influence male fertility by altering the function of the hypothalamus and/or pituitary, or by directly damaging the seminiferous tubules of the testis. A number of compounds found in the environment can reduce male fertility. These include pesticides, glycols and glycol ethers, metals (lead), and synthetic hormones (estrogen and analogues) (Milby and Whorton, 1980; Veulemans et al., 1993; Gennart et al., 1992; Harrington, 1982). High temperature is know to adversely affect spermatogenesis, and this is believed to be the reason why the testis are located outside of the body in many mammalian species (Bedford,

1991). Occupations in which high heat is common may lead to reduced fertility, i.e. welders and foundry workers (Bonde, 1992). Consideration of environmental exposure should be made in studies of male fertility, but it should be noted that environmental effects on male fertility are very hard to identify in epidemiologic studies. It is often very difficult to identify when the exposure took place in time, and consideration must be given to the fact that any effect on spermatogenesis will take 90 days to be seen, given that is the maturation time for sperm in the human male.

Finally, let us consider environmental exposures related to lifestyle; smoking, alcohol, and illegal drug use. Because of the documented fertility reducing effect of cigarette smoking in women, many researchers have looked at the effect of smoking on male fertility. However, unlike in studies of female smoking, studies of male smoking and fertility have been far from conclusive. Many studies have shown no significant effects on semen quality (Dikshit et al., 1987; Marshburn et al., 1989; Rodriguez-Rigau et al., 1982). Others have shown reduced semen quality among male smokers (Close et al., 1990; Evans et al., 1981; Vine et al., 1996; Vogt et al., 1986). Other authors have considered time to pregnancy as a measure of fertility, and have found no effect of paternal smoking (Baird and Wilcox, 1985; Joffe and Li, 1994). Overall research into the effect of smoking on male fertility appears to be inconclusive at this time. The negative effects of alcohol consumption on male fertility among chronic users are well documented. Chronic alcohol abusers suffer direct effects of alcohol on the testis, hypoplasia of the spermatogonia and seminiferous tubule atrophy; along with secondary effects of hypoandrogenism due to liver damage, and resultant testicular atrophy and impotence (Close et al., 1990; Lester and Van Theil, 1977). Conversely, light to moderate alcohol consumption has shown no adverse reproductive effects in most studies (Close et al., 1990; Marshburn et al.,

1989; Oldereid, et al., 1992). There is a severe lack of research into the effects of illegal drugs and male fertility. Regular marijuana use has been shown to lead to abnormal sperm morphology, decreased motility and sperm count, and increased seminal fluid leukocytes (Close et al., 1990; Hembree et al., 1979; Huang et al., 1978). The effects of moderate marijuana use have not been adequately studied to date. Research into the effects of other illegal drugs and male fertility is virtually non-existent at this time. Cocaine has been demonstrated to cause seminiferous tubule damage in a rat model, but its effect on humans is unknown (Rodriguez et al., 1992). As a whole the studies of exposures to smoking, alcohol, and illegal drugs and their effect on male fertility have produced inconsistent results, and continued research is needed in all of these areas.

5.1.3. Interaction of Male and Female Fertility

Reduced male or female fertility is only likely to become recognized after the man or woman has entered into a long-term relationship in which a child is desired. Unprotected intercourse for six months to a year without conception may indicate a fertility problem, since approximately 85% of normal couples conceive within one year. Because fertility is a couple based outcome, slight reductions in fertility potential in both partners can be magnified by their union as a couple. In a study by Dorr et al. (1977) 50 couples (7.5%) were found to have fertility reducing factors in both partners. Subsequently these couples had only a 14% pregnancy rate, as compared with a 54.3% pregnancy rate when only a female factor was involved. The worldwide study of fertility by Cates et al. (1985) indicated that couples in which both partners have a fertility problem make up 25% of all infertility patients. Figure 5-3 graphically illustrates the complex interplay of male and female fertility. Couples which fall into groups one and two will not generally seek medical help as they will have no apparent fertility problems. Group one is

the ideal, with both partners having optimal fertility, while in group two one partner compensates for the other's impaired fertility with their own. In seeking medical assistance, group three will concentrate on only one partner since the other's fertility is optimal. Groups represented by four and five represent the vast majority of infertile patients, and are also the most difficult to diagnose and treat because both partners have fertility problems. The interplay between male and female is very complex, and the infertile couple should always be considered as a unit in any evaluation of infertility.

