

RESEARCH ARTICLE

Umbilical cord androgens and estrogens in relation to verbal and nonverbal abilities at age 10 in the general population

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Abstract

Sex differences in verbal and nonverbal abilities are a contentious area of research. Prenatal steroids have been shown to have masculinizing effects on the brain that may affect the development of nonverbal and verbal abilities in later life. The current study examined a wide range of biologically active sex steroids (both androgens and estrogens) in umbilical cord blood at birth in a large pregnancy cohort in relation to performance on nonverbal (Raven's Coloured Progressive Matrices) and verbal (Clinical Evaluation of Language Fundamentals-3 and the Peabody Picture Vocabulary Test-III) measures at age 10 years. Overall, Androgen and Estrogen composites in cord blood were not found to be predictive of performance on verbal and nonverbal measures at age 10. These data suggest that late gestation sex steroids do not exert a major effect on nonverbal and verbal abilities in middle childhood.

Background

Sex differences in cognition have been a long standing subject of investigation [1–4]. One aspect of interest is that of sex differentiated performance in nonverbal versus verbal domains. Males have been found to outperform females in nonverbal tasks such as spatial perception, spatial visualization [5] and mental rotation tasks [6–9]. Conversely, females have been shown to outperform males in the domain of verbal abilities [1] in tasks such as verbal fluency [10] and vocabulary [11]. However, there remains significant variation in the size and presence of effects that is dependent on the task and sample involved [5, 12, 13].

Possible causes for these cognitive sex differences have seen extensive research attention [1, 14]. There has been growing interest in the impact of biological influences, such as sex steroids, on the developing brain during prenatal life [15, 16]. Sex steroids are hypothesized to exert an 'organisational effect' on the fetal brain that has long lasting effects on cognition [17, 18].

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Examining prenatal sex steroids in relation to cognition not only enhances our understanding of the mechanisms underlying typical brain development, but may also expand our knowledge of prenatal factors affecting atypical neurodevelopmental conditions such as Autism Spectrum Disorder [19–21].

Previous research has linked sex steroids to nonverbal abilities [22–24]. In particular, androgens are hypothesized to have masculinizing effects on the fetal brain [15, 25]. Androgens may therefore contribute to improved performance in tasks that are typically associated with male superiority, such as nonverbal tasks. For example, females with congenital adrenal hyperplasia (a condition associated with high prenatal androgen exposure) have been shown to exhibit superior performance on tasks involving mental rotation [26–29]. In contrast, males with CAH show impaired performance relative to controls [30]. Furthermore, direct measurement in-utero of amniotic fluid testosterone has linked higher testosterone concentrations to a faster rate of rotation on a mental rotation task in girls, but a slower rate in boys [31]. Though the relationship between nonverbal abilities and sex steroids are not widely studied, a positive linear relationship has been found linking testosterone to nonverbal ability in males [32].

Whilst androgens have received the most attention in the literature, estrogens such as estradiol may promote brain masculinisation and/or defeminisation via aromatization (the conversion of androgens to estrogen), particularly in male rodents [33]. The relationship in humans between estrogens and nonverbal abilities is not as commonly examined. Sprague Dawley rats exposed to perinatal estradiol demonstrated improved accuracy in spatial ability on a radial-arm maze task [34]. Furthermore, levels of circulating estradiol have been linked to improved mental rotation task performance in women [35].

Prenatal sex steroids have less commonly been examined in relation to verbal abilities and findings are mixed. One study demonstrated an inverted u-shaped relationship between amniotic testosterone concentration and language comprehension and classification in 4-year-old girls, but not in boys [36]. Another study found that amniotic testosterone concentration was inversely related to vocabulary size at 18 and 24 months. However, this relationship was significant only when both sexes were examined together and not separately [37]. More recent studies involving the Western Australian Pregnancy (Raine) Cohort have demonstrated inverse relationships between perinatal bioavailable testosterone concentrations in umbilical cord blood and pragmatic language at age 10 in girls [38] and expressive vocabulary in boys at age 2 [39]. Higher cord-blood testosterone concentration has also been linked to significant language delay in males between the ages of 1 and 3 [40]. Interestingly, in this last study, perinatal testosterone was a protective factor in females between the ages of 1 and 3 whereby it was associated with improved language.

