Maternal reproductive and demographic characteristics as risk factors for hypospadias

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Summary

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This study examined the association of hypospadias risk with several maternal reproductive and demographic characteristics: age, parity, body mass index (BMI), nausea and vomiting of pregnancy (NVP), multiple pregnancy, fertility treatments and procedures, education and race-ethnicity. The study included data on deliveries with estimated due dates from October 1997 to December 2000 that were part of the National Birth Defects Prevention Study, a multi-state case–control study of many birth defects. The analysis included 502 cases with second or third degree hypospadias (i.e. the urethra opened at the penile shaft, scrotum or perineum) and 1286 male, liveborn, non-malformed controls. Risks were estimated from a multivariable logistic regression model that included all exposures of interest.

Results indicated particularly elevated risks among births to women who were primiparae, aged ≥35 years and had a BMI of >26, compared with women who were multiparae, aged <30 years and had a BMI of ≤26 [adjusted OR 12.5, 95% CI 5.1, 30.8]. NVP at least once per day during the second or third month of pregnancy vs. no NVP was associated with slightly reduced risk [OR 0.8, 95% CI 0.6, 1.1]. Multiple birth, fertility treatments and college education were associated with increased risks, and Hispanic race-ethnicity was associated with reduced risk. Although the potential contribution of underlying maternal endocrine parameters to the current findings are unknown, the results do provide clues regarding hypospadias aetiology that merit further investigation.

Keywords: *hypospadias*, *congenital malformations*, *maternal BMI*, *maternal age*, *parity*, *fertility*, *maternal education*, *multiple births*, *nausea and vomiting*.

Introduction

Hypospadias, which occurs as a result of abnormal urethral closure from 8 to 14 weeks after conception, is one of the most common birth defects, with a prevalence of three to five per 1000 births.[1–3] Conversion of testosterone to dihydrotestosterone and proper androgen receptor signalling are critical to normal urethral closure. One factor known to interfere with these processes is oestrogen.[4–10] Accordingly, it has been hypothesised that increased maternal oestrogen levels may be associated with increased hypospadias risk.[11,12]

Documented support for the oestrogen hypothesis for hypospadias, however, is mixed at best. Some factors associated with maternal oestrogen levels are associated with increased hypospadias risk (e.g. primiparity and multiple births), but others are not

(e.g. smoking and oral contraceptives), and for some factors the association is not in the expected direction (e.g. birthweight).[13–19] Further, results of studies investigating whether *in utero* exposure to maternal oestrogens contributes to the occurrence of cryptorchidism and testicular cancer, which may share a common aetiology with hypospadias, have been inconsistent.[4,20–27] Alternatively, it has been suggested that maternal androgen levels, or androgen/oestrogen balance, may be associated with these outcomes.[27]

This study uses recent data from a population-based, multi-state case–control study to examine the association of hypospadias risk with several maternal reproductive and demographic characteristics: age, parity, body mass index (BMI), nausea and vomiting of pregnancy (NVP), multiple pregnancy, fertility treatments and procedures, race-ethnicity and education. These factors were selected because they may be associated with maternal endocrine function and/or with other endocrine-related outcomes. For example, lower parity, obesity, NVP and multiple birth have been shown to be associated with higher oestrogen levels[28–33] and with risk of cryptorchidism, testicular cancer and breast cancer.[22,23,25,34–41] The association of some but not all of these factors with hypospadias has been explored previously.[38,42–47] Findings have not been entirely consistent, and potential interaction or confounding among them has not been thoroughly explored.

Methods

This study includes data on deliveries that had estimated due dates from October 1997 to December 2000 and were part of the National Birth Defects Prevention Study (NBDPS), a multi-state case–control study of over 30 different birth defects. The study is an approved activity of the Institutional Review Boards of the participating study centres and the Centers for Disease Control and Prevention. Detailed study methods and descriptions of the surveillance systems in the eight states that contributed data to this analysis have been published.[48] In brief, five of the eight states included liveborn, stillborn (fetal deaths at >20 weeks gestation), and prenatally diagnosed and electively terminated cases (AR, CA, GA, IA, TX), one state included only liveborn and stillborn cases (MA), and two states included only liveborn cases (NJ, NY). Non-malformed, liveborn controls were selected randomly from birth certificates (IA, MA, NJ) or from birth hospitals (AR, CA, GA, NY, TX) that reflected the populations from which cases were derived; this analysis is restricted to male controls. Clinical information on cases and birthweight on cases and controls were obtained from multiple hospital reports and medical records, and data were entered into a standardised database.

This study included only second and third degree hypospadias, i.e. the urethra opened at the penile shaft, scrotum or perineum: British Pediatric Association codes 752.606, 752.607, 752.626, and 752.627. Medical record information (including operative reports when available) with anatomical descriptions or diagrams by paediatricians, urologists, geneticists, pathologists, or other health care providers was reviewed by a clinical geneticist at each study centre who decided about inclusion or exclusion in the NBDPS database. Cases described as chordee alone, mild (i.e. first degree, coronal, or glandular), hypospadias not otherwise specified, epispadias, or having ambiguous genitalia without further description were excluded. Infants with recognised single gene disorders or chromosomal abnormalities were excluded. Each case received a final review by one clinical geneticist (R.O.), to ensure that cases from each study centre met standard eligibility criteria. This geneticist also classified each case as isolated if there were no concurrent major anomalies or only minor anomalies (e.g. sacral/pilonidal dimple) or as non-isolated if there was at least one accompanying major anomaly.[49] Otherwise-isolated cases with accompanying genital anomalies (e.g. cryptorchidism, bifid scrotum) were classified as isolated.

