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Neonatal Hormone Concentrations and Risk of Testicular Germ Cell Tumors (TGCT)

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Abstract

Background—Testicular germ cell tumor (TGCT) incidence has increased over the last 40 years in the United States. In contrast to TGCT among infants, it is hypothesized that TGCT in adolescents and young men is the result of sex steroid hormone imbalance during early fetal development. However, little is known about the neonatal period when abrupt hormonal changes occur, and direct supporting evidence is scarce due to the difficulties in obtaining pre-diagnostic specimens.

Methods—We conducted a population-based case-control study examining hormone levels at birth among 91 infants (0–4 years) and 276 adolescents (15–19 years) diagnosed with TGCT, and 344 matched controls. Estrogen and androgen levels were quantified using liquid chromatography-tandem mass spectrometry (LC-MS/MS) from archived newborn dried blood spots. Logistic regression models were used to estimate the association between each hormone level and TGCT risk.

Results—Higher levels of androstenedione were associated with increased TGCT risk among adolescents (OR: 2.33, 95% CI: 1.37–3.97 for highest vs. lowest quartile; p-trend=0.003) but not among infants (OR: 0.70, 95% CI: 0.28–1.77). A similar pattern was observed for testosterone (OR: 1.73, 95% CI: 1.00–3.00,) although the trend was not significant (p-trend=0.12). Associations were stronger among non-Hispanic white subjects, relative to Hispanics. There was

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no difference by tumor histologic subtype. Estriol (the only detectable estrogen) was not associated with TGCT risk in either age group.

Conclusions—Higher levels of neonatal androgens were associated with increased risk of TGCT among adolescents, suggesting that early life hormone levels are related to the later development of TGCT.

Keywords

testicular cancer; testicular germ cell tumors; hormones; sex steroid hormones; biomarkers

Introduction

The incidence of testicular germ cell tumors (TGCT), the most common type of testicular malignancy, has increased during the past 40 years in the United States (US), with the most rapid recent increases observed among men of Hispanic descent (also referred to as Latinos) [1, 2]. It is the most commonly occurring cancer among male adolescents and young adults (AYA), defined by ages 15–19 years and 20–39 years, respectively[3, 4]. Globally, TGCT incidence has increased in almost every country in which it has been studied[5]. TGCT is comprised of two major histologic types: seminomas and nonseminomas. AYA develop both seminomas and nonseminomas, while infants (ages 0–4 years) are almost exclusively diagnosed with nonseminomas (either yolk sac tumors or teratomas)[6]. The increases in incidence rates are greatest among AYA males and men under age 50[7].

TGCT in AYA are characterized by high heritability and polygenic architecture, and it has been suggested that almost half of TGCT are due to inherited genetic factors[8–10]. However, the increasing incidence rates of TGCT are suggestive of environmental factors in TGCT etiology,[11, 12], alone or in combination with genetic factors, although to date, no environmental factors have been identified.

Estrogens and androgens play a central role in the development of the testis,[13] and may have a critical role in the etiology of TGCT. Germ cell neoplasia in situ (GCNIS), the precursor lesion of the seminomatous and non-seminomatous TGCT most common among AYAs, strongly resemble fetal gonocytes, supporting the hypothesis that TGCT is of fetal origin and a late-onset manifestation of the failure of normal fetal differentiation of primordial germ cells to spermatogonia[14]. Infantile TGCT is thought to differ from that of adult and AYA TGCT and be unrelated to GCNIS[15]. Another critical window for the development of TGCT may be the transition from intra-uterine to extra-uterine life, which is marked by a postnatal surge of the newborns' steroid sex hormones and other regulatory hormones.

While originally hypothesized that TGCT is related to increased exposure to estrogens during development[16], later studies contradicted this theory[17]. More recently, it has been proposed that androgen insufficiency, or an imbalance between androgens and estrogens during testis development, may be relevant[5, 17]. Supporting studies have been retrospective focusing on maternal exposure to exogenous hormones during pregnancy and tumors occurring 20–40 years later in male offspring[5, 18]. However, assessing the

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potential role of perinatal sex hormones in the neonate has proven challenging, mainly due to difficulties in obtaining perinatal biospecimens, particularly when TGCT onset is typically decades later. Regarding the postnatal period, epidemiologic studies have not indicated that external hormonal exposures are associated with TGCT[5].