Overall, the existing research examining verbal and nonverbal abilities and sex steroids is inconsistent. There is significant variation in age of participants, sample size, sex steroid sampling method, and type of verbal and nonverbal abilities measured. The current study builds on previous literature in several ways. First, we examined a range of biologically active androgens including Androstenedione (A4) and dehydroepiandrosterone (DHEA), which have been associated with weak masculinizing effects [41, 42]. Moreover, these pro-hormones are also involved in sex steroid pathways that are closely linked to the synthesis of other androgens as well as estrogens [43]. We also investigated a range of biologically active estrogens (estrone, estetrol, estriol and estradiol). This enabled us to examine biologically active estrogens and androgens in relation to cognition.

Secondly, we examined sex steroids in a large pregnancy cohort by directly measuring sex steroids in umbilical cord blood at birth. Direct methods of sampling steroids include amniotic fluid, maternal blood and umbilical cord blood sampling. Whilst these methods all have strengths and weaknesses (see [44–46]), cord blood sampling is considered to be the most

representative method whereby its comparatively less intrusive nature allows for the measurement of sex steroids in larger samples involving the general population.

Finally, no study has yet investigated sex steroids in cord blood and examined their relationship to both nonverbal and verbal cognitive abilities in middle childhood. Hence the current study attempted to do this. Androgen and estrogen composites were calculated according to a novel method proposed by Hollier et al. [47]. This method weights the biological activity of each steroid to form composites for both androgens and estrogens. These composites were then analysed in relation to verbal measures and nonverbal measures administered at age 10. Based on the existing literature it would be expected that females outperform males on verbal measures (CELF-3 and PPVT-III) and vice versa on the nonverbal measure (RCPM). In particular the RCPM has been found in a meta-analysis to demonstrate a small but significant advantage for males compared to females. The authors hypothesize that this may be due to the greater visualization component involved as compared to the original Standard Progressive Matrices in which sex differences are not apparent at age 10 [48]. In addition, the Androgen composite would be expected to negatively predict verbal scores and positively predict nonverbal scores and these effects may differ according to sex. The nature of the relationship between the Estrogen composite values and subsequent scores on verbal and nonverbal measures is unclear.

Methods

Participants

Participants were recruited from the Raine study in Western Australia. The original samples involved 2,900 women recruited from the public antenatal clinic at King Edward Memorial Hospital or surrounding private clinics between May 1989 and November 1991. The pregnant women had a gestational age of between 16 and 20 weeks, delivered at King Edward Memorial Hospital, had English language skills and had the intention to remain in Western Australia for subsequent follow ups [49]. In total, 2,868 (96%) births were available for follow-up. Written informed consent was obtained from mothers and guardians who participated, this was documented on paper and stored securely in a locked cabinet. The mothers provided consent when pregnant, parents or guardians at age 10. Participant recruitment, consent and all follow-ups were approved by the Human Ethics Committee at King Edward Memorial Hospital and/ or Princess Margaret Hospital for Children in Perth.

Procedure

Cord blood was obtained from offspring of the Raine cohort at birth. The children were seen at multiple time points (every 2–3 years) for comprehensive phenotyping. At age 10, participants completed the Clinical Evaluation of Language Fundamentals-3 (CELF-3) and the Peabody Picture Vocabulary Test third edition (PPVT-III) as verbal measures and the Raven's Coloured Progressive Matrices (RCPM) as a nonverbal measure.

