Maternal Steroid Levels and the Autistic Traits of the Mother and Infant

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Maternal steroid levels and the autistic traits of the mother and infant

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Abstract:

Background: Elevated prenatal sex steroids and maternal conditions that are related to sex steroids (e.g., polycystic ovary syndrome) have been positively associated with autism likelihood. It is unclear if this is detectable in the maternal circulation, if it relates to maternal autistic traits, and whether it is also predictive of autistic traits in infants.

Methods: Maternal serum samples were collected as part of routine prenatal screening from pregnant women taking part in the longitudinal Cambridge Ultrasound Siblings and Parents (CUSP) study (n=219) (gestational age: mean=12.7 [SD=0.8] weeks). Concentrations of the following were measured via immunoassays: testosterone (T), estradiol (E2), dehydroepiandrosterone sulphate (DHEAS), progesterone (P); sex hormone-binding globulin (SHBG). Human choriogonadotropin (hCG) and pregnancy-associated plasma protein A (PAPP-A) were collected from clinical records corresponding to the same serum samples. Participants completed the adult Autism Spectrum Quotient (AQ). Infants were followed-up with the Quantitative Checklist for Autism in Toddlers (Q-CHAT) between 18-20 months old (mean=570 days, SD=21.3 days).

Results: Maternal AQ scores significantly correlated with circulating levels of total E2 (Pearson's r=0.20, p=0.036) and the bioactive fraction of E2 (Pearson's r=0.26, p=0.008) in univariate and multiple regression models. Total E2, DHEAS and a steroidogenic factor (derived from total E2, DHEAS and T) were all associated with Q-CHAT scores in multiple regression models that controlled for covariates and for an interaction with infant sex. This interaction also had a significant effect, leading to a positive correlation between hormone levels and Q-CHAT scores in males but not in females (interaction term: semipartial correlation r=0.23, p=0.018). The opposite was found for standardised hCG

values and Q-CHAT scores, with a positive association in females but not in males (interaction term: semipartial correlation r=-0.22, p=0.009).

Limitations: This longitudinal clinical study was relatively small and statistical power was further reduced by the need to account for different effects according to sex. The findings will need to be confirmed in a larger cohort and with clinically diagnosed cases of autism.

Conclusion: In line with previous findings, this study's results suggest that increased steroid synthesis prenatally is related to autistic traits and that this is detectable in the maternal circulation.

Keywords: autism, steroids, hCG, maternal, traits, sex, interaction

Background

Autism is a neurodevelopmental condition characterised by restricted interests, repetitive behaviour and difficulties in social communication (American Psychiatric Association 2013). Co-occurring clinical conditions are frequent and include sensory hypersensitivity, learning difficulties, sleep disorders and nutritional intolerances (Croen et al. 2015). Diagnosis of autism is possible as early as 18 months of age (3). Furthermore, autistic traits exist along a spectrum in the wider population (Baron-Cohen et al. 2001; Allison et al. 2008). Autism is diagnosed more often in males than females, despite increased awareness of its differential presentation in females (6). In addition, personality traits that show sex differences on average in the neurotypical population (e.g. systemising and empathising) are shifted towards a male profile in autistic individuals of both genders (Baron-Cohen et al. 2014; Greenberg et al. 2018).

Several lines of evidence indicate that prenatal sex steroid hormonal factors may be mediating the likelihood of autism. First, a study of neuroanatomical differences between autistic and neurotypical brains revealed autistic females had atypical structure in regions that substantially overlapped with sexually dimorphic regions in neurotypical controls. This suggests that autism affects female brains in regions related to sexual differentiation, which is in turn regulated by prenatal sex steroid hormones (9). This male-shift is also evident in childhood, as indicated by studies showing that facial features of autistic boys, girls and their siblings are masculinised compared to neurotypical controls (Tan et al. 2017; Tan et al. 2020). Prenatally, autistic males have also been found to have higher levels of steroid hormones when these were assessed in a multivariate analysis, as well as higher levels of estrogens in particular in univariate analyses of the same amniotic fluid samples (Baron-Cohen et al. 2015; 2019). Estradiol levels have also

been found to be elevated in the maternal serum of pregnancies that resulted in autism in an independent cohort (Bilder et al. 2019).

Traits and developmental outcomes that are related to autism have also been linked to prenatal sex steroids. For example, prenatal levels of testosterone measured during amniocentesis are negatively correlated with eye-contact at 12 months (Lutchmaya, Baron-Cohen, and Raggatt 2002), vocabulary size at 18 and 24 months (Lutchmaya, Baron-Cohen, and Raggatt 2001), and are positively correlated with restricted interests, with attention to detail (17) and with general autistic traits at 18 and 48 months (18,19).

Epidemiological analyses in several independent populations have also revealed that maternal polycystic ovary syndrome (PCOS) increases the likelihood of autism in their children (20–22) confirming the significance of the wider prenatal hormonal environment. This effect persisted after controlling for overt clinical symptoms of the condition (e.g. obesity, insulin resistance) and for diagnosed perinatal complications (22). Controlling for familial confounding also suggested that the effect wasn't primarily driven by shared genetics and that it was further modulated by the sex of the offspring (23).

PCOS is a syndrome that is associated with higher levels of androgens, as well as other signs of hormonal dysregulation such as a reduced placental capacity to aromatise androgens into estrogens (24). Interestingly, steroidogenic conditions such as PCOS are also more common in autistic people (21,25), and autistic adults demonstrate signs of steroid imbalance in various tissues (26–28). Converging evidence from animal and longitudinal human studies indicate that PCOS may be the result of prenatal programming, whereby higher levels of sex steroids in mothers with the condition induce PCOS in the offspring, resulting in transgenerational transmission of the condition (29).

Autism is a heritable condition, with genetic factors accounting for a large percentage of variance in both twin and population studies (30). It remains unclear how genetic factors interact with hormonal factors that lead to differential effects in males and females. The genetic likelihood of autism may overlap with the genetic variance associated with sex differences in physical growth, further indicating that this interaction may be present throughout life and could start during prenatal development (31).

To evaluate the effects of several prenatal hormonal factors, such as androgens, estrogens and placental markers, we conducted a longitudinal study of pregnancies and assessed both the mothers and their infants for autistic traits.

Methods

Mothers were recruited early in their pregnancy, during or before their routine 20-week ultrasound scan, as part of the CUSP study at the Rosie Hospital, Cambridge University Hospitals NHS Foundation Trust (n=219) (32). A favourable ethical opinion for the study was given by the East of England Cambridge Central Research Ethics Committee (REC Ref 16/EE/0004) and the Research and Development Department of Cambridge University Hospitals. Eligibility inclusion criteria for the study were as follows: (1) little/no consumption of alcohol during pregnancy, (2) no smoking or recreational drug use during pregnancy, (3) a singleton fetus whose measurements indicated their size to be appropriate for gestational age, (4) the absence of any major fetal anomalies, and (5) a fetus that was not considered to have an intrauterine growth restriction (IUGR) or to be large-for-gestational age (LGA). Eligibility criteria for including the fetus in the final data analysis was the birth of a clinically healthy baby.

