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# The relationship between offspring's 2D:4D ratio and postpartum maternal circulating testosterone, estradiol, and their indices in a Ghanaian population

**Moses Banyeh** | **Nafiu Amidu** | **Lawrence Quaye**

Department of Biomedical Laboratory Science, School of Allied Health Sciences, University for Development Studies, Tamale, Ghana

**Correspondence**
Moses Banyeh, Department of Biomedical Laboratory Science, School of Allied Health Sciences, University for Development Studies, Tamale, Ghana.
Email: mosesbanyeh@gmail.com

## Abstract

**Objectives:** The 2D:4D ratio is influenced by prenatal testosterone (PT) and estrogen (PE) exposure in utero. This study sought to determine whether evidence of Manning's hypothesis can still be observed even in the postpartum period. We hypothesize that the offspring 2D:4D ratios will be inversely correlated with maternal postpartum circulating testosterone but positively correlated with estradiol.

**Methods:** This study was conducted between December 2020 and April 2021 and was cross-sectional in nature. There were 272 mother-offspring pairs; the mothers were aged between 18 and 36 years while the median (IQR) age of their offspring was 111 (44–210) days. Offspring right (2D:4DR) and left (2D:4DL) digit ratios were measured using computer-assisted analysis. Sampling was done at 111 (44–210) days postpartum and blood was analyzed for total testosterone (TT), estradiol (E2) and sex hormone-binding globulins using the enzyme-linked immunosorbent assay technique.

**Results:** The 2D:4DR of sons was significantly lower compared to daughters ($p = .031$). Mothers with sons had significantly increased levels of serum TT ($p = .001$) while mothers with daughters had significantly increased levels of E2 ($p = .000$). As hypothesized, the maternal serum free testosterone (FT%) was inversely correlated with their daughters' ($r = -0.320$, $p = .003$), and also with their sons' ($r = -0.213$, $p = .047$), 2D:4DL. Unexpectedly, daughters' 2D:4DL was inversely correlated with maternal circulating free E2 ($r = -0.255$, $p = .015$).

**Conclusions:** In humans, evidence of the relationship between maternal testosterone levels and their offspring's 2D:4D ratio may persist even into the postpartum period.

## 1 | INTRODUCTION

Sexual dimorphism in the second (2D) to fourth (4D) digit ratio (2D:4D) in humans has been reported over 120 years ago. However, the association between the 2D:4D ratio and prenatal testosterone (PT) and estrogen (PE) exposure was not predicted until the publication of Manning (Manning et al., 1998). It was observed that high PT exposure leads to lower digit ratios and vice versa, such that the average male has lower digit ratios as compared to the average female in many populations (Butovskaya et al., 2021; Ertuğrul & Özener, 2020; Williams et al., 2000).

  

Evidence of the effect of PT and PE exposure on human digit ratios is complicated because the phenomenon seems to occur at an early stage in fetal development, probably in the first trimester of pregnancy. Usually, endocrine-disrupting conditions arising from pathology, genetic aberrations or chemical exposures have served as sources of postexposure estimates of the effect of PT and PE on human digit ratios. The finding of masculinized digit ratios in subjects with congenital adrenal hyperplasia (CAH), feminized digit ratios in Klinefelter's and androgen insensitivity syndromes and the relationship between digit ratio and androgen receptor (AR) gene polymorphism, all lend credence to the hypothesis that 2D:4D ratio is a proxy for prenatal testosterone exposure (Breedlove, 2010). The Organizational Hypothesis proposed that androgens act early in life to masculinized brain development, behavior and also, digit ratios. But other sources of sexual dimorphism in digit ratios have been proposed: Digit ratios may be affected by the secretion of anti-Mullerian hormones by the testes in male fetuses, influences of the sex chromosome may also affect digit ratios and also, social factors, which influence sex-specific behaviors may affect digit ratios. Subsequent studies have, however, ruled out the others and have affirmed the role of prenatal androgen exposure in human digit development (Breedlove, 2010; Manning et al., 2013; Richards, Browne, Aydin, et al., 2020). However, recent meta-analytic studies have found that effect sizes may be reduced in comparison to earlier reports between persons with CAH and controls (Nave et al., 2021). A study among persons who had the 46, XY karyotype with complete androgen insensitivity, found considerable intra-group variability and inter-group overlap, making the authors conclude that the 2D:4D ratio was not a good indicator of prenatal androgen exposure between individuals. However, this variability may have been the consequence of the effect of prenatal estrogen (Berenbaum et al., 2009).

Males tend to have higher prenatal testosterone (PT) than prenatal estrogen (PT), with PT peaking close to the end of the second trimester. PT and PE affect the fetal digits differently, with PT levels correlating negatively with the 2D:4D ratio and PE levels correlating positively with the 2D:4D ratio (Manning, 2011; Zheng & Cohn, 2011). The precise effect of PT and PE on digit development remained controversial until experimental studies were conducted on mice (Zheng & Cohn, 2011). In the experiment, it was observed that the developing primordia of the digits were rich in androgen and estrogen receptors, particularly the 4D, which have a modulating effect on the skeletogenic gene expression and the proliferation of cells such that a higher PT to PE ratio promoted chondrocyte growth and hence longer 4D, while higher PE exposure inhibited the process (Zheng & Cohn, 2011). Digit ratios were not determined by either PT or PE alone, but a balance between them signaling within a narrow period of fetal digit developmental window. Some authors have critiqued the whole concept of the 2D:4D ratio by citing the contradictory outcomes between Zheng and Cohn (2011) and that of Huber et al. (2017). In the former, digit ratios were inversely correlated with dihydrotestosterone (DHT) while in the latter, digit ratios correlated positively with DHT. It is, however, worthy to note that different strains of the mice were used in those studies, which may have been responsible for the differences in the outcomes (Leslie, 2019).