Male Reproductive Function	Optimal	3	2	1
	Impaired	5	4	2
	Absent	5	5	3
		Absent	Impaired	Optimal
		Female Reproductive Function		

Figure 5-3: Interdependence of Male and Female Fertility

5.2. Pathophysiology of Cryptorchidism

5.2.1. Testicular Descent

Cryptorchidism is non-descent of the testis into the scrotal sac. The clinical diagnosis is defined as deviation or arrest of the testis at any point along the normal path of its descent from an origin below the kidneys down into the scrotal sac (Rezvani, 1987). The process of testicular descent is a complex one, and many theories have been put forth attempting to explain how it occurs. Recently, Hutson and Beasley (1992) have synthesized much of the earlier work to formulate a plausible theory of the process of testicular descent. Descent is believed to occur in two distinct phases. The first phase is one in which the testis is anchored to the inguinal region while the rest of the abdominal organs move away during the growth of the embryo (differential growth). This process is not mediated by androgens and the position of the testis is largely controlled by the mechanical anchoring of the gubernaculum to the inguinal region. This phase of descent across the abdomen may be stimulated by mullerian inhibiting substance (MIS). MIS is believed to promote gubernacular development thereby anchoring the testis in the inguinal region, and to promote regression of the mullerian ducts. Estrogens given to male mice inhibit MIS and result in retention of mullerian ducts and undescended testes (Jean, 1966; Hutson, 1987). Also, male children with MIS deficiency, a genetic disorder, have completely undescended testes and retained mullerian ducts (Brook, 1981). In patients with intersex disorders the degree of cryptorchidism is coincident with the degree of mullerian duct retention (Scott, 1987). This association of retained mullerian ducts and cryptorchidism is evidence for a common etiology with MIS control of both processes. Several experimental models have contradicted this idea however. In the first, conducted by Tran et al. (1986), antibodies against

bovine MIS failed to block testicular descent in rabbits. Another experiment conducted by van Vlissingen et al. (1988) showed that pig gubernacular fibroblasts did not respond to MIS when exposed in tissue culture. Future research will have to be undertaken to determine whether the natural experiments like mullerian duct syndrome or the laboratory experiments involving MIS give us the results indicating the role of MIS in testicular descent.

The second phase of testicular descent, inguinoscrotal descent, appears to be controlled by androgens, specifically testosterone and dihydrotestosterone. The means by which they act in this process is still largely unknown. Evidence for their action is provided by the observation that descent of the testis stops at the inguinal region in completely androgen resistant males (testicular feminizing syndrome) (Hutson, 1986). Also, androgen antagonists prevent inguinoscrotal descent in rats (Wensing, 1968). Androgens are believed to stimulate androgen receptors in the gubernaculum causing it to grow and migrate into the scrotum, thereby drawing the testis into the scrotal pouch. To date these androgen receptors have only been detected early in the development of the gubernaculum and not at the stage of inguinoscrotal migration of the testis (George, 1988). Hutson and Beasley (1987) have suggested that these androgens act on the gubernaculum indirectly through the genitofemoral nerve. Cutting this nerve causes cryptorchidism in neonatal rats (Lewis, 1948; Beasley 1987).

Though many of the detailed aspects of testicular descent in humans are based on animal models and are thus theoretical in nature, Hutson and Beasley (1992) have put forward a plausible theory of testicular descent that incorporates a majority of the available information. They believe that transabdominal descent is controlled by gubernacular enlargement, which is in turn regulated by MIS. Migration of the testis into the scrotum is believed to be indirectly controlled by androgens through the genitofemoral nerve. They also believe that the older ideas of relative

growth, gubernacular traction, and abdominal pressure to be important for proper descent of the testis. Overall the descent of the testis from its point of origin along the genitofemoral ridge to its final position in the scrotum is a very complex one, involving the coordination of both hormonal and mechanical mechanisms. Any perturbation in any of these mechanisms has the potential to cause cryptorchidism.

