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# Relationship of estradiol and progesterone with partnership and parity among Bangladeshi and British women of European origin.

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| **Abstract:** | Purpose : Recently, studies in social endocrinology have been exploring the effects of social relationships on female reproductive steroid hormones: estradiol and progesterone, investigating whether these are suppressed in partnered and parous women. Results have been mixed for these hormones although evidence is more consistent that partnered and women with young children have lower levels of testosterone. These studies were sequential to earlier research on men, based on Wingfield's Challenge Hypothesis, that showed men in committed relationships, and/or with young children, have lower levels of testosterone compared to unpartnered men or men with older or no children. The study here therefore further explored associations between estradiol and progesterone with partnership and parity among women from two different ethnicities: South Asian and white British. We hypothesized that both steroid hormones would be lower among partnered and/or parous women with children < 3 years old, regardless of ethnicity.<br>Methods: We analyzed data from 320 Bangladeshi and British women of European origin aged 18 to 50 who participated in two previous studies of reproductive ecology and health. Levels of estradiol and progesterone were assayed using saliva and/or serum samples and the body mass index calculated from anthropometric data. Questionnaires provided other covariates. Multiple linear regressions were used to analyze the data.<br>Results : The hypotheses were not supported.<br>Conclusion : We argue here that, unlike links between testosterone and male social relationships, theoretical foundations for such relationships with female reproductive steroid hormones are lacking, especially given the primary role of these steroids in regulating female reproductive function. Further longitudinal studies are needed to explore the bases of independent relationships between social factors and female reproductive steroid hormones. |
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# Relationship of estradiol and progesterone with partnership and parity among Bangladeshi and British women of European origin.

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## DECLARATIONS


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### Competing interests

The authors declare no competing interests. None of the funding bodies were involved in the study design, collection, analysis, interpretation of data, writing of the report or the decision to submit this article for publication.

### Availability of data and material

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

### Authors' contributions

Gillian Bentley: Conceptualization, Methodology, Validation, Data Curation, Writing – Original Draft, Writing – Review and Editing, Supervision, Project administration, Funding acquisition.

Alejandra Núñez-de la Mora: Investigation, Data Curation, Writing – Review & editing, Visualization, Funding Acquisition

Michele Freed: Conceptualization, Writing – Review and Editing

Khurshida Begum: Investigation, Data Curation, Writing – Review & Editing

Shanthi Muttukrishna: Resources, Supervision, Project administration, Funding Acquisition

Taniya Sharmeen: Investigation, Data Curation, Writing – Review & Editing

Lorna Murphy: Investigation, Data Curation, Writing – Review & Editing

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Richard Gunu: Validation, Investigation, Writing – Review & Editing

Lynnette Leidy Sievert: Conceptualization, Methodology, Validation, Data Curation, Writing – Review and Editing, Supervision, Project administration, Funding acquisition.

**Ethics approval**

For the first sample involving saliva samples, ethical approval was granted by the Ethics Committees of University College London Hospital, East London and the City Local Health Authority, Camden and Islington Local Health Authority, and Sylhet M.A.G Osmani Medical College (Núñez-de la Mora et al., 2007a, 2008). For the second sample involving plasma E2, ethical approval was granted by the Institutional Review Board for the University of Massachusetts, Amherst, the Ethics Committees of University College London, the Department of Anthropology, Durham University, and Sylhet M.A.G. Osmani Medical College. For both sets of samples, all participants provided written informed consent prior to participation, and data storage complied with the Data Protection Act in effect at that time in the UK (Begum et al. 2016; Núñez-de la Mora et al., 2007a; 2008) .

**Consent to participate (include appropriate statements)**

For both sets of samples, all participants provided written informed consent prior to participation, and data storage complied with the Data Protection Act in effect at that time in the UK.

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**ABSTRACT**

*Purpose*: Recently, studies in social endocrinology have been exploring the effects of social relationships on female reproductive steroid hormones: estradiol and progesterone, investigating whether these are suppressed in partnered and parous women. Results have been mixed for these hormones although evidence is more consistent that partnered and women with young children have lower levels of testosterone. These studies were sequential to earlier research on men, based on Wingfield's Challenge Hypothesis, that showed men in committed relationships, and/or with young children, have lower levels of testosterone compared to unpartnered men or men with older or no children. The study here therefore further explored associations between estradiol and progesterone with partnership and parity among women from two different ethnicities: South Asian and white British. We hypothesized that both steroid hormones would be lower among partnered and/or parous women with children $\leq$ 3 years old, regardless of ethnicity.

*Methods:* We analyzed data from 320 Bangladeshi and British women of European origin aged 18 to 50 who participated in two previous studies of reproductive ecology and health. Levels of estradiol and progesterone were assayed using saliva and/or serum samples and the body mass index calculated from anthropometric data. Questionnaires provided other covariates. Multiple linear regressions were used to analyze the data.

*Results*: The hypotheses were not supported.

*Conclusion*: We argue here that, unlike links between testosterone and male social relationships, theoretical foundations for such relationships with female reproductive steroid hormones are lacking, especially given the primary role of these steroids in regulating female reproductive function. Further longitudinal studies are needed to explore the bases of independent relationships between social factors and female reproductive steroid hormones.

**Keywords:** Social endocrinology; Partnership; Parity; Estradiol; Progesterone; Ethnicity; Bangladeshi; Women

## INTRODUCTION

Research on the link between reproductive steroid hormones and human social relationships has been increasing, particularly in connection with marriage and parenthood (Barrett et al., 2015, 2014, 2013; Bernstein et al., 1985; Booth and Dabbs, 1993; Gray et al., 2006, 2004, 2002; Hill et al., 1986; Kuzawa et al., 2009, 2010; Rosenbaum et al., 2018). Much has focused on how either pair-bond status or fatherhood might mediate male testosterone (T) levels, building on arguments that, due to its theoretical importance in controlling reproductive effort, T should be elevated in the context of mate-seeking but down-regulated with pair-bonding and paternal care (Ellison and Gray, 2012).

Perhaps because these studies were related to Wingfield's Challenge Hypothesis and studies of male birds (Ellison and Grey, 2012; Wingfield et al., 1990), less attention has been paid to potential social relationships and hormonal patterns among women. Those studies that do exist, however, have consistently found that marriage and motherhood are both significantly correlated with lower T levels, mirroring findings in men (Barrett et al., 2013; Kuzawa et al., 2010; van Anders and Watson, 2007).

A few studies have also attempted to link levels of estradiol (E2) and progesterone (P4) with female partnership and parity status. Barrett et al. (2015) found higher levels of salivary E2 and P4 among partnered women in a small sample of Norwegian women of reproductive age, while a few studies examining levels of these hormones among parous women have produced inconsistent results (Barrett et al., 2014, 2015; Bernstein et al., 1985; Yu et al., 1981). There are greater complexities inherent in studying endogenous reproductive hormones in women that may explain these inconsistencies (Ellison and Gray, 2012:342). These stem from the changing nature of steroid hormone profiles with menstrual cycling, pregnancy, lactation, menopause, and hormonal contraceptive use, as well as numerous ecological influences that can moderate reproductive steroid hormone levels, including nutritional, energetic, immunological and psychosocial stressors (Ellison et al., 1993; Jasienska, 2013; Núñez-de la Mora et al., 2008; Panter-Brick et al., 1993; Vitzthum, 2009; Vitzthum et al., 2004, 2002). Consequently, much less is known about levels of E2 and P4 in connection with social relationships (Barrett et al., 2015; Lebbe and Woodruff, 2013), although both hormones are implicated as role players in studies of mate attraction (Abitbol et al., 1999; Collins and Missing, 2003; Ellison and Gray, 2012; Feinberg et al., 2005; Havlicek et al., 2005; Jasieńska et al., 2004; Kuukasjärvi et al., 2004; Law Smith et al., 2006; Singh and Bronstad, 2001; Thornhill et al., 2003; Thornhill and Grammer, 1999).

Furthermore, it is not immediately evident why E2 and P4 should be mediated by partnership status and/or parity in women independently of reproductive factors such as post-partum infecundability, when reproductive steroid hormones are naturally suppressed. It is possible that E2 could play a role

in postpartum feelings of attachment toward offspring, but evidence for this is limited (Bos, 2017; Fleming et al., 1997). The most critical and well-understood role of E2 and P4 is in determining fecundity in females (Baird et al., 1997; Clancy et al., 2009; Lipson and Ellison, 1996). Endogenous levels of both these hormones, however, vary greatly within and between women across the menstrual cycle, across reproductive life, and even throughout the day (Baird et al., 1997; Ellison and Gray, 2012). Such variation, in addition to ecological factors such as nutritional stress, further complicate attempts to recognize and interpret influences derived solely from social relations such as pair-bonding.

The study presented here, therefore, aims to explore further the possibility of purported associations between social relationships and E2 and P4 levels among a total number of 320 premenopausal women aged 18 to 50 years. We also explore potential differences in social relationships and female reproductive steroid hormone levels from two different ethnic groups: 1) Bangladeshi women (n=245) and 2) British women of European origin (n=75). The Bangladeshi women included migrants of different generations (first- and second-generation) living in the UK, as well as women living in Sylhet in northeast Bangladesh (sedentees) from where the majority of British-Bangladeshis originate.

We tested the following hypotheses: 1) women who are partnered (married or 'living as married') will have E2 and P4 levels that differ from unpartnered women; and 2) women with children aged $\leq$3 will have E2 and P4 levels that differ significantly from nulliparous women, or those with older children. We predicted that in both cases, levels of E2 and P4 would be lower in partnered women and those with children aged $\leq$3 years. We also explored whether observed patterns among E2 and P4 in relation to partnership and parity might differ by ethnicity hypothesizing that, if there were significant effects of partnership, Bangladeshi women who frequently have arranged marriages would differ from British women of European origin. We therefore predicted that the effects of partnership among Bangladeshi women would be reduced compared to those that might be observed among UK women of European origin, but that the effects of having children aged $\leq$3 years would be the same.