Participating mothers gave informed consent for access to all their pregnancy-related clinical records, test results and the biological samples that were obtained during routine

clinical care. Study data were collected and managed using REDCap electronic data capture tools hosted at University of Cambridge (33,34). Serum samples were collected at the end of the 1st trimester (mean gestational age of 12.7 [SD=0.8] weeks since conception) by a specialist phlebotomist at the Rosie Hospital, Cambridge, and then stored at -80C, as part of a national screening programme for biomarkers of Down's Syndrome and other conditions. A total of n=122 samples from participants of the Cambridge Ultrasound Siblings and Parents Project (CUSP) were subsequently thawed and transferred to separate vials (1ml aliquots per sample), which were further anonymised and sent for analysis at the Core Biochemical Assays Laboratory (CBAL) at Addenbrookes Hospital, Cambridge.

Assays

The following steroids and peptides were assessed in terms of concentration: Testosterone (T), Estradiol (E2), Dehydroepiandrosterone sulphate (DHEAS), Progesterone (P), sex hormone-binding globulin (SHBG). Samples were analysed on a DiaSorin Liaison® XL automated immunoassay analyser using a one-step competitive chemiluminescence immunoassay for each hormone and two monoclonal antibodies for each peptide. All reagents, standards and consumables are those supplied by DiaSorin (DiaSorin S.p.A, 13040 Saluggia (VC), Italy).

Autistic Traits

Mothers were given a copy of the Autism Spectrum Quotient (AQ) – Adult version (Baron-Cohen et al. 2001) to complete at home or during their first visit to the maternity ward of the Rosie Hospital. In addition, parents were invited via email to complete an online version of the Quantitative Checklist for Autism in Toddlers (Q-CHAT) (5) after their infant reached 18 months of age.

Statistical Analysis

Autistic trait distributions (maternal AQ and infant Q-CHAT) were assessed for extreme outliers, as defined by an interval of three times the interquartile range. If present, these were reduced to the highest possible value within the interval to facilitate statistical testing while preserving cases of potential clinical significance. Before proceeding with any statistical testing Q-CHAT scores were also standardised according to infant age in days since birth.

All hormonal values exhibited positive skew. None of the outliers were deemed extreme according to the definition above. Values were therefore not transformed, and non-extreme outliers were not removed at any stage of the analysis in order to preserve power and to better reflect typical clinical heterogeneity. This is in accordance with proposed guidelines on the analysis of hormonal values (35). Human choriogonadotropin (hCG) and pregnancy-associated peptide alpha (PAPP-A) values were retrieved from participants' clinical records. These had been further standardised according to multiple of the median (MoM) by the Prenatal Screening Department of the Trust according to maternal age, gestational age and the national means (36,37).

Targeted multivariate analysis was conducted by calculating composite scores for bioactive estradiol, bioactive testosterone, aromatisation and overall steroidogenesis. The free testosterone index (FTI) and the free estradiol index (FEI) were calculated by dividing the corresponding sex steroids with SHBG values. The aromatisation ratio for each participant was computed by dividing estradiol (E2) with testosterone (T) values for each participant. Finally, latent factor analysis ('nFactors' package) was used to identify the optimal number of common steroidogenic factors based on the correlation matrix of the raw data. Values for the predicted steroidogenic factor were calculated for each individual via the "Bartlett" method, based on the predicted loadings.

A series of clinical characteristics and group covariates were assessed for association with autistic traits, as well as with circulating hormones in a pairwise manner. If these were continuous, Pearson's correlation coefficient was used. If these were binary, differences were tested via Student's *t*-tests. Specifically, family history of autism was defined as present if the participating mothers reported having a first-degree relative (including previous child) that had been diagnosed with autism. Additionally, a score of clinical severity of maternal hirsutism was devised based on responses in the Pregnancy History Questionnaire (PHQ). The PHQ is a self-report inventory designed to collect information on metabolic, reproductive and clinically diagnosed conditions in mothers as well as information on maternal and child health during pregnancy and at birth. Maternal hirsutism was ascertained by the question: 'During your adult years, have you found coarse, dark hair, growing in any of the following areas?', followed by drawings of multiple body areas that are prone to secondary hair growth (e.g. chest, lower face, upper or lower limbs) (38). A score of 1 denoted selection of one area of excess hair growth and 2 denoted more than one area. Hirsutism scores were then tested for association with autistic traits and hormone values via Pearson's correlation coefficient, along with other cohort variables (e.g., maternal age, BMI, infant birth weight).

Circulating hormones were assessed for association with autistic traits. For AQ, pairwise Pearson's correlations were first used and then followed-up with a linear regression model with AQ as the outcome variable and the following predictor variables: hormonal concentration, maternal age, comorbidity with PCOS and a family history of autism. To account for potential underlying effects of infant sex on Q-CHAT scores, a different linear regression model was used in which the hormonal value was the outcome variable and the following were predictors: Q-CHAT score, infant sex at birth, an interaction term of Q-CHAT and infant sex, as well as maternal age, comorbidity with

PCOS, history of hirsutism and gestational age at the time of hormone measurement. For these analyses, Q-CHAT scores were also adjusted for infant age at the time of assessment (measured in days since birth) by a separate linear regression model of the two variables (Q-CHAT, age) and adding the residuals of this on the intercept. The same approach and models were used when examining the hormone composite measures, namely FEI, FTI, aromatisation index and the predicted steroidogenic factor (for E2, DHEAS and T).

To ensure their validity, all multiple regression models for both AQ and Q-CHAT were tested for heteroscedasticity via the studentized Breusch-Pagan test and the normality of their residuals via the Shapiro-Wilk test.

Results

Of the n=219 pregnant women who consented to take part in the study, n=17 had a first-degree relative with autism, n=26 had been diagnosed with PCOS, and n=89 responded positively to having excess body hair growth in the past. Overall mean age of the mothers was 32.4 years (SD=4.54). Of this cohort, n=189 completed the Autism Spectrum Quotient (AQ), with scores ranging from 1 to 47 (mean=14.63, SD=8.11). Women with a family history of autism had a significantly higher AQ score (mean=28, SD= 14.36) compared to women without any first-degree relatives with autism (mean=13.64, SD=6.51) (Cohen's D=1.98, p=0.004) (Table 1). The extent of hirsutism also correlated with AQ (Pearson's r=0.15, p=0.04), with women reporting excess body hair growth in more than one area of the body having significantly higher AQ than women with no affected areas (Cohen's D=0.36, p=0.021). Potential covariates, such as maternal age, a previous diagnosis of PCOS, birth weight and fetal sex, were not associated with differences in AQ Scores (Table 1).

