

Maternal Hormone Levels and Risk of Cryptorchism among Populations at High and Low Risk of Testicular Germ Cell Tumors

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Abstract

Cryptorchism is one of the few well-described risk factors for testicular cancer. It has been suggested that both conditions are related to increased *in utero* estrogen exposure. The evidence supporting the "estrogen hypothesis" has been inconsistent, however. An alternative hypothesis suggests that higher *in utero* androgen exposure may protect against the development of cryptorchism and testicular cancer. In order to examine both hypotheses, we studied maternal hormone levels in two populations at diverse risks of testicular cancer; Black Americans (low-risk) and White Americans (high-risk). The study population of 200 mothers of cryptorchid sons and 200 mothers of non-cryptorchid sons was nested within the Collaborative Perinatal Project, a cohort study of pregnant women and their children. Third trimester serum levels of estradiol (total, free, bioavailable), estriol, testosterone (total, free,

bioavailable), sex hormone-binding globulin, α -fetoprotein, and the ratios of estradiols to testosterone were compared between the case and control mothers. The results found no significant differences in the levels of testosterone (total, free, bioavailable), α -fetoprotein, sex hormone-binding globulin, or in the ratios of estrogens to androgens. Total estradiol, however, was significantly lower in the cases versus the controls ($P = 0.03$) among all mothers and, separately, among White mothers ($P = 0.05$). Similarly, estriol was significantly lower among all cases ($P = 0.05$) and among White cases ($P = 0.05$). These results do not support either the estrogen or the androgen hypothesis. Rather, lower estrogens in case mothers may indicate that a placental defect increases the risk of cryptorchism and, possibly, testicular cancer. (Cancer Epidemiol Biomarkers Prev 2005;14(7):1732-7)

Introduction

Cryptorchism, affecting ~1% to 4% of liveborn boys, is one of the most common congenital anomalies among males (1). Because cryptorchism is associated with other urogenital anomalies, low sperm count, infertility, and testicular cancer (2), Skakkebaek et al. proposed that the disorders were part of a "testicular dysgenesis syndrome" that originated in fetal life (3). Although the etiology is unclear, one dominant hypothesis has suggested that increased *in utero* estrogen exposure leads to the development of the testicular dysgenesis syndrome disorders (4, 5).

The "estrogen hypothesis" has been directly examined in several prior studies of cryptorchism (6-8). Only one of the studies, however, has provided support for the hypothesis (7). The inconsistencies may have been due to somewhat limited sample sizes and/or to the examination of hormone levels only in the first trimester of pregnancy. As testicular descent is a two-phase process that takes place in both first and third trimesters, hormone levels in the third trimester may be as important in understanding the etiology of cryptorchism as hormone levels earlier in pregnancy.

Another possible limitation of previous studies is that they have only included White mothers. Although it is not clear whether rates of cryptorchism differ by ethnicity in the U.S.,

rates of testicular cancer are five times higher in White men than Black men (9). This disparity in risk prompted an earlier investigation of hormone levels in Black and White mothers (10). Examining first trimester samples, the study found no difference in estrogen levels by ethnicity. Total testosterone levels, however, were significantly higher in the Black mothers, suggesting that testosterone might protect against the development of cryptorchism and/or testicular cancer.

In addition to hormone levels, it has been suggested that maternal levels of α -fetoprotein might be related to the subsequent development of cancer. High maternal α -fetoprotein levels have been associated with decreased risk of breast cancer in several studies (11-13). As α -fetoprotein may alter the hormonal milieu by binding estrogens, it is conceivable that α -fetoprotein might be related to cryptorchism/testicular cancer as well as to breast cancer.

In order to follow up on the leads of these earlier studies, we conducted an investigation of third trimester maternal hormone levels and risk of cryptorchism among two populations at varying risks of testicular cancer, Black Americans and White Americans.