## METHODS

### Participants

*Sample 1 (Younger women aged 18-41)*

Levels of salivary E2 and P4 originating from samples collected daily across one menstrual cycle were examined in relation to marriage/partnership and parity, first, among a sample of 202 healthy volunteers (156 Bangladeshi women, and 47 British women of European origin) aged 18-41 years, with regular menstrual patterns (23-37 days in length), referred to here as Sample 1. Bangladeshi women included those who were still living in Bangladesh (sedentees, n=46), first-generation, British-

Bangladeshi migrants in the UK who came to the UK as adults (aged >16 years, post-menarche, n=54), 3) first-generation, British-Bangladeshi women who came to the UK as children (aged < 16 years pre-menarche, n=35), and second-generation women born in the UK (n=21). The sedentees were recruited using snowball techniques and local networks while migrants in the UK were recruited from local mosques, schools, community and sports centers. Women of European descent were primarily reached through advertisements placed in local newspapers. All participants were given financial compensation for their time and travel costs. Data for Sample 1 were collected between 2001 and 2002.

The adult migrants and sedentees who grew up in Bangladesh were previously established to have comparable mean levels of P4 to one another, but significantly lower levels of P4 relative to the second-generation and British women of European origin who grew up in the UK (Núñez-de la Mora et al., 2007a). Child migrants who spent varying periods of their childhood in both Bangladesh and the UK had P4 levels in between (Núñez-de la Mora et al., 2007a). In contrast, levels of E2 did not differ between any of the groups (Núñez-de la Mora et al., 2008).

*Sample 2 (Older women aged 35-50)*

The same relationships (partnership and parity) were also examined among 117 older, premenopausal women (89 Bangladeshi and 28 of British women of European origin) aged 35-50, referred to here as Sample 2, using a single sample of plasma E2 taken during days 4-6 of the menstrual cycle (there were no analyses of P4 in the luteal phase for this sample). Again, the Bangladeshi women reflected groups of sedentees (n=17), adult migrants (n=31), child migrants (35) and second-generation (n=6). Recruitment techniques mirrored those used for Sample 1 in the different locations. Women in Sample 2 had taken part in earlier studies investigating the influence of early life development on adult levels of serum reproductive steroid hormones and age at menopause (Begum et al., 2016; Murphy et al., 2013). An earlier study established that, like in Sample 1, there were no differences in levels of serum E2 between groups (Chaney et al., 2022). All data were collected between 2007 and 2010.

Women from both Samples 1 and 2 were excluded from participation if they had a history of exogenous steroid hormone use in the three months before data collection, were pregnant and/or lactating in the six months before data collection, had a history of hysterectomy or oophorectomy, or if they had clinically diagnosed infertility, thyroid problems, or polycystic ovarian syndrome, conditions which would affect hormone levels. Data obtained from extensive questionnaires and anthropometrics allowed us to examine several covariates including pair status, numbers of children, age at interview, and the body mass index (BMI).

**Hormonal Analyses**

*Sample 1 (Younger women aged 18-41)*

Saliva samples were collected daily for a complete menstrual cycle where Day 1 signified the first day of menstrual bleeding and the last day of the cycle represented the day preceding the next menstrual bleed. Saliva samples were collected in polystyrene tubes and stored at room temperature using sodium azide (0.1%) as a preservative to prevent bacterial contamination. A spearmint flavored gum that did not affect assay outcomes was used as a salivary stimulant to aid in sample collection (Lu et al., 1999). Women were instructed to wait at least one hour after betel nut use, or food or drink consumption, and/or tooth brushing to avoid saliva contamination (Núñez-de la Mora et al., 2007b). All samples were stored at -20$^{o}$C and assayed for E2 and P4 at Northwestern University, Chicago, USA, using radioimmunoassay (Lu et al., 1999; Núñez-de la Mora et al., 2007b, 2008). Intra- and interassay coefficient variations were 14.6 and 19.9%, respectively, for E2, and 11.6% and 14.7%, respectively, for P4. Assay sensitivity was 1.56 pg/ml for E2, and 15 pg/ml for P4.

*Sample 2 (Older women aged 35-50)*

A single blood sample (5 ml) was collected using venipuncture from each woman scheduled for forward days 4-6 of the menstrual cycle counting from the first day of menstrual bleeding. After transport to the laboratory, samples were centrifuged to separate the serum and stored at -20$^{o}$C at the Microbiology Laboratory at M.A.G. Osmani Medical College in Sylhet, Bangladesh, or at the Centre for Reproductive Science at University College London (UCL). Serum samples were assayed for E2 levels at UCL using an electrochemiluminescence immunoassay kit supplied by Roche Molecular, Biochemicals, Mannheim, Germany; samples from Bangladesh were transported to London by plane using dry ice. The measuring range of the assay was 5 to 4,300 pg/ml and the mean inter and intra-assay variation were both <10% (Begum et al., 2016).

**Statistics**

For both Samples 1 and 2, Pair Status was divided into two categories, "paired" and "unpaired," the former including women who were married or cohabitating with their partner, and the latter category including those who self-identified as single, divorced, separated, or widowed. In order to measure the effect of age of the youngest child on hormone levels, we created a variable called "Parity Status" which was divided into three categories: 1) no children, 2) youngest child ≤3 years of age, and 3) only children >3 years of age. A further variable divided the women according to where they had spent their childhood developmental years, which was previously shown to influence levels of reproductive hormones significantly (Núñez-de la Mora et al., 2007a, 2008). Accordingly, the sedentees and adult migrants who had grown up in Bangladesh formed one group, while child migrants, the second-

generation and British women of European origin who had grown up in the UK formed the second. The potential effects of ethnicity were measured by including a dichotomous variable that grouped all of the Bangladeshi women together while the British women of European origin formed a second group.

Daily profiles for both E2 and P4 vary markedly across a menstrual cycle and by amounts secreted in the body (Fig. 1). These relative amounts have also affected the development of sufficiently sensitive assays for each hormone, particularly E2 (Read, 2009; Wood, 2009). Levels of E2 are relatively high for approximately 7 days before and after ovulation while P4 levels begin to rise only after ovulation (the luteal phase) and begin to decline again about halfway through this phase (Fig. 1). Consequently, for Sample 1, salivary E2 samples used for analyses were only included from days -7 to +6 of the menstrual cycle (centered on the day of ovulation), while data for salivary P4 were analyzed during the luteal phase from days +2 to +10 of the cycle (Fig. 1). This sampling strategy also matches that used by Barrett et al. (2015) in their study to facilitate data comparisons.

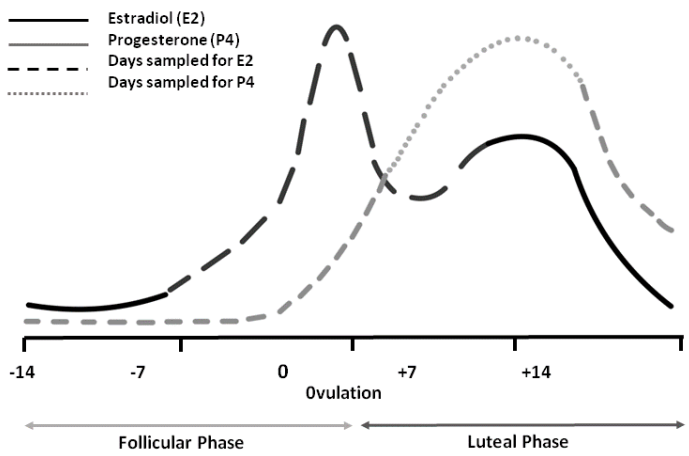



**Fig.1** Estradiol and progesterone profile across a normal menstrual cycle showing the follicular and luteal phases and sampled days averaged for Sample 1 (Younger women aged 18-41)

For Sample 1, day of ovulation was calculated for each woman using individual full menstrual profiles for both salivary E2 and P4, and plotting the characteristic fall in E2 just prior to ovulation followed by a rise in P4 just after ovulation (Núñez-de la Mora et al., 2008) (Fig. 1). Values for the sets of days for each hormone were then averaged to create single E2 and P4 values for each woman

and these averages were then used for subsequent analyses. These procedures also match the hormonal analyses run by Barrett et al. (2015).

Additionally, a separate, single E2 value was created in Sample 1 by randomly choosing a value from forward menstrual cycle days 4 to 6 for each woman (i.e., not aligned at ovulation, but counting forward in the cycle where Day 1 is the first day of menstrual bleeding). This second E2 value was generated in order to match the analyses with those of Sample 2, where a single (in this case serum) sample from women had been collected between forward cycle days 4 to 6. These days were chosen during the original study design for optimal sampling of several reproductive hormones for analyses of ovarian reserve (Begum et al., 2016). Data from Sample 2, however, did not include analyses of serum P4 meaning P4 levels cannot be compared here.

All hormonal data were $\log^{10}$ transformed to normalize the distributions. Multiple linear regression models were created to examine the relationship between hormone levels and both Pair and Parity Status and included additional, independent covariates that are known to affect reproductive steroid hormone levels, specifically, Age, and BMI as continuous variables, and two dichotomous covariates: Group, referring to the developmental environments where women had grown up (Bangladesh or the UK), and Ethnicity (being either of Bangladeshi or European ancestry). Model numbers are preceded by an "E" where they refer to analyses of E2, and "P" where they refer to analyses of P4.

In order to examine the effects of Pair Status on levels of averaged E2 and P4, we first constructed models restricted to nulliparous women in Sample 1 (Models E1.1 and P1.1, n=91) where we would expect many women might be entering their first, potentially long-term partnerships and to rule out any possible confounding effects of parity. Second-generation women were removed from the sample as no partnered women in this group were nulliparous. An interaction term between Ethnicity and Pair Status explored the potential moderating effect of the former on the latter.

Secondly, Models E1.2 and P1.2 (n=97) examined the effects of Parity Status on E2 and P4 levels among parous women only in Sample 1 using the covariate "Child aged $\leq$3 years or >3 years" along with the other covariates identified above; second-generation women were included in these Models. Models E1.2 and P1.2 also included an interaction term between Ethnicity and Parity Status. Sample 2 could not be analysed in this way because very few of the older women had children aged $\leq$3 years old.