5.2.2. Etiology of Cryptorchidism

Testicular descent is believed to be the result of a complex series of hormonal and mechanical events. Any disruption of this process has the potential to cause cryptorchidism in the human male. Attention has therefore been focused on defects in the hypothalamic-pituitary-testicular (H-P-T) axis and on structural abnormalities as causes for cryptorchidism. Defects in the H-P-T axis, which result in clinical syndromes (Kallmann's, Prader-Willi's), have often been associated with cryptorchidism. Figure 5.3 shows the hypothalamic-pituitary-testicular axis and the associated defects in the axis that can cause cryptorchidism. As can be seen in the figure, there are also a number of syndromes which affect the H-P-T axis by disrupting gonadotropin synthesis, androgen production, or androgen function. Figure 5-4 also shows some of the more than 40 congenital malformations and developmental syndromes which have been associated with cryptorchidism.

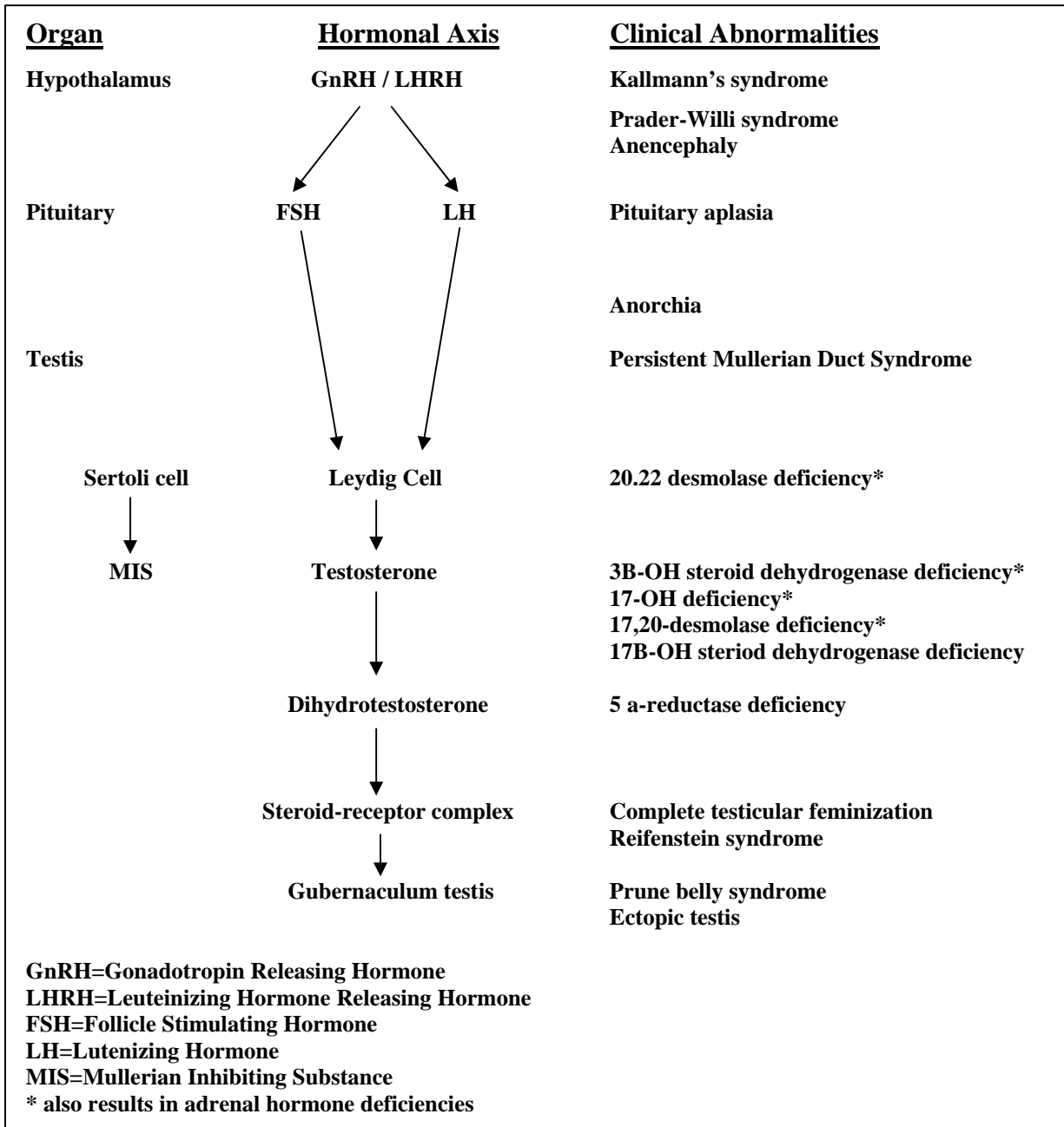


Figure 5-4: The hypothalamic-pituitary-testicular axis and clinical abnormalities associated with cryptorchidism.

Abnormalities of the hypothalamus and pituitary, testicular insufficiency, and primary failure of the testis are all causes of testicular maldescent (Hutson, 1990). These syndromes account for many cases of bilateral cryptorchidism, but in most cases a unilateral undescended testis is an unexplained abnormality. A small proportion (~6%) of unilateral patients may have an underlying defect in androgen synthesis or action which causes maldescent (Hortling, 1967). A common cause for the majority of cases of cryptorchidism is not known. We do know that the transabdominal phase of descent is rarely affected. Intraabdominal testes comprise a small group of about 5-10% of all cases, and in the majority of cases the testes have descended to or below the upper inguinal ring (Rozanski, 1995). Androgens are believed to play an important role in testicular descent through the inguinal canal into the scrotum. This leads researchers to believe that defects in androgen secretion may be a primary cause of undescended testes. Other causes of maldescent may be mechanical or structural in nature. A common example of a mechanical cause of cryptorchidism is presence of an inguinal hernia that is frequently associated with undescended testes (70% + in the current study, unpublished data). Any abnormality that can cause the inguinal canal to be closed prior to testicular descent or fail to close after descent has the potential to cause cryptorchidism.