The direct mechanism of action of PT and PE are difficult to replicate in humans due to ethical reasons. To overcome this, previous and current studies rely on amniotic fluid, cord and maternal blood as proximate samples to glean into what happens during the narrow prenatal window (Lutchmaya et al., 2004; Mitsui et al., 2016; Ventura et al., 2013). In this current study, postpartum maternal blood samples were used to determine the relationships between maternal testosterone, estradiol and offspring's 2D:4D ratios. This study is the first of its kind to be conducted in a Ghanaian population. The study sought to determine whether evidence of Manning's hypothesis can still be observed even in the postpartum period. We hypothesize that the offspring 2D:4D ratios will be inversely correlated with maternal postpartum circulating testosterone but positively correlated with estradiol.

## 2 | MATERIAL AND METHODS

### 2.1 | Sample

The study took place from December 2020 to April 2021 and was cross-sectional in nature. In this study, 132 mother-daughter pairs and 140 mother-son pairs were recruited, totaling 272 mother-offspring pairs. The participants were recruited from the Reproductive and Child Health (RCH) unit, in Tamale. To be included in the study, the mothers should have given birth for the first time with a singleton birth. Their offspring should have been less than 730 days (≤2 years) old to qualify for inclusion in the study. All the mothers were not to be on any form of hormonal treatment and nonmenstruating (without the use of contraceptives). Both mothers and their offspring were to be devoid of any limb, finger, and spinal deformities and also without any known endocrine-disrupting conditions (e.g., CAH, AIS or chemical exposure). Sampling was performed within a specified period between 8.00- and 12.00-hours Greenwich

Mean Time (GMT) to minimize diurnal variations in hormonal and anthropometric variables.

## 2.2 | Data collection

### 2.2.1 | Sociodemographic and anthropometrics

The detailed method for the collection of sociodemographic and anthropometric variables of mother-offspring pair can be found in a previously published work by the same authors (Banyeh et al., 2021). A validated structured questionnaire was used to collect the medical and demographic data of the studied population including offspring weight at birth, weight at sampling, fetal length, and fetal condition at birth. The digit ratios were measured from palmar surfaces of hand scans following the guidelines of Neyse and Brañas-Garza (2014). The scanned hand images were analyzed using a computer-assisted program [GIMP (v 2.10.22), www.gimp.org]. The intraclass correlation coefficients (ICC) were calculated using the two-way mixed, single measures with absolute agreement technique. The ICC for the 2D:4DL was 0.996 (95% CI: 0.994–0.998), while the ICC for the 2D:4DR was 0.984 (95% CI: 0.980–0.989). The maternal standing height (cm) and body weight (Kg) were measured following the guidelines of Best and Shepherd (2020).

### 2.2.2 | Laboratory analysis

*Sample collection*

After an overnight fast (12 h), a venous blood sample was collected at a median (IQR) of 111 (44–210) days postpartum into an ethylene diamine tetra-acetic acid and a red top vacutainer tube. The blood samples meant for serum were allowed to clot for 30 min at 4°C before both tubes were then centrifuged at 1500 rpm for 10 min to separate the serum/plasma. To appropriately store the samples for later analysis, serum and plasma samples were aliquoted into 1.5 ml plastic cryotubes and then frozen at −25°C. The samples were not previously thawed and refrozen beofre they were analysed.

*Enzyme-linked immunosorbent assay*

Maternal serum TT, E2, and sex hormone-binding globulins (SHBG) were analyzed in duplicates using the AccuBind® Microplate enzyme-linked immunosorbent assay test system (Monobind Inc., Lake Forest, CA 92630, USA). Serum TT was measured using the competitive enzyme immunoassay (product code: 3725–300). The within and between assay coefficient of variation (CV) for TT were ≤ 5.6% and ≤ 7.9% respectively with a sensitivity of 0.0576 ng/ml (Accuracy range: 0.29–21.9 ng/mL). Serum E2 was measured using a delayed competitive immunoassay (product code: 4925–300). The sensitivity of the test kit was 8.2 pg/mL with an accuracy range of 10–4300 pg/mL. The within and between assay CV of the E2 test kit were ≤ 7.5% and ≤ 8.2% respectively. The SHBG in serum samples were measured using an immunoenzymometric assay (product code: 9125–300). The test kit had a sensitivity of 0.0122 nmol/L within a 4.6–184.0 nmol/L accuracy range. The intra-assay precision of the test kit ranged from 1.5% to 2.6%.

*Calculated indices*

The indices of TT and E2 were calculated using standardized formulae. Free testosterone (FT%) and bioavailable testosterone (BioT%) were calculated from the website (http://www.issam.ch/freetesto.html) based on the recommended formula by Vermeulen et al. (1999). The free androgen index (FAI) and testosterone-estradiol ratio (TT/E2) were calculated by dividing TT by SHBG and E2 respectively (all in the same unit). Free E2 (FE2%) was estimated on the website (https://hrt.cafe/free-e2-estimator/) based on the recommended formula of Södergard et al. (1982).

*Routine biochemistry*

The BT 1500 automated biochemistry analyzer (Biotechnica Instruments, SPA, Italy) was used to analyzed maternal postpartum serum/plasma samples for albumin and total cholesterol. The authors followed the manufacturer's instructions and used the manufacturer's recommended reagents.

## 2.3 | Statistical analysis

Data collection was done using an Excel spreadsheet (www.microsoft.com). The data was sorted before statistical analysis was performed in SPSS (v23) and GraphPad Prism (v 8) statistical software. Descriptive statistics were performed for each variable. Parametric (normally distributed) variables were presented as mean ± SD, non-parametric variables (non-normally distributed) as median (IQR) while categorical variables were presented as numbers (percent). The differences between means were determined using the student t-test while differences between medians were determined using the Mann–Whitney *U* test. The within-group differences between the left and right digit ratios for daughters and sons were performed using the paired student *t*-test. The relationships between digit ratios and maternal variables

were performed using linear regression and Spearman correlation analysis. All the statistical analyses were 2-tailed at a $p$ value of <.050.