Finally, we ran models comparing the effects of both Pair Status and Parity Status on E2 levels among all women in each of Samples 1 (Models E1.3, n=184) and 2 (E2.1, n= 117). To match the sampling strategy in Sample 2 (that used a single sample of serum E2 between days 4-6 of the cycle), we ran

Model E1.3 using the single randomized rather than the averaged E2 value. Independent covariates for these models included Pair Status, Parity Status (coded as No Children, Children aged ≤3 years, or Children aged >3 years), Age, BMI, Group, and Ethnicity. We included interaction terms between both Pair Status and Ethnicity, and Parity Status and Ethnicity.

All statistical analyses were run using IBM SPSS Statistics Version 27. Adjusted models refer to regressions where additional covariates were added. Significance levels were set at $p<0.05$.

**Data availability**

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

**Ethics**

For Sample 1 involving saliva collection, ethical approval was granted by the Ethics Committees of University College London Hospital, East London and the City Local Health Authority, Camden and Islington Local Health Authority, and Sylhet M.A.G Osmani Medical College (Núñez-de la Mora et al., 2007a, 2008). For the second sample involving plasma E2, ethical approval was granted by the Institutional Review Board for the University of Massachusetts, Amherst, the Ethics Committees of University College London, the Department of Anthropology, Durham University, and Sylhet M.A.G. Osmani Medical College. For both sets of samples, all participants provided written informed consent prior to participation, and data storage complied with the Data Protection Act in effect at that time in the UK (Begum et al. 2016; Núñez-de la Mora et al., 2007a; 2008).

**RESULTS**

**Descriptive Data**

*Sample 1 (Younger women aged 18-41)*

Relevant descriptive statistics are presented in Table 1. For Sample 1, the majority of participants were Bangladeshi (77%), and women were on average 29 ± 5.5 years old, close to the mean age of 30.8 reported for women studied by Barrett et al. (2015). Forty-nine percent of women were married or cohabitating. Only 7% of participants identified themselves as separated, divorced, or widowed, with "single" constituting the majority of unpaired individuals. Fifty-two percent of women did not have any children, while 18% had children up to 3 years of age; only 30% had children older than 3

years. For women with children, the age of their youngest child ranged from <1 year to 23 years of age, while parity ranged from 1 to 5 children (mean 1.2 ± 1.4). Parous women were significantly older (32 ± 4.7) than nulliparous women (26 ± 4.6) regardless of pair status and were more likely to be married (83%). British women of European origin were significantly more likely to be single (60% vs 39%) and nulliparous (64% vs. 48%) than Bangladeshi women. The latter were significantly ($p < 0.05$) more likely to have children ≤3 years of age (21%) compared to British women of European origin (9%), while the former were generally older, had lower BMIs and fewer children than Bangladeshi women.

*Table 1 about here*

*Sample 2 (Older women aged 35-50)*

Descriptive statistics for participants in Sample 2 are also presented in Table 1. The majority of participants were Bangladeshi (76%), and women were on average 41 years old. Seventy six percent of women were married or cohabitating, while 10% identified as divorced, separated, or widowed, and 14% identified as single. Seventy eight percent of women had children, but only 6% had children ≤3 years of age, and 72% had only older children (>3 years old). For parous women, the age of the youngest child ranged from 1 to 29 years of age, and parity ranged from 1 to 8 (mean 2.6 ± 1.8) . In general, women with children had higher BMIs, while paired women with children had the highest. Parous women were, on average, more likely to be married than unmarried. Women of European origin tended to be older, have a lower BMI, a higher age at first pregnancy, and were significantly more likely to be unpaired (75% vs. 5%, $p<0.01$) and nulliparous (68% vs. 10%, $p<0.01$) compared to Bangladeshi women.

In Sample 1, there were no significant correlations between salivary E2 and ethnicity, age, BMI, or total number of children. There was, however, a significant association between P4 and Ethnicity ($p<0.01$), BMI ($p<0.05$) and total number of children ($p <0.05$). In Sample 2, associations between plasma E2 and ethnicity approached significance ($p=0.056$) while correlations with BMI ($p=0.06$) and total number of children ($p=0.058$) were also approaching significance.

**Multiple Regression Models**

*Pair Status*

Adjusted Model E1.1, examining relationships in Sample 1 between Pair Status and averaged E2 among all nulliparous women, was not significant and had no significant covariates. Adjusted Model

P1.1 examining the role of P4 with Pair Status was significant ($r^2 = 0.310$, $F_{5,85} = 7.650$, $p<0.001$) and maintained significance with the addition of the interaction term between Pair Status and Ethnicity ($r^2 = 0.310$, $F_{6,84} = 6.300$, $p<0.001$). Here, Group was a significant covariate in the adjusted model (t=4.205, p<0.001) and in the Model including the interaction term (t=4.079, p<0.001). Sedentees and adult migrant who grew up in Bangladesh had significantly lower levels of P4 compared to the other groups as reported in other publications (Núñez-de la Mora et al., 2007a). The interaction term between Ethnicity and Pair Status was not significant for either model (Table 2).

*Table 2 about here*

*Parity Status*

Model E1.2 that examined the effects of Parity Status on E2 levels was not significant and had no significant covariates. Adjusted Model P1.2 without the interaction term was significant overall ($r^2 = 0.121$, $F_{5,90} = 2.467$, $p<0.038$) having Ethnicity as a significant covariate (t= -2.095, p=0.038) but, once the Model P1.2 was adjusted with the interaction term, it was no longer significant (Table 2).

*Pair and Parity Status*

Unadjusted Model E1.3 (examining both Pair and Parity Status among women in Sample 1 in relation to randomized E2 levels) was significant ($r^2 = 0.044$, $F_{3,181} = 2.822$, $p<0.04$), with both Parity Status (children $\leq$3, t=2.785, p=0.006; and children >3, t=2.203, p=0.029) being significant (Table 2). In both cases, Parity Status was associated with higher E2 levels. In the adjusted model, the Model was no longer significant overall, while Children $\leq$3 remained significant (t=2.525, p=0.012), but not Children >3. When the interaction term for both Pair and Parity Status with Ethnicity was added to the adjusted model, it was no longer significant overall and no covariates were significant (Table 2).

Adjusted Model P1.3 examining both Pair and Parity Status among all women in Sample 1 in relation to averaged P4 levels) was significant ($r^2 = 0.192$, $F_{7,192} = 6.228$, $p<0.001$), with significant covariates of Group (t=3.274, p=0.001) and Ethnicity (t= -2.087, p=0.038). The adjusted model including the interaction terms between Pair and Parity Status and Ethnicity remained significant ($r^2 = 0.192$, $F_{9,190} = 5.016$, $p<0.001$) with Group as the only significant covariate (t=3.257, p=0.001) (Table 2).

Adjusted Model E2.1 (using Sample 2) was borderline significant overall ($r^2 = 0.117$, $F_{7,109} = 2.072$, $p<0.053$) with Ethnicity as a significant covariate (t=2.190, p=0.031). The adjusted model with interaction terms was also significant overall ($r^2 = 0.182$, $F_{9,107} = 2.649$, $p = 0.008$) with both Ethnicity (t=2.417, p = 0.017) and the interaction between Ethnicity and Pair Status (t = -2.353, p = 0.02) being significant. Being both Bangladeshi and partnered was associated with having higher E2 levels.

## DISCUSSION

In evaluating the associations between social relationships (partnership and parity) with ovarian steroid hormone levels, we examined the following hypotheses among a sample of Bangladeshi and British women of European origin: 1) women who are partnered (married or 'living as married') would have E2 and P4 levels that differ from unpartnered women and 2) women with children aged <3 would have E2 and P4 levels that differ significantly from nulliparous women, or those with older children. We predicted that partnered women and those with children aged <3 years old would have lower levels of E2 and P4. We also hypothesized that that the effects of social relationships on reproductive steroid hormone levels would differ by ethnicity, predicting that Bangladeshi women would show less effect if partnered but the same effect compared to British women of European origin if they had young children aged <3 years.

We found no effect of Pair Status on either E2 or P4 among nulliparous women in Sample 1 and no effect of Parity Status on these hormones among all parous women in the same sample. The only significant covariates in these models related to Group or Ethnicity with P4, which has previously been shown to differ among women in Sample 1 depending on their developmental environments in Bangladesh or the UK (Núñez-de la Mora et al., 2007a). When we examined both Pair Status and Parity Status in all of Sample 1, having children $\leq$3 years was significant in the adjusted model that included all other covariates. However, once we added the interaction terms between Ethnicity and Pair Status and Ethnicity and Parity Status, the model was no longer significant. This finding may be explained by the fact that many more Bangladeshi women were parous overall compared to the British women of European origin.

The only adjusted model that showed any significant relationships between hormone levels and social relationships was in Sample 2 where Ethnicity and the interaction between Ethnicity and Pair Status were both significant. In this case, however, we believe these results likely reflect the disparity in numbers of individuals who were partnered between Bangladeshi women and British women of European origin. The former group were much more likely to be married compared to the latter which had greater numbers of women who were divorced or separated. More than half (54%) of the women classified as "single" in Sample 2 were British women of European origin despite comprising a smaller numerical group overall.

Only one study to date has previously examined the relationship between ovarian steroid hormone concentrations and pair status (Barrett et al., 2015). Unlike the results from our study here, Barrett and colleagues found a significant relationship between partnership status and reproductive hormone

levels, with higher salivary E2 and P4 levels in women who were married or partnered. Their study, however, was relatively limited in scope due to a small and ethnically homogenous sample of 185 white Norwegian women, as well as a narrow age-range of 25-35 years. Barrett et al. (2015) suggested two possibilities to explain their findings: first, that it might be adaptive for E2 and P4 to increase following partnership because of the prospect of "more stable, long-term access to resources" (pg. 505). No mechanism, however, was suggested as to how these prospects might influence hormone levels. A second, more plausible suggestion is that, in cultures where individuals can choose their own partner, women with higher levels of E2, signalling greater fecundity and attractiveness (Jasieńska et al., 2004), might be more likely to marry.