Maternal interviews were conducted using a standardised, computer-based questionnaire, primarily by telephone, in English or Spanish, no earlier than 6 weeks after the infant's estimated date of delivery, and no later than 24 months after the estimated due date. Participation in the study was 71% among case mothers and 68% among control mothers.

Potential risk factors included maternal age, parity, prepregnancy BMI [weight (kg)/height (m^2)], NVP during the second and third months, plurality, fertility treatments and procedures, education (categorised as less than high school graduation, equal to high school graduation, 1–3 years of college, 4 or more years of college) and race-ethnicity (non-Hispanic white, non-Hispanic black, Hispanic, other). All exposures were based on maternal self-report. BMI was categorised as underweight, normal weight, overweight or obese, based on Institute of Medicine categories.[50] The subfertility variable was based on a positive response to any of three questions: 'Did you have any surgical

Table 1. Association of maternal reproductive and demographic characteristics with risk of hypospadias: unadjusted risk estimates

	No. cases	No. controls	Unadjusted OR [95% CI]
Parity[a]			
0	295 (59%)	507 (40%)	2.7 [2.0, 3.6]
1	134 (27%)	442 (34%)	1.4 [1.0, 1.9]
2 or more	72 (14%)	333 (26%)	1.0 Reference
Age (years)			
<25	124 (25%)	437 (34%)	1.0 Reference
25–29	106 (21%)	347 (27%)	1.1 [0.8, 1.4]
30–34	165 (33%)	320 (25%)	1.8 [1.4, 2.4]
35 or more	107 (21%)	182 (14%)	2.1 [1.5, 2.8]
Body mass index (kg/m^2)[50]			
Underweight (<19.8)	71 (15%)	200 (16%)	0.9 [0.7, 1.3]
Normal weight (19.8–26.0)	261 (53%)	694 (56%)	1.0 Reference
Overweight (>26.0–29.0)	69 (14%)	136 (11%)	1.3 [1.0, 1.9]
Obese (>29.0)	88 (18%)	199 (16%)	1.2 [0.9, 1.6]
Nausea and vomiting in the second month of pregnancy			
None	239 (48%)	565 (44%)	1.0 Reference
Nausea <1/day, no vomiting	44 (9%)	93 (7%)	1.1 [0.8, 1.7]
Nausea ≥1/day, no vomiting	83 (17%)	179 (14%)	1.1 [0.8, 1.5]
Nausea <1/day, vomiting <1/day	22 (4%)	89 (7%)	0.6 [0.4, 1.0]
Nausea ≥1/day, vomiting <1/day	33 (7%)	115 (9%)	0.7 [0.4, 1.0]
Any nausea, vomiting ≥1/day	77 (15%)	238 (19%)	0.8 [0.6, 1.0]
Nausea and vomiting in the third month of pregnancy			
None	246 (49%)	586 (46%)	1.0 Reference
Nausea <1/day, no vomiting	40 (8%)	88 (7%)	1.1 [0.7, 1.6]
Nausea ≥1/day, no vomiting	69 (14%)	174 (14%)	0.9 [0.7, 1.3]
Nausea <1/day, vomiting <1/day	29 (6%)	104 (8%)	0.7 [0.4, 1.0]
Nausea ≥1/day, vomiting <1/day	37 (7%)	94 (7%)	0.9 [0.6, 1.4]
Any nausea, vomiting ≥1/day	78 (16%)	230 (18%)	0.8 [0.6, 1.1]
Plurality			
Singleton	456 (91%)	1239 (96%)	1.0 Reference
Multiple	45 (9%)	46 (4%)	2.7 [1.7, 4.1]
Sub-fertility treatments and procedures			
None	438 (87%)	1226 (96%)	1.0 Reference
Any	63 (13%)	55 (4%)	3.2 [2.2, 4.7]
Education			
<High school graduation	53 (11%)	211 (16%)	0.9 [0.6, 1.3]
=High school graduation	95 (19%)	350 (27%)	1.0 Reference
1–3 years of college	136 (27%)	357 (28%)	1.4 [1.0, 1.9]
4 or more years of college	215 (43%)	353 (28%)	2.2 [1.7, 3.0]
Race-ethnicity			
Non-Hispanic white	355 (71%)	793 (62%)	1.0 Reference
Non-Hispanic black	69 (14%)	145 (11%)	1.1 [0.8, 1.5]
Hispanic	51 (10%)	280 (22%)	0.4 [0.3, 0.6]
Other	20 (4%)	53 (4%)	0.8 [0.5, 1.4]

[a]Parity 0 refers to women with no live birth previous to the index delivery.

