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# **Foetal testosterone and vocabulary size in 18- and 24-month-old infants**

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## **ABSTRACT**

Amniotic fluid, obtained from 87 pregnant women for routine amniocentesis, was analysed for foetal testosterone (FT) level. Their infants (40 girls and 47 boys) were followed up 18 and 24 months after birth and their vocabulary size was assessed. Girls were found to have a significantly larger vocabulary than boys at both ages. This replicates previous findings of a female advantage in language ability, but reveals this sex difference at the earliest point of development. Additionally, FT was an inverse predictor of vocabulary size when data from both sexes was examined together, but not within sex. The lack of a significant correlation between FT and vocabulary within each sex may reflect the relatively small sample size. However, the significant correlation between FT and vocabulary when the sexes were combined suggests FT might be involved in shaping the neural mechanisms underlying communicative development.

Foetal testosterone (FT) acts on the developing brain to influence cerebral lateralisation (Kimura, 1999; Wilson, Foster, Kronenberg, & Larsen, 1998). Evidence for this derives from both animal studies (Arnold & Gorski, 1984; Harris & Levine, 1962; Williams, Barnett, & Meck, 1990), and the effects of abnormal hormonal environments during human pregnancy, such as Congenital Adrenal Hyperplasia or synthetic hormone injections (Collaer & Hines, 1995; Hines & Shipley, 1984).

There is reason to believe that sex hormones might be involved in the development of language delay (Geschwind & Galaburda, 1987; Geschwind & Galaburda, 1985a; Geschwind & Galaburda, 1985b; Geschwind & Galaburda, 1985c). This is not only because sex differences (female superiority) have been found in studies of normal language development (Hyde & Linn, 1988; Maccoby & Jacklin, 1974), but also because some postulated right hemisphere functions such as mental rotation (spatial) ability are better in males than in females and correlates significantly with FT (Grimshaw, Bryden, & Finegan, 1995). Geschwind's theory was that this right hemisphere superiority in males might be caused by early testosterone levels, and occur at the expense of the left hemisphere, which is usually dominant for language functions. The aim of the current study was therefore to test if there was a sex difference in the language development of infants at 18 and 24 months of age, and also to test if language development was related to FT levels. 18 months of age represents one of the earliest points in development at which to test for sex differences in language ability. Retesting this at 24 months of age allowed for a within-sample replication test of any sex difference found at 18 months old.

To examine language development at 18 and 24 months, we used the Communicative Development Inventory (CDI) which assesses vocabulary size (Hamilton, Plunkett, & Schafer, 2000). The CDI is a checklist of 416 words divided into 19 categories, including animal names, body parts, quantifiers and pronouns. Parents are instructed to mark each word their child can say (recognition format). The CDI is an adaptation by British researchers in Oxford of the American MacArthur CDI (Fenson et al., 1994). Fenson et al developed two vocabulary checklists – one for infants and one for toddlers – in order to assess communicative development in children. The Oxford version replaces American words with their British equivalents, which made it more suitable for our (British) sample. In addition, the Oxford version focuses solely on single words, whereas the American version includes gestures and early language structure. This gave us a very simple, easily quantifiable outcome measure. CDI scores for 20-month-olds correlate closely (.6 to .8) to laboratory based measures at 28 months (Dale, Bates, Reznick, & Morisset, 1989).

Amniocentesis is a routine antenatal test offered during the 2<sup>nd</sup> trimester of pregnancy, to mothers at high risk of carrying a foetus with Down Syndrome. Amniocentesis also provides a way to access information about foetal hormones such as FT (Abramovich & Rowe, 1973; Finegan, Bartleman, & Wong, 1991). We therefore studied mothers who had undergone amniocentesis, so that we could test the relationship between FT and postnatal language development.

The predictions were as follows: (1) Girls would have a larger vocabulary than boys. (2) Vocabulary size would be inversely related to FT levels. (3) A non-linear relationship between FT level and vocabulary size would result if, for example, there were a different relationship within each sex (Grimshaw et al., 1995).