## 3 | ETHICAL CONSIDERATIONS

The study complied with the guidelines of the 1964 Helsinki Declaration and its later amendments regarding human subject studies. The study also complied with all national and institutional guidelines for human subject studies. The study received approval from the Institutional Review Board of the University for Development Studies, Tamale. All mothers verbally gave informed consent to participate on their own and on behalf of their offspring before the study.

## 4 | RESULTS

### 4.1 | Mothers' characteristics

The sociodemographic and anthropometric characteristics of the mothers in the study are summarized in Table 1. The mothers were 272, aged between 18 and 36 years, with a mean ± SD of 23.9 ± 3.67. Their BMI ranged from 17.3 to 34.8 Kg/m$^2$. The number of mothers with daughters was 132 (48.5%) while the rest had sons (51.5%).

### 4.2 | Offspring's characteristics

The age and digit (2D:4D) ratios of daughters and sons are shown in Table 2 and Figure 1. There was no significant difference in age between daughters and sons. The 2D:4DL was significantly higher compared to the 2D:4DR in both daughters and sons ($p < .010$). The 2D:4DR of daughters was significantly higher compared to sons ($p = .031$).

### 4.3 | Maternal blood TT, E2, and their indices stratified by offspring sex

Maternal TT, E2, and their indices were compared by offspring sex (Table 3). There were no significant differences in age and BMI between mothers with daughters and those with sons ($p > 0.05$). However, maternal blood TT, FT%, FAI, BioT%, FE2%, and TT/E2 were significantly higher among mothers with sons while E2 and SHBG were significantly higher among mothers with daughters ($p < .010$).

**TABLE 1** Sociodemographic and anthropometric characteristics of the mothers

| Variable | Min–Max/*n* (%) |
|---|---|
| *Continuous* | |
| Age (years) | 18.0–36.0 (23.9 ± 3.67) |
| Height (cm) | 145–176 (162.5 ± 5.63) |
| Weight (Kg) | 45.3–34.8 (61.9 ± 9.44) |
| BMI (Kg/m$^2$) | 17.3–34.8 (23.6 ± 3.56) |
| *Categorical* | |
| Number of mothers | 272 (100) |
| Ethnicity | |
| Mole-Dagomba | 260(95.6) |
| Other-ethnicity | 12(4.4) |
| Religion | |
| Islam | 240(88.2) |
| Christianity | 32(11.8) |
| Marital status | |
| Married | 258(94.9) |
| Other partnership | 14(5.1) |
| Educational status | |
| None/basic | 44(16.2) |
| JHS | 48(17.6) |
| SHS/vocational | 118(43.4) |
| Tertiary | 62(22.8) |
| Employment status | |
| Unemployed | 152(55.9) |
| Self-employed | 84(30.9) |
| Salaried work | 36(13.3) |
| Sex of child | |
| Female | 132(48.5) |
| Male | 140(51.5) |

*Note*: Results were presented as minimum-maximum (mean ± SD) for continuous variables and as number (percent) for categorical variables.

**TABLE 2** Sociodemographic and anthropometric variables of the daughters and sons

| Variable | Daughters | Sons | *p* value |
|---|---|---|---|
| Age (days) | 116 (51–240) | 134 (51–240) | 0.808 |
| 2D:4DR | 0.91 ± 0.04‡ | 0.90 ± 0.04† | .031 |
| 2D:4DL | 0.92 ± 0.05 | 0.92 ± 0.05 | 0.555 |
| Dr-l | −0.013 ± 0.048 | −0.021 ± 0.052 | .193 |

*Note*: Results presented as mean ± SD or median (IQR). The mean values of sons were compared with those of daughters using the student *t*-test (unpaired, 2-tailed) while the median values were compared using the Mann-witney *U* test (unpaired, 2-tailed). Also, an intra-sex comparison of the right and the left digit ratio was performed using the paired student *t*-test (2-tailed). ‡Significant at $p = .002$ when compared to 2D:4DL. †Significant at $p < .001$ when compared to 2D:4DL.

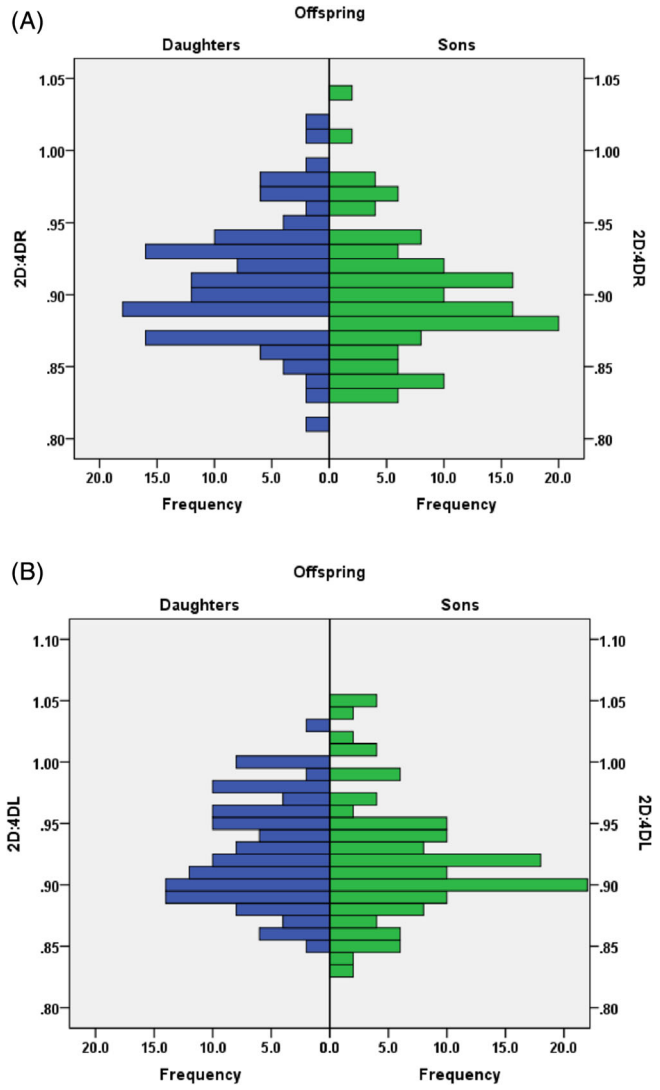


FIGURE 1 The distribution of digit (2D:4D) ratios among daughters and sons of the study population: Distribution of 2D:4DR (A), distribution of 2D:4DL (B)