Measures

Sex steroid measurement. Mixed arterial and venous umbilical cord blood was collected at the birth of 860 deliveries as previously described [21]. Immediately after delivery, mixed umbilical arterial-venous (UA:UV) cord blood was collected, allowed to clot and the resulting serum was frozen at -80°C and stored without thawing until the present study was performed. Eight hundred and three cord blood samples (92.3%) representing 396 female and 407 male infants had sufficient serum (after removal, aliquoting and archiving of 1 ml for future studies)

for steroid analysis. In January 2010, these serum samples were thawed and analysed for sex steroid content by liquid chromatography-tandem mass spectrometry described by Keelan et al. [50]. SHBG was measured by ELISA according to the manufacturer's instructions (IBL International, Hamburg, Germany). The inter-assay imprecision was <4.5% (n = 25). Intra-assay variation was 5.2% (n = 860). Samples with an initial replicate coefficient of variation (CV) of >10% were reanalyzed [50]. The androgens measured were testosterone, androstenedione (A4) and dehydroepiandrosterone (DHEA). The estrogens examined were estrone (E1), estradiol (E2), estriol (E3) and estetrol (E4).

Calculation of bioavailable testosterone, estradiol and estrone. The following formula was used to calculate BioT (nmol/L), representing the fraction of total testosterone either free (unsequestered by SHBG) or bound to serum albumin [50].

$$BioT = [free\ testosterone] + [albumin - bound\ testosterone]$$

Free testosterone was calculated using the empirical method and formula described by [51]. Albumin levels were adjusted using published reference values to take into account the decrease in serum albumin concentrations with gestational age [52]. Bioavailable concentrations of E₁ (BioE₁) and of E₂ (BioE₂) were calculated using the method described by Mazer [53] and adjusted accordingly as described in detail by Hollier et al. (2014).

Composite measures. The calculated composites take into account the biological potency, binding affinity and unbound proportion of the sex steroids. Each steroid is weighted according to its biological potency and the following formulae are used:

$$\begin{aligned} Androgen\ Composite &= BioT + 0.1[A4] + 0.01[DHEA] \\ Estrogen\ Composite &= BioE2 + 0.5[BioE1] + 0.1[E3] + 0.02[E4] \end{aligned}$$

The formulae used to calculate the composites are explained in detail in Hollier et al. (2014).

The Clinical Evaluation of Language Fundamentals-3. The Clinical Evaluation of Language Fundamentals-3 (CELF-3; [54]) is a widely used language assessment that measures expressive and receptive language ability in individuals aged 6 to 21. The assessment takes approximately 30 to 45 minutes to complete. In children older than 9, the test includes two domains, with four subscales measuring receptive language (Concepts and Directions, Semantic Relations, Word Classes, Recalling Sentences) and three subscales measuring expressive language (Sentence Assembly, Formulated Sentences, Recalling Sentences). The CELF-3 has been shown to have sound psychometric properties including high internal consistency (.83 - .95). As part of the analyses of the current study, sex differences in CELF-3 scores are examined. For this reason we elected to use raw scores as opposed to the sex-specific standard scores.

The Peabody Picture Vocabulary Test third edition. The Peabody Picture Vocabulary Test third edition (PPVT-III; [55]) is a commonly administered standardised test examining verbal ability including receptive vocabulary knowledge and comprehension. It is an individually administered, norm referenced test designed for individuals aged 2.5 to 90. There are 204 items and each page consists of four black and white line drawings. The examiner instructs the participant to "Put your finger on ____, Show me ____, or Find" the named target stimulus. A raw score is calculated by summing scores across items and then a standard score (M = 100, SD = 15) is derived. The PPVT-III has sound reliability and validity [56, 57].

Raven's Coloured Progressive Matrices. Raven's Coloured Progressive Matrices (RCPM; [58]) is a widely used measure of nonverbal intelligence for individuals between the ages of 5 and 11. The test consists of 36-items that increase in difficulty. Each item consists of a matrix

of geometric figures with one figure missing. Individuals then select an item from options below that best fits the visual pattern of the matrix. The psychometric properties of this measure in children are considered generally sound [59].

Sample characteristics

Sociodemographic variables (maternal age at conception, maternal education, family income) were recorded at 18 weeks' pregnancy, antenatal variables (maternal smoking and alcohol consumption during pregnancy) at 34 weeks' pregnancy and obstetric variables (gestational age, offspring gender, parity, and Apgar scores) at birth. Proportion of optimal birth weight (POBW) was also calculated to provide an indicator of appropriateness of fetal growth [60]. This was based on the ratio of the observed birth weight to the optimal birth weight for that individual [61].