The current study is the first to examine the relationship between sex steroid hormone levels at birth and risk of TGCT among infant and adolescent males.

Methods

Study Population

TGCT cases and controls were selected from the Childhood Cancer Record Linkage Project (CCRLP). Details of the CCRLP have been described previously[19]. Briefly, the CCRLP was created using a probabilistic record linkage of cancer registry records from the California Cancer Registry (CCR) to birth records maintained by the Vital Statistics unit of the California Department of Public Health (CDPH). Cases were diagnosed from 1988 (the earliest year the CCR data were electronically available) through 2011 (when the linkage was conducted) and born in or after 1982 (the earliest year the California birth data were electronically available and archived newborn blood spots are available). By comparing CCRLP cases to California SEER registry data for years 2000–2010 (years with complete SEER coverage of California), we estimated that ~70% of pediatric (0–19 years of age at diagnosis) cancer cases were linked to California birth certificates. Cases included in this analysis were diagnosed with testicular germ cell cancer (International Classification of Childhood Cancer, 3rd edition recode 103; International Classification of Diseases for Oncology, 3rd edition [ICD-O-3], morphology codes 9060–9065 [germ cell tumors] 9070–9072 [embryonal carcinoma] 9080–9085 [teratomas] 9100, 9101, 9105 [choriocarcinoma]; topography codes C62.0, C62.1, C62.9). Only subjects with invasive cancer were included. To increase power to detect associations in the two age groups of interest for childhood TGCT, we selected only cases aged 0–4 years (“infants”) and 15–19 years (“adolescents”) at diagnosis. These age groups have the highest incidence of pediatric TGCT and comprise 95% of the cases in our pediatric population. Cases were sampled to roughly reflect the age distribution in the population, with 25% being infants and 75% being adolescents.” Control subjects were randomly selected from the statewide birth records and matched to the case on year and month of birth and race/ethnicity (Hispanic, non-Hispanic white, non-Hispanic black, non-Hispanic Asian/Pacific Islander, non-Hispanic other). Eligible controls were those who were cancer-free by age 19, or by the year 2011 (whichever came first). A total of 370 TGCT cases and 370 matched controls were selected for this study. This study was approved by the State of California’s Committee for the Protection of Human Subjects (protocol #12-07-0529) under the US Common Rule, where information and biospecimens may be obtained and used for research by the Department or Department-approved scientific researchers without identifying the person or persons from whom these results were obtained. Research protocols were approved by Institutional Review Boards at the CDPH and the University of California, Berkeley.

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Data Collection

Biologic specimens were obtained through the CDPH California Biobank Program (CBP), the entity that represents the biospecimen and data resources of the CDPH California Genetic Disease Screening Program (GDSP), including the newborn screening program (NBS). The California NBS is a public health program initiated in 1966 that screens all babies shortly after birth for serious but treatable genetic disorders. Shortly after birth, a few drops of blood from the newborn's heel are collected on filter paper and sent to a state-contracted regional laboratory for testing. The residual dried blood spot (DBS) samples are sent to the CDPH laboratory for archiving.

DBS specimens were located for 370 cases and 344 controls. For each specimen, CBP supplied information on the age of the blood spot in hours (time from birth until blood draw). Information on sociodemographics and birth characteristics were obtained from birth records. Data on birthweight, gestational age, race, maternal and paternal age at delivery, maternal and paternal educational status at delivery, mode of delivery, plurality (single vs. multiple births), birth order, maternal birthplace, maternal history of miscarriage, and maternal history of stillbirth were abstracted to account for potential confounding and/or to examine potential effect modification.

