Original Research Article

Prenatal Hormones in First-Time Expectant Parents: Longitudinal Changes and Within-Couple Correlations

ROBIN S. EDELSTEIN,[1]* BRITNEY M. WARDECKER,[1] WILLIAM J. CHOPIK,[1] AMY C. MOORS,[1,2] EMILY L. SHIPMAN,[1] AND NATALIE J. LIN[1]
[1]*Department of Psychology, University of Michigan, Ann Arbor, Michigan 49109*
[2]*Department of Women's Studies, University of Michigan, Ann Arbor, Michigan 48109*

Objectives: Expectant mothers experience marked hormone changes throughout the transition to parenthood. Although similar neuroendocrine pathways are thought to support maternal and paternal behavior, much less is known about prenatal hormone changes in expectant fathers, especially in humans.

Methods: We examined longitudinal changes in salivary testosterone, cortisol, estradiol, and progesterone in 29 first-time expectant couples ($N = 58$). Couples were assessed up to four times throughout the prenatal period, at approximately weeks 12, 20, 28, and 36 of pregnancy. We also examined within-couple correlations in hormones. Data were analyzed using dyadic growth curve modeling.

Results: As expected, women showed large prenatal increases in all four hormones. Men showed significant prenatal declines in testosterone and estradiol, but there were no detectable changes in men's cortisol or progesterone. Average levels of cortisol and progesterone were significantly positively correlated within couples.

Conclusions: The current study represents one of the most extensive investigations to date of prenatal hormones in expectant couples. It is also the first study to demonstrate prenatal testosterone changes in expectant fathers and within-couple correlations in progesterone. We discuss implications of these findings for parental behavior and adjustment. Am. J. Hum. Biol. 27:317–325, 2015.

The transition to parenthood is a major developmental milestone that brings significant, long-lasting changes for new parents and their intimate relationships (Cowan and Cowan, 2000). Expectant mothers, in particular, experience marked physiological changes during pregnancy and the postpartum, including large prenatal increases in hormones such as testosterone, cortisol, estradiol, and progesterone. Many of these hormonal changes have long-term implications for women and their families. For instance, very high levels of prenatal cortisol have been linked with poor infant and child outcomes (Davis and Sandman, 2010).

Much less is known about prenatal hormone changes among expectant fathers, however, especially in humans. Wynne-Edwards and colleagues propose that paternal behavior involves the activation of the same neuroendocrine pathways generally associated with maternal care (Wynne-Edwards, 2001; Wynne-Edwards and Reburn, 2000). Insofar as similar hormones are involved in both maternal and paternal behavior (Storey and Walsh, 2011), men's hormones might also show changes during the transition to parenthood. Extant research provides some support for this hypothesis. For instance, new fathers typically show pre- to postnatal declines in testosterone (Berg and Wynne-Edwards, 2001; Gettler et al., 2011).

However, of the few studies that have examined prenatal hormones among human fathers, most are cross-sectional, and all have included relatively small samples of fathers (ranging from 9 to 37) and/or hormones (often only testosterone). Moreover, to our knowledge, only one study has examined men's hormone changes longitudinally throughout the prenatal period (Berg and Wynne-Edwards, 2001). Thus, it is not yet clear whether hormone changes associated with fatherhood are limited to the postnatal period or whether they might begin prenatally.

In addition, because only two studies have included both expectant mothers and fathers (Berg and Wynne-Edwards, 2001; Storey et al., 2000), very little is known about the correspondence between expectant parents' hormone levels. Evidence of within-couple correlations would provide further support for the idea that similar neuroendocrine pathways support maternal and paternal behavior. Such evidence might also suggest a synchrony or interdependence between partners' prenatal physiological responses (Berg and Wynne-Edwards, 2001).

The primary goal of the current study was to examine longitudinal changes in prenatal hormones among first-time expectant parents. We extended and addressed limitations of prior research by: (1) including a larger sample of expectant couples ($N = 29$) than in prior longitudinal research, (2) assessing salivary testosterone, cortisol, estradiol, and progesterone in both couple members, and (3) examining changes over multiple prenatal time points (up to 4 and beginning in the first trimester). Our second goal was to examine the extent to which couple members' hormones were correlated throughout the prenatal period.

We focused on testosterone, cortisol, estradiol, and progesterone because these hormones show large prenatal changes in pregnant women and have important

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*Correspondence to: Robin Edelstein, Department of Psychology, University of Michigan, 530 Church Street, Ann Arbor, MI 48109.
E-mail: redelste@umich.edu

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implications for parental behavior (Fleming et al., 1997; Wynne-Edwards and Reburn, 2000). In the following sections, we briefly describe the evidence for prenatal changes in each hormone, as well as any evidence for within-couple correlations in hormone levels.

