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Maternal hormone levels in early gestation of cryptorchid males: a case-control study

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Summary A case-control study was conducted to assess maternal hormonal factors associated with increased risk of bearing a cryptorchid son. Serum samples were collected during the first trimester of pregnancy from participants in the US Collaborative Perinatal Study. Twenty-five mothers of normal offspring (controls) were individually matched on medical center, age, parity, weight and length of gestation at the time of sampling to women bearing sons who had a diagnosis of cryptorchidism at one year of age or older. Compared with controls, mothers of cryptorchid sons (cases) had significantly greater percentages of non-protein bound ($P=0.010$) and albumin-bound ($P=0.014$) estradiol during the first trimester of the index pregnancy. On average, cases had 16% more bioavailable oestradiol than controls. Levels of human chorionic gonadotropin, testosterone, non-protein bound testosterone and sex-hormone binding globulin did not differ between the two groups. The data presented support the hypothesis that cryptorchidism results from elevated maternal oestrogen levels early in pregnancy.

Cryptorchidism, a relatively common abnormality of the male genitourinary system, is the major known risk factor for testicular cancer (Henderson *et al.*, 1979; Schottenfeld *et al.*, 1980; Depue *et al.*, 1983). Although the causes of both testicular cancer and cryptorchidism are largely unknown, their epidemiology (Depue *et al.*, 1983; Swerdlow *et al.*, 1983; Depue, 1984; Brown *et al.*, 1986) and the higher rates of urogenital developmental anomalies, including cryptorchidism, in testicular cancer cases and male family members of testicular cancer cases (Tollerud *et al.*, 1985) suggest common aetiological factors.

Higher maternal levels of oestrogen in early pregnancy may play a role in the aetiology of cryptorchidism. Testicular maldescent can be produced experimentally in animals by administering diethylstilbestrol (DES) or other forms of oestrogen during gestation (Jean, 1973; McLachlin *et al.*, 1975; Nomura & Kanzaki, 1977; Yasuda *et al.*, 1985). A number of clinical studies have found an increased frequency of cryptorchidism in males with a history of DES exposure *in utero* (Cosgrove *et al.*, 1977; Whitehead & Leiter, 1981). In epidemiologic studies of cryptorchidism, the abnormality has been associated with maternal use of exogenous oestrogens including DES during gestation (Gill *et al.*, 1979; Depue, 1984).

High maternal body weight also has been associated with an increased risk of cryptorchidism (Depue, 1984). Increased levels of 'free' (non-protein bound) oestradiol (E_2) are associated with high maternal body weight (Bernstein *et al.*, 1986). In pregnancy, plasma E_2 is mostly bound to sex-hormone binding globulin (SHBG) and the remainder to albumin, with only about 1% being free (Anderson, 1974). It is generally accepted that the non-protein bound E_2 is free to reach intracellular receptors, and there is now increasingly persuasive evidence that the E_2 bound to albumin may also be 'bioavailable' (Pardridge, 1986). The observed effect of high maternal body weight on risk of cryptorchidism may result from higher maternal levels of bioavailable E_2.

Another possible mechanism that has been suggested is that higher maternal E_2 levels exert an effect on testicular descent by lowering testosterone (T) levels in the foetus (Hadziselimovic & Herzog, 1980). If this is an important mechanism, high maternal T levels may protect against cryptorchidism.

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Davies *et al.* (1986) recently suggested that impaired placental function may play a role in cryptorchidism. They theorized that when levels of human chorionic gonadotropin (hCG) are reduced, there may be changes in foetal testicular function and hence an increased risk of maldescent.

The purpose of the present study was to determine whether first trimester maternal hormone levels in the index pregnancy differ between mothers of cryptorchid sons (cases) and mothers of 'normal' offspring (controls). Here, we compare levels of free and bound E_2, free and bound T, SHBG and hCG.

Subjects and methods

Subjects

Study subjects were participants in the Collaborative Perinatal Project of the National Institute of Neurological and Communicative Disorders and Stroke (Bethesda, MD, USA), a prospective study conducted to identify etiologic factors related to adverse pregnancy outcome (Niswander & Gordon, 1972). In this project, more than 55,000 pregnancies were registered at 12 university-affiliated medical centers in the United States between 1958 and 1965. The majority of these pregnancies were beyond 20 weeks (measured from day 1 of the last menstrual period) at the time of registration. Blood samples were collected at each prenatal visit. The samples have been stored at a central repository at −20°C. A detailed medical history was obtained from each participant and detailed obstetric and delivery records were kept.