Hormone Measures

Hormone assays in DBS specimens were carried out using liquid chromatography-tandem mass spectrometry (LC-MS/MS) at the ZRT Laboratory (Beaverton, OR), and blinded to case-control status. The ZRT laboratory has shown that steroids and peptide hormones in DBS specimens are stable for at least 10 years when maintained frozen at −70°C [20, 21]. Prior to processing study samples, a pilot study using 25 randomly selected and freshly collected neonatal DBS specimens (from infants born within the last 30 days) from CBP was conducted to 1) help determine the minimum amount of specimen that can be used to produce valid and reproducible measures, and 2) evaluate the stability of steroid hormones in DBS specimens that were up to 20 years old. All methods were optimized during this pilot study. Four 3-mm punches (~12 ul of blood) from each DBS spot were rehydrated, partially purified by solid phase extraction (SPE) column chromatography, eluted into solvent, dried, reconstituted and derivatized to increase sensitivity (estrogens only), and then run by LC-MS/MS. Methods for sex steroid measures by LC-MS/MS have been described and validated previously[22]. In brief, LC-MS/MS was carried out using a Shimadzu Prominence UFLC system equipped with an InfinityLab Poroshell 120 EC-C8 (Agilent) column (3.0 mm × 50 mm × 2.7 μm) coupled to a Sciex 5500 tandem quadrupole mass spectrometer with APCI source. All analytes were monitored in a single analytic run using a mobile phase variably consisting of 20% – 95% methanol. Internal standards (deuterated or C13) were included in the extraction solvent for each steroid being extracted. Mass transitions and retention times for each hormone are listed in Supplementary Table 1. Assay detection limits for each analyte are shown in Table 1. All samples were run with Biorad serum controls (low, intermediate, and high levels of hormones) that were prepared by mixing (1:1) with washed red blood cells that were then spotted onto Whatman filter cards, dried overnight, and stored with a desiccant in plastic bags at −70°C. Each control was confirmed by repeat testing using eleven assays over a sixty day period, to establish intra-

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and inter-assay coefficients of variability. The CVs for each hormone at various concentrations are given in Supplementary Table 2. The cutoff for the lower limit of quantification (LLOQ) was 20% CV, similar to the cutoff used in other studies[22], and each hormone had a signal to noise ratio of at least 10 at the LLOQ..

Valid ranges for each hormone were established by comparing our measures in controls to corresponding hormones in DBS from other populations of infants[22, 23], as well as published reference ranges from commercial diagnostic laboratories (www.questdiagnostics.com, www.mayomedicallaboratories.com, www.esoterix.com) for infants. In general, androstenedione, testosterone, and DHEA measures in our population were similar to those in reference populations,[22, 23] with some variation due to our larger sample size (n=714 vs. n=105–147 males) and tighter time frame from birth to blood sampling (~80% of our subjects had their blood drawn in the first 48 hours after birth, while the reference population blood draws were as much as 33 weeks after birth). There were no comparable published reference ranges for estrogens and progesterone in newborn infants; however, because estrogen levels in newborns at birth are very elevated (reflective of levels in late pregnancy and in placenta [24, 25]), then drop precipitously after birth and reach pre-pubertal levels during the first week of life[26], the wide range of estrogen measures in our population is expected. Progesterone follows a similar pattern[27].

The final hormone panel of sex steroid hormones measured included estradiol, estriol, estrone, testosterone, progesterone, androstenedione, dehydroepiandrosterone (DHEA), and dihydrotestosterone (DHT).

Statistical Analysis

Of the 716 DBS that were obtained from GDSP, two controls were removed for mismatched gender, leaving a total sample size of 370 cases and 344 controls. Pearson's chi-square tests were used to compare cases and controls on sociodemographic and reproductive characteristics. Odds ratios (ORs) and 95% confidence intervals (CIs) were estimated using unconditional logistic regression. Two-way scatterplots and statistical models plotting neonate age (in hours) at blood draw against each hormone among controls suggested a correlation with all hormones (r^2 range: 0.0199–0.2642; p-value range: 0.01–<0.0001) except estradiol and estrone (r^2 range: 0.001–0.009; p-value range: 0.0804–0.4265) (Supplementary Table 3). Adjusted models included year of birth, race/ethnicity, and neonate age at blood draw (except for estradiol). All hormones were modeled categorically (centiles above LOD as defined among controls); p-trends were calculated for grouped categorical variables. Because infant and adolescent germ cell tumors are pathogenically different[15], all analyses were stratified by age at diagnosis (0–4 years vs. 15–19 years). Additionally, stratified analyses by histologic subtype (seminomas vs. non-seminomas), race/ethnicity (non-Hispanic white vs. Hispanic), and method of delivery (vaginal vs. caesarian) were conducted for adolescents only. The small number of infants in this study precluded their examination in stratified analyses. SAS (version 9.4, SAS Institute, Cary, North Carolina) and STATA (release 13, StataCorp, College Station, TX) were used for all analyses. All tests were two-sided, and $p < 0.05$ indicated statistical significance. Evidence of effect modification was defined as p-interaction < 0.20.