Table 2. Association of maternal parity[a], age and body mass index (BMI, kg/m^2) in combination with risk of hypospadias

	No. cases (%)	No. controls (%)	Unadjusted OR [95% CI]
Parity and age (years) combined			
Parity 1+, Age <30	63 (13%)	402 (31%)	1.0 Reference
Parity 1+, Age 30–34	86 (17%)	229 (18%)	2.4 [1.7, 3.4]
Parity 1+, Age 35+	57 (11%)	144 (11%)	2.5 [1.7, 3.8]
Parity 0, Age <30	167 (33%)	378 (29%)	2.8 [2.0, 3.9]
Parity 0, Age 30–34	79 (16%)	91 (7%)	5.5 [3.7, 8.3]
Parity 0, Age 35+	49 (10%)	38 (3%)	8.2 [5.0, 13.6]
Parity, age and BMI combined			
Parity 1+, Age <30, BMI ≤26	32 (7%)	256 (21%)	1.0 Reference
Parity 1+, Age <30, BMI >26	30 (6%)	120 (10%)	2.0 [1.2, 3.4]
Parity 1+, Age 30–34, BMI ≤26	59 (12%)	154 (13%)	3.1 [1.9, 4.9]
Parity 1+, Age 30–34, BMI >26	21 (4%)	74 (6%)	2.3 [1.2, 4.2]
Parity 1+, Age 35+, BMI ≤26	38 (8%)	104 (8%)	2.9 [1.7, 4.9]
Parity 1+, Age 35+, BMI >26	19 (4%)	36 (3%)	4.2 [2.2, 8.2]
Parity 0, Age <30, BMI ≤26	117 (24%)	285 (23%)	3.3 [2.1, 5.0]
Parity 0, Age <30, BMI >26	45 (9%)	77 (6%)	4.7 [2.8, 7.9]
Parity 0, Age 30–34, BMI ≤26	57 (12%)	65 (5%)	7.0 [4.2, 11.7]
Parity 0, Age 30–34, BMI >26	22 (4%)	19 (2%)	9.3 [4.5, 18.9]
Parity 0, Age 35+, BMI ≤26	29 (6%)	29 (2%)	8.0 [4.2, 15.1]
Parity 0, Age 35+, BMI >26	20 (4%)	9 (1%)	17.8 [7.5, 42.3]

[a]Parity 0 refers to women with no live birth previous to the index delivery.

procedures . . . [to help you become pregnant]?'; 'In the two months before you became pregnant with [baby's name], did you take any medications to help you become pregnant?'; or 'Did you have any other procedures to help you become pregnant . . . ?'

To estimate risks, maximum likelihood estimates of odds ratios (OR) and their corresponding 95% confidence intervals [CI] were calculated from logistic regression models using SAS (version 9.1, 2002–03, SAS Institute, Cary, NC, USA).[51,52] We first examined crude ORs for each exposure. We then examined several factors – maternal age, parity, BMI and NVP – in combination, to explore potential effect modification, because of their potential association with maternal oestrogen levels or endocrine function; multiple births and sub-fertility treatments were too rare to examine in combination with the other factors. We then examined the independent effects of each studied risk factor, by including all variables in a single, multivariable model. We examined final risk estimates separately for: subjects with birthweight <2500 g vs. ≥2500 g, because it is possible that cases of normal and low birthweight may be aetiologically distinct;[16,53] cases that were isolated; and cases with no family history of hypospadias in the father or any brothers.

Results

Interviews were available for mothers of 502 hypospadias cases and 1286 male controls. The median time between the actual date of delivery and the interview was 12.9 months among case mothers and 7.3 months among control mothers. Among case infants, 26% were low birthweight, whereas 6% of control infants were

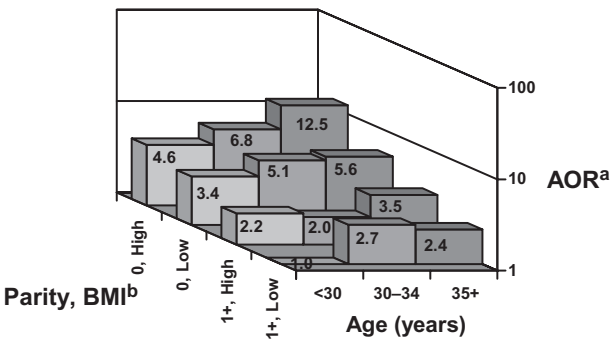

Figure 1. Association of maternal parity, body mass index (BMI, kg/m^2) and age with risk of hypospadias. [a]AOR, adjusted odds ratio; adjusted for race-ethnicity, education, fertility treatments, nausea and vomiting of pregnancy and plurality; see Table 3 for 95% confidence intervals associated with the AORs. [b]Parity of 0 refers to women with no live birth previous to the index delivery. BMI 'Low' if ≤26.0, and 'High' if >26.0.