PRENATAL HORMONES

Testosterone

Testosterone is associated both with aggression and parental care (at higher vs. lower levels, respectively; van Anders et al., 2011; Wingfield et al., 1990). Women's testosterone increases during pregnancy and declines gradually after birth (Fleming et al., 1997). Increases in testosterone are thought to contribute to the maintenance of pregnancy and the initiation of parturition (Makieva et al., 2014); higher levels of maternal testosterone may also facilitate infant protection (Wynne-Edwards and Reburn, 2000).

Cross-sectional research consistently demonstrates that fathers have lower testosterone than non-fathers (Gray et al., 2006; Perini et al., 2012). These findings have been bolstered by longitudinal research indicating that men's testosterone in fact declines when they become fathers (Berg and Wynne-Edwards, 2001; Gettler et al., 2011), and that such changes are most pronounced among men who are more directly involved in infant care (Gettler et al., 2011). Post-birth declines in testosterone are thought to support paternal care by reducing aggression toward infants, focusing attention away from mating effort, and/or facilitating paternal attachment (Wynne-Edwards, 2001). However, prior research cannot definitively speak to *prenatal* changes in testosterone. In a relatively small subsample of expectant fathers ($n = 11$) who had data available throughout the prenatal period, Berg and Wynne-Edwards (2001) did not find evidence for prenatal changes in salivary testosterone (including in their reanalysis of nine fathers from this sample; Berg and Wynne-Edwards, 2002). Gettler et al. (2011) assessed longitudinal changes pre- to postnatally, but included only one prenatal assessment of salivary testosterone. Thus, the extent and timing of men's testosterone changes during the prenatal period are not yet clear.

To date, the only study that has assessed between-partner correlations in prenatal testosterone revealed no significant correlations in expectant couples (Berg and Wynne-Edwards, 2002). However, other research on couples with children provides some evidence that testosterone levels may be positively associated between partners (Booth et al., 2005).

Cortisol

Cortisol is a stress hormone that is particularly responsive to social stressors and challenges (Dickerson and Kemeny, 2004). In women, cortisol increases throughout pregnancy and gradually declines postpartum (Davis et al., 2007; Fleming et al., 1997). Higher levels of postpartum maternal cortisol have been associated with affectionate and approach-related behavior toward infants (Fleming et al., 1987), suggesting that cortisol may facilitate maternal behavior by preparing mothers for the challenge of caregiving (Mileva-Seitz and Fleming, 2011).

In their cross-sectional study of expectant fathers, Storey et al. (2000) found that serum cortisol levels were lower among men whose partners were earlier in their pregnancy (weeks 16–35; $n = 12$) compared with those who were later in their pregnancy (weeks 35–40; $n = 8$). A separate longitudinal sample of expectant fathers ($n = 10$) revealed a similar increase in men's salivary cortisol levels during the last week of pregnancy (Berg and Wynne-Edwards, 2001; see also Berg and Wynne-Edwards, 2002). Thus, there is some evidence that expectant fathers' cortisol levels may increase close to the delivery, perhaps in preparation for caregiving (Storey and Walsh, 2011).

Both Storey et al. (2000) and Berg and Wynne-Edwards (2002) found that prenatal cortisol levels were positively correlated within couples. Research on couples with children similarly suggests significant within-couple correlations in cortisol (Saxbe and Repetti, 2010; Rodriguez and Margolin, 2013).

Estradiol

Estradiol is associated with caregiving and bonding in humans and other mammals (Mileva-Seitz and Fleming, 2011). Estradiol has also been linked with individual differences in desire for and responses to emotional closeness (Edelstein et al., 2010, 2012). In women, estradiol increases markedly during pregnancy, spikes just before birth, and drops precipitously thereafter (Fleming et al., 1997; Storey et al., 2000). Pre-birth increases in estradiol are thought to be important for the onset of maternal behavior and for maternal attachment (Wynne-Edwards and Reburn, 2000).

In their longitudinal sample, Berg and Wynne-Edwards (2001) did not find evidence for prenatal changes in men's estradiol; however, there was an increase in the number of men with detectable estradiol levels in the weeks following delivery (but not in the subsample reported in Berg and Wynne-Edwards, 2002). New fathers also had higher estradiol levels than a comparison sample of men without children (Berg and Wynne-Edwards, 2001), again suggesting an increase in estradiol as a function of fatherhood. Such findings are consistent with evidence that estradiol facilitates paternal behavior in some animal species (California mice, Trainor and Marler, 2002); however, estradiol can inhibit paternal behavior in other species (prarie voles, Cushing et al., 2008). Moreover, the role of estradiol in human paternal behavior is not yet well understood (Wynne-Edwards and Reburn, 2000), limiting our ability to make strong predictions about prenatal changes in men's estradiol.