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Sensitivity analyses were conducted to ensure that 1) potential outlier hormone measures, 2) subjects whose blood was drawn more than three days after birth, and 3) the inclusion of multiple births, did not bias results. For the first sensitivity analysis, subjects whose androgen measures were higher than those observed from commercial laboratories (androstenedione> 290 ng/dL, testosterone>400 ng/dL, or DHEA>12.92 ng/dL) were excluded, removing 51 (14%) cases/59 (17%) controls, 4 (1%) cases/7 (2%) controls, 83 (22%) cases/89 (26%) controls from analyses, respectively. For the second sensitivity analysis, subjects who were older than 72 hours when their blood sample was drawn were excluded, removing 23 (6%) cases and 25 (8%) controls from analyses. For the third sensitivity analysis, twelve subjects who were non-singleton births (4 cases and 8 controls) were removed. Point estimates all sensitivity analyses were very similar to those using full data with somewhat wider confidence intervals; therefore, only results using full data are presented.

Results

Of the eight sex steroids examined, seven were present in the newborn samples (androstenedione, DHEA, estradiol, estriol, estrone, testosterone, and progesterone). DHT was not detected or below the LOD in all samples and are therefore not included in any tables. The 2.5% – 97.5% range for all hormone measures among controls were similar to those observed from the freshly collected DBS specimens measured in the pilot study, suggesting that major degradation was not observed in blood spots archived for up to 20 years. Similarly, hormone levels were similar across birth years among controls (Supplementary Table 4), demonstrating the relative stability of steroid hormones in DBS specimens up to 20 years old. Descriptive statistics (mean, median, standard deviation, minimum value, maximum value, and range) and the number of samples with measures below the LOD, for each of the 7 measured hormones, are shown in Table 1. The proportion of measures that fell below the LOD ranged from 1.3% (progesterone) to 98.2% (estradiol). Because there were so few subjects with estradiol and estrone measures above the LOD (n=13 and n=39, respectively), they were not included in subsequent analyses. Hormone levels by case-control status, race/ethnicity, and decade of birth are shown in Supplementary Table 4.

The 370 TGCT cases and 344 age and race/ethnicity-matched controls were similar with respect to all socio-demographic and birth characteristics compared (Table 2). As sampled, 25% of cases were diagnosed between ages 0–4 years, and 75% were diagnosed between ages 15–19 years. Eight percent of cases were classified as seminomas, and 92% were non-seminomas, including 35% mixed germ cell tumors, 21% yolk sac tumors, and 11% embryonal carcinomas. All cases were neoplasms of the testis, with 54% arising from descended testes, 1% from undescended testes, and 44% from unspecified descended or undescended testes.

High androstenedione levels were associated with TGCT among boys diagnosed during adolescence (15–19 years) (Q4 vs Q1 OR: 2.33; 95% CI: 1.37–3.97; p-trend<0.01) but not among boys diagnosed in infancy (0–4 years) (Q4 vs Q1 OR: 0.70; 95% CI: 0.28–1.77) (p-value for interaction<0.01) (Table 3). A similar finding was observed for testosterone (15–19

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years, Q4 vs Q1 OR: 1.73; 95% CI: 1.00–3.00 vs 0–4 years, Q4 vs Q1 OR: 0.67; 95% CI: 0.26–1.72 (p-value for interaction=0.17). Androstenedione and testosterone exhibit a weak positive linear relationship (r2=0.32). In models mutually adjusting for each hormone (androstenedione and testosterone), the observed association with androstenedione among adolescents remained (Q4 vs Q1 OR: 2.2; 95% CI: 1.25–3.91), while the association with testosterone was attenuated (Q4 vs Q1 OR: 1.28; 95% CI: 0.71–2.33). The associations between high androstenedione and adolescent TGCT appeared to be stronger among non-Hispanic whites (Q4 vs. Q1 OR: 3.29; 95% CI: 1.33–8.12 vs. 1.95; 95% CI: 0.97–3.94 in Hispanics; p-value for interaction = 0.15). Similarly, the association with testosterone was limited to non-Hispanic whites (Q4 vs. Q1 OR: 2.97; 95% CI: 1.21–7.30, compared to 0.82; 95% CI: 0.39–1.74 in Hispanics; p-value for interaction = 0.03) (Table 4). There was no evidence that the relationship between any hormone and adolescent TGCT differed by major histologic subtype (seminoma vs. non-seminoma) or method of delivery (vaginal vs. caesarian birth).