87 mothers were recruited, who had undergone amniocentesis in the Cambridge region between June 1996 and June 1997 and who had given birth to healthy singleton infants (40 girls and 47 boys) between December 1996 and December 1997. When the infants reached 18 months of age, parents were sent a copy of the Oxford CDI. Parents were instructed to indicate which of the words their child could say (out of a possible 416).

The infant's amniotic fluid sample was retrieved from frozen storage at Addenbrooke's Hospital, Cambridge, where FT levels were measured by radioimmunoassay, by a technician blind to the CDI scores<sup>1</sup>. For boys, the FT level ranged from 0.13 to 2.0 nmol/l with mean 1.01 nmol/l (sd = 0.4 nmol/l). For girls, the FT level ranged from 0.2 to 0.8 nmol/l with mean 0.4 nmol/l (sd = 0.2 nmol/l). For both sexes together, the FT level

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<sup>1</sup> Assays were carried out by the Department of Clinical Biochemistry, Addenbrooke's Hospital, Cambridge. Amniotic Fluid was extracted with diethyl ether. Recovery experiments have demonstrated 95 percent recovery of testosterone via this method. The ether was evaporated to dryness at room temperature and the extracted material redissolved in assay buffer. The testosterone was assayed by the DPC 'Count-a-Coat' method (Diagnostic Products Corp, Los Angeles, CA90045-5597). This uses an antibody to testosterone coated onto propylene tubes and a 125-I labelled testosterone analogue. The detection limit of the assay is approximately 0.1 nmol/l. This method thus measures total extractable testosterone.

ranged from 0.1 to 2.0 nmol/l with mean 0.7 nmol/l (sd = 0.5 nmol/l). The FT data was positively skewed, so a natural logarithmic transformation was carried out.

We also included the following control variables in the analysis. (1) Amniotic fluid oestradiol, the most potent oestrogen. This is known to have masculinising as well as feminising effects on development and is synthesised in vivo via aromatisation of testosterone and related precursors. Testosterone is known to have a number of its masculinising effects by first being converted to oestradiol in the brain (see (MacLusky & Naftolin, 1981) so it is important to consider oestradiol when looking at the biological activity of testosterone. (2) Alpha-foetoprotein (AFP), is thought to be a general marker for severe foetal ill-health and also provides a specific control for any unexpected abnormalities of amniotic fluid dilution. (3) Sex, (4) number of siblings, (5) maternal age, (6) paternal age, and (7) educational level attained by the parents. The latter measure was attained by classifying parents according to a 5 point scale: 1 = no formal qualifications, 2 = 'O' level / G.C.S.E. or equivalent, 3 = 'A' level, HND or vocational qualification, 4 = university degree, 5 = postgraduate qualification. The score for both parents was added together.

There was no relationship between gestational age at amniocentesis and levels of FT (Spearman's  $\rho = .1$ ,  $p > .05$ ) or oestradiol level (Spearman's  $\rho = .1$ ,  $p > .05$ ). AFP level was significantly related to gestational age at amniocentesis (Spearman's  $\rho = -.6$ ,  $p < .01$ ) so this factor was entered into subsequent analyses involving AFP level. Also included was the age in months at the time of data collection.

Results at 18 months old showed that girls scored significantly higher on vocabulary size than boys. For boys, vocabulary size ranged from 0.0 to 222.0,  $M = 41.8$  ( $SD = 50.1$ ). For girls vocabulary size ranged from 2.0 to 318.0,  $M = 86.8$  ( $SD = 83.2$ ). For both sexes together the vocabulary size ranged from 0.0 to 318.0,  $M = 62.5$  ( $SD = 70.7$ ). There was no need to obtain inter-rater reliability scores, as the CDI data were unambiguous. A significant sex difference (female superiority) was found for vocabulary size ( $t = -3.1$ ,  $p = .001$ ).

Regarding the relationship between FT level and vocabulary size, initially we undertook analysis of data for both sexes together. The data was positively skewed, so a natural log transformation was carried out. Use of the log transformation resulted in the loss of three subjects whose vocabulary size score was 0 (as  $\ln 0$  is not defined). In addition, 2 outliers were removed. These were the 2 lowest scores, both vocabulary size = 2.0.