### 4.4 | Daughters' 2D:4D ratio and maternal blood TT, E2, and their indices

Maternal blood TT, E2, and their indices were regressed on the 2D:4DR and 2D:4DL of their daughters (Figure 2). Maternal blood TT ($r = -0.236$, $p = .026$), FT% ($r = -0.320$, $p = .003$), BioT% ($r = -0.319$, $p = .003$), FAI ($r = -0.321$, $p = .002$), FE2% ($r = -0.255$, $p = .015$) were inversely correlated while SHBG ($r = 0.214$, $p = .045$) was significant but positively correlated with the 2D:4DL of their daughters ($p < .050$).

### 4.5 | Sons' 2D:4D ratio and maternal blood TT, E2, and their indices

When maternal blood TT, E2, and their indices were regressed on their sons' digit ratios (Figure 3), FT% ($r = -0.213$, $p = .047$) was significant but inversely correlated with their son's 2D:4DL while FAI was significant but positively correlated with 2D:4DL ($r = 0.232$, $p = .032$).

## 5 | DISCUSSION

The study aimed to determine the relationships between maternal postpartum TT, E2 and their calculated indices and offering's 2D:4D ratios. The 2D:4DR of sons was significantly lower, compared to that of daughters. It was also observed that mothers with sons had significantly higher TT, FT%, BioT%, FAI, and TT/E2 ratio while mothers with daughters had significantly higher levels of circulating E2 and SHBG. There was a significant but inverse relationship between daughters' 2D:4DL and maternal circulating TT, FT%, BioT%, FAI, and FE2%. Also, maternal FT% inversely correlated with their sons' 2D:4DL.

The study observed that the 2D:4DR of sons was significantly lower, compared to that of daughters (Hönekopp & Watson, 2010). It is suggested that the right hand is more responsive to the effect of prenatal androgen exposure, AR gene polymorphism and clinical conditions than the left hand (Breedlove, 2010). Studies among monozygotic and dizygotic twins have shown that the heritability of digit ratios in males was higher in the left than the right hand, this was a further indication of the high responsiveness of the right to prenatal androgen exposure than the left-hand (Gobrogge et al., 2008). More details on the heritability of digit ratios can be found in an earlier population by the same authors (Banyeh et al., 2021).

It was observed in this study that mothers who had sons at birth had higher circulating TT, while mothers with daughters had significantly higher levels of circulating E2. Although in this study, postpartum blood samples were used, previous studies have indicated that parental hormones around the time of conception have an influence on offspring sex ratios at birth (James, 1996; Mascaro et al., 2014). The hormonal hypothesis suggests that elevated parental blood testosterone and estrogen around the time of conception is significantly associated with sons at birth while parental blood progesterone and gonadotrophins, with daughters (James, 1998). This hypothesis is supported in conditions of pathology, chemical or occupational exposures, that disrupt the endocrine system in humans and other mammals (James, 2006). The hormones in the hormonal hypothesis satisfy the "condition" in the Trivers-Willard Hypothesis (TWH), which proposed that mothers in good "conditions" tend to produce sons as opposed to daughters in other to have more grand offspring. According to the TWH, sons have a reproduction advantage over females, because, in

**TABLE 3** Comparing maternal blood testosterone, estradiol and their indices by offspring sex at birth

| Variable | Mothers of daughters *n* (132) | Mothers of sons *n* (140) | *p* value |
|---|---|---|---|
| Age (years) | 23.0(21.0–26.0) | 25.0(21.0–27.0) | 0.228 |
| BMI (Kg/m$^2$) | 22.7(20.8–25.3) | 22.7(20.9–25.6) | 0.621 |
| TT (nmol/L) | 1.2(1.0–1.4) | 3.5(2.7–5.1) | 0.000 |
| FT (%) | 0.8(0.6–1.0) | 1.0(0.7–1.3) | 0.001 |
| BioT (%) | 23.4(15.7–29.5) | 28.3(21.0–34.4) | 0.001 |
| FAI | 1.4(0.7–1.9) | 4.7(3.2–7.4) | 0.000 |
| E2 (pmol/L) | 90.9(54.0–205.9) | 54.5(35.2–80.5) | 0.000 |
| FE2 (%) | 1.7(1.3–2.0) | 2.0(1.6–2.3) | 0.001 |
| TT/E2 (both in pmol/L) | 10.3(7.0–21.7) | 56.2(37.9–104.5) | 0.000 |
| SHBG (nmol/L) | 92.0(67.9–149.0) | 71.2(51.3–107.0) | 0.001 |
| TCHOL (mmol/L) | 5.2(4.6–6.0) | 5.1(4.5–6.0) | 0.416 |
| ALB (g/L) | 51.5(46.2–58.3) | 50.1(47.5–55.7) | 0.040 |

Abbreviations: ALB, albumin; BMI, body mass index; BioT, bioavailable testosterone; FT, free testosterone; FAI, free androgen index; E2, estradiol; FE2, free estradiol; SHBG, sex hormone-binding globulin; TT, total testosterone; TCHOL, total cholesterol.