Discussion

In the first TGCT study with direct measures of sex steroid hormones at birth, we found that elevated androgens (androstenedione and testosterone) were associated with an increased risk of TGCT, while estriol, the predominant estrogen during pregnancy and the only estrogen present in detectable concentrations at birth, was unrelated. The association of androstenedione and testosterone was observed only in TGCT diagnosed in adolescence and not TGCT diagnosed in infancy. The marked difference in risk by age at diagnosis (infants vs. adolescents) was not unexpected; germ cell tumors arising in infants are predominantly teratomas and yolk sac tumors[6], are not preceded by GCNIS, and are thought to be etiologically distinct from those arising in AYA[15]. The association between androgens and TGCT was stronger among non-Hispanic white males, relative to those reporting Hispanic ethnicity. While non-Hispanic white men have higher incidence rates than other ethnic groups within the same geographic region[28], this result does little to further explain the observed recent increase in incidence rates among Hispanic adolescents in the United States[1, 2].

Androgen insufficiency and/or an imbalance between androgens and estrogens during critical windows of testis development have been posited as a factor in the development of TGCT[5]. Direct evidence of these relationships in humans is sparse, however, due to the protracted time between perinatal life and disease onset, and to the rarity of TGCT. Supporting studies have been retrospective, focusing on maternal exposure to exogenous hormones during pregnancy[5, 18].

The results from the current study are based upon hormone levels at birth and reflect the hormone environment during late gestation/extremely early postnatal life; they suggest that biologic mechanisms operating during this period and related to androgens promote germ cell pathogenesis. However, since sex steroid hormone levels vary during and after pregnancy, the measures in our study do not necessarily reflect the hormonal *in utero* milieu during germ cell differentiation and initiation/promotion of germ cell neoplasia in situ in

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early pregnancy. Therefore our study may not adequately test the hypothesis that TGCT arises from hormone imbalance during early gestation.

The steady increases in TGCT strongly point to environmental causes, and it has been hypothesized that exposure to endocrine disrupting chemicals (EDCs) may play a fundamental role[4, 29–31]. Sources of exposure to EDCs are diverse and vary widely around the world, and in humans, can disrupt reproductive and sexual development. Fetuses, infants, and children may have greater susceptibility than adults, thus the impact of EDCs during gametogenesis, fetal development, and early life can be particularly important, even though effects may not become apparent until adolescence and adulthood[32]. Male reproductive disorders thought to be related to TGCT, including undescended testes, hypospadias, and poor semen quality, have been induced in rodents after perinatal exposure to EDCs[5, 29, 30], and exposure to EDCs has been associated with shortened anogenital distance, a sensitive marker of androgen action in utero among male newborns[33]. Although it has been speculated that exposure to EDCs during fetal development plays a role in TGCT development[34], the lack of prospectively collected samples from the prenatal period has hindered direct examination of this relationship in humans. [34–36]

Strengths of this study include its inclusion of neonatal pre-diagnostic specimens, unbiased data collection from cases and controls, large sample size, ethnic diversity, and validated laboratory methods. There are some limitations, however, that should be considered. First, given the design of the study, our study did not include TGCT cases that were diagnosed in California but born elsewhere. In addition, it is possible that some of the controls could have moved from California prior to a diagnosis of TGCT, and therefore not linked to the CCR. Given the rarity of TGCT, however, the chance of this is low. More important, there are no data to suggest that such out-migration is associated with hormonal status. Sex steroid hormone measures in this study represent a single point in time; specifically, the window immediately after birth, when the hormonal milieu is a mixture of those being produced by the newborn testis and the residual maternal hormones. As such, these levels do not necessarily reflect levels during other potentially important windows of exposure, such as periods of gonocyte differentiation early in pregnancy and puberty. However, as the first study with direct measures during the neonatal period, the information obtained in this investigation provides the first piece in defining the relationships between these hormones and future risk of TGCT. Due to the low concentrations of some sex steroid hormones at birth and the small amount of biospecimen used for assays, some compounds of interest were below the limit of detection for most or all of the samples, including estradiol, estrone and dihydrotestosterone. As technology and methods for more precise quantification of steroid hormones in dried blood spots are developed, the role of these, and potentially other, compounds can be more clearly defined. Cryptorchidism and personal/familial history of testicular cancer are known risk factors for TGCT, although contributing to a small fraction of testicular cancer[28]. While our record linkage study is the first to measure sex hormones at birth, data for cryptorchidism was not available on California birth record. Future studies measuring sex hormones levels at birth should attempt to collect and account for these characteristics. Finally, the relatively small sample size, particularly for infant TGCT, hindered our ability to examine some associations,