Materials and Methods

Study Participants. The study participants were enrolled in the Collaborative Perinatal Project, a prospective study of pregnant women and their children (14). The women were recruited between 1959 and 1965 at 12 U.S. study centers [in Baltimore, Boston, Buffalo, Memphis, Minneapolis, New Orleans, New York (two), Philadelphia, Portland, Providence, and Richmond]. Eleven study centers recruited patients from the participating University Hospital's prenatal clinic, and one

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study center (Buffalo) recruited patients from 13 participating private practices. Women were ineligible if they were incarcerated, if they were planning to leave the area or to give up the child for adoption, or if they gave birth on the day they were recruited into the study. Once enrolled, the mothers' nonfasting blood was collected approximately every 8 weeks, at delivery, and 6 weeks postpartum. Sera were stored in glass at -20°C . Approximately 42,000 women were enrolled, and 55,000 children were born in the study. The children were systematically assessed for the presence of birth defects and other outcomes through age 7 years.

For the current research, a nested case-control design was used to study the association of maternal serum hormone levels and risk of cryptorchism among sons. Mothers' eligibility criteria were: (a) gave birth to a singleton boy, (b) baby lived at least 28 days, (c) gestational age at delivery of at least 26 weeks but no more than 48 weeks, (d) date of third trimester blood draw between 182 days gestation and 1 week prior to date of delivery, and (e) availability of a 1 mL aliquot of third trimester maternal serum. Gestational age at birth was calculated by subtracting the date of the mother's last menstrual period from the date of delivery. Among the 28,444 boys born in the Collaborative Perinatal Project, 267 were not liveborn, 441 were not singletons, and for 5,389, no maternal blood samples were available.

Among the remaining 22,347 boys, 241 were classified as having cryptorchism based on the original study record noting either unilateral or bilateral undescended testicles at any time during the first year of life. Of the 241 cryptorchid boys, the study record indicated that the testicles were descended at birth in 103. Because the cremasteric reflex (causes retraction of the testicles) is not well-developed in the first year of life (15), it was assumed that these 103 boys had been misclassified as noncryptorchid on their birth examination. Among those with at least one undescended testicle noted at birth ($n = 138$), all but one also had a subsequent observation of the abnormality on at least one of the three subsequent physical examinations (4 months, 1 year, and 7 years of age), or orchiopexy. Boys first observed to have an undescended testicle(s) after the first year of life were not classified as cryptorchid because of the impossibility of distinguishing true cryptorchism from retractile testes.

All mothers of cryptorchid sons were matched on ethnicity (Black, White) and baby's gestational age at birth to mothers of noncryptorchid sons. The 200 case-control pairs with the closest gestational ages of sons were selected for the study.

Maternal variables that had previously been reported to be associated with either cryptorchism or maternal hormone levels were included in the study. These variables included gestational age at blood draw, age at blood draw, height, prepregnancy weight, parity, smoking status at study enrollment, preeclampsia, and socioeconomic index. The socioeconomic index calculated for subjects in the Collaborative Perinatal Project was the mean of three percentile scores (for education, occupation, and family income), where education was for the head of the household, occupation was for the head of the household or chief wage earner, and the score used to calculate the percentile for an occupation was based on the percentiles of education and income among those with the same occupation (16).

Laboratory Analysis. All hormone assays were conducted in the laboratory of one of the authors (F.Z. Stanczyk) at the Reproductive Endocrine Research Laboratory of the University of Southern California Keck School of Medicine. Estriol, estradiol and testosterone were determined by well-established and validated RIA methods as previously reported (17, 18). Estriol was first extracted with hexane/ethyl acetate (10:1) to remove estrone and estradiol. Estriol in the aqueous fraction was then extracted with hexane/ethyl acetate (3:2). A separate

serum sample was used to extract testosterone and estradiol, using ethyl acetate/hexane (3:2). This was followed by isolation of the two steroids using Celite column partition chromatography using ethylene glycol as the stationary phase. Testosterone and estradiol were eluted with 15% ethyl acetate in isooctane and 40% ethyl acetate in isooctane, respectively. Appropriate tritiated internal standards were added to each serum sample prior to organic solvent extraction to follow procedural losses. After drying each fraction, the estriol fraction was reconstituted in ethanol and the other fractions in assay buffer. An aliquot of each was taken to determine procedural loss, and duplicate aliquots were taken for the RIA of each hormone. Each RIA uses an iodinated radioligand in conjunction with a specific antiserum (Diagnostic Systems Laboratories, Webster, TX). A seven-point standard was included in each assay. After an overnight incubation (16-18 hours), antibody-bound steroid was separated from unbound steroid by precipitation of the first antibody with a second antibody (Diagnostic Systems Laboratories), and subsequent centrifugation.