*Note*: Results were presented as median (IQR). The difference in median values between the two groups was determined using the Mann–Whitney *U* test (unpaired, 2-tailed).

favorable conditions, sons will have more offspring in polygamous systems. However, an unsuccessful male in polygamous systems has fewer chances of mating when conditions are bad mainly due to competition. But an unsuccessful female in adverse conditions may still produce at least one or two offspring, and thus guaranteeing future grand offspring (Trivers & Willard, 1973). Previous studies have observed that the proportion of sons among women who were infected with *Toxoplasma gondii* or carriers of the hepatitis B virus around the time of conception or developed preeclampsia during pregnancy, was significantly higher. This was due to the relatively high levels of testosterone around the time of conception in such individuals since females usually respond to stress by increased secretions of adrenal androgens (James, 2006; Kaňková et al., 2007; Oster, 2005; Yuan et al., 1995). Although the fathers were not considered in this study, it was reported that there was an inverse correlation, independent of parental sex or ethnicity, between parental 2D:4D ratio and the proportion of sons at birth (Manning et al., 2002). Other hypotheses proffering an explanation for SRB include Krackow's hypothesis of developmental asynchrony (Krackow, 1995), the maternal dominance ranking hypothesis (Grant, 2006), the maternal stress hypothesis (Catalano, 2003) and the maternal glucose hypothesis (Cameron et al., 2008). However, attempts at finding the mechanism for all the various hypotheses on SRB are pointing to the effect of parental hormones. High SRB was found among both low-rank and high-rank females contrary to the maternal dominance rank hypothesis. It was later shown that stress may be associated with both low-rank and high-rank females. Female mammalians usually respond to stress by excess secretion of adrenal hormones and if this occurs around the time of conception, then a high SRB will be observed. Also, the outcome of studies among women with diabetes mellitus regarding SRB were mixed. It was explained that excess adrenal hormones may stimulate the release of glucose in stressful conditions and the adrenal hormones and not the glucose may be the determining factor of the high SRB (Brown et al., 2020). Although the hormonal hypothesis has some credibility, some have critiqued that it is only supported by correlational or observational but not experimental evidence (James & Grech, 2017).

In this study, daughters 2D:4DL was inversely correlated with maternal TT while maternal FT% was inversely correlated with sons' 2D:4DL. Whitehouse et al. (2015) found an inverse correlation between BioT% and 2D:4DL but not 2D:4DR among females. Most studies that investigated the effect of maternal environment, on offspring digit ratios most often, assayed amniotic fluid or cord blood androgens (Lutchmaya et al., 2004; Mitsui et al., 2016). Only a few studies have examined the relationship between maternal blood androgens' concentration and offspring's digit ratios (Ventura et al., 2013). A study found an inverse correlation between male 2D:4D ratio and cord blood dehydroepiandrosterone (DHEA) in a Japanese population while an earlier study found significantly higher levels of cord blood TT, and

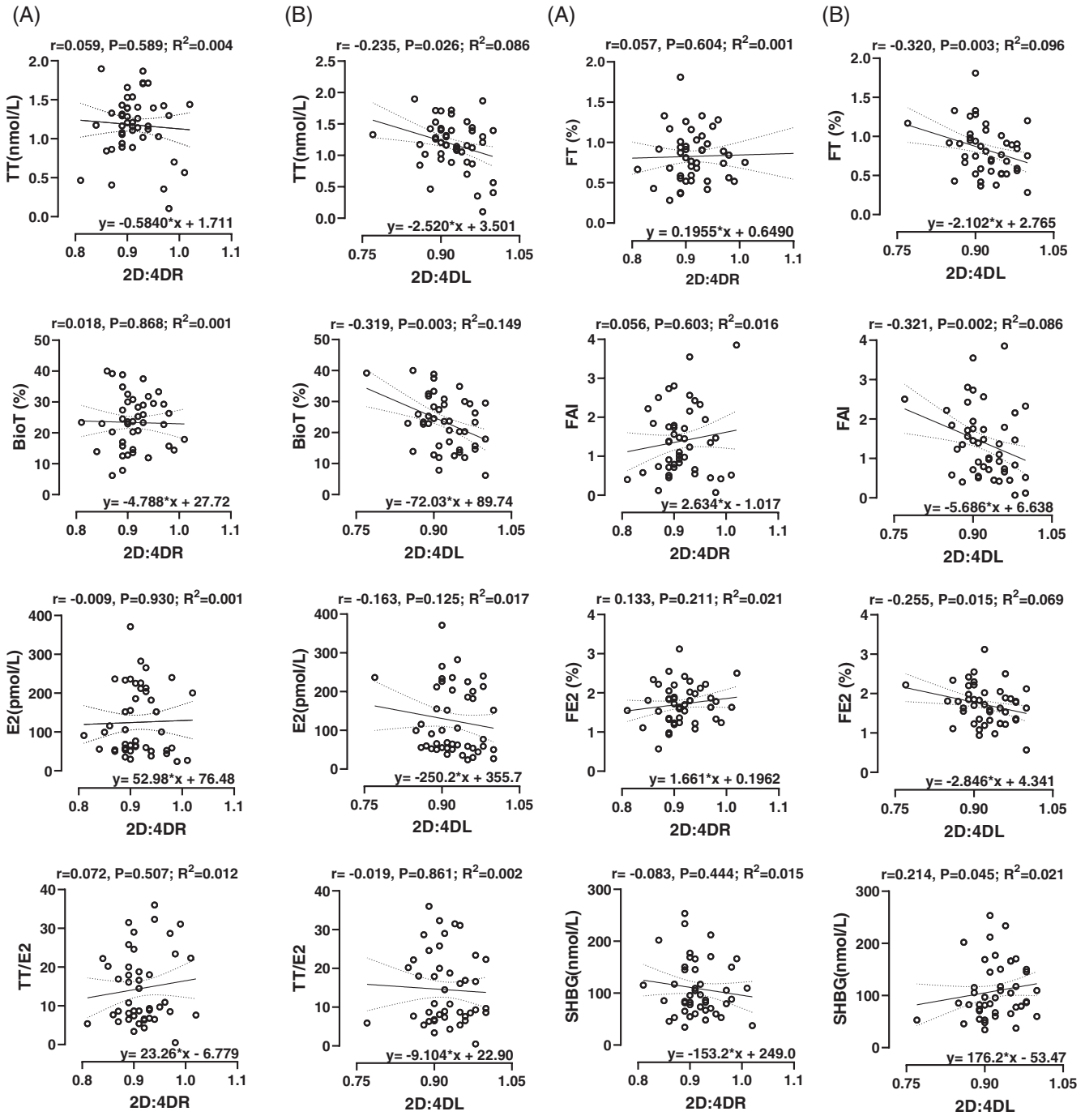


**FIGURE 2** Simple linear regression graphs with spearman rank correlation coefficients (r). Maternal blood total testosterone (TT), estradiol (E2) and their indices were regressed on their daughters' 2D:4DR (column A), and 2D:4DL (column B). FT; free testosterone, BioT; bioavailable testosterone, FAI; free androgen index, FE2; free estradiol, SHBG; sex hormone-binding globulin