Statistical analyses

Scores on the CELF-3 and PPVT-III were combined to form a composite verbal score. This composite score along with scores on RCPM were transformed to z-scores to provide standardized verbal and nonverbal ability scores. Those who completed measures at age 10 and those who did not were compared on sex steroid composites, and on obstetric, antenatal and sociodemographic variables. Independent-samples t-tests were conducted to compare the sexes on key sex steroid and verbal and nonverbal measures. Any subsequent analyses were sex specific. Correlation analyses were conducted to examine relationships between sex steroid and verbal and nonverbal measures in each sex. Spearman's rho was used to examine monotonic relationships and curve estimation regression analyses were conducted in order to account for any quadratic relationships. Any significant correlations or regression models were followed up with a stepwise multiple regression to determine the predictive ability of the sex steroid composite in relation to the outcome variables. Any antenatal, obstetric or sociodemographic variable that significantly correlated ($p < .05$) with the verbal or non-verbal measure was entered in the first block. The composite was entered in the second block and any quadratic relationships were also investigated by entering the quadratic term of the sex steroid composite in the third block of the regression analysis. An alpha level of .05 was used for all statistical analyses.

Results

There were 860 children who had available sex steroid data of which 464 (227 females and 237 males) provided follow-up information at age 10 years on the PPVT-III, RCPM and the CELF-3. There was no significant difference in age between males ($M = 10.63$, $SD = 0.17$) and females ($M = 10.61$, $SD = .18$) at the 10 year follow up, $t(462) = .67$, $p = .50$. Chi-squared analyses were conducted to examine any differences between those who did and did not complete the measures at age 10 –these are presented in [Table 1](#). Those individuals who did not complete the measures at age 10 were more likely to have younger mothers, mothers who smoked during pregnancy and have a family income below the poverty line. In contrast, there was no significant difference found in maternal education at pregnancy, maternal alcohol intake during pregnancy, gestational age, POBW, sex, 5-minute Apgar score or parity. There were also no significant differences between completers and non-completers for females on the Androgen, $t(426) = 1.14$, $p = .26$, or Estrogen, $t(426) = 0.42$, $p = .67$, composite, and also for males on the Androgen, $t(429) = 1.03$, $p = .31$, or Estrogen, $t(429) = .66$, $p = .51$, composite.

Table 1. Characteristics of those who did and did not complete age 10 measures.

Categorical variables	Completed age 10 measures (n = 464)		Did not complete age 10 measures (n = 396)		p value
	N	n (%)	N	n (%)	
Maternal age at birth	453		376		<.01
<20		31 (6.8)		47 (12.5)	
20–24		84 (18.5)		93 (24.7)	
25–29		124 (27.4)		111 (29.5)	
30–34		140 (30.9)		84 (22.3)	
35+		74 (16.3)		41 (10.9)	
Maternal education at pregnancy	453		379		.51
Completed secondary school		170 (37.5)		134 (35.4)	
Did not complete secondary school		283 (62.5)		245 (64.6)	
Family income below poverty line	452		369		<.01
Yes		167 (36.9)		181 (49.1)	
No		285 (63.1)		188 (50.9)	
Maternal smoking in pregnancy	454		372		<.05
None		352 (77.5)		262 (70.4)	
1–10 cigarettes daily		62 (13.7)		55 (14.8)	
11+ cigarettes daily		40 (8.8)		55 (14.8)	
Maternal alcohol intake during pregnancy	454		372		.30
None		273 (60.1)		242 (65.1)	
Once a week or less		156 (34.4)		109 (29.3)	
Several times a week or more		25 (5.5)		21 (5.6)	
Gestational age	453		376		.38
< 32 weeks		8 (1.8)		9 (2.4)	
32 to 37 weeks		72 (15.9)		68 (18.1)	
38 to 40 weeks		291 (64.2)		246 (65.4)	
> 40 weeks		82 (18.1)		53 (14.1)	
Proportion of optimal birthweight	462		395		.45
< 90		133 (28.8)		127 (32.2)	
90 to 110		263 (56.9)		208 (52.7)	
> 110		66 (14.3)		60 (15.2)	
Sex	464		395		.52
Male		233 (50.2)		198.2 (50.1)	
Female		231 (49.8)		197 (49.9)	
Apgar score	453		375		.10
Generally normal		441 (97.4)		357 (95.2)	
Fairly low		12 (2.6)		18 (4.8)	
Critically low		0		0	
Parity	464		394		.06
0		203 (43.8)		198 (50.3)	
1		154 (33.2)		103 (26.1)	
>1		107 (23.1)		93 (23.6)	