Sex hormone-binding globulin was quantified by direct chemiluminescent immunoassay using the Immulite analyzer (Diagnostic Products Corporation, Inglewood, CA). The sex hormone-binding globulin concentration was then used in a validated algorithm to calculate free testosterone or free estradiol, in conjunction with the total testosterone or total estradiol concentration, respectively, and an average albumin concentration of pregnant women (19).

α -Fetoprotein was quantified in serum by a solid-phase, two-site sequential chemiluminescent immunometric assay on the Immulite Analyzer (Diagnostic Products Corporation). The calibration range was up to 300 IU/mL, using the WHO First International Standard 72/225. The analytical sensitivity of the assay was 0.2 IU/mL. Various dilutions of samples ($n = 3$) with high α -fetoprotein levels showed linearity, with observed/expected values ranging from 97% to 114%. Evaluation of assay specificity showed nondetectable cross-reactivity with a variety of relevant proteins, e.g., albumin, hemoglobin, transferrin, and IgG.

Serum samples from the same blood pool were prepared for the purpose of quality control. Each sample was assigned a unique, random identification number in order to blind identity. Samples were analyzed in a total of 20 batches with each batch containing two quality control samples. The coefficients of variation were 4% for α -fetoprotein, 7% for sex hormone-binding globulin, 11% for estriol, 21% for estradiol, and 22% for testosterone.

Statistical Analysis. Ratios of sex hormones were calculated using serum levels of total, free, and albumin-bound estradiol divided by serum levels of total, free, and albumin-bound testosterone, respectively. The ratios and serum hormone levels were logarithmically transformed to achieve approximately normal distributions and the transformed values were used in all statistical analyses. χ^2 Analyses and matched t tests were employed to compare the distributions of the categorical covariates and the means of the continuous covariates. Conditional logistic regression was used to adjust for the matching variables in comparing serum levels of hormones and ratios of steroid hormones between case and control mothers, further adjusting for maternal age, gestational age at blood draw, and prepregnancy body weight as continuous variables. P values were calculated based on two-tailed tests of significance. Statistical analyses were done using the SAS system, version 8.02 (SAS Institute, Inc., Cary, NC).

Results

The study population originally included 200 cases and 200 matched controls. One matched set (a case and her matched control) were dropped from the analysis after examination of

Table 1. Distribution of maternal and neonatal characteristics in case and control mothers

	Black mothers			White mothers			All mothers		
	Cases (n = 77)	Controls (n = 77)	P	Cases (n = 122)	Controls (n = 122)	P	Cases (n = 199)	Controls (n = 199)	P
Age at blood draw (y)*	23.9 (6.0)	24.4 (6.3)	0.65	26.3 (7.0)	25.3 (6.1)	0.24	25.4 (6.7)	24.9 (6.2)	0.49
Height (in.)*	63.5 (2.8)	63.6 (2.5)	0.97	63.6 (2.6)	63.7 (2.4)	0.88	63.6 (2.7)	63.6 (2.4)	0.93
Prepregnancy weight (lbs)*	138.9 (31.8)	133.3 (26.8)	0.28	130.8 (24.8)	129.4 (20.2)	0.75	134.0 (28.0)	130.9 (23.0)	0.31
Gestational age (wk) [†]	30.3 (2.7)	31.1 (2.7)	0.10	31.3 (2.4)	30.9 (2.6)	0.25	30.9 (2.6)	31.0 (2.6)	0.78
Birth weight (g)	3,138.0 (548)	3,182.6 (527)	0.60	3,370.8 (501)	3,383.8 (507)	0.83	3,281 (531)	3,306 (523)	0.61
Placental weight (g)*	442.0 (101.9)	422.9 (95.3)	0.46	449.8 (91.8)	460.1 (86.9)	0.32	447.0 (95.4)	447.2 (91.4)	0.72
Prior pregnancies (n, %)									
No	21 (27%)	16 (21%)	0.28	23 (19%)	35 (29%)	0.07	44 (22%)	51 (26%)	0.46
Yes	53 (69%)	61 (79%)		98 (80%)	86 (70%)		151 (76%)	147 (73%)	
Unknown	3 (4%)			1 (1%)	1 (1%)		4 (2%)	1 (1%)	
Smoking (n, %)									
No	38 (49%)	43 (55%)	0.42	50 (41%)	60 (50%)	0.24	88 (44%)	103 (52%)	0.18
Yes	39 (51%)	34 (45%)		72 (59%)	61 (50%)		111 (56%)	95 (48%)	
Unknown					1				
Preeclampsia (n, %)									
No	74 (96%)	73 (95%)	0.99	117 (96%)	120 (98%)	0.25	191 (96%)	193 (97%)	0.40
Yes	3 (4%)	3 (4%)		5 (4%)	2 (2%)		8 (4%)	5 (3%)	
Unknown		1					0	1 (1%)	
Socioeconomic index (n, %)									
Low	18 (24%)	21 (27%)	0.14	10 (8%)	11 (9%)	0.49	28 (14%)	32 (16%)	0.14
Medium	50 (65%)	40 (52%)		51 (42%)	42 (34%)		101 (51%)	82 (41%)	
High	8 (10%)	16 (21%)		60 (49%)	68 (56%)		68 (34%)	84 (42%)	
Unknown	1 (1%)	0		1 (1%)	1 (1%)		2 (1%)	1 (1%)	