Prior results from studies examining ovarian steroid hormones in relation just to parity have been inconsistent. Some studies have found no significant differences in estrogen levels between parous and nulliparous women similar to our findings in the adjusted model here (Barrett et al., 2015; Yu et al., 1981). In contrast, Bernstein et al. (1985) found that both plasma and urinary estrogens (particularly E2) were lower in parous than nulliparous women. Similarly, Barrett et al. (2014) found that women who had given birth within the previous three years were characterized by urinary estrogen metabolites (estrone sulfate and estrone glucuronide—E1C) that were on average 22% lower compared to nulliparous women, and 13% lower than women who had given birth more than three years previously. The same study also found that, among parous women, time since last birth was positively correlated with an increase in these metabolites up to nine years after last birth (Barrett et al., 2014).

There are several potential reasons why parous women might have lower estrogen levels. Although the studies cited above found a significant inverse relationship between parity and estrogen levels, they did not control for length of lactation of the last-born child or take into account coital frequency. Women with young children may demonstrate a lingering effect of lactation which dampens E2 and P4 levels (McNeilly, 2001), depending on when they were sampled post-partum and if they had breastfed. Secondly, women with young children may engage in coitus less frequently due to tiredness or lack of opportunity; some research suggests higher coital frequency may contribute to higher estrogen levels (Prasad et al., 2014; Wilcox et al., 2004). Thirdly, women with younger children may be more tired and stressed, leading to suppression of reproductive steroid levels (Joseph and Whirledge, 2017). The finding by Barrett et al. (2014), that urinary estrogens increase with time since last birth, may likely show recovery by women from all of these post-partum factors.

All the aforementioned studies looked exclusively at Western women, mostly of European descent. The only study to date examining parity in relation to ovarian steroid hormones among non-Western populations found that, among Japanese women, parous women had higher plasma E2 than

nulliparous women (Hill et al., 1986). However, the women in this study were aged 35-45 and, while apparently matched by age group, weight and diet, the comparisons did not control for age or pair status and it is possible that some other factor may have influenced the results. Where our findings demonstrated differences by Ethnicity or Group, we could explain these either by previously demonstrated hormonal differences between women dependent on their developmental environments, or by differences in proportions of women with and without children rather than a purported effect of social relations such as having young children.

As mentioned briefly in the Introduction, it is less clear from a theoretical viewpoint, why one would predict an effect of partnership and/or parity on either E2 or P4 levels in women from the viewpoint of social endocrinology, especially given the complex and crucial roles of these hormones in regulating female reproductive capabilities. Although levels of E2 are implicated in mate attraction (Jasieńska et al., 2004), this may be due to an evolved ability of men to detect subtle cues of a woman's fecundity or ovulatory status (Gildersleeve et al., 2012; Lobmaier et al., 2018; Puts et al., 2013), and may bear little relationship to aspects of partnering or parity. Gettler et al. (2018) have suggested that studies in social endocrinology should analyze multiple hormones and the complex, dynamic relationships between them in order to understand more fully how they relate to parental and mating investments. Such studies should also account for specific geographical and cultural contexts. Other research has, in fact, focused on hormones such as oxytocin and prolactin and their role in promoting bonding to partners and children (Bos, 2017; Rilling, 2013; Snowdon and Ziegler, 2015; Storey and Ziegler, 2016). It has proven difficult to measure these protein hormones easily in humans in any medium other than blood, thus precluding multiple sampling regimes. As assays develop in sensitivity, however, we should expect such analyses to become easier and more frequent and they may prove to be more compelling than those focusing on reproductive ovarian steroids.

In contrast to E2 and P4, and following the Challenge Hypothesis (Reburn and Wynne-Edwards, 1999; Wingfield et al., 1990; Ziegler, 2000), there is a clear rationale for why T levels may be modulated when men are in committed partnerships or have dependent children. T is an anabolic hormone that is important for both mating effort (Bhasin et al., 2012; Herbst and Bhasin, 2004) and intra-sex competition (Wingfield et al., 1990). It requires significant resource investment and represents part of an evolved trade-off between mating effort and parenting (Barrett et al., 2015; Ellison and Gray, 2012). T may be similarly required for women although there is less written specifically on this topic (Barrett et al., 2013; Kuzawa et al., 2010; van Anders and Watson, 2007).

There is some preliminary evidence to suggest that E2 may also be involved in parenting effort in males, with one study (Berg and Wynne-Edwards, 2001) showing new fathers had higher E2 levels than men without children. However, recently, Gettler et al. (2018) showed that, although partnered

men with children demonstrated a greater decline in levels of E2 relative to unpartnered, childless men, these differences were not significant after controlling for longitudinal changes in T levels. The role of both E2 and P4 in human paternal behavior is, however, still not well understood, with studies of these hormones in relation to fatherhood providing equivocal results (Berg and Wynne-Edwards, 2001; Edelstein et al., 2015; Wynne-Edwards and Reburn, 2000).

Analyzing any potential declines in E2 and/or P4 specifically among women with young children is complicated by the natural suppression of both hormones with postpartum, lactational infecundability, the length of which differs between individuals depending on their nutritional status and period of lactation (Barrett et al., 2015; Valeggia and Ellison, 2009; Vitzthum, 1994). When one adds the numerous other factors that are known to suppress ovarian steroids, including developmental influences, age, physical exercise, and psychosocial stress, unravelling any additional effects from having a partner or young children represents a difficult task. We have demonstrated the difficulties of this using a sample with different ethnicities and social relationships that included many of the covariates known to affect levels of E2 and P4. We found no compelling results to suggest that being partnered or having young children have independent effects on the levels of these hormones.

**Limitations of the Study**

The principal limitation of this study was relatively small sample sizes, particularly in Sample 2 (n = 117). For both Samples 1 and 2, group sizes within the two paired categories and three parity categories used in analyses were small and often unbalanced (Table 1). In particular, the number of women with children $\leq$3 years of age was very small for Sample 2 (n = 7). There were significantly more Bangladeshi women in both Samples 1 and 2. As was also the case in Barrett et al. (2015), small sample sizes prevented the examination of whether being separated, divorced, or widowed rather than never married affected E2 concentrations.

In terms of E2 analyses, there is debate regarding the use of blood versus saliva. Saliva sampling provides measures of free E2, unbound by SHBG, which indicate hormone concentrations available for diffusion into the reproductive tract and other tissues (Barrett et al., 2015; Chatterton et al., 2006; Pollard et al., 2009). A single serum E2 sample is less robust and more variable than saliva samples collected from multiple days throughout the menstrual cycle, particularly when trying to ascertain individual basal E2 concentrations (Barrett et al., 2015; Pollard et al., 2009). Moreover, as Barrett et al. (2015) pointed out, hormone concentrations measured over a single cycle cannot account for inter-cycle variation among individual women, and therefore possibly provide a less-than-accurate picture of individual basal E2 levels. In future studies, it would be ideal to collect hormonal data over the

course of multiple cycles in order to mitigate this concern and provide the most accurate possible assessment of individual E2 levels.

In relation to some covariates of interest, we did not have direct data on whether Bangladeshi women were in arranged or love marriages nor on aspects of romantic feelings for women's partners. Any future work examining the relationship between female reproductive hormones, partnerships and parity should therefore explore issues of attachment within relationships since this may be an important mediator of hormone levels. This could be achieved using psychometric instruments to measure adult attachments (e.g., Eastwick and Finkel, 2012).

As the present study was a secondary analysis of samples used in previous studies (Begum et al., 2016; Núñez-de la Mora et al., 2007a, 2008), some of the hormonal data available were not ideal for comparing basal E2 concentrations. In particular, the hormonal data for Sample 2, comprising single blood samples taken between cycle days 4–6, are not the most robust indicator of differences in average E2 between individuals due to large inter-individual and inter-cycle variability in E2 patterns throughout the menstrual cycle (Pollard et al., 2009). In their 2016 study, Begum et al. collected their samples as one of many indicators of ovarian reserve for which purpose days 4-6 of the menstrual cycle were optimal. For determining average total E2 over the whole cycle, however, the most accurate single serum measures are those taken between days 9 and 11 (Ahmad et al., 2002). Serum measures, however, are still considered inferior to daily salivary E2 sampling. Discrepancies in some of the results found between E2 measures may further illustrate these methodological differences. Finally, Sample 2 did not provide serum measures for P4, which could have been useful in terms of comparison with the salivary P4 from Sample 1.

## CONCLUSIONS

This study examined relationships between social characteristics (pair bonding and having young children) and levels of both serum/salivary E2 and salivary P4 in Bangladeshi and British women of European origin. We found no compelling evidence that levels of these two reproductive steroid hormones were influenced consistently by partnership or parity. Instead, we argue here that, given the complex roles of these hormones in regulating reproductive function and their susceptibility to many ecological factors, it would be difficult to disentangle additional influences suggested to operate in the context of partnership and having young children. While a wealth of cross-species and human data support evolutionary arguments for the role of partnership and parity affecting T levels, especially in men, there are so far no persuasive theoretical arguments or even consistent results from other studies to suggest similar relationships with either E2 or P4 in women. Further studies, particular longitudinal ones, could help to disentangle the many factors that complicate these relationships.

## REFERENCES


Abitbol, J., Abitbol, P., & Abitbol, B. (1999). Sex hormones and the female voice. *Journal of Voice*, *13*(3), 424–446. https://doi.org/10.1016/S0892-1997(99)80048-4

Ahmad, N., Pollard, T., & Unwin, N. (2002). The optimal timing of blood collection during the menstrual cycle for the assessment of endogenous sex hormones: can interindividual differences in levels over the whole cycle be assessed on a single day? *Cancer Epidemiol Biomarkers Prev*, *11*(1), 147–151.