Table 3. Association of maternal reproductive and demographic characteristics with risk of hypospadias: adjusted risk estimates

Maternal exposures	No. cases (%)	No. controls (%)	Adjusted OR [95% CI][a]
Parity, age and BMI combined[b]			
Parity 1+, Age <30, BMI ≤26	31 (6%)	254 (21%)	1.0 Reference
Parity 1+, Age <30, BMI >26	29 (6%)	118 (10%)	2.2 [1.3, 3.9]
Parity 1+, Age 30–34, BMI ≤26	58 (12%)	151 (12%)	2.7 [1.6, 4.5]
Parity 1+, Age 30–34, BMI >26	21 (4%)	74 (6%)	2.0 [1.1, 3.8]
Parity 1+, Age 35+, BMI ≤26	38 (8%)	103 (9%)	2.4 [1.4, 4.1]
Parity 1+, Age 35+, BMI >26	18 (4%)	36 (3%)	3.5 [1.8, 7.1]
Parity 0, Age <30, BMI ≤26	114 (24%)	278 (23%)	3.4 [2.2, 5.2]
Parity 0, Age <30, BMI >26	44 (9%)	76 (6%)	4.6 [2.7, 7.8]
Parity 0, Age 30–34, BMI ≤26	56 (12%)	64 (5%)	5.1 [3.0, 8.8]
Parity 0, Age 30–34, BMI >26	22 (5%)	19 (2%)	6.8 [3.2, 14.5]
Parity 0, Age 35+, BMI ≤26	29 (6%)	29 (2%)	5.6 [2.8, 10.9]
Parity 0, Age 35+, BMI >26	20 (4%)	9 (1%)	12.5 [5.1, 30.8]
Nausea and vomiting in the second or third month of pregnancy			
None	229 (48%)	534 (44%)	1.0 Reference
Nausea <1/day, no vomiting	44 (9%)	92 (8%)	1.2 [0.8, 1.8]
Nausea ≥1/day, no vomiting	81 (17%)	172 (14%)	1.1 [0.8, 1.5]
Nausea <1/day, vomiting <1/day	21 (4%)	84 (7%)	0.6 [0.4, 1.1]
Nausea ≥1/d, vomiting <1/day	33 (7%)	110 (9%)	0.7 [0.5, 1.1]
Any nausea, vomiting ≥1/day	72 (15%)	219 (18%)	0.8 [0.6, 1.1]
Plurality			
Singleton	436 (91%)	1167 (96%)	1.0 Reference
Multiple	44 (9%)	44 (4%)	1.9 [1.1, 3.1]
Education			
<High school graduation	47 (10%)	173 (14%)	1.3 [0.8, 1.9]
=High school graduation	92 (19%)	335 (28%)	1.0 Reference
1–3 years of college	133 (28%)	352 (29%)	1.3 [0.9, 1.8]
4 or more years of college	208 (43%)	351 (29%)	1.5 [1.1, 2.1]
Race-ethnicity			
Non-Hispanic white	352 (73%)	786 (65%)	1.0 Reference
Non-Hispanic black	66 (14%)	143 (12%)	1.2 [0.9, 1.7]
Hispanic	44 (9%)	232 (19%)	0.6 [0.4, 0.8]
Other	18 (4%)	50 (4%)	0.9 [0.5, 1.6]
Sub-fertility treatments and procedures			
None	417 (87%)	1156 (95%)	1.0 Reference
Any	63 (13%)	55 (5%)	1.5 [0.9, 2.3]

[a]All variables are adjusted for each other, in a single, multivariable model; $n = 480$ cases and 1212 controls.

[b]Parity 0 refers to women with no live birth previous to the index delivery.

low birthweight. A majority of study subjects were non-Hispanic white and had some college education (Table 1).

Unadjusted results indicated that women who were aged 35 years or older were 2.1 times more likely than women who were less than 25 years old to deliver an infant with hypospadias (Table 1). Women who were primiparae were 2.7 times more likely than women with two or more previous live births to deliver an infant with hypospadias. Overweight and obese women were associated with slightly elevated risks of delivering infants with hypospadias. We examined reported NVP in the second and third months of pregnancy. Most ORs indicated slightly reduced risks associated with vomiting, but not nausea in the absence of vomiting. Multiple births were associated with a 2.7-fold increased risk, and maternal sub-fertility treatments with a 3.2-fold increased risk. Case mothers were less likely to be Hispanic [OR 0.4] and more likely to have at least four years of college education [OR 2.2].

We examined the potential combined effects of age, parity, BMI and NVP. We collapsed categories that suggested similar risk estimates in the univariable results, in order to maximise cell sizes for these comparisons

(Table 1); for example, for NVP, we created a variable reflecting any vs. no vomiting during the second *or* third month during pregnancy, and we dichotomised the BMI variable to compare women who were underweight or normal weight (i.e. BMI $\leq$ 26.0 kg/m^2) vs. overweight or obese (i.e. BMI > 26.0). The results for age and parity suggested effect modification, with the highest risk [OR 8.2, 95% CI 5.0, 13.6] observed among women who were primiparae and aged 35 years or older (Table 2). BMI further increased risks associated with age and parity. For example, compared with multiparae of age <30 years and BMI $\leq$ 26, primiparae of age 35 years or older and BMI > 26 had a 17.8-fold increased risk of delivering an infant with hypospadias [95% CI 7.5, 42.3] (Table 2 and Fig. 1). Vomiting in combination with age and parity did not appear to be associated with further modification in risk when combined with age and parity; that is, ORs for women with any vs. no vomiting closely paralleled the overall risks among the age and parity groupings (data not shown).