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Sex differences on sex steroids and verbal and non-verbal measures

Table 2 presents the outcome of independent-samples t-tests conducted to examine sex differences on sex steroid composites and verbal and nonverbal measures and subscales. Males had a significantly higher Androgen composite than females, while there was no sex difference in

Table 2. Mean (SD) for sex steroid variables and verbal and non-verbal measures at age 10 for males and females.

	Females (N = 231)	Males (N = 233)	p value	Cohen's d
Androgen composite	0.38 (.16)	0.43 (.16)	.001**	0.31
Estrogen composite	94.73 (46.83)	97.68 (47.64)	.50	0.06
CELF-3 total score	97.31 (14.81)	94.75 (15.15)	.07	0.17
• Concepts and directions	24.53 (4.15)	24.24 (4.31)	.45	0.07
• Word classes	23.46 (4.57)	22.33 (5.41)	.02*	0.23
• Semantic relations	15.30 (4.17)	14.81 (4.59)	.23	0.11
• Sentence assembly	11.55 (4.20)	10.43 (4.63)	.01*	0.25
• Formulated sentences	29.43 (6.61)	28.82 (6.81)	.33	0.09
• Recalling sentences	47.47 (12.57)	46.32 (12.62)	.34	0.09
PPVT-III total standard score	103.17 (12.36)	103.65 (12.38)	.68	0.04
RCPM score	31.19 (3.57)	31.17 (3.35)	.96	0.01
Total verbal z-score	0.08 (1.82)	-0.052 (1.81)	.45	0.07

Note: PPVT-III = Peabody Picture Vocabulary Test—III, CELF-3 = Clinical Evaluation of Language Fundamentals -3, RCPM = Raven's Coloured Progressive Matrices.

*p <.05,

**p <.01

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the estrogen composite. Females scored significantly higher than males on the Word Classes and Sentence Assembly subscales of the CELF-3 (all *ps* <.05). No other measures exhibited a significant sex difference (all *ps* >.05).

Relationships between sex steroid composite values and verbal and nonverbal measures

Table 3 shows the outcome of Spearman rank correlations conducted on sex steroid composites and nonverbal and verbal measures separately for each sex. In females, Androgen composite values were weakly negatively correlated with Total verbal standard scores and with the Concepts and Directions subscale scores of the CELF-3 (all *ps* <.05). For males, Androgen composite values were weakly negatively correlated with the Formulated Sentences subscale scores. All other correlations in males and females were non-significant (all *ps* >.05). Sex specific curve estimation regression analyses were carried out on all main outcome variables with no statistically significant effects (all *ps* >.05).

Further hierarchical multiple linear regression analyses were conducted as a follow-up to the significant correlations. Covariates that significantly correlated with the outcome variable were included in the first step, the Androgen composite at the second step, and the quadratic Androgen composite at the third step of the model to account for possible non-linear effects. In females, Androgen composite values did not significantly predict total Verbal scores (Table 4) or scores on the Concepts and Directions subscale (see Table 5) over and above the variance accounted for by covariates. In males, the quadratic Androgen composite did significantly predict scores on the Formulated Sentences subscale over and above the variance accounted for by covariates (see Table 6).