178 of the corresponding infants were followed-up with the Q-CHAT when they were older than 18 months of age (range: 541 to 671 days after birth), with most being assessed between 18 and 20 months (mean=570 days, SD=21.3 days). One extreme outlier was noted in the distribution (Q-CHAT=71), which was reduced to the highest value within an interval of three times the interquartile range (Q-CHAT=53), in order to reduce skewness and facilitate statistical comparisons. Q-CHAT scores were marginally higher in males (mean=30.35, SD=8.13) than females (mean =29.63, SD=7.58), but this difference was not statistically significant (Cohen's D=0.09, Student's t=-0.62, p=0.54). Finally, Q-CHAT scores were correlated with maternal AQ scores (Pearson's r=0.21, p=0.008) (Figure 1).

		AQ			**Q-CHAT**	
Categorical	*Mean*	*Effect*	*p*	*Mean*	*Effect*	*p*
History of autism						
• With: n=17	28.00	**D=1.98**	**0.0036**	31.00	D=0.14	0.720
• Without: n=202	13.64			29.90		
Maternal PCOS						
• With: n=26	14.96	D=0.05	0.8043	27.86	D=0.31	0.115
• Without: n=193	14.58			30.28		
Hirsutism						
• None: n=130	13.81	**r=0.15**	**0.039**	29.19	r=0.09	0.25
• 1 area: n=33	13.93			31.46		
• >1 area: n=56	16.78			30.63		
Fetal sex						
• Male: n=104	14.15	D=0.11	0.453	31.00	D=0.09	0.54
• Female: n=115	15.04			29.63		
Continuous	*Mean*	*Effect*	*p*	*Mean factor*	*Effect*	*p*
Maternal age	32.41 years	r=-0.05	0.462	32.41 years	r=-0.14	0.065
Birth Weight	3410.53 g	r=-0.04	0.596	3410.53 g	r=-0.03	0.727
Infant age				570,2 days	r=-0.09	0.250

Table 1: Cohort characteristics and associations between clinical/demographic factors and autistic traits. Test coefficients are Cohen's D and Pearson's correlation coefficient - r.

Hormone covariates and factor analysis

The analysed maternal serum samples corresponded to a narrow period of gestation between the late 1st and early 2nd trimester (mean=12.7 weeks, SD=0.8 weeks). Circulating hormones showed varying degrees of correlation with each other (Figure 2) and with other demographic and clinical variables (Suppl. Table 1). Testosterone, DHEAS and progesterone were all positively correlated with maternal age. Women with PCOS also had significantly higher levels of estradiol and progesterone, but lower levels of SHBG, compared to women without the condition. Estradiol levels correlated significantly and positively with the degree of hirsutism in the cohort (Pearson's r=0.20, p=0.029).

Unsupervised factor analysis showed that a common latent factor could be derived from estradiol, testosterone and DHEAS and account for 32% of the total variance in hormone levels (Suppl. Figure 1, Suppl. Table 2). Factor analysis also showed that hCG was largely independent compared to the other hormones assayed. The values of the first 'steroidogenic factor' were predicted for each participant based on the steroids assayed and introduced to subsequent analyses along with the two other composite measures: the free estradiol index (FEI) and the free testosterone index (FTI).

Association of hormones with maternal AQ score

The association between circulating hormones in maternal serum was investigated via univariate Pearson's correlation coefficient, as well as with multiple regression models in which the effect on AQ score was controlled for maternal age and family history of autism (first-degree relative or diagnosed child) (Table 2). Of all the hormones assessed in maternal serum, only estradiol was significantly and positively correlated with AQ scores in both the direct comparison and the regression model (β=0.20, p=0.036). A similar trend was noted for DHEAS, which was not statistically significant (p<0.1). With regard

to hormone composite scores, FEI was significantly and more strongly correlated with maternal AQ score than estradiol alone (β=0.255, 0.008) (Table 3). Non-significant trends for FTI and a latent steroidogenic factor for the assayed sex steroids were also noted ($p<0.15$). Progesterone, hCG and PAPP-A did not correlate with maternal AQ score.

Association of hormones with infant Q-CHAT score

To study the relationship between circulating maternal hormones and the early autistic traits of their infants, two statistical methods were used: univariate regression using Pearson's correlation coefficient, as well as multiple linear regression controlling for covariates related to hormone levels (maternal age, maternal PCOS and hirsutism). In the latter case, the interaction between Q-CHAT score and infant sex was also used as a covariate in the same model, with hormone levels as the outcome variable to allow for this (Table 2). Q-CHAT scores were previously adjusted for infant age at the time of assessment. These analyses were also conducted with hormonal composite variables, such as those derived from hormone factor analysis and hirsutism scores (Table 3). No hormonal variable was associated with infant Q-CHAT score in univariate regression with both sexes.

In the multiple regression models, estradiol, DHEAS and the composite steroidogenic factor were all significantly associated with Q-CHAT scores, as well as with the interaction term between Q-CHAT score and infant sex (Tables 2 & 3). The effect for Q-CHAT score was more positive and more pronounced in males than females (Figure 3A). These effects were independent of maternal age, diagnosis of PCOS, family history of autism or clinical history of hirsutism. Standardised hCG levels were also significantly associated with Q-CHAT score and the interaction of Q-CHAT score and infant sex. This effect was in the opposite direction as the association with Q-CHAT was positive in females and not in males (Figure 3B).

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	Maternal AQ			Infant Q-CHAT	
	Effect size	p		Effect size	p
Estradiol					
Pearson's correlation	**r=0.20**	**0.036**	Pearson's correlation	r=0.05	0.617
MR model[1]:			MR model [2]:		
• AQ with hormone	**r=0.20**	**0.032**	• Hormone with Q-CHAT	**r=-0.20**	**0.040**
			• Interaction with sex:	**r=0.21**	**0.034**
Testosterone					
Pearson's correlation	r=0.16	0.097	Pearson's correlation	r=-0.01	0.910
MR model [1]:			MR model [2]:		
• AQ with hormone	r=0.14	0.150	• Hormone with Q-CHAT	r=-0.15	0.115
			• Interaction with sex	r=0.07	0.453
DHEAS					
Pearson's correlation	r=0.18	0.058	Pearson's correlation	r=0.05	0.645
MR model [1] :			MR model [2]:		
• AQ with hormone	r=0.16	0.089	• Hormone with Q-CHAT	**r=-0.19**	**0.040**
			• Interaction with sex	**r=0.19**	**0.049**
Progesterone					
Pearson's correlation	r=-0.08	0.396	Pearson's correlation	r=-0.07	0.486
MR model [1]:			MR model [2]:		
• AQ with hormone	r=-0.04	0.684	• Hormone with Q-CHAT	r=0.01	0.890
			• Interaction with sex	r=0.05	0.578
HCG MoM					
Pearson's correlation	r=-0.03	0.665	Pearson's correlation	r=0.05	0.556
MR model [1]:			MR model [2]:		
• AQ with hormone	r=-0.03	0.684	• Hormone with Q-CHAT	**r=0.20**	**0.015**
			• Interaction with sex	**r=-0.22**	**0.009**
PAPP-A MoM					
Pearson's correlation	r=-0.04	0.588	Pearson's correlation	r=0.05	0.552
MR model [1]:			MR model [2]:		
• AQ with hormone	r=-0.04	0.603	• Hormone with Q-CHAT	r=0.10	0.218
			• Interaction with sex	r=-0.04	0.613