Thus, potential causes of cryptorchidism include defects in the hypothalamic-pituitary-testicular axis or structural abnormality. Defects in the H-P-T axis need not cause overt syndromes to cause cryptorchidism. Normal secondary sexual characteristics and puberty can occur when testosterone levels are 1/16th of normal. Cryptorchidism may be a subtle variant of hypogonadotropic hypogonadism, which may be detected by a blunted or absent post-natal hormonal surge normally seen at 2-3 months of age (Rozanski, 1995). Cryptorchidism can result in a complex series of hormonal and testicular abnormalities that may lead to infertility or

testicular carcinoma. The next section will discuss the histology of the undescended testis and the potential negative outcomes associated with cryptorchidism.

5.2.3. Pathology of Cryptorchidism

The negative outcomes of infertility and testicular cancer have been associated with cryptorchidism. The etiologic mechanism behind these outcomes is not well understood at this point. What is understood however is that there is a rapid deterioration of germ cells in the undescended testis. There is also damage to the contralateral descended testis in men with unilateral cryptorchidism. Whether the deterioration of the testis is due to exposure to higher temperatures inside the body or to a congenital lesion associated with cryptorchidism is poorly understood.

The first suggestion that the higher temperatures found inside the body might be the cause of testicular degeneration was made by F.A.E. Crew in 1921. Since this time a number of investigators have provided evidence using animal models that testes are damaged by being inside the body. Moore (1926) showed, that in the guinea pig, a cryptorchid testis shows damage as soon as 6 days after being placed in the abdomen. Further experiments conducted in the rat showed that placement of the testis in the abdomen leads to a progressive loss of all spermatogenic cells (Nelson, 1951). Additional evidence that increased temperature is the cause of the damage is provided by experiments conducted by Moore and colleagues. Moore and Oslund (1923) demonstrated that there was testicular degeneration in rams whose testicles were wrapped in linen cloth for 90 days. This established that it was something other than simply the position of the testicle inside the body causing the degeneration. Further clarification of temperature being the cause is provided by Moore and Chase's (1923) experiments in which heat applied to the scrotum under a variety of conditions consistently produced damage in the testis.