In the Perinatal Project, offspring were examined at birth, 4 months, 1 year and 7 years of age; records were kept on congenital abnormalities and development. White women bearing sons who had a diagnosis of cryptorchidism at the 1 year or 7 year examination were considered eligible as 'cases' for the present study: no woman who had taken hormones or had experienced severe nausea or vomiting during the index pregnancy was considered eligible. There were 25 such women with sera available for evaluation who registered with the Perinatal Project by week 13 (measured from day 1 of the last menstrual period) of the relevant pregnancy. One control woman was individually matched to each case by medical center, race, parity, weight (within 4 kg), age at time of index pregnancy (within 5 years), and length of gestation at registration and sampling (within 11 days). Twenty-two of the 25 control mothers bore male offspring; for the remain-

ing 3 cases, we were forced to use mothers of female offspring. All of the offspring of control mothers were followed for 7 years with no malformations noted.

Assays

Serum samples were shipped on dry ice to London (JM) for measurement of total E_2, percentage of E_2 bound to SHBG, percentage of free E_2, total T, percentage of free T, SHBG, and hCG. The identity of specimens was not known to the processing laboratory. The only identifier was a coded number unique for each submission of a specimen.

Ostradiol levels were measured by direct radioimmunoassay (Steranti Research Limited, St Albans, Herts., UK). The percentage of free E_2 was measured by centifugal-ultrafiltration-dialysis in undiluted serum at 37°C (Hammond *et al.*, 1980). The percentage of E_2 which was bound to albumin was calculated from the measured free E_2 (%) in native serum and the free E_2 (%) observed in serum which had been heated at 60°C for 1 h (Hammond *et al.*, 1982; Siiteri *et al.*, 1982). SHBG was measured by a liquid phase immunoradiometric assay (Hammond *et al.*, 1985) using antisera kindly supplied by Dr G.L. Hammond, University of Western Ontario, Canada. Levels of free T were measured by the 'Coat-A-Count' free testosterone kit method (Diagnostic Products Corporation, Los Angeles, CA, USA) and total T by direct assay using the Gamma-B ^{125}I-testosterone kit (RIA UK, Washington, Tyne and Wear, UK). hCG was assayed by the double antibody kit method supplied by Diagnostic Products Corporation, Los Angeles, CA, USA.

Statistical analysis

The amount of free E_2 was computed as the product of total E_2 and the percentage of free E_2; other amounts were calculated in a similar manner. Hormone and SHBG values followed a lognormal distribution and logarithmic (base 10) values of these variables were used in all statistical analyses. Statistical analyses were performed using paired *t* tests and repeated measures analysis of covariance. Adjustments for differences in length of gestation assumed a linear relationship between length of gestation and log hormone values. One-sided *P* values are presented for these comparisons because the hormonal hypotheses to be tested predicted higher total (and bioavailable) E_2 which would be the stimulus for greater amounts of SHBG (Pearlman *et al.*, 1967) and lower T and hCG levels.

Results

One case-control pair was eliminated from the analyses that follow because the control's values for hCG and E_2 were low and not consistent with a 7 week gestation. Inclusion of this pair would have accentuated the differences presented below.

Relevant pregnancy characteristics and assay results for the remaining 24 matched case-control pairs are presented in Table I. Cases and controls were closely matched on age, weight and length of gestation at sampling. Subjects ranged in age from 18 to 39. Length of gestation at the time of sampling ranged from 46 to 93 days from the first day of the last menstrual period.

Although total E_2 concentrations of cases and controls did not differ, cases had significantly greater percentages of free E_2 ($P=0.010$) and of albumin-bound E_2 ($P=0.014$) than controls. The E_2 fractions that are considered biologically available were correspondingly greater in cases than in controls. On average, the cases had 16% more free E_2 ($P=0.066$) and 16% more albumin-bound E_2 ($P=0.038$) than controls. Levels of SHBG in cases and in controls were not significantly different. Total and free T levels did not differ significantly between cases and controls. Although not statistically significant, hCG levels were 15% higher in cases than in control women and this difference was consistently found across gestational ages.

Adjusting for length of gestation, parity, and, in the case of SHBG, for weight had no effect on the results presented in Table I. Results of analyses restricted to the 21 matched pairs in which all of the offspring were male did not alter the results presented.