*Mean and SD of variable is shown.

[†]Gestational age at blood draw.

the hormone values revealed extreme outlying values for one member of the pair. Thus, the final population included 199 cases and 199 controls. Demographic and maternal characteristics of the cases and controls are shown in Table 1.

There were no significant differences in maternal age at blood draw, height, prepregnancy weight or gestational age at blood draw between all cases and controls, or between the cases and controls stratified on ethnicity. Similarly, there were no significant differences in birth weight of the babies or in placental weight. In addition, there were no significant differences in parity, smoking at the time of study registration, preeclampsia, or socioeconomic status. An examination of the covariates and the hormone values found that, as anticipated, maternal weight and age were significantly correlated with levels of total estradiol, whereas maternal weight, age and son's birth weight were significantly correlated with estriol (data not shown).

Hormone levels of the cases and controls, adjusted for gestational age at blood draw, are shown in Table 2. There were no significant differences in levels of α -fetoprotein, sex hormone-binding globulin, testosterone, free testosterone, bioavailable (free plus albumin-bound) testosterone, free estradiol, bioavailable estradiol or in any of the ratios of estradiol-to-testosterone among all case and control participants, or among the Black and White participants separately. In contrast, levels of total estradiol were significantly lower ($P = 0.03$) among the cases than the controls. Similarly, estriol levels were significantly lower ($P = 0.05$) among the cases than the controls. Among all mothers, the odds ratio for the increase in estradiol from the 25th to the 75th percentile was 0.74 [95% confidence interval (CI), 0.56-0.97]. The corresponding odds ratio for estriol was 0.73 (95% CI, 0.53-0.99).

After stratification on ethnicity, the results for the White mothers were very similar to the results for all mothers combined. The White case mothers had significantly lower estradiol ($P = 0.05$) and estriol ($P = 0.05$) levels than did their matched controls. Although the results for estradiol ($P = 0.38$) and estriol ($P = 0.71$) among the Black case mothers and their controls were not statistically significant, the differences were in the same direction as in the White mothers. Among the

White mothers, the odds ratio for the increase in estradiol from the 25th to the 75th percentile was 0.72 (95% CI, 0.52-0.99). The corresponding odds ratio for estriol was 0.64 (95% CI, 0.40-0.99).

Further adjustment of the hormone values for the correlated covariates did not greatly alter the results (Table 2). After adjustment for gestational age at blood draw, maternal age, and maternal prepregnancy weight, estriol levels remained significantly lower among all case mothers ($P = 0.04$; odds ratio, 0.71; 95% CI, 0.51-0.98) and among White case mothers ($P = 0.04$; odds ratio, 0.59; 95% CI, 0.36-0.97). Total estradiol levels also remained significantly lower among all case mothers ($P = 0.05$; odds ratio, 0.74; 95% CI, 0.55-0.99), although the effect was somewhat attenuated among the White mothers ($P = 0.09$; odds ratio, 0.72; 95% CI, 0.49-1.04). Further adjustment of the model, to include parity and smoking, did not affect the results.