Discussion

The current study examined the relationship between sex steroids in umbilical cord blood and nonverbal and verbal abilities in middle childhood. As expected, androgen composite values

Table 3. Spearman’s correlations (p value) between sex steroid values and scores on measures for males and females.

	Androgen composite		Estrogen composite	
	Males	Females	Males	Females
CELF-3 total score	-.11 (.12)	-.13 (.05)	-.01 (.89)	.03 (.71)
• Concepts and directions	-.07 (.29)	-.16 (0.01)*	-.02 (.81)	-.01 (.86)
• Word classes	-.09 (.17)	-.09 (.20)	.06 (.37)	.01 (.84)
• Semantic relations	-.04 (.55)	-.10 (.13)	-.02 (.79)	.01 (.88)
• Sentence assembly	-.09 (.19)	-.10 (.15)	.03 (.69)	-.02 (.82)
• Formulated sentences	-.15 (.02)*	-.06 (.36)	-.01 (.94)	-.02 (.82)
• Recalling sentences	-.06 (.36)	-.10 (.15)	.08 (.22)	.05 (.47)
PPVT-III total standard score	-.10 (.12)	-.12 (.08)	-.09 (.90)	.01 (.87)
RCPM score	-.002 (.98)	.01 (.85)	-.06 (.40)	.06 (.33)
Total verbal z-score	-.11 (.10)	-.14 (.04)*	.04 (.51)	.01 (.84)

Note: PPVT-III = Peabody Picture Vocabulary Test—III, CELF-3 = Clinical Evaluation of Language Fundamentals -3, RCPM = Raven’s Coloured Progressive Matrices.

*p <.05

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were significantly higher in males than females. This supports commonly acknowledged sex differences in testosterone concentrations found prenatally and at birth, in both animal and human studies [62–64]. However, the Estrogen composite was not found to differ according to sex. This too is unsurprising given the limited effects found so far in the existing literature when examining estrogens both prenatally and perinatally [65, 66].

Raw scores on the Word Classes and Sentence Assembly subscales of the CELF-3 were significantly higher for females than males, though this effect was small in magnitude. Whilst the CELF-3 subscale scores appear to trend towards a slight female advantage, statistically the majority of the verbal measures do not support a significant sex difference in performance. Age factors may explain, in part, the lack of sex differences in these results. For example, the variation between sexes in language ability appears to be most prominent at early preschool ages. A meta-analysis found that verbal superiority in females was apparent in those younger than age 5 and older than 26 [10]. It may be that age effects interact with the type of verbal ability being assessed. For example, sex differences appear to be more pronounced in early

Table 4. Outcomes of hierarchical multiple regression analyses predicting the total verbal scores in females.

		<i>B</i>	<i>SE B</i>	<i>B</i>	R ²	R ² change
Step 1	Covariates only				.08	
Step 2	Constant	.66	.33			
	Maternal education	.65	.26	.17		
	Maternal income	-.76	.26	-.20		
	Androgen composite	-1.35	.74	-.12		
					.10	.01, <i>p</i> = .07
Step 3	Constant	1.22	.51			
	Maternal education	.68	.26	.18		
	Maternal income	-.73	.26	-.19		
	Androgen composite	-4.27	2.17	-.38		
	Quadratic androgen composite	3.10	2.16	.27		
					.10	.01, <i>p</i> = .15

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Table 5. Outcomes of hierarchical multiple regression analyses predicting the concepts and directions subscale score in females.

		<i>B</i>	<i>SE B</i>	<i>B</i>	<i>R</i> ²	<i>R</i> ² change
Step 1	Covariates only				.09	
Step 2	Constant	23.44	1.40			
	Maternal education	1.24	.58	.15		
	Maternal income	-1.40	.57	-.16		
	Maternal language	.70	1.17	.04		
	Maternal race	1.86	.94	.14		
	Androgen composite	-2.96	1.23	-.16		
					.11	.01, <i>p</i> = .08
Step 3	Constant	24.01	1.62			
	Maternal education	1.27	.59	.15		
	Maternal income	-1.37	.58	-.16		
	Maternal language	.75	1.17	.05		
	Maternal race	1.86	.94	.14		
	Androgen composite	-6.14	4.84	-.24		
	Quadratic androgen composite	3.45	4.85	.14		
					.11	.002, <i>p</i> = .48

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vocabulary development [39, 67, 68]. Overall, these results support recent suggestions of the inconsistency of female superiority on language measures [13, 69].