Histologic changes in the contralateral descended testis in unilateral cryptorchidism could also be caused by elevated temperatures. Rapaport (1969) and Fernandez (1972) showed that thermal injury to the testes can cause autoimmune reactions. These reactions have the potential to affect the descended partners of undescended testes.

Evidence for the congenital nature of the defect which causes cryptorchidism comes from a number of sources. This evidence includes impaired fertility in both unilaterally and bilaterally cryptorchid men, evidence from biopsy specimens that germ cell numbers do not decline after early childhood, and evidence of damage to the contralateral descended testis in many cases of unilateral cryptorchidism. Unilaterally cryptorchid men who present with azoospermia provide evidence that there is damage to both testes even when one is descended normally. Men who are otherwise normal, but have only one testicle because of injury or torsion have reduced sperm counts, but do not commonly present with azoospermia (Ferreira et al., 1991). Evidence for the lack of a decline in germ cell numbers after early childhood comes from histologic examinations of biopsies from undescended testes and their descended partners. Gracia et al. (1995) found no difference in total fertility index (TFI), a proportion indicating the numbers of germ cells in a histological sample, in children who had orchidopexy before the age of two years and those who had surgery after age seven. Experiments with induced cryptorchidism in mice have shown that an undescended testis can induce damage in the descended partner. Salman et al. (1988) induced cryptorchidism by surgically elevating one testicle of the mouse into the abdomen. They found that there was significant damage to the contralateral descended testis by three weeks into the experiment. Based this data, they and others postulate that the damage is the result of an autoimmune response to the cryptorchid testis. Similar observations have been made in humans, with reduced sperm counts from descended testes observed in unilateral cryptorchid men (Shirai,

1966; Nagler, 1982). There is evidence for both damage from heat and a congenital defect resulting in reduced germ cell numbers in cryptorchid men, and it likely that reduced spermatogenesis is the result of both an inherent defect and increased temperature. These etiologies may act together, or be separate causes, but it seems likely that there are multiple causes of the reduction of fertility in cryptorchidism. Further research in this area is needed to uncover the primary cause, or causes, of testicular damage in cryptorchidism.

5.3. Epidemiology and Outcomes of Cryptorchidism

5.3.1. Incidence and Prevalence

Three large studies have reported on the incidence and prevalence of cryptorchidism (Scorer, 1964; Radcliffe Hospital Study Group, 1992; Berkowitz et al, 1993) and their results are summarized in table 5-1. The Scorer study was a cohort study of 3600 infants born in London in the late 1950's. The incidence of cryptorchidism was 4.3% at birth, declining to a prevalence of 0.97% at three months of age, and declining further to 0.78% at one year. The second study, conducted by the John Radcliffe Hospital Cryptorchidism Study Group (1992), was a prospective study of 7500 consecutive male births between November 1984 and October 1988. Incidence at birth was 5% and prevalence at 3 months was 1.78%. The third large cohort study was conducted by Berkowitz et. al. (1993). They assessed cryptorchidism prospectively in a cohort of 6935 males born between October 1987 and October 1990 at Mount Sinai Hospital in New York City. Incidence of cryptorchidism at birth was 3.68%, falling to 1% at three months of age, and 1.06% at one year. As in the two earlier studies, cryptorchidism rates were higher for low birth weight infants (<2500 g), with 19.83% cryptorchid at birth, and 1.94% cryptorchid at three months and one year after expected delivery date. Normal weight babies (2500+ g) had cryptorchidism rates of 2.22%, 0.91%, and 0.95% at birth, three months, and one year

respectively. There were significant differences between the low and normal birthweight babies only at birth (19.83% vs. 2.22%, $p < 0.001$). By three months and at one year there were no significant differences between the cryptorchidism rates. Overall, the rates for cryptorchidism in these three cohorts were very similar with a 3-5% incidence at birth declining to 1-2% prevalence in the first year of life.