testosterone-estradiol ratio among boys (Mitsui et al., 2016). In another study, maternal blood TT and digit ratios showed a significant but weak negative correlation while amniotic fluid TT was strongly but inversely correlated with digit ratios but only in female newborns. Although Lutchmaya et al. (2004) found significant negative relationships between digit ratios and amniotic fluid TT and E2 a follow-up replicative study and similar studies did not find any significant relationships (Çetin et al., 2016; Hollier et al., 2015; Richards, Browne, & Constantinescu, 2020). It is however thought that amniotic fluid and cord blood analysis is an assessment of the perinatal but not the prenatal period since these samples can only be collected from the second and third trimesters respectively, by which time the effect of prenatal androgens on digit development would have occurred

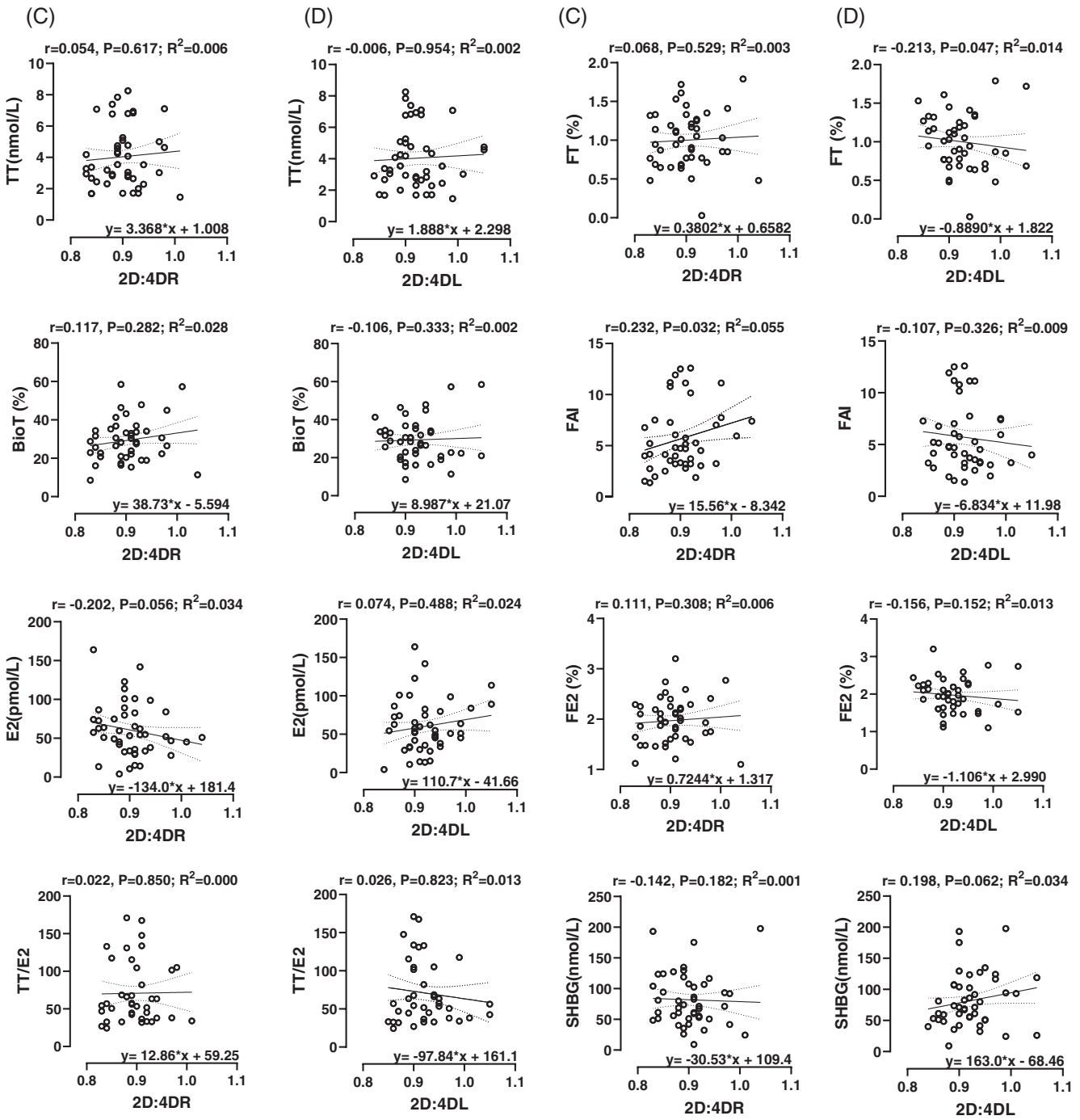


**FIGURE 3** Simple linear regression graphs with spearman rank correlation coefficients (r). Maternal blood total testosterone (TT), estradiol (E2) and their indices were regressed on their sons' 2D:4DR (column C), and 2D:4DL (column D). FT; free testosterone, BioT; bioavailable testosterone, FAI; free androgen index, FE2; free estradiol, SHBG; sex hormone binding globulin

probably in the first trimester (Richards, 2017). Although postpartum samples were used in this study, significant findings were made as hypothesized by Manning et al. (1998). These observations may probably show that the relationship between PT and digit ratios may persist even into the postpartum period.