All variables were examined simultaneously in a single, multivariable model. Some ORs were reduced, compared with unadjusted ORs, but the overall pattern of results was unchanged (Table 3), including the increasing risk observed among women with lower parity, higher age and higher BMI in combination (Fig. 1). Exclusion of subjects who were low birthweight (129 cases and 71 controls) or non-isolated (41 cases) or who had a family history of hypospadias (22 cases and one control) from the multivariable model did not substantially change the results, with one exception. In the model that excluded low birthweight subjects, the OR for multiple vs. singleton birth was 0.7 [95% CI 0.3, 1.6], vs. 1.9 in the model that included all subjects regardless of birthweight.

Discussion

Maternal primiparity, age 35 years or older and BMI > 26 were associated with increased risk of hypospadias among offspring, especially when present in combination. This finding remained after adjustment for other potential risk factors, including reported subfertility treatments and procedures. We are aware of two previous studies that examined maternal age and parity in combination;[45,54] both studies observed twofold to threefold higher risks of hypospadias among older, primiparous women, as compared with younger, multiparous women. Our analyses suggested that BMI may further modify the increased risk we observed among older primiparae. We are unaware of any previous studies examining the association of hypospadias with maternal BMI.

One potential explanation for our findings related to maternal age, parity and BMI is that these variables may be predictive of the production and action of maternal oestrogens. However, we were unable to confirm whether this was true in our subjects. Furthermore, the specific association of these variables, either individually or in combination, with maternal hormone levels is somewhat unclear. Only a few studies have examined the association of these factors with oestrogen levels during the specific time period of early pregnancy. One previous study, which utilised samples from the Collaborative Perinatal Study, found that free oestradiol levels during early pregnancy were higher in women's first pregnancies than during their second pregnancies.[28] Results stratified by prepregnancy weight indicated that levels were particularly high among women who were experiencing their first pregnancy and had prepregnancy weight >125 lbs. Other studies examining correlates of maternal oestrogen levels have focused on later pregnancy[29,55] or multiparae,[56] thus their applicability to our findings is less clear.

Maternal sub-fertility has been proposed to contribute to higher risks of hypospadias observed among older and primiparous women.[57,58] Our observed associations with increased age and primiparity remained after adjustment for reported fertility treatments or procedures. A more inclusive measure of sub-fertility, such as time to conception, was not available.

Several studies have reported that maternal oestradiol levels are higher among women with NVP.[30,31,59] Our finding of reduced risk of hypospadias among women with pregnancy-related vomiting therefore contradicts hypotheses regarding higher oestrogen levels. However, in addition to oestradiol, NVP is associated with a variety of other hormones and peptides that contribute to fetal growth and development and maternal immune function,[30,60–64] and in general terms NVP may be considered a marker for normal production of pregnancy-related hormones and growth factors.[60] The only previous finding regarding a potential association between hypospadias and NVP of which we are aware reported that intake of antiemetic medications was associated with higher hypospadias risk, but specific medications were not described.[65]

In agreement with previous studies,[16,43] multiple birth was associated with increased risk in univariable

analyses but with reduced risk in analyses that were restricted to subjects with birthweight ≥2500 g. This finding supports the hypothesis that the association of hypospadias with multiple birth stems from its association with growth retardation.[42] Multiple gestation is associated with elevated oestradiol during early pregnancy,[32] but also with elevated levels of other hormones such as testosterone, progesterone and hCG.[32]

Our study is strengthened by its population-based design, consistent method of case ascertainment, and multivariable analysis, but it is also limited in several ways. The applicability of our results to less severe cases (i.e. first degree, or coronal or glandular) is unknown, and a potential underlying physiological explanation for our results could not be explored directly. We were unable to evaluate recall bias, but reporting for most of the exposures we examined is relatively straightforward (e.g. age, parity, multiple gestation). Similarly, data to compare maternal exposures among participants and non-participants were not available, but we have no evidence to suggest that these exposures would have differentially influenced participation among cases and controls and thus contribute to selection bias. Data on maternal and paternal fertility were also limited; for example, general indicators such as time to conception were not available.

Previous analyses of data from this study found that oral contraceptive intake[14] and smoking[18] during early pregnancy were not substantially associated with hypospadias risk, thus not adding support to the oestrogen hypothesis. A previous analysis also found that intake of progestins for the purpose of improving fertility or preventing pregnancy complications was associated with two- to threefold increased risk.[14] Exclusion of women who took pregnancy-related progestins from the current analysis did not substantially alter our findings (data not shown). An analysis of other potentially oestrogenic, exogenous exposures (e.g. phytoestrogens, pesticides) was beyond the scope of the present analysis. Finally, given that hypospadias is associated with growth retardation,[42,43] the inverse association of fetal growth with maternal oestrogen levels[66,67] does not support the oestrogen hypothesis. However, fetal growth parameters have minimal discriminatory ability as predictors of maternal oestrogen levels,[66,67] and the biological significance of oestrogen levels is likely to be complex.