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We consider this study to be the first step in a more expansive investigation into the rising incidence of TGCT observed worldwide. Future investigations should include 1) the confirmation of the observed associations with androgens, perhaps in a larger population that includes young men with the highest incidence, 2) environmental in utero and postnatal exposures, including EDCs, and their impact on the developing fetus and future TGCT risk, and 3) an examination of sex steroid hormones at other critical periods of development. In conclusion, this study fills a gap in the current knowledge of early life origins of TGCT, as well as quantifies sex hormone levels at birth among males.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Descriptive statistics of sex steroid hormones measures at birth, among 714 males from the Childhood Cancer Record Linkage Project (CCRLP) (1988–2011)

		Cases (n=370)						Controls (n=344)					
Hormone	Limit of Detection (LOD)	Below LOD n (%)	mean	med	std dev	min	max	Below LOD n (%)	mean	med	std dev	min	max
Androstenedione (ng/mL)	1	63 (17.0)	1.93	1.68	1.07	0.41	6.60	83 (24.1)	1.98	1.65	1.42	0.18	8.96
Dehydroepiandrosterone (ng/dL)	4	60 (16.2)	11.6	7.18	14.1	0.29	150	60 (17.4)	12.3	7.19	15.2	0.13	106
Estradiol (pg/mL)	50	366 (98.9)	13.6	10.4	13.1	0.02	129	335 (97.4)	18.7	14.3	23.6	0.24	359
Estriol (pg/mL)	200	58 (15.7)	2132	1102	3283	14	29214	41 (11.9)	1916	950	2930	1	26582
Estrone (pg/mL)	100	356 (96.2)	29213.8	26.2	34.6	0.81	336	319 (92.7)	48.7	34.2	56.3	0.12	414
Testosterone (ng/dL)	50	91 (24.6)	113	88.7	88.0	0.7	527	99 (28.8)	112	90.9	93.9	5.6	587
Progesterone (ng/mL)	1	2 (0.5)	20.9	14.2	21.2	0.5	125	7 (2.0)	21.9	12.4	25.6	0.6	187

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Table 2

Comparison of demographic and birth characteristics at birth, among 370 cases and 344 controls from the California Cancer Registry Linkage Project (CCRLP) (1988–2011)

Variable	Category	Controls n (%)	Cases n (%)
Age at Diagnosis	0–4 years	88 (26)	94 (25)
	15–19 years	256 (74)	276 (75)
Histologic Subtype	Seminomas		
	Seminoma, NOS		23 (6)
	Seminoma, anaplastic		1 (0)
	Germinoma		7 (2)
	Non-seminomas		
	Germ cell tumor, non-seminomatous		12 (3)
	Embryonal carcinoma		41 (11)
	Yolk sac tumor		77 (21)
	Teratoma, malignant		28 (8)
	Teratocarcinoma		22 (6)
	Mixed Germ Cell Tumor		131 (35)
	Choriocarcinoma		7 (2)
	Choriocarcinoma combined with other germ cell elements		21 (6)
Birthweight (in grams)	Low Birthweight (<2500g)	19 (6)	20 (5)
	Normal Birthweight (2500–4000g)	281 (82)	291 (79)
	High Birthweight (>4000g)	44 (13)	59 (16)
	mean(g)	3421.6	3412.4
Gestational Age (in weeks)	Preterm (< 37 weeks)	39 (11)	42 (11)
	Normal (37–40 weeks)	278 (81)	297 (80)
	Overdue (> 40 weeks)	11 (3)	11 (3)
	Missing	16 (5)	20 (5)
	mean(weeks)	41.8	42.1
Race/Ethnicity	Non-Hispanic White	111 (32)	128 (35)
	Non-Hispanic Black	3 (1)	3 (1)
	Hispanic	200 (58)	207 (56)
	Non-Hispanic Asian	25 (7)	27 (7)
	Non-Hispanic Other	5 (1)	5 (1)
Mother's Age At Delivery (in years)	Less than 20	35 (10)	34 (9)
	20–29	195 (57)	204 (55)
	30–39	110 (32)	128 (35)
	40+	4 (1)	4 (1)
Father's Age At Delivery (in years)	Less than 20	15 (4)	7 (2)
	20–29	163 (47)	176 (48)
	30–39	132 (38)	136 (37)
	40+	24 (7)	30 (8)
	Unknown	10 (3)	21 (6)