Table 2: Associations between maternal serum hormones and autistic traits. Effect sizes are Pearson's correlation coefficient - r for univariate regression, or the semipartial correlation coefficient for the specific independent variable for multiple regression (MR) models. These were controlled for the following covariates:
1.For AQ with hormones: maternal age, family history of autism.
2.For hormones with Q-CHAT: infant sex, interaction of sex with Q-CHAT, maternal age, maternal PCOS and hirsutism.

Composite scores	Maternal AQ		Infant Q-CHAT		
	Effect size	p		Effect size	p
Free Estradiol					
Pearson's correlation	**r=0.26**	**0.008**	Pearson's correlation	r=0.06	0.547
MR model [1]: • **AQ with hormone**	**r=0.25**	**0.009**	MR model [2]: • Hormone with Q-CHAT	r=0.23	0.233
			• Interaction with sex	r=0.10	0.103
Free Testosterone					
Pearson's correlation	r=1.55	0.123	Pearson's correlation	r=0.07	0.510
MR model [1]: • **AQ with hormone**	r=0.15	0.124	MR model [2]: • Hormone with Q-CHAT	r=-0.05	0.565
			• Interaction with sex	r=-0.02	0.870
Aromatisation ratio					
Pearson's correlation	r=0.09	0.384	Pearson's correlation	r=-0.06	0.569
MR model [1]: • **AQ with hormone**	r=0.13	0.176	MR model [2]: • Hormone with Q-CHAT	r=-0.02	0.834
			• Interaction with sex	r=0.07	0.459
Steroid Factor					
Pearson's correlation	r=0.18	p=0.061	Pearson's correlation	r=0.02	p=0.840
MR model [1]: • **AQ with hormone**	r=0.17	p=0.085	MR model [2]: • Hormone with Q-CHAT	**r=-0.22**	**p=0.027**
			• Interaction with sex	**r=0.23**	**p=0.018**

Table 3: Associations between composite hormonal scores and autistic traits. Effect sizes are Pearson's correlation coefficient - r for univariate regression or the semipartial correlation coefficient for the specific independent variable for multiple regression (MR) models. These were controlled for the following covariates:
1.For AQ with hormones: maternal age, family history of autism.
2.For hormones with Q-CHAT: infant sex, interaction of sex with Q-CHAT, maternal age, comorbidity with PCOS and hirsutism.

Discussion

This is the first clinical longitudinal study to report a detailed investigation of the endocrine profile of pregnant women and to relate this to their own autistic traits and to those of their children. Although the size of the cohort was small, this study resulted in several findings that are in line with the prenatal sex steroid theory of autism and with previously reported findings (Baron-Cohen et al. 2019). First, we found that circulating estradiol levels correlate positively with autistic traits of pregnant neurotypical women.

Second, we found that the levels of prenatal estradiol and DHEAS, as well as a latent steroidogenic factor, were linked to a sex-dependent association with infant Q-CHAT score; more specifically, higher maternal steroid concentrations corresponded to higher Q-CHAT scores at 18 months in males rather than females. Third, we found the opposite is true for standardised hCG levels, which negatively correlated with Q-CHAT score in males (Figure 3). Finally, this was the first longitudinal study to show a significant positive correlation between maternal and infant autistic traits.

The association between circulating estradiol and maternal AQ was independent of age or having a first degree relative with a diagnosis of autism. When assessing the bioactive fraction of estradiol (FEI), this effect was more pronounced. In addition, when examining clinical history, both AQ score and estradiol levels were associated with a history of excess body hair. Higher estradiol was also found in the PCOS subgroup of the cohort. This is in accordance with previous studies relating estradiol levels, PCOS and hirsutism to autism (13,21,25).

There was no association between testosterone levels and autistic traits in this study. Testosterone correlated positively with estradiol but did not differ between women with and without PCOS, or correlate with a clinical history of hirsutism. Previous studies have also shown that circulating testosterone levels do not always correlate closely with these clinical parameters (39) (Suppl. Table 1). Particularly during pregnancy, estradiol levels may be more clinically informative, as testosterone is rapidly aromatised into estradiol by the placenta. Estradiol may thus be interpreted as the end-product of wider steroidogenesis and may be a better biomarker of the 'steroidopathy' previously indicated by epidemiological studies of autistic women (25). The association between AQ score and hirsutism, as well as estradiol levels, in this study further reinforces the argument that increased estradiol levels may be associated with indirect androgenic

316 effects, adding more insight to the previous findings of elevated fetal estrogens in autistic
317 males (Baron-Cohen et al. 2019).

318 This is also the first study to investigate circulating hormones in maternal serum in
319 relation to autistic traits in their infants measured between 18 and 20 months of age via
320 the Q-CHAT. Even though there was no significant sex difference in Q-CHAT scores and
321 no detectable difference in maternal serum hormone levels, this study found significant
322 interactions with sex in the associations of these hormones with neurodevelopment.
323 Specifically, increased maternal estradiol had a more pronounced predictive effect on the
324 Q-CHAT scores of males than females. This was independent of potential confounding
325 variables, such as maternal age, diagnosis of PCOS, a history of hirsutism and any effects
326 of fetal sex on maternal hormone levels. The same association was found for DHEAS, as
327 well as for a latent steroidogenic factor derived from the levels of estradiol, DHEAS and
328 testosterone. The correlation, between the predicted steroidogenic factor levels and Q-
329 CHAT scores, was more pronounced than that of estradiol alone, in accordance with
330 previous findings of multivariate analysis of steroidogenesis in autism (Baron-Cohen et
331 al. 2015).

332 Contrary to the results with the AQ, bioactive estradiol (FEI) estimated via serum
333 SHBG was not more predictive of Q-CHAT scores. SHBG is a peptide and it does not cross
334 the placenta as easily as steroids, as shown in rare cases of partial deficiency in mothers
335 but not their fetus (40). Maternal SHBG may therefore not accurately capture the
336 bioavailability of steroids in the fetal circulation or accurately predict their effects on
337 infant neurodevelopment.