Baird, D. D., Wilcox, A. J., Weinberg, C. R., Kamel, F., McConnaughey, D. R., Musey, P. I., & Collins, D. C. (1997). Preimplantation hormonal differences between the conception and non-conception menstrual cycles of 32 normal women. *Human Reproduction*, *12*(12), 2607–2613. https://doi.org/10.1093/humrep/12.12.2607

Barrett, E. S., Parlett, L. E., Windham, G. C., & Swan, S. H. (2014). Differences in ovarian hormones in relation to parity and time since last birth. *Fertility and Sterility*, *101*(6), 1773-1780.e1. https://doi.org/10.1016/j.fertnstert.2014.02.047

Barrett, E. S., Tran, V., Thurston, S., Jasienska, G., Furberg, A. S., Ellison, P. T., & Thune, I. (2013). Marriage and motherhood are associated with lower testosterone concentrations in women. *Hormones and Behavior*, *63*(1), 72–79. https://doi.org/10.1016/j.yhbeh.2012.10.012

Barrett, E. S., Tran, V., Thurston, S. W., Frydenberg, H., Lipson, S. F., Thune, I., & Ellison, P. T. (2015). Women who are married or living as married have higher salivary estradiol and progesterone than unmarried women. *American Journal of Human Biology*, *27*(4), 501–507. https://doi.org/10.1002/ajhb.22676

Begum, K., Muttukrishna, S., Sievert, L. L., Sharmeen, T., Murphy, L., Chowdhury, O., et al. (2016). Ethnicity or environment: Effects of migration on ovarian reserve among Bangladeshi women in the United Kingdom. *Fertility and Sterility*, *105*(3), 744-754.e1. https://doi.org/10.1016/j.fertnstert.2015.11.024

Berg, S., & Wynne-Edwards, K. (2001). Changes in Testosterone, Cortisol, and Estradiol Levels in Men Becoming Fathers. *Mayo Clinic Proceedings*, *76*(6), 582–592. https://doi.org/10.4065/76.6.582

Bernstein, L., Pike, M., Ross, R., Judd, R., Brown, J., & Henderson, H. (1985). Estrogen and sex hormone-binding globulin levels in nulliparous and parous women. *J Natl Cancer Inst. , 74*(4), 741–745.

Bhasin, S., Travison, T. G., Storer, T. W., Lakshman, K., Kaushik, M., Mazer, N. A., et al. (2012). Effect of testosterone supplementation with and without a dual 5α-reductase inhibitor on fat-free mass in men with suppressed testosterone production: A randomized controlled trial. *JAMA - Journal of the American Medical Association*, *307*(9), 931–939. https://doi.org/10.1001/jama.2012.227

Booth, A., & Dabbs, J. (1993). Testosterone and Men's Marriages. *Social Forces*, *72*, 463–477. https://doi.org/https://doi.org/10.2307/2579857

Bos, P. A. (2017). The endocrinology of human caregiving and its intergenerational transmission. *Development and Psychopathology*, *29*(3), 971–999. https://doi.org/10.1017/S0954579416000973

Burnham, T., Chapman, J., Gray, P., McIntyre, M., Lipson, S., & Ellison, P. (2003). Men in committed, romantic relationships have lower testosterone. *Hormones and Behavior*, *44*(2), 119–122. https://doi.org/10.1016/S0018-506X(03)00125-9

Chatterton, R. T., Mateo, E. T., Lu, D., & Ling, F. J. H. (2006). Interpopulational differences in the concentrations and ratios of salivary and serum progesterone. *Fertility and Sterility*, *86*(3), 723–

725. https://doi.org/10.1016/j.fertnstert.2006.01.034

Chaney, C., Begum, K., Núñez-de la Mora, A., Sievert, L.L., Muttukrishna, S., Harries, V., Sharmeen, T., Murphy, L., Gunu, R., Chowdhury, O., Bentley, G.R. 2022. No impact of developmental conditions on serum estradiol levels among Bangladeshi women in the UK and Bangladesh. *American Journal of Human Biology*, 34(3), e23631. https://doi.org/10.1002/ajhb.23631

Clancy, K. B. H., Ellison, P. T., Jasienska, G., & Bribiescas, R. G. (2009). Endometrial thickness is not independent of luteal phase day in a rural Polish population. *Anthropological Science*, *117*(3), 157–163. https://doi.org/10.1537/ase.090130

Collins, S. A., & Missing, C. (2003). Vocal and visual attractiveness are related in women. *Animal Behaviour*, *65*(5), 997–1004. https://doi.org/10.1006/anbe.2003.2123

Eastwick, P. W., & Finkel, E. J. (2012). The evolutionary armistice: Attachment bonds moderate the function of ovulatory cycle adaptations. *Personality and Social Psychology Bulletin*, *38*(2), 174–184. https://doi.org/10.1177/0146167211422366

Edelstein, R. S., Wardecker, B. M., Chopik, W. J., Moors, A. C., Shipman, E. L., & Lin, N. J. (2015). Prenatal hormones in first-time expectant parents: Longitudinal changes and within-couple correlations. *American Journal of Human Biology*, *27*(3), 317–325. https://doi.org/10.1002/ajhb.22670

Ellison, P., & Gray, P. (2012). *Endocrinology of social relationships*. Cambridge: Harvard University Press.

Ellison, P., Panter-Brick, C., Lipson, S., & O'rourke, M. (1993). The ecological context of human ovarian function. *Human Reproduction*, *8*(12), 2248–2258. https://doi.org/10.1093/oxfordjournals.humrep.a138015

Feinberg, D. R., Jones, B. C., DeBruine, L. M., Moore, F. R., Law Smith, M. J., Cornwell, R. E., et al. (2005). The voice and face of woman: One ornament that signals quality? *Evolution and Human Behavior*, *26*(5), 398–408. https://doi.org/10.1016/j.evolhumbehav.2005.04.001

Fleming, A. S., Ruble, D., Krieger, H., & Wong, P. Y. (1997). Hormonal and experiential correlates of maternal responsiveness during pregnancy and the puerperium in human mothers. *Hormones and Behavior*, *31*(2), 145–158. https://doi.org/10.1006/hbeh.1997.1376

Gettler, L.T., Kuo, P.X., Bechayda, S.A., 2018. Fatherhood and psychobiology in the Philippines: Perspectives on joint profiles and longitudinal changes of fathers' estradiol and testosterone. *American Journal of Human Biology*, 30(6), e23150 https://doi.org/10.1002/ajhb.23150

Gildersleeve, K. A., Haselton, M. G., Larson, C. M., & Pillsworth, E. G. (2012). Body odor attractiveness as a cue of impending ovulation in women: Evidence from a study using hormone-confirmed ovulation. *Hormones and Behavior*, *61*(2), 157–166. https://doi.org/10.1016/j.yhbeh.2011.11.005

Gray, P., Chapman, J., Burnham, T., McIntyre, M., Lipson, S., & Ellison, P. (2004). Human male pair bonding and testosterone. *Human Nature*. Springer New York LLC. https://doi.org/10.1007/s12110-004-1016-6

Gray, P., Kahlenberg, S., Barrett, E., Lipson, S., & Ellison, P. (2002). Marriage and fatherhood are associated with lower testosterone in males. *Evolution and Human Behavior*, *23*(3), 193–201. https://doi.org/10.1016/S1090-5138(01)00101-5

Gray, P., Yang, C., & Pope, H. (2006). Fathers have lower salivary testosterone levels than unmarried men and married non-fathers in Beijing, China. *Proceedings of the Royal Society B: Biological Sciences*, *273*(1584), 333–339. https://doi.org/10.1098/rspb.2005.3311

Havlicek, J., Roberts, S. C., & Flegr, J. (2005). Women's preference for dominant male odour: Effects

of menstrual cycle and relationship status. *Biology Letters*, *1*(3), 256–259. https://doi.org/10.1098/rsbl.2005.0332

Herbst, K., & Bhasin, S. (2004). Testosterone action on skeletal muscle. *Current Opinion in Clinical Nutrition and Metabolic Care*, *7*(3). https://doi.org/10.1097/00075197-200405000-00006

Hill, P., Garbaczewski, L., Kasumi, K., & Wynder, E. L. (1986). Plasma hormones in parous, nulliparous and postmenopausal Japanese women. *Cancer Letters*, *33*(2), 131–136. https://doi.org/10.1016/0304-3835(86)90017-0

Jasienska, G. (2013). *The Fragile Wisdom*. *The Fragile Wisdom*. Harvard University Press. https://doi.org/10.4159/harvard.9780674067196

Jasieńska, G., Ziomkiewicz, A., Ellison, P. T., Lipson, S. F., & Thune, I. (2004). Large breasts and narrow waists indicate high reproductive potential in women. *Proceedings of the Royal Society B: Biological Sciences*, *271*(1545), 1213–1217. https://doi.org/10.1098/rspb.2004.2712

Joseph, D., & Whirledge, S. (2017). Stress and the HPA axis: Balancing homeostasis and fertility. *International Journal of Molecular Sciences*, *18*(10). https://doi.org/10.3390/ijms18102224

Kuukasjärvi, S., Eriksson, C. J. P., Koskela, E., Mappes, T., Nissinen, K., & Rantala, M. J. (2004). Attractiveness of women's body odors over the menstrual cycle: The role of oral contraceptives and receiver sex. *Behavioral Ecology*, *15*(4), 579–584. https://doi.org/10.1093/beheco/arh050

Kuzawa, C. W., Gettler, L. T., Huang, Y. yen, & McDade, T. W. (2010). Mothers have lower testosterone than non-mothers: Evidence from the Philippines. *Hormones and Behavior*, *57*(4–5), 441–447. https://doi.org/10.1016/j.yhbeh.2010.01.014

Kuzawa, C. W., Gettler, L. T., Muller, M. N., McDade, T. W., & Feranil, A. B. (2009). Fatherhood, pairbonding and testosterone in the Philippines. *Hormones and Behavior*, *56*(4), 429–435. https://doi.org/10.1016/j.yhbeh.2009.07.010