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Variable	Category	Controls n (%)	Cases n (%)
Mother's Education Status	High School or less	129 (38)	136 (37)
At Delivery	At least some college	59 (17)	63 (17)
	Unknown	156 (45)	171 (46)
Father's Education Status	High School or less	128 (37)	129 (35)
At Delivery	At least some college	56 (16)	61 (16)
	Unknown	160 (47)	180 (49)
Mode of Delivery	Cesarian	74 (22)	91 (25)
	Vaginal	268 (78)	278 (75)
	Unknown	2 (1)	1 (0)
Plurality	Singleton	336 (98)	366 (99)
	Multiple Birth	8 (2)	4 (1)
Birth Order	First child	126 (37)	158 (43)
	Second child	116 (34)	101 (27)
	Third child	61 (18)	55 (15)
	Fourth or higher child	41 (12)	56 (15)
Mother History of Miscarriage	Never	288 (84)	321 (87)
(prior to index birth)	Ever	56 (16)	49 (13)
Mother History of Stillbirth	Never	338 (98)	363 (98)
(prior to index birth)	Ever	6 (2)	7 (2)
DBS Age of Collection	Less than 22 hours	82 (24)	82 (22)
	22 to 31 hours	76 (22)	100 (27)
	31 to 45 hours	75 (23)	84 (23)
	More than 45 hours	92 (27)	93 (25)
	Missing	19 (6)	11 (3)
	mean (hrs)	37.5	38.1

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Table 3

Risk of testicular cancer among infants (0–4 years) and adolescents (15–19 years), California

Variable		0 to 4 years				14 to 19 years				
		Cases	Controls	OR[1]	(95% CI)	Cases	Controls	OR[1]	(95% CI)	P-interaction
Androstenedione[3]	Q1[3]	16	17	1.00		47	69	1.00		
	Q2	36	23	1.35	(0.55–3.36)	75	64	1.73	(1.03–2.89)	
	Q3	19	21	0.83	(0.31–2.19)	71	66	1.77	(1.05–2.99)	<0.01
	Q4	23	30	0.70	(0.28–1.77)	83	57	2.33	(1.37–3.97)	
	p-trend[2]				0.18				<0.01	
Testosterone[4]	Q1[3]	23	19	1.00		80	68	1.00		
	Q2	27	24	0.99	(0.42–2.31)	58	76	1.86	(1.13–3.07)	
	Q3	24	20	1.09	(0.43–2.74)	62	62	1.39	(0.82–2.36)	0.17
	Q4	20	25	0.67	(0.26–1.72)	56	70	1.73	(1.00–3.00)	
	p-trend[2]				0.44				0.12	
DHEA[5]	Q1[3]	17	9	1.00		43	51	1.00		
	Q2	25	31	0.41	(0.15–1.09)	80	64	1.48	(0.86–2.54)	
	Q3	30	28	0.54	(0.20–1.44)	83	67	1.44	(0.85–2.46)	0.61
	Q4	22	20	0.58	(0.21–1.67)	70	74	1.15	(0.66–2.00)	
	p-trend[2]				0.70				0.80	
Estriol[6]	Q1[3]	9	9	1.00		49	32	1.00		
	Q2	23	27	0.83	(0.28–2.47)	57	74	0.52	(0.28–0.96)	
	Q3	27	31	0.89	(0.30–2.63)	79	70	0.76	(0.41–1.41)	0.29
	Q4	35	21	1.61	(0.54–4.79)	91	80	0.76	(0.42–1.41)	
	p-trend[2]				0.18				0.85	
Progesterone[7]	Q1[4]	14	19	1.00		77	71	1.00		
	Q2	23	20	1.40	(0.51–3.83)	66	70	1.13	(0.65–1.99)	
	Q3	30	28	1.77	(0.63–4.98)	71	50	1.71	(0.94–3.14)	0.62
	Q4	27	21	2.22	(0.72–6.81)	65	65	1.34	(0.68–2.65)	
	p-trend[2]				0.15				0.25	