338 The findings with hCG suggest a potential regulating role of the placenta for infant
339 outcomes. hCG is produced by the developing trophoblast cells in the placenta and
340 regulates early implantation as well as steroid production (41). Specifically, we found

that standardised hCG MoM values were associated with Q-CHAT scores, but this was moderated by fetal sex. More specifically, this resulted in higher scores in females but lower scores in males when controlling for various covariates. hCG was measured late in the 1st trimester, during the first ultrasound visit, as part of the screening programme for Down's syndrome. Atypical levels are thought to be associated with placental dysfunction that can often be indicative of genomic instability due to aneuploidies (37). In cases of clinically diagnosed autism, both very low and very high levels of hCG have been found in maternal serum, leading to a "U-shaped" association when studying both males and females (42). Furthermore, autistic traits in the children have been associated with the severity of nausea and 'morning sickness' during pregnancy, symptoms that are also linked to high hCG levels (43,44).

Similarly to autism likelihood, placental function shows sex differences as early as the 1st trimester (45). Placental pathologies (e.g. trophoblast inclusions, intrauterine growth restriction) are more frequent in pregnancies of fetuses that developed autism (46–48). hCG levels may also be affected by sex, as indicated in the multiple regression model of this study as well as by studies showing higher levels in pregnancies of females (49). Further studies into placental functionality could offer insight into this effect, and may help determine whether it is an adaptive response that is more pronounced in females.

Finally, this study also found that prenatal DHEAS was associated with neurodevelopmental outcomes. Produced by both the maternal and fetal adrenals, DHEAS interacts with the placenta, providing the steroid substrate for synthesis of androgens and estrogens (Jaffe 2001; Bilder et al. 2019). Interestingly, a recent investigation of molecular sex differences in human placentas showed that DHEAS is significantly higher in male placentas (52). DHEAS levels are often higher in the brain than in circulation and may increase androgenic effects by upregulating the androgen

366 receptor in neurons (53,54). Higher levels of DHEAS have also been found in the saliva of
367 prepubertal autistic children compared to controls (55). In addition to androgenic and
368 estrogenic effects, DHEAS functions as a neurotrophin, acting through the brain-derived
369 neurotrophic factor (BDNF) receptor in the brain to inhibit apoptosis (56,57). Estradiol
370 and testosterone may also have anabolic effects on the developing brain, as they have
371 been shown to have neurotrophic properties in *in vitro* cultures and animal models
372 (58,59). This would be consistent with signs of brain overgrowth in neonates with a
373 family history of autism and in those who were later diagnosed with autism (Hazlett et
374 al. 2011; 2017).

375 Limitations of the current study include the relatively small sample size, as well as
376 potential ascertainment bias given the voluntary process of recruitment. In addition, AQ
377 and Q-CHAT scores were, in most cases, both rated by the mother herself. A relatively
378 high mean Q-CHAT score compared to that of previous studies could also be attributed to
379 this, or related to a comparatively younger age range in this cohort (5). Nevertheless, the
380 items of the AQ-Adult and Q-CHAT are substantially different, with the latter dealing with
381 behavioural and developmental milestones that are specific to infants rather than to
382 interests and personality traits that are more evident in adulthood. Furthermore, the
383 findings of this study may have been inflated by Type I errors, as p-value thresholds were
384 not corrected for multiple testing. However, the findings are consistent with those
385 observed in other cohorts (Baron-Cohen et al. 2019; Bilder et al. 2019). The high degree
386 of correlation between many of the assessed hormones (Figure 1) also indicates a
387 common functional and regulatory framework. The association tests for individual
388 hormones may then, not be entirely independent, but instead affected by a common
389 steroidogenic factor as previously reported (Baron-Cohen et al. 2015). Replication of
390 these findings in a larger, independent cohort is warranted to confirm their validity.

In conclusion, this is the first longitudinal study to report associations between maternal steroid levels and the autistic traits of both mother and child, and our findings are in accordance with previous observations (Baron-Cohen et al. 2019; Bilder et al. 2019). Additional research is needed to clarify how steroids differentially affect neurodevelopment in males and females, how placental function may be affected by genetic and environmental factors, and how these processes interact to disproportionately increase the liability for clinical autism in males.

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612

Figures

Figure 1: Association between maternal AQ score and infant Q-CHAT score stratified by infant sex