Table 5-1: Comparison of Cryptorchidism Rates (%) at Birth, 3 Months, and 1 Year of Age

Age at Evaluation	Birth Weight (g)	Scorer ¹ (n=3612)	John Radcliffe Hospital ² (n=7400)	Mount Sinai Hospital ³ (n=6935)
Birth	<2500	21.00	22.83	19.83
	2500+	2.70	4.08	2.22
	Overall	4.30	5.01	3.68
3 Months	<2500	1.74	5.16	1.94
	2500+	0.91	1.61	0.91
	Overall	0.97	1.78	1.00
1 Year	<2500	1.67	No data	1.94
	2500+	0.69	No data	0.95
	Overall	0.78	No data	1.06

¹Scorer (1964)

²John Radcliffe Hospital Cryptorchidism Study Group (1992)

³Berkowitz et. al. (1993)

5.3.2. Outcomes of Cryptorchidism

There are two potential negative outcomes of cryptorchidism, testicular cancer and infertility. Cryptorchidism at birth, whether corrected or uncorrected, is widely accepted to increase risk of testicular cancer in adulthood. Cryptorchidism as a cause of infertility is however not as widely accepted by researchers or clinicians. Bilateral cryptorchidism is a cause of infertility, but the results of studies of unilateral cryptorchidism fail to show that fertility is reduced.

5.3.2.1. Cryptorchidism and Cancer

Men with a history of cryptorchidism have elevated rates of testicular carcinoma. Reports suggested that patients with cryptorchidism have a significantly increased risk of tumors, and this increase is generally felt to be 4 to 10 fold (Giwerzman, et al, 1988). Among all men who have

cryptorchidism, the lifetime risk of a testicular cancer has been reported to be 2 to 3 % and at least 4 times greater than the general population (Cortes, 1998, Moller et al, 1998). Based upon the development of testicular tumors among men with a history of cryptorchidism, the risk was 4.7 times greater as compared with the general population (Giwerzman, et al, 1988). The odds ratio among men with bilateral cryptorchidism was 9.3 and among the unilateral group 2.4. Even the contralateral descended testis seems at higher risk. Furthermore, a much greater cancer risk is present among men with uncorrected cryptorchidism (RR 15.9) (Pike et al, 1986). Furthermore, it is likely that many of the rates reported are falsely lowered by the inclusion of many patients who had retractile testes rather than truly cryptorchid testes.

5.3.2.2. Infertility

Fertility after cryptorchidism and orchiopexy has been evaluated by two measures, paternity and semen analysis. Paternity has the advantage of proving the man's potential for fertility by the birth of a child, while semen analysis allows for the assessment of a far larger proportion of formerly cryptorchid men as it can include those men who have not yet attempted paternity. Both study outcomes have their shortcomings as well. Use of paternity data has several potential problems including paternity certainty, the influence of the fertility of the female partner, and the requirement that paternity must be attempted in order to evaluate fertility. Semen analysis on the other hand is only a proxy measure of fertility. There are problems with the standardization of measurements, and with the determination of what constitutes a potentially fertile semen sample. Men with very low sperm counts, low motility, and poor morphology are still able to father children. It seems that studies of fertility after cryptorchidism based on paternity are more reliable measures of the true potential for fertility than semen analysis studies. The use of semen analysis as a proxy measure for fertility is useful however as it allows us to assess fertility in

relation to a number of other biological measures such as androgenic hormone levels, and to assess fertility potential in those who have never or will never attempt paternity.

Table 5-2 summarizes the results from studies which used paternity to assess fertility among cryptorchid men. Overall, paternity rates are markedly reduced among men with a history of bilateral cryptorchidism and slightly lower among men with unilateral cryptorchidism. However, all of these results must be viewed with caution since many of the studies have small sample size and lack of a control group for comparison purposes.