Law Smith, M. J., Perrett, D. I., Jones, B. C., Cornwell, R. E., Moore, F. R., Feinberg, D. R., et al. (2006). Facial appearance is a cue to oestrogen levels in women. *Proceedings of the Royal Society B: Biological Sciences*, *273*(1583), 135–140. https://doi.org/10.1098/rspb.2005.3296

Lebbe, M., & Woodruff, T. K. (2013, December 1). Involvement of androgens in ovarian health and disease. *Molecular Human Reproduction*. Oxford Academic. https://doi.org/10.1093/molehr/gat065

Lipson, S., & Ellison, P. (1996). Comparison of salivary steroid profiles in naturally occurring conception and non-conception cycles. *Human Reproduction*, *11*(10), 2090–2096. https://doi.org/10.1093/oxfordjournals.humrep.a019055

Lobmaier, J. S., Fischbacher, U., Wirthmüller, U., & Knoch, D. (2018). The scent of attractiveness: Levels of reproductive hormones explain individual differences in women's body odour. *Proceedings of the Royal Society B: Biological Sciences*, *285*(1886). https://doi.org/10.1098/rspb.2018.1520

Lu, Y.C., Bentley, G.R., Gann, P.H., Hodges, K.R., Chatterton, R.T., 1999. Salivary estradiol and progesterone levels in conception and nonconception cycles in women: Evaluation of a new assay for salivary estradiol. Fertil. Steril. 71, 863–868. https://doi.org/10.1016/S0015-0282(99)00093-

McNeilly, A. S. (2001). Lactational control of reproduction. *Reproduction, Fertility and Development*, *13*(7–8), 583–590. https://doi.org/10.1071/rd01056

Murphy, L., Sievert, L., Begum, K., Sharmeen, T., Puleo, E., Chowdhury, O., Muttukrishna, S., Bentley, G., (2013). Life course effects on age at menopause among Bangladeshi sedentees and migrants to the UK. *American Journal of Human Biology*, 25, 83–93.

https://doi.org/10.1002/ajhb.22345

Núñez-de la Mora, A., Bentley, G. R., Choudhury, O. A., & Napolitano, D. A. (2008). The impact of developmental conditions on adult salivary estradiol levels: Why this differs from progesterone? *American Journal of Human Biology*, *20*(1). https://doi.org/10.1002/ajhb.20698

Núñez-de la Mora, A., Chatterton, R., Choudhury, O., Napolitano, D., & Bentley, G. (2007a). Childhood conditions influence adult progesterone levels. *PLoS Medicine*, *4*(5), 0813–0821. https://doi.org/10.1371/journal.pmed.0040167

Núñez-de la Mora, A., Chatterton, R.T., Jesmin, T., Mateo, E., Bentley, G.R., (2007b). The effect of chewing betel nut on measurements of salivary progesterone and estradiol. *American Journal of Physical Anthropology* 132(2), 311-5. https://doi.org/10.1002/ajpa.20513

Panter-Brick, Catherine, Panter-Brick, C, Lotstein, D.S., Ellison, P.T., (1993). The Ecological Context of Human Ovarian-Function Seasonally of reproductive function and weight loss in rural Nepali women. *Human Reproduction*, 8, 684–690. https://doi.org/10.1093/oxfordjournals.humrep.a138015

Pollard, T. M., Unwin, N., Fischbacher, C., & Chamley, J. K. (2009). Total estradiol levels in migrant and British-born British Pakistani women: Investigating early life influences on ovarian function. *American Journal of Human Biology*, *21*(3), 301–304. https://doi.org/10.1002/ajhb.20859

Prasad, A., Mumford, S. L., Buck Louis, G. M., Ahrens, K. A., Sjaarda, L. A., Schliep, K. C., et al. (2014). Sexual activity, endogenous reproductive hormones and ovulation in premenopausal women. *Hormones and Behavior*, *66*(2), 330–338. https://doi.org/10.1016/j.yhbeh.2014.06.012

Puts, D. A., Bailey, D. H., Cárdenas, R. A., Burriss, R. P., Welling, L. L. M., Wheatley, J. R., & Dawood, K. (2013). Women's attractiveness changes with estradiol and progesterone across the ovulatory cycle. *Hormones and Behavior*, *63*(1), 13–19. https://doi.org/10.1016/j.yhbeh.2012.11.007

Read, G. F., (2009). Salivary steroid assays: Still awaiting a killer application. *Annals of Clinical Biochemistry*. 46, 181–182. https://doi.org/10.1258/acb.2009.200901

Reburn, C. J., & Wynne-Edwards, K. E. (1999). Hormonal changes in males of a naturally biparental and a uniparental mammal. *Hormones and Behavior*, *35*(2), 163–176. https://doi.org/10.1006/hbeh.1998.1509

Rilling, J. K. (2013, March 1). The neural and hormonal bases of human parentalcare. *Neuropsychologia*. Pergamon. https://doi.org/10.1016/j.neuropsychologia.2012.12.017

Rosenbaum, S., Gettler, L. T., McDade, T. W., Bechayda, S. S., & Kuzawa, C. W. (2018). Does a man's testosterone "rebound" as dependent children grow up, or when pairbonds end? A test in Cebu, Philippines. *American Journal of Human Biology*, *30*(6), e23180. https://doi.org/10.1002/ajhb.23180

Singh, D., & Bronstad, P. M. (2001). Female body odour is a potential cue to ovulation. *Proceedings of the Royal Society B: Biological Sciences*, *268*(1469), 797–801. https://doi.org/10.1098/rspb.2001.1589

Snowdon, C. T., & Ziegler, T. E. (2015). Variation in prolactin is related to variation in sexual behavior and contact affiliation. *PLoS ONE*, *10*(3), e0120650. https://doi.org/10.1371/journal.pone.0120650

Storey, A. E., & Ziegler, T. E. (2016). Primate paternal care: Interactions between biology and social experience. *Hormones and Behavior*, *77*, 260–271. https://doi.org/10.1016/j.yhbeh.2015.07.024

Thornhill, R., Gangestad, S. W., Miller, R., Scheyd, G., McCollough, J. K., & Franklin, M. (2003).

Major histocompatibility complex genes, symmetry, and body scent attractiveness in men and women. *Behavioral Ecology*, *14*(5), 668–678. https://doi.org/10.1093/beheco/arg043

Thornhill, R., & Grammer, K. (1999). The body and face of woman: One ornament that signals quality? *Evolution and Human Behavior*, *20*(2), 105–120. https://doi.org/10.1016/S1090-5138(98)00044-0

Valeggia, C., & Eellison, P. (2009). Interactions between metabolic and reproductive functions in the resumption of postpartum fecundity. *American Journal of Human Biology*, *21*(4), 559–566. https://doi.org/10.1002/ajhb.20907

van Anders, S. M., & Watson, N. V. (2007). Testosterone levels in women and men who are single, in long-distance relationships, or same-city relationships. *Hormones and Behavior*, *51*(2), 286–291. https://doi.org/10.1016/j.yhbeh.2006.11.005

Vitzthum, V. (1994). Causes and Consequences of Heterogeneity in Infant Feeding Practices among Indigenous Andean Women. *Annals of the New York Academy of Sciences*, *709*(1), 221–224. https://doi.org/10.1111/j.1749-6632.1994.tb30408.x

Vitzthum, V. J. (2009). The ecology and evolutionary endocrinology of reproduction in the human female. *American Journal of Physical Anthropology*, *140* (SUPPL. 49), 95–136. https://doi.org/10.1002/ajpa.21195

Vitzthum, V. J., Bentley, G. R., Spielvogel, H., Caceres, E., Thornburg, J., Jones, L., et al. (2002). Salivary progesterone levels and rate of ovulation are significantly lower in poorer than in better-off urban-dwelling Bolivian women. *Human Reproduction*, *17*(7), 1906–1913. https://doi.org/10.1093/humrep/17.7.1906

Vitzthum, V. J., Spielvogel, H., & Thornburg, J. (2004). Interpopulational differences in progesterone levels during conception and implantation in humans. *Proceedings of the National Academy of Sciences of the United States of America*, *101*(6), 1443–1448. https://doi.org/10.1073/pnas.0302640101

Wilcox, A. J., Day Baird, D., Dunson, D. B., McConnaughey, D. R., Kesner, J. S., & Weinberg, C. R. (2004). On the frequency of intercourse around ovulation: Evidence for biological influences. *Human Reproduction*, *19*(7), 1539–1543. https://doi.org/10.1093/humrep/deh305

Wingfield, J., Hegner, R., Dufty, A., & Ball, G. (1995). The "Challenge Hypothesis": Theoretical Implications for Patterns of Testosterone Secretion, Mating Systems, and Breeding Strategies. *The American Naturalist*, *136*(6), 829–846.