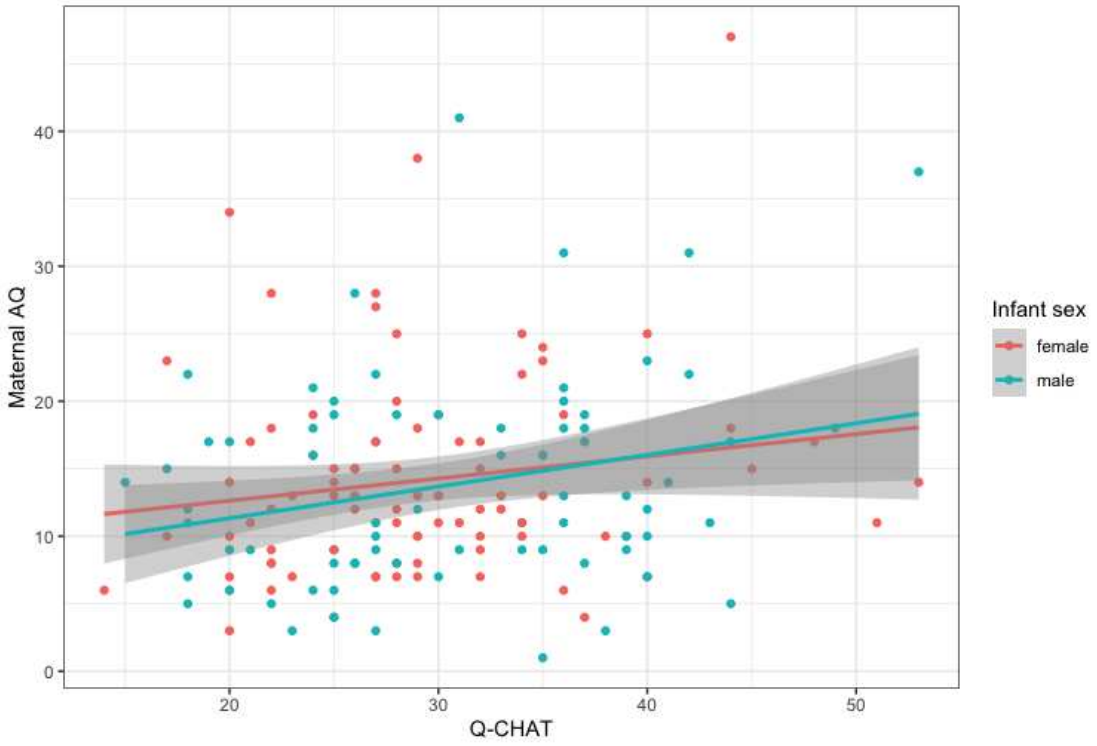
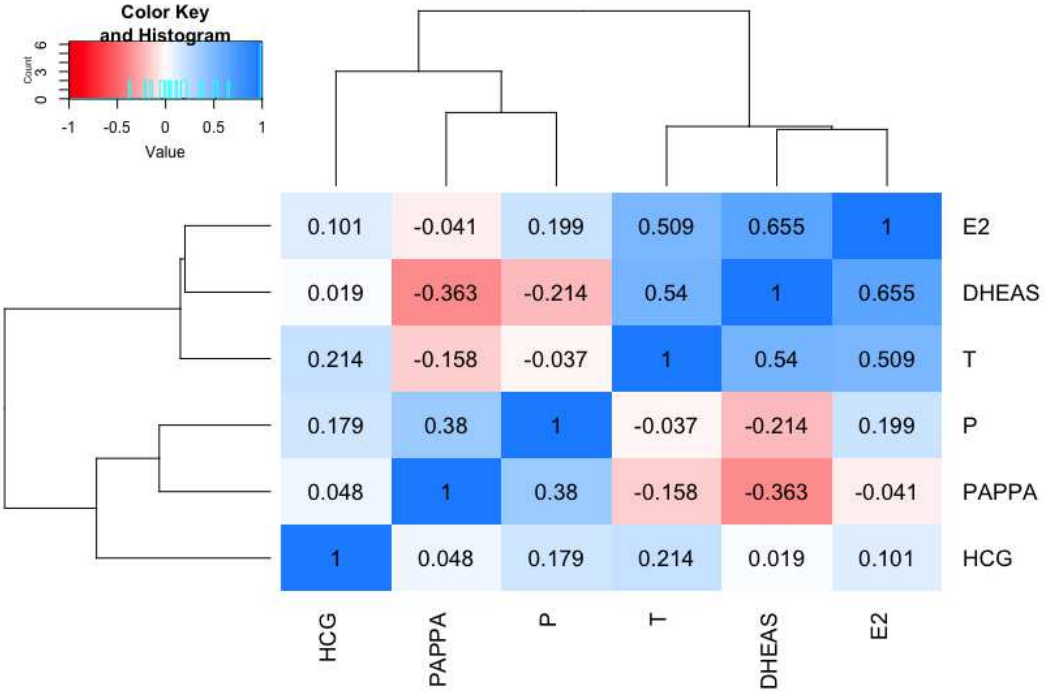
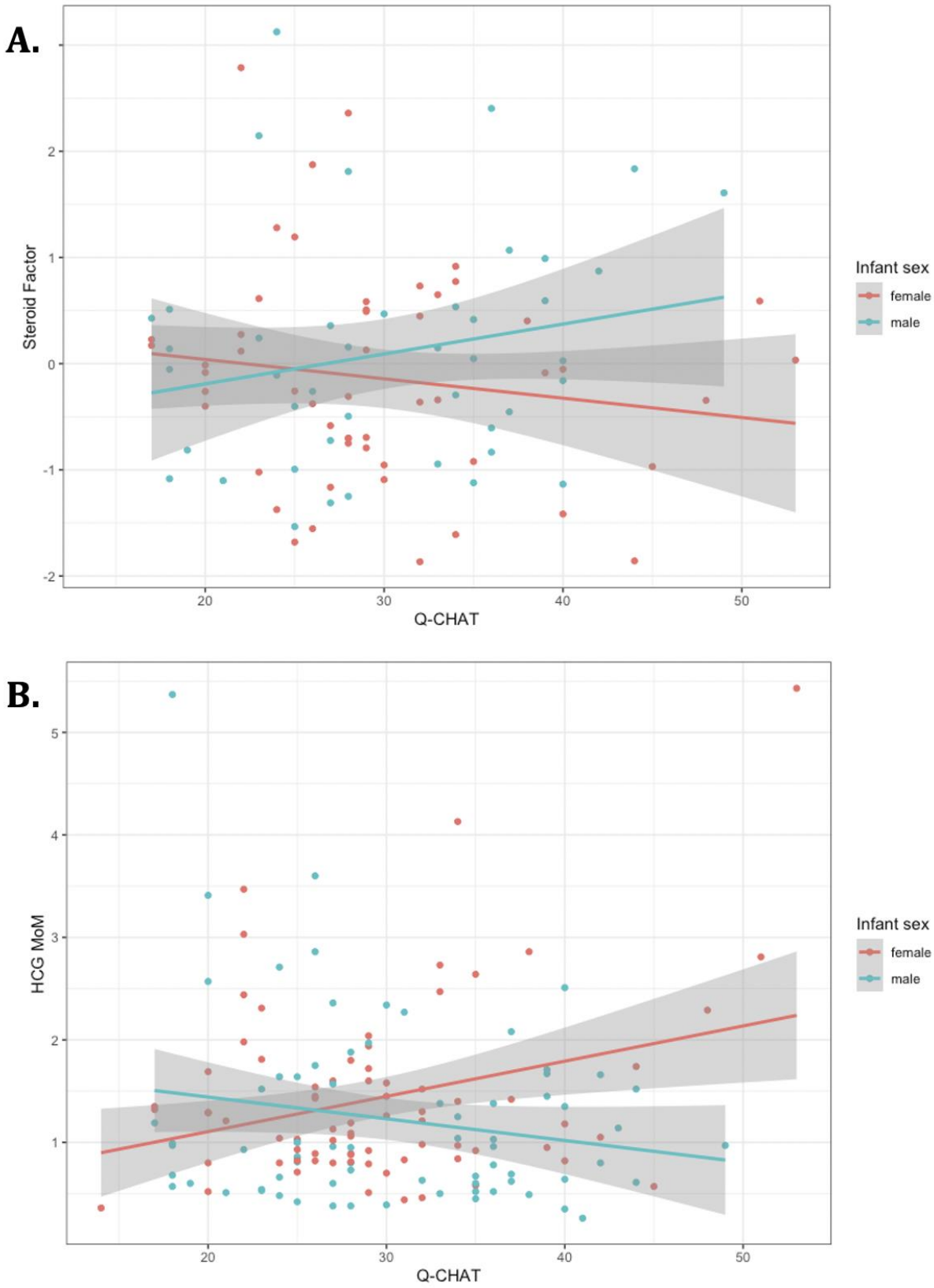


Figure 2. Heatmap and dendrogram showing the pairwise (Pearson's) correlations between the tested hormones/peptides



620 Figure 3: Scatterplots for the association between the latent steroidogenic factor and Q-
621 CHAT score (A) and hCG and Q-CHAT (B); separate linear models are presented for each
622 sex, and show significant interactions with infant sex.