The first step was to look for correlations between vocabulary size and each of the predictor variables. If the relationship was significant at the  $p < .2$  level, that predictor was entered into the model (Altman, 1991). The following predictors were found to be related to vocabulary size, at the  $p < .2$  level. FT level (Spearman's  $\rho = -.2$ ,  $p = .08$ ), sex (Mann-Whitney  $U = 569.5$ ,  $p = .001$ ), oestradiol level (Spearman's  $\rho = -.3$ ,  $p = .02$ ), paternal age (Spearman's  $\rho = .2$ ,  $p = .2$ ), parental education level (Spearman's  $\rho = .3$ ,  $p = .01$ ) and age at time of data collection (Spearman's  $\rho = .3$ ,  $p = .001$ ).

Additionally, if a predictor was entered into the model, any other predictor found to be significantly correlated to it was also entered. The following relationships were found between predictors at the  $p = .01$  level. FT level was significantly related to sex (Mann-Whitney  $U = 158.5$ ,  $p < .01$ ). Maternal age was significantly correlated to paternal age (Spearman's  $\rho = .7$ ,  $p < .01$ ) and to number of siblings (Spearman's  $\rho = .3$   $p < .01$ ). Paternal age was significantly correlated to educational level attained by parents (Spearman's  $\rho = .4$ ,  $p < .01$ ). Therefore, the following predictors were included in the model: maternal age, which was correlated to paternal age, and number of siblings which was related to maternal age.

A backward stepwise linear regression was used (entry criteria  $p = .05$ , removal criteria  $p = .1$ ) to find the best fit for the dependence of vocabulary size on the predictor variables. The regression discarded from the model all predictors except for sex, ln FT, oestradiol level and parental education level (See Table 1).

insert Table 1 here

We then undertook analysis of data within each sex (using the same procedure as described above), to find out if the previous result was simply due to a sex difference. No significant relationship between FT and vocabulary size was observed within sex for boys or girls. In summary, a significant inverse relationship was found between FT and vocabulary size with both sexes together, but not within either sex.

As many of the same subjects were followed up at age 24 months. We obtained language data on 38 girls and 43 boys. (ie 75 of the 81 subjects who had taken part in Experiment 1). When the infants reached 24 months of age, parents were sent a copy of the Oxford CDI.

For boys, the FT level ranged from 0.1 to 2.0 nmol/l with mean 1.02 nmol/l (sd = 0.4 nmol/l). For girls, the FT level ranged from 0.2 to 0.8 nmol/l with mean 0.4 nmol/l (sd = 0.2 nmol/l). For both sexes together, the FT level ranged from 0.1 to 2.0 nmol/l with mean 0.7 nmol/l (sd = 0.5 nmol/l). The FT data was positively skewed, so a natural logarithmic transformation was used.

At 24 months old, girls scored significantly higher on vocabulary size than boys. For boys, vocabulary size ranged from 0.0 to 414.0,  $M = 196.8$  ( $SD = 126.8$ ). For girls vocabulary size ranged from 15.0 to 415.0,  $M = 275.1$  ( $SD = 121.6$ ). For both sexes together the vocabulary size ranged from 0.0 to 415.0,  $M = 231.4$  ( $SD = 130.4$ ). A significant sex difference (female superiority) was found for vocabulary size ( $t = -2.8$ ,  $p = .01$ ).

Regarding the relationship between FT level and vocabulary size, analytic methods were as reported for Study 1 above. The following predictors were found to be related to vocabulary size, at the  $\alpha = .2$  level. FT level (Spearman's  $\rho = -.2$ ,  $p = .2$ ), sex (Mann-

Whitney  $U = 531.0$ ,  $p = .01$ ), paternal age (Spearman's  $\rho = .2$ ,  $p = .10$ ) and parental education level (Spearman's  $\rho = .4$ ,  $p = .01$ ).