The current study has some strengths: this study is the first to come from Ghana that sought to determine the effect of maternal TT, E2, and their calculated indices on offspring digit ratios. Also, digit ratios were measured using computer-assisted analysis, which is more precise than direct measurements or from photocopies (Fink & Manning, 2018). Moreover, the study did not examine TT and E2 alone, but also their calculated indices. However, the authors acknowledge the use of postpartum maternal samples instead of amniotic fluid, first-trimester samples,

or cord blood since the effect of prenatal androgen exposure seem to take effect earlier in pregnancy (Richards, 2017). The effect of DHT and other androgens on digit ratios were not assessed due to cost implications. We conclude that evidence of the hypothesis that PT exposure has a relationship with offspring's 2D:4D ratio may probably persist even into the postpartum period.

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## CONFLICT OF INTEREST

The authors declare no conflict of interest. Also, no external funds were used for this study.

## DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

## AUTHOR CONTRIBUTIONS

**Moses Banyeh:** Conceptualization (equal); data curation (lead); formal analysis (lead); investigation (lead); writing – original draft (lead); writing – review and editing (equal). **Nafiu Amidu:** Conceptualization (equal); supervision (equal); validation (equal); writing – review and editing (equal). **Lawrence Quaye:** Conceptualization (equal); supervision (equal); validation (supporting); writing – review and editing (equal).

## ORCID

*Moses Banyeh* https://orcid.org/0000-0003-3594-6077

## REFERENCES

Banyeh, M., Amidu, N., & Quaye, L. (2021). Second to fourth (2D:4D) digit ratio and their relationships among a mother and child population in Ghana. *Scientific Reports*, *11*(1), 13028. https://doi.org/10.1038/s41598-021-92358-x

Berenbaum, S. A., Bryk, K. K., Nowak, N., Quigley, C. A., & Moffat, S. (2009). Fingers as a marker of prenatal androgen exposure. *Endocrinology*, *150*(11), 5119–5124. https://doi.org/10.1210/en.2009-0774

Best, C., & Shepherd, E. (2020). Accurate measurement of weight and height 2: Calculating height and BMI. *Nursing Times*, *116*(5), 43–45.

Breedlove, S. M. (2010). Minireview: Organizational hypothesis: Instances of the fingerpost. *Endocrinology*, *151*(9), 4116–4122.

Brown, J., Kapurubandara, S., & McGee, T. M. (2020). Confounding effect of ethnic diversity on booking-in body mass index and prevalence of gestational diabetes and hypertensive disorders in pregnant women in western Sydney 1997-2016. *The Australian & New Zealand Journal of Obstetrics & Gynaecology*, *60*(3), 369–375. https://doi.org/10.1111/ajo.13077

Butovskaya, M., Burkova, V., Apalkova, Y., Dronova, D., Rostovtseva, V., Karelin, D., Mkrtchyan, R., Negasheva, M., & Batsevich, V. (2021). Sex, population origin, age and average digit length as predictors of digit ratio in three large world populations. *Scientific Reports*, *11*(1), 1–17.

Cameron, E. Z., Lemons, P. R., Bateman, P. W., & Bennett, N. C. (2008). Experimental alteration of litter sex ratios in a mammal. *Proceedings of the Royal Society B: Biological Sciences*, *275*(1632), 323–327.

Catalano, R. A. (2003). Sex ratios in the two Germanies: A test of the economic stress hypothesis. *Human Reproduction*, *18*(9), 1972–1975.

Çetin, R., Can, M., & Özcan, E. (2016). The relationship between testosterone and oestrogen level of the cord blood and length of fingers of newborns 2D: 4D. *Balıkesır Health Sciences Journal*, *5*, 75–82.

Ertuğrul, B., & Özener, B. (2020). Relationship between parental consanguinity and digit ratios (2D:4D) among Turkish newborns in Sivas. *American Journal of Human Biology*, *32*(3), e23369. https://doi.org/10.1002/ajhb.23369

Fink, B., & Manning, J. T. (2018). Direct versus indirect measurement of digit ratio: New data from Austria and a critical consideration of clarity of report in 2D:4D studies. *Early Human Development*, *127*, 28–32. https://doi.org/10.1016/j.earlhumdev.2018.09.007

Gobrogge, K. L., Breedlove, S. M., & Klump, K. L. (2008). Genetic and environmental influences on 2D:4D finger length ratios: A study of monozygotic and dizygotic male and female twins. *Archives of Sexual Behavior*, *37*(1), 112–118. https://doi.org/10.1007/s10508-007-9272-2

Grant, V. J. (2006). *Maternal personality, evolution and the sex ratio: Do mothers control the sex of the infant?*. Routledge.

Hollier, L. P., Keelan, J. A., Jamnadass, E. S., Maybery, M. T., Hickey, M., & Whitehouse, A. J. (2015). Adult digit ratio (2D:4D) is not related to umbilical cord androgen or estrogen concentrations, their ratios or net bioactivity. *Early Human Development*, *91*(2), 111–117. https://doi.org/10.1016/j.earlhumdev.2014.12.011

Hönekopp, J., & Watson, S. (2010). Meta-analysis of digit ratio 2D:4D shows greater sex difference in the right hand. *American Journal of Human Biology*, *22*(5), 619–630. https://doi.org/10.1002/ajhb.21054

Huber, S. E., Lenz, B., Kornhuber, J., & Müller, C. P. (2017). Prenatal androgen-receptor activity has organizational morphological effects in mice. *PLoS One*, *12*(11), e0188752. https://doi.org/10.1371/journal.pone.0188752

James. (1996). Evidence that mammalian sex ratios at birth are partially controlled by parental hormone levels at the time of conception. *Journal of Theoretical Biology*, *180*(4), 271–286.

James. (1998). Hypotheses on mammalian sex ratio variation at birth. *Journal of Theoretical Biology*, *192*(1), 113–116.

James. (2006). Are there preconceptional determinants of mammalian sex? A response to Boklage (2005). *Human Reproduction*, *21*(10), 2486–2490.