In summary, this study lends minimal, if any, support for a role for oestrogen in hypospadias aetiology. The need for alternatives to the oestrogen hypothesis is apparent. Hypothesising a role for oestrogens admittedly oversimplifies the complex endocrine milieu that the mother, embryo and placenta must regulate together. For example, the production and action of oestrogens is intricately tied to the production, receptor binding and activation of other sex hormones (e.g. androgen precursors, human chorionic gonadotropin), and the balance among the various endocrine parameters is also critical to proper endocrine function. Although the potential contribution of maternal endocrine factors to the current findings are unknown, the results certainly provide clues regarding hypospadias aetiology that merit further investigation.

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References

1 Paulozzi LJ. International trends in rates of hypospadias and cryptorchidism. *Environmental Health Perspectives* 1999; **107**:297–302.
2 Paulozzi LJ, Erickson JD, Jackson RJ. Hypospadias trends in two US surveillance systems. *Pediatrics* 1997; **100**:831–834.
3 Dolk H, Vrijheid M, Scott JE, Addor MC, Botting B, de Vigan C, *et al.* Toward the effective surveillance of hypospadias. *Environmental Health Perspectives* 2004; **112**:398–402.
4 Nonneman DJ, Ganjam VK, Welshons WV, Vom Saal FS. Intrauterine position effects on steroid metabolism and steroid receptors of reproductive organs in male mice. *Biology of Reproduction* 1992; **47**:723–729.
5 Lee DK, Young JC, Tamura Y, Patterson DC, Bird CE, Clark AF. In vitro effects of estrogens on the 4 -reduction of testosterone by rat prostate and liver preparations. *Canadian Journal of Biochemistry* 1973; **51**:735–740.
6 Abney TO. The potential roles of estrogens in regulating Leydig cell development and function: a review. *Steroids* 1999; **64**:610–617.
7 McKinnell C, Atanassova N, Williams K, Fisher JS, Walker M, Turner KJ, *et al.* Suppression of androgen action and the induction of gross abnormalities of the reproductive tract in male rats treated neonatally with diethylstilbestrol. *Journal of Andrology* 2001; **22**:323–338.
8 Rivas A, McKinnell C, Fisher JS, Atanassova N, Williams K, Sharpe RM. Neonatal coadministration of testosterone with diethylstilbestrol prevents diethylstilbestrol induction of most reproductive tract abnormalities in male rats. *Journal of Andrology* 2003; **24**:557–567.

9 Manson JM, Carr MC. Molecular epidemiology of hypospadias: review of genetic and environmental risk factors. *Birth Defects Research. Part A, Clinical and Molecular Teratology* 2003; **67**:825–836.

10 Baskin LS, Himes K, Colborn T. Hypospadias and endocrine disruption: is there a connection? *Environmental Health Perspectives* 2001; **109**:1175–1183.

11 Sharpe RM, Skakkebaek NE. Are oestrogens involved in falling sperm counts and disorders of the male reproductive tract? *Lancet* 1993; **341**:1392–1395.

12 McLachlan JA, Newbold RR, Burow ME, Li SF. From malformations to molecular mechanisms in the male: three decades of research on endocrine disrupters. *APMIS* 2001; **109**:263–272.

13 Kallen B, Mastroiacovo P, Lancaster PA, Mutchinick O, Kringelbach M, Martinez-Frias ML, *et al.* Oral contraceptives in the etiology of isolated hypospadias. *Contraception* 1991; **44**:173–182.

14 Carmichael SL, Shaw GM, Laurent C, Croughan MS, Olney RS, Lammer EJ, *et al.* Maternal progestin intake and risk of hypospadias. *Archives of Pediatrics and Adolescent Medicine* 2005; **159**:957–962.

15 Harris EL. Genetic epidemiology of hypospadias. *Epidemiologic Review* 1990; **12**:29–40.

16 Carmichael SL, Shaw GM, Nelson V, Selvin S, Torfs CP, Curry CJ. Hypospadias: trends and descriptive epidemiology in a California population. *Epidemiology* 2003; **14**:701–706.

17 Fredell L, Kockum I, Hansson E, Holmner S, Lundquist L, Lackgren G, *et al.* Heredity of hypospadias and the significance of low birth weight. *Journal of Urology* 2002; **167**:1423–1427.

18 Carmichael SL, Shaw GM, Laurent C, Lammer EJ, Olney RS. Hypospadias and maternal exposures to cigarette smoke. *Paediatric and Perinatal Epidemiology* 2005; **19**:406–412.

19 Kallen K. Role of maternal smoking and maternal reproductive history in the etiology of hypospadias in the offspring. *Teratology* 2002; **66**:185–191.

20 Bernstein L, Pike MC, Depue RH, Ross RK, Moore JW, Henderson BE. Maternal hormone levels in early gestation of cryptorchid males: a case-control study. *British Journal of Cancer* 1988; **58**:379–381.

21 Depue RH. Maternal and gestational factors affecting the risk of cryptorchidism and inguinal hernia. *International Journal of Epidemiology* 1984; **13**:311–318.