[1] Odds ratios are adjusted for year of birth, race, and age at collection

[2] p-trend for ordinal variable

[3] Androstenedione (ng/mL): Q1=<1.00; Q2=1.00–1.43; Q3=1.43–1.93; Q4=1.93+

[4] Testosterone (ng/dL): Q1=<50.00; Q2=50.00–92.93; Q3=92.93–153.57; Q4=153.57+

[5] DHEA (ng/dL): Q1=<4.00; Q2=4.00–5.57; Q3=5.57–7.81; Q4=7.81+

[6] Estriol (pg/mL): Q1=<200.00; Q2=200.00–750.42; Q3=750.42–1797.24; Q4=1797.24+

[7] Progesterone (ng/mL): Q1=<5.733; Q2=5.73–12.45; Q3=12.45–28.11; Q4=28.11+

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Table 4

Risk of testicular cancer among adolescents (ages 15–19), stratified by race (non-Hispanic white vs Hispanic), California Cancer Registry Linkage Project

		Non-Hispanic White				Hispanic				
Variable		**cases**	**controls**	**OR[1]**	**(95% CI)**	**cases**	**controls**	**OR[1]**	**(95% CI)**	**P-interaction**
Androstenedione[3]	Q1[3]	17	28	1.00		27	38	1.00		
	Q2	30	32	1.55	(0.70–3.45)	40	29	1.97	(0.96–4.02)	0.15
	Q3	33	22	2.63	(1.11–6.21)	35	39	1.47	(0.73–2.97)	
	Q4	33	17	3.29	(1.33–8.12)	44	34	1.95	(0.97–3.94)	
	p-trend[2]	113	99		0.01	146	140		0.14	
Testosterone[4]	Q1[3]	23	33	1.00		43	41	1.00		
	Q2	28	20	2.47	(1.05–5.77)	43	32	1.51	(0.78–2.90)	0.03
	Q3	26	22	2.29	(0.93–5.62)	33	37	0.92	(0.47–1.83)	
	Q4	36	24	2.97	(1.21–7.30)	27	30	0.82	(0.39–1.74)	
	p-trend[2]				0.04				0.47	
DHEA[5]	Q1[3]	16	22	1.00		25	27	1.00		
	Q2	37	23	2.23	(0.96–5.21)	39	37	1.13	(0.55–2.35)	0.99
	Q3	36	28	1.71	(0.75–3.86)	41	33	1.36	(0.65–2.83)	
	Q4	24	26	1.29	(0.53–3.12)	41	43	1.07	(0.52–2.23)	
	p-trend[2]				0.90				0.80	
Estriol[6]	Q1[3]	23	14	1.00		23	15	1.00		
	Q2	31	33	0.58	(0.24–1.40)	21	37	0.41	(0.17–1.03)	0.89
	Q3	27	29	0.55	(0.22–1.39)	48	39	0.90	(0.37–2.20)	
	Q4	32	23	0.81	(0.32–2.03)	54	49	0.80	(0.33–1.93)	
	p-trend[2]				0.91				0.54	
Progesterone[7]	Q1[4]	43	37	1.00		26	28	1.00		
	Q2	25	28	0.77	(0.35–1.70)	37	36	1.94	(0.82–4.58)	0.74
	Q3	22	16	1.21	(0.48–3.10)	45	33	2.56	(1.00–6.57)	
	Q4	23	18	1.04	(0.37–2.93)	38	43	1.89	(0.66–5.42)	
	p-trend[2]				0.75				0.39	

[1] Odds ratios are adjusted for year of birth, race, and age at collection

[2] p-trend for ordinal variable

[3] Androstenedione (ng/mL): Q1=<1.00; Q2=1.00–1.43; Q3=1.43–1.93; Q4=1.93+

[4] Testosterone (ng/dL): Q1=<50.00; Q2=50.00–92.93; Q3=92.93–153.57; Q4=153.57+

[5] DHEA (ng/dL): Q1=<4.00; Q2=4.00–5.57; Q3=5.57–7.81; Q4=7.81+

[6] Estriol (pg/mL): Q1=<200.00; Q2=200.00–750.42; Q3=750.42–1797.24; Q4=1797.24+

[7] Progesterone (ng/mL): Q1=<5.73; Q2=5.73–12.45; Q3=12.45–28.11; Q4=28.11+

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