List of abbreviations

AQ: Autism Quotient

BDNF: Brain-derived neurotrophic factor

CBAL: Core Biochemical Assays Laboratory

CUSP: Cambridge Ultrasound Siblings and Parents Project

DHEAS: Dehydroepiandrosterone sulphate

E2: Estradiol

FEI: Free Estradiol Index

FTI: Free Testosterone Index

hCG: Human chorionic gonadotropin

IUGR: Intrauterine Growth Restriction

LGA: Large for Gestational Age

MoM: Multiple of the Median

P: Progesterone

PAPP-A: Pregnancy-associated peptide alpha

PCOS: Polycystic ovaries syndrome

PHQ: Pregnancy History Questionnaire

Q-CHAT: Quantitative Checklist of Autism in Toddlers

SHBG: Sex hormone binding globulin

Ethics approval and consent to participate

A favourable ethical opinion for the study's protocol, recruitment criteria and related materials, including the consent form, was given by the East of England Cambridge Central Research Ethics Committee (REC Ref 16/EE/0004) and the Research and Development Department of Cambridge University Hospitals.

Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests

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673

674 ## Author Contributions

675 AT conducted the analysis, interpreted the data and drafted the manuscript. EA and CA
676 contributed significantly to the organisation and design of the study and to data
677 acquisition. EG and GR helped with the statistical analysis and interpretation of the data.
678 GH and TA provided guidance with and supervised the use of clinical data. SBC and RH
679 contributed equally to study design, study supervision, data interpretation and to the
680 revisions of the manuscript.
681

682 ## Availability of Data and Materials

683 The datasets generated during and/or analysed during the current study are not publicly
684 available due to limited Ethics approval for the wider clinical study (CUSP) by CUH and
685 to the specific consent provided by the participants. They may be available from the
686 corresponding author on reasonable request and pending approval of any future analyses
687 by CUH.
688