Table 5-2: Studies of Fertility after Cryptorchidism: Paternity

Authors	Year	Study Type	Population (Total N)	Bilateral # Fertile (%)	Unilateral # Fertile (%)	Control # Fertile (%)
Hand	1956	Prospective Cohort	Cryptorchidism (n=85)	15/24 (62%)	47/61 (77%)	N/A
			and Orchiopexy Patients (n=21)	4/9 (44%)	11/12 (92%)	
Atkinson	1975	Retrospective Cohort	Orchiopexy Patients (n=60)	8/18 (44%)	32/42 (76%)	N/A
Gilhooly et al.	1984	Retrospective Cohort	Orchiopexy Patients (n=145)	16/45 (36%)	80/100 (80%)	N/A
Fallon and Kennedy	1985	Retrospective Cohort	Orchiopexy Patients And Controls (n=99)	2/15 (13%)	35/38 (92%)	37/46 (80%)
Kumar et al.	1989	Retrospective Cohort	Orchiopexy Patients (n=71)	9/15 (60%)	47/56 (84%)	N/A
Cendron et al.	1989	Retrospective Cohort	Orchiopexy Patients (n=32)	3/9 (33%)	20/23 (87%)	N/A
Lee et al.	1997	Retrospective Cohort	Orchiopexy Patients And Controls (n=633)	24/39 (62%)	246/276 (89%)	298/318 (94%)
Combined Totals				66/150 (44%)	471/547 (86%)	335/364 (92%)

A summary of the studies which utilized semen analysis to measure fertility potential among cryptorchid men is shown as table 5-3. Again, the samples are relatively small and only one study collected data for a control group. Once again bilaterals show markedly reduced fertility potential as indicated by the low percentage of men with normal sperm densities. Sperm density is also reduced among unilaterals, but the reduction is not as pronounced as for bilateral men.

Table 5-3: Studies of Fertility after Cryptorchidism: Semen Analysis

Authors	Year	Study Type	Population	N	Bilateral w/ Normal Density	Unilateral w/ Normal Density	Control w/ Normal Density
Svend-Hansen	1949	Retrospective Cohort	Formerly Cryptorchid Men	25 Bilat 36 Unilat	28% (7/25) >10 Mill/mL	81% (29/36) >10 Mill/mL	N/A
Albescu et al.	1971	Retrospective Cohort	Formerly Cryptorchid Men	22 Bilat 21 Unilat	50% (11/22) > 60 Mill/mL	95% (20/21) >60 Mill/mL	N/A
Bramble et al.	1974	Retrospective Cohort	Formerly Cryptorchid Men	21 Bilat	48% (10/21) >20 Mill/mL	N/A	N/A
Lipshultz et al.	1976	Retrospective Cohort	Formerly Cryptorchid Men and Controls	29 Unilat 30 Control	N/A	72% (21/29) >20 Mill/mL Mean= 26.8 Mill/mL	100% (30/30) >20 Mill/mL Mean=73.6 Mill/mL
Retief	1977	Retrospective Cohort	Formerly Cryptorchid Men	29 Bilat 76 Unilat	35% (10/29) >20 Mill/mL	70% (53/76) > 20 Mill/mL	N/A
Okuyama et al.	1989	Retrospective Cohort	Formerly Cryptorchid Men	61 Bilat. 149 Unilat.	0% (0/61) >20 Mill/mL 77% (47/61) Azoospermic	72% (107/149) >20 Mill/mL 7% (10/149) Azoospermic	N/A
Mandat et al.	1994	Retrospective Cohort	Formerly Cryptorchid Men	23 Bilat. 112 Unilat.	26% (6/23) >20 Mill/mL	53% (59/112) >20 Mill/mL	N/A

If we consider fertility after cryptorchidism looking at both the data from the paternity and semen analysis studies, we can begin to see the overall effect of cryptorchidism on male fertility. It is readily apparent from both types of studies that fertility is greatly compromised by bilateral cryptorchidism. Paternity rates are between 13% and 62% in the seven paternity studies

reviewed, and sperm density is far below normal in each of the six semen analysis studies. Among formerly unilaterally cryptorchid men this reduction of fertility is not as readily observed, but it does appear to be present in many of the studies utilizing semen analysis. In the studies of paternity, it appears that unilaterals have reduced fertility, but that the reduction is not large, and that large samples of formerly cryptorchid men need to be studied in order to observe any statistically significant difference in paternity rates. The reduction in fertility potential seen in the studies measuring sperm density may not be evident when included in the complex process of actually conceiving and bearing a child. This process is a complex one that includes numerous mitigating factors in both the man and the woman attempting to have a child.