Wood, P., (2009). Salivary steroid assays – Research or routine? *Annals Clinical Biochemistry*, 46, 183–196. https://doi.org/10.1258/acb.2008.008208

Wynne-Edwards, K. E., & Reburn, C. J. (2000). Behavioral endocrinology of mammalian fatherhood. *Trends in Ecology and Evolution*. Elsevier. https://doi.org/10.1016/S0169-5347(00)01972-8

Yu, M. C., Gerkins, V. R., Henderson, B. E., Brown, J. B., & Pike, M. C. (1981). Elevated levels of prolactin in nulliparous women. *British Journal of Cancer*, *43*(6), 826–831. https://doi.org/10.1038/bjc.1981.121

Ziegler, T. E. (2000). Hormones associated with non-maternal infant care: A review of mammalian and avian studies. *Folia Primatologica*, *71*(1–2), 6–21. https://doi.org/10.1159/000021726

Table 1 Descriptive statistics and frequencies for Sample 1 (Younger women aged 18-41) and Sample 2 (Older women aged 35-50); mean (SD)

| **SAMPLE 1: Younger women aged 18-41** | | | | | |
|---|---|---|---|---|---|
| | Whole sample[a] (*n* = 203) | Paired with children (*n* = 82) | Paired without children (*n* = 17) | Unpaired with children (*n* = 15) | Unpaired without children (*n* = 88) |
| **Demographic information** | | | | | |
| Age (years)* | **29 (5.5)** | **32 (4.7)** | **27.3 (3.8)** | **31.7 (4.9)** | **26.1 (4.9)** |
| BMI (kg/m$^2$)† | **24.4 (4.9)** | **26.3 (4.3)** | **23.6 (5.5)** | **23.5 (4.1)** | **22.8 (4.9)** |
| % British women of European origin | 23.2 | 11.0 | 41.2 | 53.3 | 26.1 |
| % Bangladeshis | 76.9 | 89.0 | 58.8 | 46.7 | 73.8 |
| Mean number of children | **1.2 (1.4)[b]** | **2.6 (1.2)** | | **1.8 (0.7)** | |
| Mean age of youngest child (years) | 5.4 (3.9) | 5.2 (4.2) | | 6.1 (2.0) | |
| % with child ≤ 3 years of age | 18.3[b] | 42.7 | | 13.3 | |
| Mean estradiol, days 4 to 6 (pg/ml, log10) | 0.76 (0.6) | 0.84 (0.5) | 0.46 (0.8) | 0.95 (0.5) | 0.72 (0.6) |
| Mean estradiol, avg. days -7 to 6 (pg/ml, log10) | 0.87 (0.4) | 0.89 (0.4) | 0.71 (0.4) | 0.96 (0.4) | 0.87 (0.4) |
| Mean progesterone, days 2 to 10 (pg/ml, log 10) | 1.25 (0.5) | 1.17 (0.5) | 1.54 (0.4) | 1.27 (0.5) | 1.27 (0.6) |

[a]Missing information on parity or pair status for 1 woman.
[b]Includes both women with and without children.
*Paired and unpaired with children differ significantly from paired and unpaired without children (F=24.397, p<0.001)
† Paired with children differs significantly from unpaired without children (F=7.701, p<0.001).

| **SAMPLE 2: Older women aged 35-50** | | | | | |
|---|---|---|---|---|---|
| | Whole sample (*n* = 117) | Paired with children (*n* = 80) | Paired without children (*n* = 9) | Unpaired with children (*n* = 17) | Unpaired without children (*n* = 11) |
| **Demographic information** | | | | | |
| Age (years) | 40.54 (3.9) | 40.13 (3.6) | 39.40 (4.0) | 42.70 (4.7) | 41.16 (4.6) |
| BMI (kg/m$^2$) | 26.71 (4.2) | 27.44 (4.4) | 24.00 (2.9) | 26.15 (3.5) | 24.53 (3.4) |
| % British women of European origin | 24.0 | 3.8 | 66.7 | 47.1 | 100.0 |
| % Bangladeshi | 76.0 | 96.2 | 33.3 | 52.9 | 0.0 |
| Number of children | 2.62 (1.8)[b] | 3.28 (1.4) | | 2.65 (1.7) | |
| Age of youngest child (years) | 9.32 (4.7) | 9.04 (4.7) | | 11.20 (4.3) | |
| % w/ child ≤ 3 years of age | 6.0%[b] | 7.5 | | 5.9 | |
| Mean estradiol, days 4 to 6 (pmol/L, log10) | 1.68 (0.2) | 1.68 (0.2) | 1.56 (0.2) | 1.68 (0.3) | 1.76 (0.2) |

Table 2 Multiple Linear Regressions for Sample 1 (Younger women aged 18-41) and Sample 2 (Older women aged 35-50).

| | **Unadjusted Estimate** | | **Adjusted Estimate[a] Without Interaction Terms** | | **Adjusted Estimatea With Interaction Terms** | |
|---|---|---|---|---|---|---|
| | B | 95% CI | B | 95% CI | B | 95% CI |
| **SAMPLE 1: Younger women aged 18-41** | | | | | | |
| **MODEL E1.1 (Averaged E2) Nulliparous Women** | | | | | | |
| **Covariates** | | | | | | |
| Pair Status | -0.198 | -0.427, 0.031 | -0.172 | -0.413, 0.069 | -0.305 | -1.137, 0.526 |
| Age | | | -0.010 | -0.032, 0.013 | -0.011 | -0.034, 0.013 |
| Body Mass Index | | | 0.000 | -0.019, 0.020 | 0.000 | -0.019, 0.020 |
| Group | | | -0.128 | -0.391, 0.136 | -0.138 | -0.409, 0.134 |
| Ethnicity | | | -0.239 | -0.530, 0.052 | -0.353 | -1.094, 0.388 |
| Interaction Pair Status * Ethnicity | | | | | 0.085 | -0.421, 0.590 |
| | | | | | | |
| **MODEL P1.1 (Averaged P4) Nulliparous Women** | | | | | | |
| Pair Status | 0.209 | -0.039, 0.619 | 0.090 | -0.206, 0.386 | 0.081 | -0.944, 1.105 |
| Age | | | 0.001 | -0.027, 0.029 | 0.001 | -0.028, 0.030 |
| Body Mass Index | | | -0.019 | -0.043, 0.005 | -0.019 | -0.043, 0.005 |
| Group | | | **0.686†** | **0.362, 1.011** | **0.686†** | **0.351, 1.020** |
| Ethnicity | | | 0.010 | -0.349, 0.368 | 0.002 | -0.911, 0.915 |
| Interaction Pair Status * Ethnicity | | | | | 0.006 | -0.617, 0.628 |
| | | | | | | |
| **MODEL E1.2 (Averaged E2) Parous Women** | | | | | | |
| Parity Status | 0.009 | -0.176, 0.194 | 0.008 | -0.206, 0.221 | 0.612 | -0.563, 1.786 |
| Age | | | 0.013 | -0.011, 0.036 | 0.011 | -0.012, 0.035 |
| Body Mass Index | | | -0.008 | -0.032, 0.016 | -0.008 | -0.032, 0.016 |
| Group | | | -0.065 | -0.297, 0.168 | -0.069 | -0.301, 0.164 |
| Ethnicity | | | 0.166 | -0.156, 0.488 | 0.735 | -0.399, 1.870 |
| Interaction Parity Status * Ethnicity | | | | | -0.317 | -0.923, 0.289 |
| | | | | | | |
| **MODEL P1.2 (Averaged P4) Parous Women** | | | | | | |
| Parity Status | 0.083 | -0.142, 0.308 | 0.109 | -0.141, 0.360 | 0.138 | -1.249 1.525 |
| Age | | | -0.016 | -0.043, 0.012 | -0.016 | -0.044, 0.012 |
| Body Mass Index | | | -0.012 | -0.040, 0.016 | -0.012 | -0.041, 0.017 |
| Group | | | -0.025 | -0.297, 0.248 | -0.025 | -0.299, 0.250 |
| Ethnicity | | | **-0.399*** | **-0.776, -0.021** | -0.371 | -1.711, 0.968 |
| Interaction Parity Status * Ethnicity | | | | | -0.015 | -0.731, 0.701 |
| | | | | | | |
| **MODEL E1.3 (Randomized E2) All Women** | | | | | | |
| Pair Status | -0.228 | -0.470, 0.013 | -0.238 | -0.493, 0.016 | -0.267 | -1.123, 0.588 |
| Parity Status | | | | | | |
| Children ≤3 year | **0.429**** | **0.125, 0.733** | **0.405**** | **0.088, 0.721** | 0.108 | -0.366, 0.582 |
| Children >3 years | **0.284*** | **0.030, 0.538** | 0.214 | -0.080, 0.509 | -0.381 | -1.183, 0.420 |
| No Children | REFERENCE | | REFERENCE | | REFERENCE | |
| Age | | | 0.003 | -0.020, 0.025 | 0.000 | -0.022, 0.023 |
| Body Mass Index | | | 0.017 | -0.003, 0.038 | 0.015 | -0.006, 0.036 |
| Group | | | -0.151 | -0.380, 0.077 | -0.144 | -0.372, 0.084 |
| Ethnicity | | | 0.216 | -0.503, 0.071 | -0.188 | -0.971, 0.596 |
| Interaction Pair Status * Ethnicity | | | | | -0.009 | -0.523, 0.505 |
| Interaction Parity Status * Ethnicity | | | | | 0.190 | -0.053, 0.432 |
| | | | | | | |
| **MODEL P1.3 (Averaged P4) All Women** | | | | | | |
| Pair Status | 0.061 | -0.161, 0.282 | 0.182 | -0.031, 0.395 | -0.206 | -0.933, 0.521 |
| Parity Status | | | | | | |
| Children ≤3 year | 0.200 | -0.479, 0.079 | -0.169 | -0.433, 0.096 | -0.245 | -0.644, 0.155 |

| | | | | | | |
|---|---|---|---|---|---|---|
| Children >3 years<br>No Children | -0.133<br>REFERENCE | -0.364, 0.097 | -0.218<br>REFERENCE | -0.370, 0.114 | -0.220<br>REFERENCE | -0.903, 0.464 |
| Age | | | -0.002 | -0.020, 0.017 | -0.004 | -0.022, 0.015 |
| Body Mass Index | | | -0.015 | -0.032, 0.002 | -0.017 | -0.034, 0.000 |
| Group | | | **0.305†** | **0.121, 0.488** | **0.304**** | **0.120, 0.488** |
| Ethnicity | | | **-0.250*** | **-0.486, -0.014** | -0.572 | -1.227, 0.084 |
| Interaction Pair Status * Ethnicity | | | | | **0.022†** | **-0.182, 0.226** |
| Interaction Parity Status * Ethnicity | | | | | **0.233†** | **-0.201, 0.667** |
| | | | | | | |
| **SAMPLE 2: Older women aged 35-50** | | | | | | |
| **MODEL E2.1 (E2 Sampled Days 4-6) All Women** | | | | | | |
| Pair Status | -0.039 | -0.169, 0.090 | 0.016 | -0.121, 0.153 | 0.168 | -0.007, 0.343 |
| Parity Status<br>Children ≤3 year<br>Children >3 years<br>No Children | <br>-0.213<br>0.002<br>REFERENCE | <br>-0.444, 0.019<br>-0.133, 0.138 | <br>-0.026<br>0.129<br>REFERENCE | <br>-0.290, 0.239<br>-0.044, 0.302 | <br>-0.076<br>0.077<br>REFERENCE | <br>-0.351, 0.199<br>-0.116, 0.269 |
| Age | | | 0.002 | -0.011, 0.015 | 0.000 | -0.013, 0.013 |
| Body Mass Index | | | -0.009 | -0.020, 0.003 | -0.009 | -0.020, 0.003 |
| Group | | | -0.087 | -0.200, 0.027 | -0.090 | -0.200, 0.020 |
| Ethnicity | | | **0.217*** | **0.021, 0.413** | **0.630*** | **0.113, 1.147** |
| Interaction Pair Status * Ethnicity | | | | | **-0.320*** | **-0.590, -0.050** |
| Interaction Parity Status * Ethnicity | | | | | 0.073 | -0.040, 0.186 |