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Figures

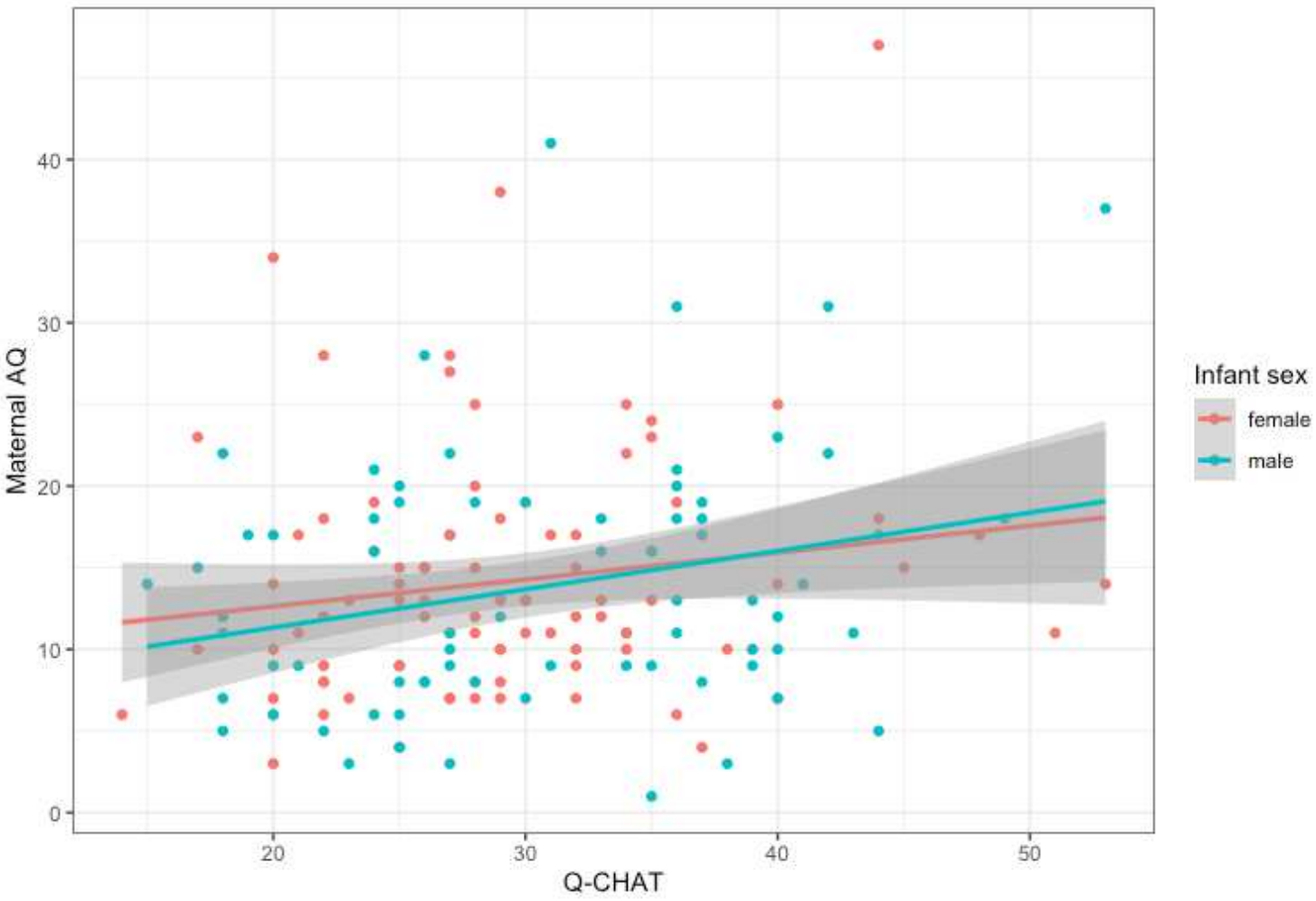

Figure 1

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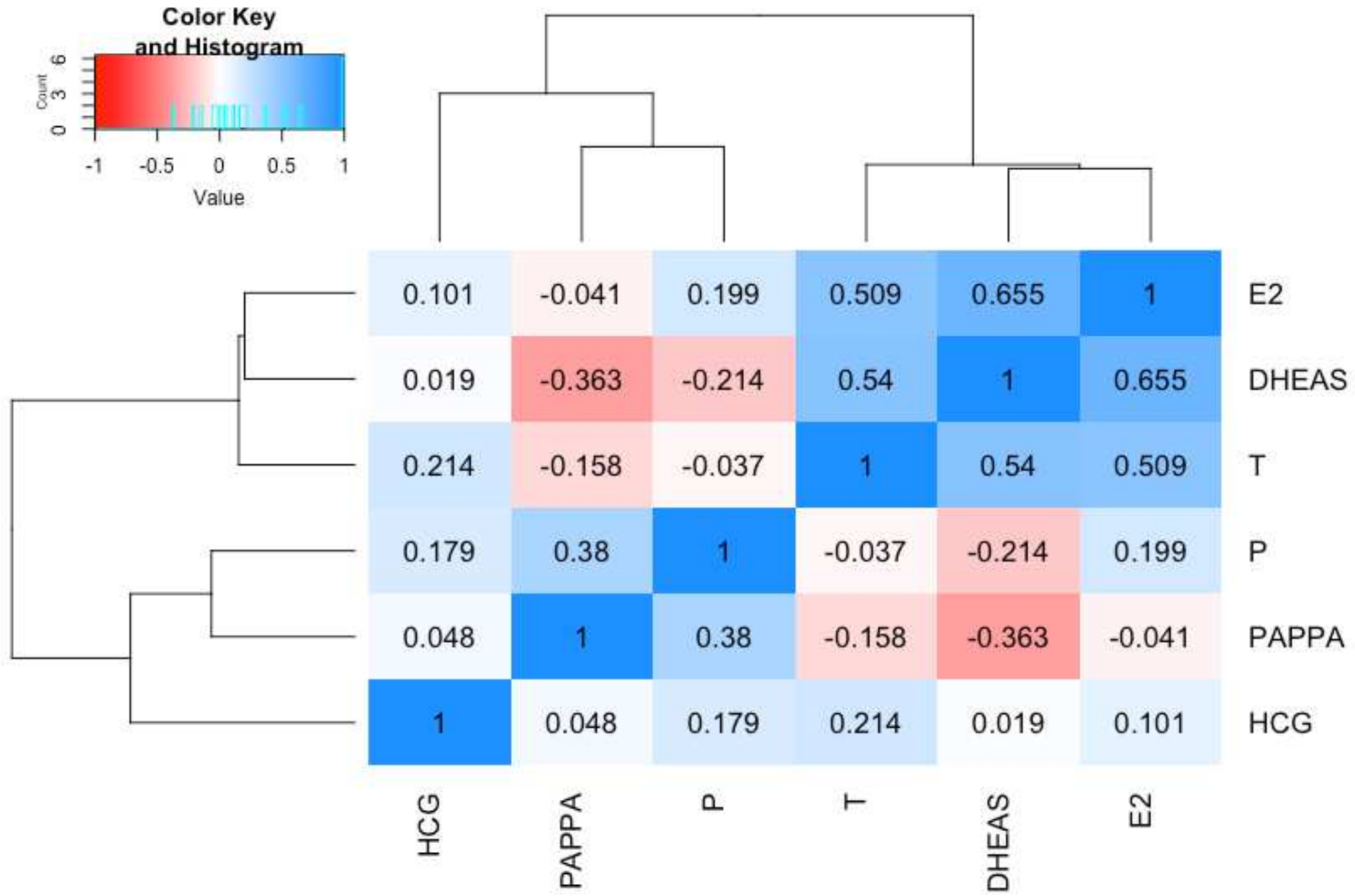

Figure 2

Heatmap and dendrogram showing the pairwise (Pearson's) correlations between the tested hormones/peptides

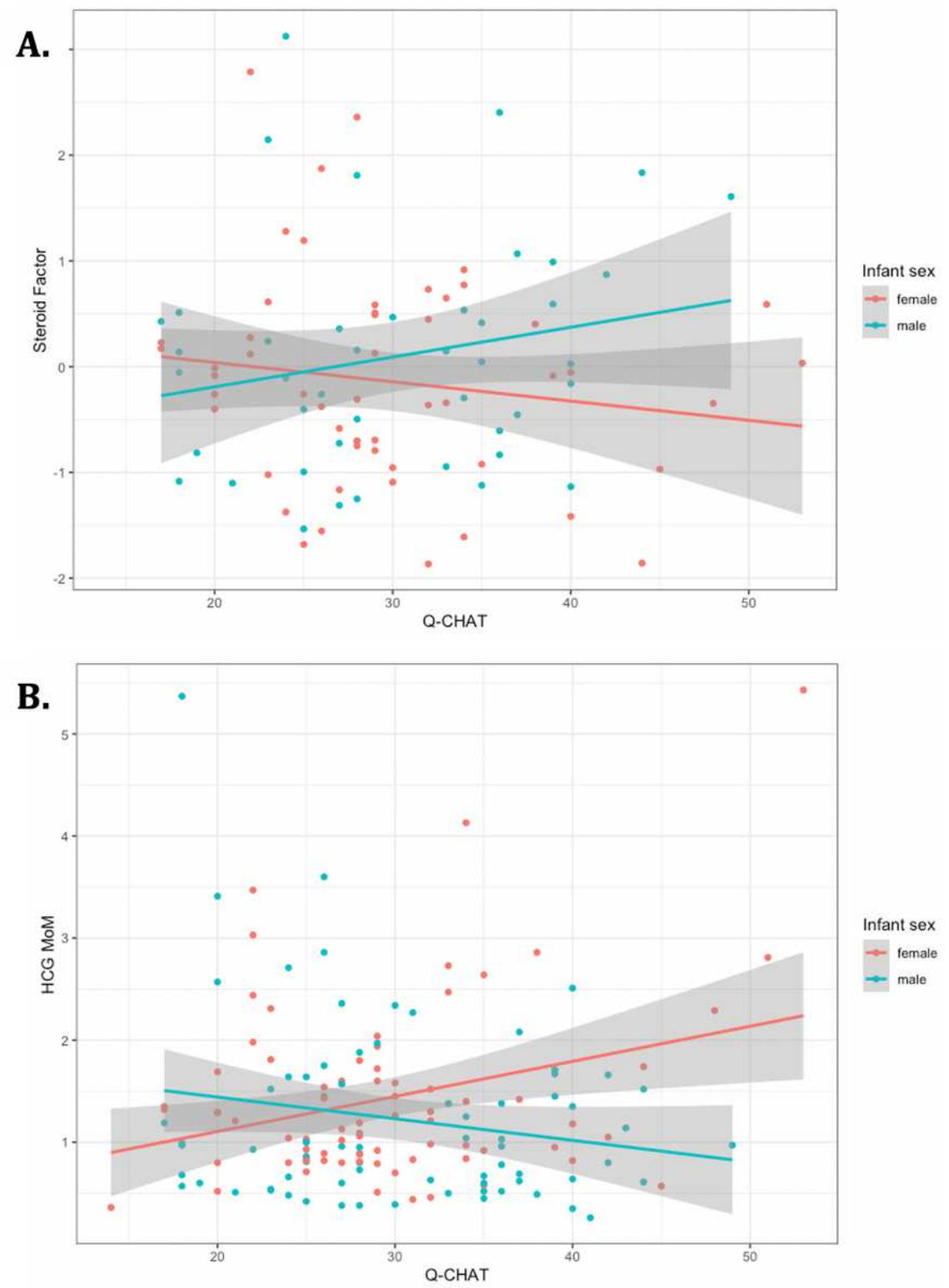

Figure 3

Scatterplots for the association between the latent steroidogenic factor and Q-CHAT score (A) and hCG and Q-CHAT (B); separate linear models are presented for each sex, and show significant interactions with infant sex.

Supplementary Files

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- SupplementaryTablesandFigures.pdf