6. Cryptorchidism as a Public Health Problem

With an incidence of 3-5% at birth and a prevalence of 1% at one year of age, cryptorchidism affects a significant number of male children born worldwide. It represents the single most common defect at birth among human males and leads to significantly increased risks for both testicular cancer and infertility. Knowing these facts, investigators have attempted to better understand the etiology and pathophysiology of cryptorchidism by studying cohorts of men with the disorder. The Children's Hospital of Pittsburgh Male Fertility Study, upon which this work is based, represents one of the largest cohorts of formerly cryptorchid men investigated to date and it has allowed us to better understand the impact of cryptorchidism on male fertility. Cryptorchidism and its causes will need to be much better understood before it can be viewed as a problem with potential for public health intervention. Today we know too little about the causes of cryptorchidism to ever hope to impact it significantly. That said, in the developed world management of cryptorchidism utilizing orchiopexy by 1 year of age represents best current medical practice and appears to minimize the impact of the disorder on both fertility and

testicular cancer. In the developing world cryptorchidism may have a much greater impact on fertility and occurrence of testicular cancer since very few boys (if any) will undergo orchiopexy (Okeke and Osegbe, 2001). Future research should ideally focus on identifying any potential environmental and behavioral causes of cryptorchidism and on optimizing treatment of the disorder. In this way we can attempt to find areas in which intervention is possible in order to prevent cryptorchidism and also to maximize the fertility potential for those affected males while minimizing the impact of testicular cancer.

7. Summary

The manuscripts presented as articles 1-3 represent a portion of the results from the Children's Hospital of Pittsburgh Male Fertility study. Article 1 presents data on time to conception among formerly cryptorchid and control men, and is an attempt to examine the subtle influence that cryptorchidism can have on male fertility. Article 2 examines the impact of the placement of a transparenchymal suture during orchiopexy on subsequent fertility. Finally, article 3 looks at the effect of age at orchiopexy on hormone levels and sperm count in adulthood. These three manuscripts present data from each of three different data sources utilized as part of the Children's Hospital study, questionnaire data, medical record data, and clinical data. The data on time to conception were entirely derived from the 16-page questionnaire completed by study subjects. Data for the testicular suture manuscript were from both the questionnaire and from abstraction of subject medical records to determine if a testicular suture was placed at the time of orchiopexy. Finally, data on hormone levels and sperm count were collected from the secondary phase of the study in which subjects were asked to return to Children's Hospital for a clinical evaluation including a blood draw and semen analysis. The articles presented represent a cross section of the study and show the variety of data sources ascertained. The primary strength of the study is the large and well documented Children's

Hospital of Pittsburgh Male Fertility Study cohort from which the data for the three articles was drawn. The large study population provides us with data on a large group of formerly cryptorchid men who underwent orchiopexy and a matched set of controls. The population from which the subjects were drawn is believed to be a representative sample of the Allegheny County, PA population, and this is what we would want for the study results to be generalizable with respect to the U.S. population as a whole. Cryptorchidism is the most common congenital malformation among newborn males, and it is known to affect fertility in adulthood. Overall, the Children's Hospital of Pittsburgh study is large, comprehensive, and answers important questions concerning cryptorchidism's effect on male fertility.

Cryptorchidism has the potential to impact the health of the human male. Reduced fertility and increased risk of testicular cancer are the two negative outcomes associated with this congenital disorder. Formerly bilateral cryptorchid men have markedly decreased fertility (41% infertile vs. 6% in controls), while formerly unilateral cryptorchid men also have significantly reduced fertility. However, this deficit may not be readily apparent since the reduction is not large in absolute numbers (12% infertile vs. 6% control). Orchiopexy, while not a cure for the disorder, is currently best clinical practice and should be undertaken by one year of age. Surgery by this early age minimizes damage to the undescended testis and facilitates ongoing examination of the testis for signs of testicular carcinoma.

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