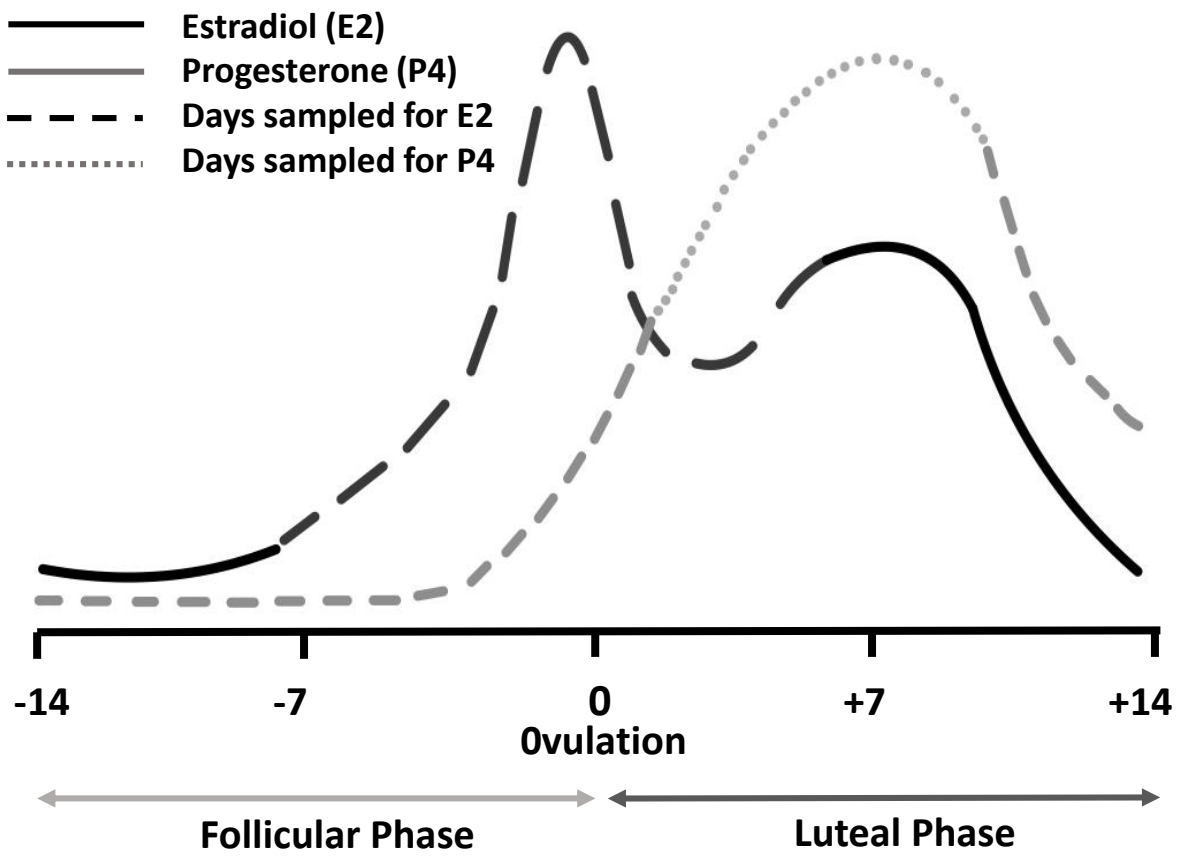
*P<0.05; **p<0.01; † p<0.001

[a] Adjusted models include other covariates known to affect reproductive steroid hormone levels

Categorical variables: Pair status coded as 1=unpartnered, 2=partnered; Parity is coded as 0=no children, 1=children ≤3, 2=children >3; Group is coded as 1=child migrants, second-generation British-Bangladeshis and women of European origin, and 2=sedentees and adult migrants; Ethnicity is coded as 1=women of European origin and 2=of Bangladeshi origin. Both Age and BMI are linear variables

Figure

Click here to access/download;Figure;Fig 1_R3.pptx



8 September 2022

Professor Louis Calistro Alvarado
Main Editor
Human Nature

**Re: HUNA-D-21-00047R1 Review**

Dear Professor Calistro Alvarado,

On behalf of my co-authors and myself, I am submitting a revised version of our manuscript "Relationship of estradiol and progesterone with partnership and parity among Bangladeshi and British women of European origin".

We would like to thank the reviewers for their thoughtful comments and suggestions towards improving our manuscript. We address comments specific to each reviewer below.

**Reviewer #1:**

The authors have responded fairly thoroughly to my requests and comments on the original version of the manuscript. I have only a couple comments remaining.

I understand that the authors made some changes to their introduction in response to Reviewer 2, who suggested leading with the lack of theoretical rationale for expecting links between social relationship status and ovarian hormones. I find this framing a little strange—a reader might reasonably ask what the point of this paper is, if the premise is "we don't think this link has reason to exist, but we're going to look anyway". Implicit in what the authors write is the fact that other researchers HAVE argued there are reasons to expect these associations, and in some cases they've found them. The authors also follow these other researchers closely in their methods (e.g. Barrett et al., 2015). I won't ask the authors to reverse their revisions, but the introduction does need to explain why others hypothesize there might be a link between ovarian hormones and social relationship status, and briefly summarize those results. This then naturally leads into a) the hypotheses the
authors test and b) the authors' skepticism and the value of their present analyses. This should be a straightforward edit, as the authors already have a good bit of this in their discussion on p. 14—this element just needs to be moved to the intro and framed appropriately so a reader can understand the rationale for the present study.

***We have now revised the Introduction following these suggestions to make more clear the history of research into this area of social endocrinology.

I asked the authors in my first review to include some simple bar graphs for model-estimated hormone concentrations by group. They responded "since none of the models revealed significant results in relation to social relationships that could not be explained from our wider knowledge of the data we did not feel it was necessary to make these kinds of graphs". I have a couple of responses to this. First, this makes the mistake of assuming that there is no information to be gleaned from a non-significant parameter estimate—a fallacy I also pointed out in my last review. Given that past studies have shown results in either direction (either higher or lower ovarian hormones as a function of parity and/or

relationship status), I maintain it would be valuable for the reader to be able to see how similar these groups are, and/or how much variation there is within group—this is, after all, central to the authors' argument. Second, now that there are interaction terms in these
models, some of which are significant in certain models, it's no longer the case that these group differences are simply non-significant. In some cases, interaction terms suggest contingently significant group differences (e.g. P1.3; though sometimes significance changes depending on e.g. inclusion of covariates—I leave it to the authors to decide which models are most worth highlighting)—what is the form of these interactions? Marginal means and standard errors could be reconstructed from the tables by a patient reader, but a paper should make the reader's job easy, and long regression tables are much less intuitive than some simple graphs showing model-estimated means and standard errors by group. I repeat my request, which I don't consider very onerous, that these figures be included.

***We consulted with two sets of statisticians in two different institutions concerning these requests since we remained uncertain about the rationale for this request above. We received the same response, namely, that regressions are not normally modelled in this way and that it would not be particularly meaningful to plot the marginal means and SEs for non-significant models. We have therefore followed the advice from these independent statisticians not to provide additional plots when the information is provided here by the standard regression tables.

**Reviewer #2:**

Thank you for your careful revisions. The paper ( reported methods specifically) is significantly improved and much more focused. I think it would be helpful in the abstract to state your hypotheses (rather than the lack of theoretical foundation) so the reader know what hypotheses were not supported.

***The abstract has now been revised to include the hypotheses more explicitly.

I also think when you discuss 'mate choice' it would be helpful to discuss who is choosing whom.. For example in the introduction in the third paragraph, line 36 on the references are mostly on studies about males being able to detect ovulatory cues in females - that might be slightly different than mate choice.

***In this paragraph, we used the term “mate attraction” which is different from mate choice. We also did not feel that further detail on this was particularly relevant to our paper here and so decided not to elaborate further.

I would also appreciate more detailed table labels. I had to flip back and forth to remember who made up each sample and which analysis. It might be more clear to label the groups as grew up in Bangladesh and Grew up in UK then add the words pair status and parity (rather than numbers) it just makes for easier reading.

***We added the description for samples 1 and 2 in both, tables and in relevant sections of the paper for easier reading. Description of each model in Table 2 is self-explanatory (hormone measured, sample strategy and group of women in relation to pair status and parity included).

Lastly this recent ( pre-print) evaluation of the validity of salivary hormones may salient to your discussion: ( I have no affiliation with it)

Arslan RC, Blake K, Botzet L, Bürkner PC, DeBruine L, Fiers T, Grebe N, Hahn A, Jones BC,

Mumford SL, Penke L. Not within spitting distance: salivary immunoassays of estradiol have subpar validity for cycle phase.

***We read this paper and because it discusses salivary analyses where daily samples were not assayed for specific phases of the cycle, it is of less relevance for our purposes, but thank you for bringing it to our attention.

I do value studies with negative findings so I appreciate you bringing this research to press

***Thank you for this kind comment.

Thank you for your consideration,

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