22 Depue RH, Pike MC, Henderson BE. Estrogen exposure during gestation and risk of testicular cancer. *Journal of the National Cancer Institute* 1983; **71**:1151–1155.

23 Weir HK, Marrett LD, Kreiger N, Darlington GA, Sugar L. Pre-natal and peri-natal exposures and risk of testicular germ-cell cancer. *International Journal of Cancer* 2000; **87**:438–443.

24 Gill WB, Schumacher GF, Bibbo M, Straus FH, Schoenberg HW. Association of diethylstilbestrol exposure in utero with cryptorchidism, testicular hypoplasia and semen abnormalities. *Journal of Urology* 1979; **122**:36–39.

25 English PB, Goldberg DE, Wolff C, Smith D. Parental and birth characteristics in relation to testicular cancer risk among males born between 1960 and 1995 in California (United States). *Cancer Causes and Control* 2003; **14**:815–825.

26 Skakkebaek NE, Rajpert-De Meyts E, Main KM. Testicular dysgenesis syndrome: an increasingly common developmental disorder with environmental aspects. *Human Reproduction* 2001; **16**:972–978.

27 McGlynn KA, Graubard BI, Nam JM, Stanczyk FZ, Longnecker MP, Klebanoff MA. Maternal hormone levels and risk of cryptorchism among populations at high and low risk of testicular germ cell tumors. *Cancer Epidemiology, Biomarkers and Prevention* 2005; **14**:1732–1737.

28 Bernstein L, Depue RH, Ross RK, Judd HL, Pike MC, Henderson BE. Higher maternal levels of free estradiol in first compared to second pregnancy: early gestational differences. *Journal of the National Cancer Institute* 1986; **76**:1035–1039.

29 Panagiotopoulou K, Katsouyanni K, Petridou E, Garas Y, Tzonou A, Trichopoulos D. Maternal age, parity, and pregnancy estrogens. *Cancer Causes and Control* 1990; **1**:119–124.

30 Lagiou P, Tamimi R, Mucci LA, Trichopoulos D, Adami HO, Hsieh CC. Nausea and vomiting in pregnancy in relation to prolactin, estrogens, and progesterone: a prospective study. *Obstetrics and Gynecology* 2003; **101**:639–644.

31 Depue RH, Bernstein L, Ross RK, Judd HL, Henderson BE. Hyperemesis gravidarum in relation to estradiol levels, pregnancy outcome, and other maternal factors: a seroepidemiologic study. *American Journal of Obstetrics and Gynecology* 1987; **156**:1137–1141.

32 Thomas HV, Murphy MF, Key TJ, Fentiman IS, Allen DS, Kinlen LJ. Pregnancy and menstrual hormone levels in mothers of twins compared to mothers of singletons. *Annals of Human Biology* 1998; **25**:69–75.

33 Michnovicz JJ, Bradlow HL. Dietary and pharmacological control of estradiol metabolism in humans. *Annals of the New York Academy of Sciences* 1990; **595**:291–299.

34 Dieckmann KP, Endsin G, Pichlmeier U. How valid is the prenatal estrogen excess hypothesis of testicular germ cell cancer? A case control study on hormone-related factors. *European Urology* 2001; **40**:677–683.

35 Henderson BE, Benton B, Jing J, Yu MC, Pike MC. Risk factors for cancer of the testis in young men. *International Journal of Cancer* 1979; **23**:598–602.

36 Kelsey JL, Gammon MD, John EM. Reproductive factors and breast cancer. *Epidemiologic Reviews* 1993; **15**:36–47.

37 Carmichael AR, Bates T. Obesity and breast cancer: a review of the literature. *Breast* 2004; **13**:85–92.

38 Akre O, Lipworth L, Cnattingius S, Sparén P, Ekbom A. Risk factor patterns for cryptorchidism and hypospadias. *Epidemiology* 1999; **10**:364–369.

39 Jones ME, Swerdlow AJ, Griffith M, Goldacre MJ. Prenatal risk factors for cryptorchidism: a record linkage study. *Paediatric and Perinatal Epidemiology* 1998; **12**:383–396.

40 Berkowitz GS, Lapinski RH, Godbold JH, Dolgin SE, Holzman IR. Maternal and neonatal risk factors for cryptorchidism. *Epidemiology* 1995; **6**:127–131.

41 Potischman N, Troisi R. In-utero and early life exposures in relation to risk of breast cancer. *Cancer Causes and Control* 1999; **10**:561–573.