The lack of sex differences found on the RCPM is inconsistent with the common perception of superior nonverbal ability in males. Research on sex differences in this area tap into multiple components of nonverbal ability and tend to show the most pronounced effects for mental rotation tasks [5]. Whilst previous meta-analyses found a small effect of male superiority in children aged 5–11 on the RCPM [48]. This may be attributed to age specific effects and

Table 6. Outcomes of hierarchical multiple regression analyses predicting the formulated sentences subscale score in males

		<i>B</i>	<i>SE B</i>	<i>B</i>	<i>R</i> ²	<i>R</i> ² change
Step 1	Covariates only				.13	
Step 2	Constant	25.43	2.65			
	Maternal education	1.57	.95	.11		
	Maternal income	-3.57	.96	-.25		
	Maternal race	2.97	1.67	.12		
	Birth weight category	.92	.70	.09		
	Maternal alcohol consumption	1.24	.75	.11		
	Androgen composite	-1.82	2.83	-.04		
					.13	.002, <i>p</i> = .52
Step 3	Constant	29.85	19.59	3.298.77	.49	
	Maternal education	1.61	.94	.11		
	Maternal income	-3.32	.96	-.23		
	Maternal race	3.11	1.66	.12		
	Birth weight category	.95	.69	.09		
	Maternal alcohol consumption	1.31	.74	.11		
	Androgen composite	-22.36	9.61	-.51		
					.15	.02, <i>p</i> = .03

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ongoing maturation of the brain. For example, much of the prefrontal cortex development is thought to occur in middle childhood [70].

Higher Androgen composites were related to lower scores on the Concepts and Directions subscale and Total verbal z-score in girls. However, these relationships did not remain significant when covariates were taken into account. Language development does not occur solely in response to genetic and biological influences. Whilst the current study attempted to control for possible environmental covariates such as socioeconomic status, maternal language and race, language development occurs in response to complex environmental factors which influence subsequent performance [71–73]. Higher Androgen Composite values did demonstrate a u-shaped relationship to Formulated Sentences subscale scores in males suggesting that the ability to produce grammatically correct sentences is highest in individuals with either high or low concentrations of androgens. However this effect was small as quadratic Androgen composite values accounted for approximately 2% of additional variance. There were no relationships found between estrogen or androgen composite values and the PPVT-III or RCPM scores. Overall, these results suggest that at age 10, perinatal androgen and estrogen were not related in any substantial way to verbal or nonverbal measures.

There are a number of alternative explanations for the null results found in the current study. First, few of the outcome variables demonstrated sex differences. Prenatal sex steroids are more likely to be related to outcome measures that demonstrate clear differences between sexes [18]. The verbal and spatial measures used in the current study may therefore tap into areas in which cognitive sex differentiation is less pronounced and where the differentiation may be age or task-related, as previously discussed. It should also be noted that verbal and nonverbal abilities are umbrella terms that encompass a range of sub-constructs that are, at present, still unclearly differentiated [74]. The current study is therefore only indicative of the specific abilities which these tasks measure.

Future research could attempt to compartmentalize these areas further in order to untangle the areas of cognition in which sex steroids may play a part. This study therefore does not undermine the previous findings in cord blood linking prenatal testosterone to early language development [38, 39]. It should be noted that there is merit in continuing to use less invasive, more representative methods such as cord blood sampling and the examination of a wide scope of sex steroids, particularly using reliable methods such as mass spectrometry [50]. Further research of this kind would allow us to more accurately define the parameters under which these late gestation levels of hormones may influence certain aspects of cognition, such as verbal and nonverbal abilities. This research is informative in exploring the traditional male-female divide in verbal and nonverbal abilities and the possible mechanisms influencing early neurocognitive development.

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