Discussion

The estrogen hypothesis has postulated that increased estrogen levels *in utero* are related to increased rates of a group of disorders known collectively as the testicular dysgenesis syndrome (3). Among these disorders are cryptorchism and testicular germ cell tumors. The failure of the current study to find higher maternal estrogen levels in cases compared with controls lends little support to this hypothesis. In the current study, in fact, maternal estrogen levels were significantly higher in the control mothers than in the case mothers.

The estrogen hypothesis in cryptorchism has been examined in several prior studies. Also evaluating mothers from the Collaborative Perinatal Project, Bernstein et al. (7) studied first trimester serum levels of estradiol, testosterone, sex hormone-binding globulin, and human chorionic gonadotropin in 24 White case mothers and 24 White control mothers. The study found no difference in total estradiol levels, but did find that the case mothers had significantly higher percentages of free ($P = 0.01$) and albumin-bound estradiol ($P = 0.014$) than did the control mothers. A similar study in the United

Table 2. Crude and adjusted hormone values of case and control mothers

	<i>n</i>	Cases, mean (SD)	Controls, mean (SD)	<i>P</i> *	<i>P</i> †
α-Fetoprotein (IU/mL)					
Total	199	205.3 (90.9)	207.6 (102.5)	0.90	0.96
Black	77	190.4 (83.2)	197.4 (122.6)	0.83	0.77
White	122	214.7 (94.6)	214.1 (87.4)	0.81	0.95
Sex hormone-binding globulin (nmol/L)					
Total	198	541.1 (176.2)	545.3 (162.8)	0.88	0.88
Black	77	564.8 (198.2)	566.4 (161.9)	0.78	0.47
White	121	526.1 (159.6)	531.9 (162.6)	0.83	0.71
Testosterone (pg/mL)					
Total	199	142.3 (86.2)	152.0 (99.9)	0.24	0.65
Black	77	180.5 (104.8)	197.8 (133.3)	0.36	0.65
White	122	118.1 (61.1)	123.1 (54.8)	0.52	0.87
Free testosterone (pg/mL)					
Total	198	4.5 (2.9)	4.6 (2.8)	0.74	0.72
Black	77	5.4 (3.2)	5.7 (3.6)	0.58	0.69
White	121	3.8 (2.5)	3.8 (1.6)	0.85	0.44
Bioavailable testosterone (ng/dL)					
Total	198	9.3 (6.1)	9.6 (5.8)	0.74	0.72
Black	77	11.4 (6.8)	12.0 (7.6)	0.58	0.69
White	121	8.0 (5.3)	8.0 (3.5)	0.84	0.43
Estradiol (ng/mL)					
Total	199	18.8 (7.8)	20.8 (9.7)	0.03	0.05
Black	77	18.1 (7.3)	19.9 (9.5)	0.38	0.50
White	122	19.2 (8.1)	21.3 (9.8)	0.05	0.09
Free estradiol (pg/mL)					
Total	198	114.8 (52.7)	125.0 (59.2)	0.10	0.17
Black	77	106.5 (47.2)	115.7 (57.6)	0.45	0.39
White	121	120.1 (55.5)	130.8 (59.8)	0.11	0.30
Bioavailable estradiol (pg/mL)					
Total	198	2,496.3 (1,146.3)	2,717.7 (1,288.3)	0.09	0.17
Black	77	2,316.2 (1,024.8)	2,516.8 (1,252.4)	0.45	0.39
White	121	2,610.8 (1,207.5)	2,845.6 (1,299.6)	0.11	0.29
Estradiol (ng/mL)					
Total	197	11.2 (5.0)	12.0 (4.8)	0.05	0.04
Black	76	10.9 (6.5)	12.0 (5.3)	0.71	0.74
White	121	11.4 (3.9)	12.0 (4.4)	0.05	0.04
Estradiol/testosterone					
Total	199	15.6 (7.1)	15.8 (7.1)	0.81	0.63
Black	77	12.1 (6.1)	11.9 (5.1)	0.54	0.61
White	122	17.8 (6.9)	18.3 (7.1)	0.36	0.30
Free estradiol/free testosterone					
Total	198	31.1 (15.0)	31.7 (15.5)	0.66	0.55
Black	77	23.9 (12.8)	23.8 (10.7)	0.58	0.64
White	121	35.7 (14.5)	36.7 (16.0)	0.28	0.27
Bioavailable estradiol/bioavailable testosterone					
Total	198	322.7 (156.0)	328.1 (160.5)	0.68	0.57
Black	77	248.3 (132.7)	246.2 (111.1)	0.55	0.62
White	121	370.0 (151.7)	380.3 (165.6)	0.29	0.28

*Adjusted for gestational age at blood draw.