42 Moller JH, Weidner IS. Epidemiology of cryptorchidism and hypospadias. *Epidemiology* 1999; **10**:352–354.
43 Weidner IS, Moller H, Jensen TK, Skakkebaek NE. Risk factors for cryptorchidism and hypospadias. *Journal of Urology* 1999; **161**:1606–1609.
44 Sweet RA, Schrott HG, Kurland R, Culp OS. Study of the incidence of hypospadias in Rochester, Minnesota, 1940–70, and a case-control comparison of possible etiologic factors. *Mayo Clinic Proceedings* 1974; **49**:52–58.
45 Hay S, Barbano H. Independent effects of maternal age and birth order on the incidence of selected congenital malformations. *Teratology* 1972; **6**:271–279.
46 Porter MP, Faizan MK, Grady RW, Mueller BA. Hypospadias in Washington State: maternal risk factors and prevalence trends. *Pediatrics* 2005; **115**: e495–e499.
47 Ahmed SF, Dobbie R, Finlayson AR, Gilbert J, Youngson G, Chalmers J, *et al.* Prevalence of hypospadias and other genital anomalies among singleton births, 1988–97, in Scotland. *Archives of Disease in Childhood. Fetal and Neonatal Edition* 2004; **89**:F149–F151.
48 Yoon PW, Rasmussen SA, Lynberg MC, Moore CA, Anderka M, Carmichael SL, *et al.* The National Birth Defect Prevention Study. *Public Health Reports* 2001; **116** (Suppl. 2):32–40.
49 Rasmussen SA, Olney RS, Holmes LB, Lin AE, Keppler-Noreuil KM, Moore CA, *et al.* Guidelines for case classification for the National Birth Defects Prevention Study. *Birth Defects Research. Part A, Clinical and Molecular Teratology* 2003; **67**:193–201.
50 Institute of Medicine. Committee on Nutritional Status During Pregnancy and Lactation Food and Nutrition Board. *Nutrition During Pregnancy. Part I, Weight Gain; Part II, Nutrient Supplements*. Washington, DC: National Academy Press, 1990.
51 Selvin S. Measures of risk: rates and probabilities. In: *Statistical Analysis of Epidemiologic Data*. New York: Oxford University Press, 1991;pp. 1–35.
52 Selvin S. *Practical Biostatistical Methods*. Belmont, CA: Wadsworth Publishing Co., 1995.
53 Van Der Werff JFA, Nievelstein RAJ, Brands E, Luijsterburg AJM, Vermeij-Keers C. Normal development of the male anterior urethra. *Teratology* 2000; **61**:172–183.
54 Kallen B, Bertollini R, Castilla E, Czeizel A, Knudsen LB, Martinez-Frias ML, *et al.* A joint international study on the epidemiology of hypospadias. *Acta Paediatrica Scandinavica* 1986; **324**:1–52.
55 Troisi R, Potischman N, Roberts J, Siiteri P, Daftary A, Sims C, *et al.* Associations of maternal and umbilical cord hormone concentrations with maternal, gestational and neonatal factors (United States). *Cancer Causes and Control* 2003; **14**:347–355.
56 Kaijser M, Jacobsen G, Granath F, Cnattingius S, Ekbom A. Maternal age, anthropometrics and pregnancy oestriol. *Paediatric and Perinatal Epidemiology* 2002; **16**:149–153.
57 Kallen B, Castilla EE, Kringelbach M, Lancaster PAL, Martinez-Frias ML, Mastroiacovo P, *et al.* Parental fertility and infant hypospadias: an international case-control study. *Teratology* 1991; **44**:629–634.
58 Czeizel A, Toth J. Correlation between the birth prevalence of isolated hypospadias and parental subfertility. *Teratology* 1990; **41**:167–172.
59 Jarnfelt-Samsioe A, Bremme K, Eneroth P. Steroid hormones in emetic and non-emetic pregnancy. *European Journal of Obstetrics, Gynecology, and Reproductive Biology* 1986; **21**:87–99.
60 Boneva RS, Moore CA, Botto L, Wong L-Y, Erickson JD. Nausea during pregnancy and congenital heart defects: a population-based case-control study. *American Journal of Epidemiology* 1999; **149**:717–725.
61 Panesar NS, Li CY, Rogers MS. Are thyroid hormones or hCG responsible for hyperemesis gravidarum? A matched paired study in pregnant Chinese women. *Acta Obstetricia et Gynecologica Scandinavica* 2001; **80**:519–524.
62 Yoneyama Y, Kobayashi H, Kato M, Chihara H, Yamada T, Otsubo Y, *et al.* Plasma 5′-nucleotidase activities increase in women with hyperemesis gravidarum. *Clinical Biochemistry* 2002; **35**:561–564.
63 Kaplan PB, Gucer F, Sayin NC, Yuksel M, Yuce MA, Yardim T. Maternal serum cytokine levels in women with hyperemesis gravidarum in the first trimester of pregnancy. *Fertility and Sterility* 2003; **79**:498–502.
64 Gadsby R, Barnie-Adshead A, Grammatoppoulos D, Gadsby P. Nausea and vomiting in pregnancy: an association between symptoms and maternal prostaglandin E2. *Gynecologic and Obstetric Investigation* 2000; **50**:149–152.
65 Neto RM, Castilla EE, Paz JE. Hypospadias: an epidemiological study in Latin America. *American Journal of Medical Genetics* 1981; **10**:5–19.
66 Peck JD, Hulka BS, Savitz DA, Baird D, Poole C, Richardson BE. Accuracy of fetal growth indicators as surrogate measures of steroid hormone levels during pregnancy. *American Journal of Epidemiology* 2003; **157**:258–266.
67 Kaijser M, Granath F, Jacobsen G, Cnattingius S, Ekbom A. Maternal pregnancy estriol levels in relation to anamnestic and fetal anthropometric data. *Epidemiology* 2000; **11**:315–319.