James W. H., & Grech, V. (2017). A review of the established and suspected causes of variations in human sex ratio at birth. *Early*

*Human Development*, *109*, 50–56. https://doi.org/10.1016/j.earlhumdev.2017.03.002

Kaňková, Š., Šulc, J., Nouzová, K., Fajfrlík, K., Frynta, D., & Flegr, J. (2007). Women infected with parasite toxoplasma have more sons. *Naturwissenschaften*, *94*(2), 122–127.

Krackow, S. (1995). The developmental asynchrony hypothesis for sex ratio manipulation. *Journal of Theoretical Biology*, *176*(2), 273–280. https://doi.org/10.1006/jtbi.1995.0197

Leslie, M. (2019). The mismeasure of hands?. Science, 364(6444), 923–925.

Lutchmaya, S., Baron-Cohen, S., Raggatt, P., Knickmeyer, R., & Manning, J. T. (2004). 2nd to 4th digit ratios, fetal testosterone and estradiol. *Early Human Development*, *77*(1–2), 23–28.

Manning, J. T. (2011). Resolving the role of prenatal sex steroids in the development of digit ratio. *Proceedings of the National Academy of Sciences*, *108*(39), 16143–16144.

Manning, J. T., Kilduff, L. P., & Trivers, R. (2013). Digit ratio (2D: 4D) in Klinefelter's syndrome. *Andrology*, *1*(1), 94–99. https://doi.org/10.1111/j.2047-2927.2012.00013.x

Manning, J. T., Martin, S., Trivers, R. L., & Soler, M. (2002). 2nd to 4th digit ratio and offspring sex ratio. *Journal of Theoretical Biology*, *217*(1), 93–95. https://doi.org/10.1006/jtbi.2002.3014

Manning, J. T., Scutt, D., Wilson, J., & Lewis-Jones, D. I. (1998). The ratio of 2nd to 4th digit length: A predictor of sperm numbers and concentrations of testosterone, luteinizing hormone and oestrogen. *Human Reproduction (Oxford, England)*, *13*(11), 3000–3004.

Mascaro, J. S., Hackett, P. D., Gouzoules, H., Lori, A., & Rilling, J. K. (2014). Behavioral and genetic correlates of the neural response to infant crying among human fathers. *Social Cognitive and Affective Neuroscience*, *9*(11), 1704–1712. https://doi.org/10.1093/scan/nst166

Mitsui Araki, A., Goudarzi, H., Miyashita, C., Ito, S., Sasaki, S., ... Morioka, K. (2016). Effects of adrenal androgens during the prenatal period on the second to fourth digit ratio in school-aged children. *Steroids*, *113*, 46–51.

Nave, G., Koppin, C. M., Manfredi, D., Richards, G., Watson, S. J., Geffner, M. E., Yong, J. E., Kim, R., Ross, H. M., Serrano-Gonzalez, M., & Kim, M. S. (2021). No evidence for a difference in 2D:4D ratio between youth with elevated prenatal androgen exposure due to congenital adrenal hyperplasia and controls. *Hormones and Behavior*, *128*, 104908. https://doi.org/10.1016/j.yhbeh.2020.104908

Neyse, L., & Brañas-Garza, P. (2014). Digit ratio measurement guide

Oster, E. (2005). Hepatitis B and the case of the missing women. *Journal of Political Economy*, *113*(6), 1163–1216.

Richards, G. (2017). What is the evidence for a link between digit ratio (2D: 4D) and direct measures of prenatal sex hormones? *Early Human Development*, 112, 71–72.

Richards, G., Browne, W. V., Aydin, E., Constantinescu, M., Nave, G., Kim, M. S., & Watson, S. J. (2020). Digit ratio (2D: 4D) and congenital adrenal hyperplasia (CAH): Systematic literature review and meta-analysis. *Hormones and Behavior*, *126*, 1–20.

Richards, G., Browne, W. V., & Constantinescu, M. (2020). Digit ratio (2D: 4D) and amniotic testosterone and estradiol: An attempted replication of Lutchmaya et al. (2004). *Journal of developmental Origins of Health and Disease*, 1–6.

Södergard, R., Bäckström, T., Shanbhag, V., & Carstensen, H. (1982). Calculation of free and bound fractions of testosterone and estradiol-17β to human plasma proteins at body temperature. *Journal of Steroid Biochemistry*, *16*(6), 801–810.

Trivers, R. L., & Willard, D. E. (1973). Natural selection of parental ability to vary the sex ratio of offspring. *Science*, *179*(4068), 90–92.

Ventura, T., Gomes, M. C., Pita, A., Neto, M. T., & Taylor, A. (2013). Digit ratio (2D:4D) in newborns: Influences of prenatal testosterone and maternal environment. *Early Human Development*, *89*(2), 107–112. https://doi.org/10.1016/j.earlhumdev.2012.08.009

Vermeulen, A., Verdonck, L., & Kaufman, J. M. (1999). A critical evaluation of simple methods for the estimation of free testosterone in serum. *The Journal of Clinical Endocrinology & Metabolism*, *84*(10), 3666–3672.

Whitehouse, A. J., Gilani, S. Z., Shafait, F., Mian, A., Tan, D. W., Maybery, M. T., ... Goonawardene, M. (2015). Prenatal testosterone exposure is related to sexually dimorphic facial morphology in adulthood. *Proceedings of the Royal Society B: Biological Sciences*, *282*(1816), 20151351.

Williams, T. J., Pepitone, M. E., Christensen, S. E., Cooke, B. M., Huberman, A. D., Breedlove, N. J., Breedlove, T. J., Jordan, C. L., & Breedlove, S. M. (2000). Finger-length ratios and sexual orientation. *Nature*, *404*(6777), 455–456.

Yuan, J. M., Ross, R. K., Stanczyk, F. Z., Govindarajan, S., Gao, Y. T., Henderson, B. E., & Yu, M. C. (1995). A cohort study of serum testosterone and hepatocellular carcinoma in Shanghai, China. *International Journal of Cancer*, *63*(4), 491–493.

Zheng, Z., & Cohn, M. J. (2011). Developmental basis of sexually dimorphic digit ratios. *Proceedings of the National Academy of Sciences*, *108*(39), 16289–16294.

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