†Adjusted for gestational age at blood draw, maternal age, and maternal prepregnancy weight.

Kingdom by Burton et al. (6) examined 30 case mothers and 30 control mothers. The study found no significant difference between first trimester estradiol levels in the cases versus the controls. In fact, as with the current study, the case mothers had lower estradiol levels than did the control mothers, although the difference was not statistically significant. In a third study, also conducted in the United Kingdom, Key et al. (8) studied 28 case mothers and 108 control mothers. Samples were collected between weeks 6 and 20 of gestation. The investigators found no significant differences in estradiol when examining all samples. When examining only the samples collected during the first trimester, the study also found no significant difference in estradiol level, although the case levels were lower than the control levels. Thus, in three of the four reported studies, including the current study, maternal estradiol levels were lower in the cases than in the controls (6, 8), although the difference was statistically significant only in the current study.

It is worth noting that the estrogen hypothesis has evolved over time to propose that other mechanisms, such as estrogen/androgen imbalance or antiandrogen effects, might also be related to cryptorchism (20). The "androgen hypothesis" has also been suggested by several studies (8, 10, 21-23). Most studies did not directly examine cryptorchism, but rather compared maternal hormone levels in populations at high- and low-risk of testicular cancer. Examining Black and White mothers from the Collaborative Perinatal Project, Henderson et al. reported that Black mothers had significantly higher testosterone levels than did White mothers in the first trimester (10). Given the low rate of testicular cancer and high rate of prostate cancer among Black men relative to White men, Henderson et al. suggested that high *in utero* testosterone exposure might be related to both cancers. Higher testosterone levels among Black mothers in comparison with White mothers have also been reported by Troisi et al. (21) and Zhang et al. (22). Indeed, the current study also found that Black mothers had higher testosterone levels than White mothers. Similarly, in comparing Chinese mothers to White mothers, Lipworth et al. (23) reported higher testosterone levels in Chinese mothers. Like Black men, Chinese men have much lower incidence of testicular cancer than do White men.

Although the results of these hormone comparisons in low- and high-risk populations suggest that increased maternal testosterone might protect against cryptorchism and/or testicular cancer, the direct examinations of the hypothesis are not as supportive. Although Key et al. (8) reported lower testosterone levels in case mothers in the first trimester, the results were only of borderline significance ($P = 0.06$). In contrast, neither the Bernstein et al. study (7) nor the current study found significant differences in testosterone levels between cases and controls.

Why the results have varied across studies is not clear but may be related to several factors. The three studies previous to the current one each included ≤ 30 cases. Because all studies, except that of Burton et al. (6), examined several hormones, chance findings may have arisen. In addition, the definitions of cryptorchism have also varied across studies, thus possibly complicating interpretation of the results. Cryptorchism cannot be reliably diagnosed in the delivery room because a nontrivial number of boys have testes that descend after birth. In addition, retractile testes (testes that can be manipulated into the scrotum, but can also spontaneously ascend) appear in some boys after birth. Thus, it is important to have boys evaluated at some time during the first year of life to determine whether a diagnosis of cryptorchism is warranted as most testes that will descend spontaneously have done so by age 6 months. The Bernstein et al. (7) study included boys identified as cryptorchid at either the 1-year or 7-year examination, thus possibly misclassifying as cryptorchid some boys who had retractile testes. The Burton et al. study (6) identified cryptorchid boys via referral to surgeons, but did not indicate the age or certainty of diagnosis. The Key et al. (8) study identified boys who were diagnosed as cryptorchid in the delivery room and had a confirmatory diagnosis at 3 months, thus including mainly truly cryptorchid boys. In the current study, the case boys were required to have a diagnosis of cryptorchism at any time during the first year of life. Boys who were first noted to be cryptorchid subsequent to age 1 year, were excluded from the study to reduce the possibility of misclassifying retractile testes as cryptorchism.