The following relationships were found between predictors at the  $p = .01$  level. FT level was significantly correlated to sex (Mann-Whitney  $U = 158.5$ ,  $p < .01$ ). Maternal age was significantly correlated to paternal age (Spearman's  $\rho = .7$ ,  $p < .01$ ) and to number of siblings (Spearman's  $\rho = .3$ ,  $p < .01$ ). Paternal age was significantly correlated to educational level attained by parents (Spearman's  $\rho = .4$ ,  $p < .01$ ). Therefore, the following predictors were included in the model: maternal age, which was correlated to paternal age, and number of siblings which was related to maternal age.

A backward stepwise linear regression was used (entry criteria  $p = .05$ , removal criteria  $p = .1$ ) to find the best fit for the dependence of vocabulary size on the predictor variables. The regression retained all predictors except for paternal age (See Table 2).

insert Table 2 here

We then undertook analysis of data within each sex (using the same procedure as described above), to find out if the previous result was simply due to a sex difference. No significant relationship between FT and vocabulary size was observed within sex for boys or girls. In summary, a significant inverse relationship was observed between FT and vocabulary size when the data from both sexes was analysed together, but not within sex for either sex.

This experiment showed that, at both 18 and 24 months of age, girls had a significantly larger vocabulary than boys. In addition, at both ages, a significant relationship was found between FT level and vocabulary size with both sexes together, but not within either sex. The observed sex difference in vocabulary size (female superiority) is as expected based on previous studies. The sex difference may be mediated in part prenatally by the sex difference in FT level. It could also be due to social factors, such as parents talking more to girls than to boys. However, the propensity of the girls to engage in this dyadic interaction may itself have its foundations in prenatal biology. So if parents are talking to girls more, it may be because girls are eliciting this. This idea is supported by the biological findings of this experiment.

When the data from both sexes was kept together, FT level was a significant predictor of vocabulary size at both 18 and 24 months old. In the regression model the coefficient for  $\ln$  FT is positive. This is because both the FT data and the vocabulary size data were logged, resulting in a change to the shape of the observed relationship. No significant relationship was observed between FT level and vocabulary size within sex. One possible conclusion is that the significant FT result for both sexes together was simply describing a sex difference. However, the regression method should tell us about the effect of FT with knowledge of the other predictors (which included sex). To detect a large effect size with 4 predictor variables (as with both sexes together) we would need at least 40 girls. Therefore, lack of power may explain the negative finding for girls.

When the data from both sexes was kept together, sex appeared to be the best predictor of vocabulary size (with girls scoring higher than boys). The next most significant predictor was parental education level. This could mean that parents with a higher level of education attainment talk to their children more, or that there is a genetic effect. In this sample, parental education level is positively correlated to parental age and number of siblings, so the finding could be a reflection of environmental effects caused by having more siblings and older parents. There could also be an effect of parents overestimating their child's vocabulary. Oestradiol level was an inverse predictor of vocabulary size. This fits with findings that oestradiol can have masculinising effects.

In conclusion, we have found a sex difference in vocabulary size and an inverse relationship to FT level at both 18 months and 24 months. Because this effect was only found when the two sexes were pooled, and not when they were examined separately, this needs to be tested further before firm conclusions can be drawn. However, the present results suggest there may be endocrine influences on this essentially human aspect of neurocognitive development.

**Table 1: Regression model at age 18 months**

Model	B	Std error	Sig
(constant)	1.3	.9	.13
Sex	1.3	.4	.00
Oestradiol	-.00	.0	.04
Parental education	.2	.1	.00
ln (FT)	.6	.3	.05

Dependent variable: ln (vocabulary size).  $R^2 = .3$   $F = 6.2$ ,  $p = .001$

Power > .99

**Table 2: Regression model at age 24 months**

Model	B	Std error	Sig
(constant)	-311.8	129.1	.02
Ln FT	58.9	29.6	<b>.05</b>
Sex	124.6	42.5	<b>.01</b>
Parental education level	27.0	8.7	<b>.00</b>
Maternal age	6.9	3.5	<b>.05</b>
Number of siblings	-28.2	15.5	<b>.08</b>

Dependent variable: vocabulary size.  $R^2 = .18$  (Medium effect size)  $F = 5.02$ ,  $p = .001$

.88 < Power > .97

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