Table I Relevant pregnancy characteristics (±s.d.) of study subjects and geometric mean hormone levels (log 10±s.d.) in early gestation for 24 mothers of cryptorchid sons (cases) and 24 mothers of normal offspring (controls)

Variable	*Cases*	*Controls*	P-*value*[a]
Age (yr)	25.7 (±5.4)	25.0 (±5.0)	0.080
Weight (kg)	60.2 (±17.1)	59.1 (±13.0)	0.497
Days of gestation at sampling	71.6 (±10.9)	70.9 (±10.4)	0.497
Weeks of gestation at birth	39.9 (±2.2)	40.0 (±2.0)	0.719
Birth weight of offspring (g)	3090 (±571)	3313 (±632)	0.146
Oestradiol			
Total ($ng\,dl^{-1}$)	969.2 (2.986±0.118)	949.8 (2.978±0.167)	0.413
Binding percentages			
SHBG-bound[b]	61.3 (±9.8)	66.5 (±8.4)	0.014
Albumin-bound[b]	37.6 (±9.6)	32.5 (±8.3)	0.014
Free	1.2 (±0.2)	1.0 (±0.2)	0.010
Non-SHBG bound ($ng\,dl^{-1}$)			
Albumin-bound[b]	348.1 (2.542±0.155)	300.0 (2.477±0.163)	0.038
Free	11.0 (1.042±0.157)	9.5 (0.977±0.161)	0.066
Testosterone			
Total ($ng\,dl^{-1}$)	125.9 (2.100±0.183)	127.1 (2.104±0.204)	0.471
Free ($ng\,dl^{-1}$)	3.4 (0.530±0.128)	3.6 (0.561±0.130)	0.219
SHBG ($nmol\,l^{-1}$)	212.3 (2.327±0.222)	205.8 (2.313±0.216)	0.392
hCG ($IU\,ml^{-1}$)	85.1 (1.930±0.278)	74.2 (1.870±0.273)	0.276

[a]Paired *t*-test, 2-sided *P* values reported for pregnancy characteristics and 1-sided *P* values reported for hormone and protein levels; [b]based on 22 pairs (insufficient samples available for 2 cases).

Discussion

It is commonly accepted that testicular descent is under hormonal control (Hutson & Donahoe, 1986). We have previously hypothesized that excess endogenous maternal oestrogens play a role in the risk of cryptorchidism. In this study, we have found significantly greater percentages of free and albumin-bound E_2 in the first trimester sera of mothers bearing cryptorchid sons. This resulted in greater concentrations of non-SHBG bound E_2 in these pregnancies.

Burton *et al.* (1987) found no significant difference in maternal E_2 levels between mothers of cryptorchid boys and control mothers. They did not consider bioavailable E_2.

Two theories have been proposed to explain the effect of oestrogen on testicular descent. Hadziselimovic & Herzog (1980) proposed that the mechanism responsible for this effect is the suppression of foetal androgen secretion by oestrogen. It has, however, recently been shown in studies of hypogonadal mice that foetal testosterone production in early gestation is not relevant to the aetiology of cryptorchidism (Charlton, 1986; Grocock *et al.*, 1988). Current thinking (Hutson & Donahoe, 1986) considers a biphasic model for the hormonal control of testicular descent. In this model, separate hormones and mechanisms control the two stages of descent, the initial transabdominal phase, occurring prior to the twelfth week of gestation in man, and the transinguinal phase, occurring during the third trimester in man. The first stage is thought to be regulated by mullerian inhibiting substance, whereas the second, later stage is androgen dependent. Based on animal data, it appears that estrogens inhibit mullerian inhibiting substance (Newbold *et al.*, 1984; Hutson *et al.*, 1985) and cause atrophy of the gubernaculum (Wensing, 1973; Grocock *et al.*, 1988).

Although cryptorchidism is a risk factor for testis cancer, the risk of testis cancer is not confined to the involved testis in unilaterally cryptorchid men (Depue *et al.*, 1983). Cryptorchidism may be secondary to oestrogen inhibition of mullerian inhibiting substance resulting in intra-abdominal arrest of descent. Excess maternal oestrogen may mediate risk of testicular cancer more directly by interrupting the progression of primitive germ cells to mature germ cells. These primitive cells, persisting into puberty, would multiply under stimulation by gonadotropins and give rise to germ cell tumours of a variety of histological types depending on their particular stage of 'developmental arrest' (Henderson *et al.*, 1983).

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