The results may also have varied because the three prior studies obtained blood samples during the first, or first and second, trimesters rather than during the third trimester. Although hormone levels vary greatly throughout pregnancy, a companion study to the current investigation in the Collaborative Perinatal Project found very good correlation between hormone levels in first and third trimesters (22). In addition, third trimester hormone levels may be more

etiologically important than early pregnancy levels as 75% to 90% of undescended testes are found in the inguinal canal, which suggests a failure during the second phase of testicular descent occurring late in third trimester (24). As a minority of undescended testes can also be found intraabdominally, a placement consistent with failure in the first phase of testicular descent, it is also possible that the inconsistent results are due to heterogeneity in the etiology of cryptorchism.

In addition to not supporting the estrogen hypothesis in cryptorchism, the current study also did not find that α -fetoprotein levels were related to cryptorchism. Several studies have reported that increased maternal α -fetoprotein levels are associated with decreased risk of breast cancer (11-13) and have suggested α -fetoprotein, because of its estrogen-binding ability, inhibits the growth of estrogen-sensitive cells. In support of this argument, it has been reported that α -fetoprotein levels are higher in preeclamptic births (25) and in multiple births (26) and that preeclampsia (27) and multiple birthing (28) themselves have been associated of a reduced risk of breast cancer. In contrast, both preeclampsia and multiple birthing have been reported to increase the risk of testicular dysgenesis syndrome disorders (29, 30), thus suggesting that increased α -fetoprotein level might be associated with increased risk of cryptorchism/testicular cancer. The current study did not find an association of α -fetoprotein and cryptorchism, however. The current study also did not find an association between preeclampsia and cryptorchism, although the power to detect an association was very low. Nevertheless, preeclampsia was twice as common among White cases (4%) as among White controls (2%).

One question raised by the current results is whether the significantly higher levels of estradiol and estriol among control mothers in the third trimester suggest that estrogens might protect against the development of cryptorchism. In support of such a supposition are several studies demonstrating that populations at low-risk of testicular cancer have higher levels of maternal estradiol in the first trimester. For example, Zhang et al. (22) and Lipworth et al. (23) reported that Black mothers and Chinese mothers, respectively, have higher estradiol levels than White mothers in the first trimester. An alternative explanation of the increased estrogen levels among the controls, however, is that the placentae of the controls are better at converting fetal and maternal precursors into estrogens. Such an explanation is consistent with the findings of previous studies which suggested that cryptorchism was the result of an underlying placental defect (31-35). Based on the association between cryptorchism and intrauterine growth retardation, Davies et al. (31) hypothesized that an impaired placenta might limit the secretion of human chorionic gonadotropin, which would adversely affect the secretion of fetal testosterone, thereby leading to defective testicular descent. Subsequent studies have lent support to the hypothesis (32-34) and even expanded upon it (35). The findings that preterm birth, fetal growth retardation, twin birth, low parity, low Apgar score and preeclampsia are related to risk of cryptorchism also support a role for placental insufficiency in the etiology of cryptorchism.

The current study has strengths as well as weaknesses. Its strengths are its relatively large sample size, inclusion of two ethnic groups and ascertainment of cryptorchism outside the delivery room. Potential weaknesses include the study's reliance on stored samples and the determination of hormone levels in maternal serum rather than in fetal serum. Although long-term storage of samples is a logical concern, the current results indicate that degradation of values was not great as the hormone levels reported in the current study are similar to levels reported in studies of contemporaneously collected samples (23). The reliance on maternal serum might be a more substantive concern as recent findings have drawn into question whether maternal hormone levels accurately reflect

levels experienced by the fetus (36, 37). One study found little correlation between umbilical cord hormone levels and maternal hormone levels (36). A second study, however, found significant correlations between both umbilical cord levels and amniotic fluid levels of estradiol and third trimester maternal estradiol levels among male fetuses (37). Correlations between amniotic fluid, umbilical cord, and maternal testosterone levels were not significant, however. These results suggest, minimally, that our estradiol results may be a good approximation of levels experienced by the fetus.

In summary, the current findings suggest that increased maternal estrogens are not related to increased risk of cryptorchism and may be related to decreased risk. Further study of the relationship between hormones and cryptorchism or hormones and testicular cancer is clearly warranted and may be particularly informative if amniotic fluid, umbilical cord samples, or neonatal blood samples could be examined.

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