Berg and Wynne-Edwards (2002) did not find significant between-partner correlations in estradiol in their sample of expectant parents. To our knowledge, there are no other data on such correlations among expectant parents or couples more generally.

Progesterone

Progesterone is associated with social closeness, maternal behavior, and affiliation in humans and other mammals (Numan and Insel, 2003). In the laboratory, manipulations that increase people's desire for affiliation or need for social closeness also increase progesterone levels (Brown et al., 2009; Schultheiss et al., 2004). Progesterone also increases in response to stress, and is thought to down-regulate physiological stress responses (Wirth, 2011). Like estradiol, progesterone shows large increases in pregnant women, followed by sharp postpartum declines (Fleming et al., 1997). Perinatal declines in

progesterone, in concert with increases in estradiol, are thought to facilitate the onset of maternal behavior in many mammals (Wynne-Edwards and Reburn, 2000).

Despite the importance of progesterone for maternal behavior and social bonding, relatively little is known about the role of progesterone among new or expectant fathers, particularly in humans (Fernandez-Duque et al., 2009; Wynne-Edwards, 2001). To our knowledge, there are no existing data on prenatal changes in progesterone among expectant fathers and no data on between-partner correlations in progesterone.

THE CURRENT STUDY

In this study, we examined neuroendocrine changes among first-time expectant parents. Couples were assessed between two and four times throughout the prenatal period (ranging from weeks 10 to 38). We focused on first-time parents because previous research suggests that their experiences differ in important ways from those of more experienced parents (Condon and Esuvaranathan, 1990) and because most prior studies of perinatal hormone changes were restricted to first-time parents (Berg and Wynne-Edwards, 2002; Fleming et al., 1997). Prior research provides strong support for the hypothesis that expectant mothers would show prenatal increases in testosterone, cortisol, estradiol, and progesterone; however, this body of research is less clear regarding changes among expectant fathers. We expected prenatal declines in testosterone and increases in cortisol among expectant fathers; examination of prenatal changes in men's estradiol and progesterone were considered exploratory.

We also examined whether mean levels of each hormone were correlated between partners throughout the prenatal period. Given prior findings, we expected that testosterone and cortisol levels would be positively correlated within dyads, but we did not have a basis for predicting within-couple correlations in estradiol and progesterone.

METHOD

Participants

Participants were 58 individuals (29 couples) who were part of a larger study of neuroendocrine and psychological changes among first-time parents; other data from this project have not yet been published. Couples were recruited via online and print advertisements and they received $25 per session ($50/couple) for participating. To be eligible, both partners had to be between the ages of 18 and 45 (because of age-related changes in hormones; Leifke et al., 2000), living together, expecting their first child, and within the first two trimesters of pregnancy. Two male participants had a child from a previous relationship, but this was the first child together for all couples and the first pregnancy for all female participants. In addition, all but one pregnancy was singleton; results were virtually identical when data from the one couple expecting twins were excluded.

Smokers, people with medical conditions that could influence hormones (e.g., autoimmune disorders), and/or those taking hormone-altering medications (e.g., some psychiatric medications) were not eligible (see Schultheiss and Stanton, 2009). Three additional couples began the study but are not included here because they: (1) were not in fact first-time parents, (2) terminated the pregnancy because of chromosomal abnormalities, or (3) did not respond to our requests to schedule subsequent sessions.

Women in the current sample ranged in age from 20 to 38 ($M = 29.41$ years, $SD = 3.70$); men ranged in age from 21 to 42 ($M = 30.48$ years, $SD = 4.01$). Participants self-reported their race/ethnicity as 74.1% Caucasian, 3.4% Black or African American, 6.9% Asian American, 5.2% Hispanic, and 5.2% mixed or other ethnicities (5.2% did not report their race/ethnicity). The majority of couples were married or engaged (90%). Median household income was $50,000-$75,000 and 69% of participants had at least a college degree.

Procedure

All procedures were reviewed and approved by the University of Michigan Institutional Review Board. Prenatal laboratory sessions were scheduled, according to couples' due dates, at approximately 8-week intervals (roughly weeks 12, 20, 28, and 36 gestation). These intervals were modeled after those used by Fleming et al. (1997), who aimed to encompass each trimester and the very end of pregnancy (assessing women at 0–16 weeks, 20–27 weeks, 28–35 weeks, and 36–42 weeks); however, we began our study at 12 weeks (because of difficulty recruiting couples earlier in the first trimester) and we targeted the beginning of the ranges used by Fleming for subsequent sessions. Couples were tested throughout the year, with initial sessions occurring between July 2011 and November 2012. Several couples began the study during the second trimester of pregnancy, and some did not complete the last session because their baby was born before their scheduled session, so there was some variability in the number of sessions completed by each couple ($M = 3.62$ sessions; $SD = 0.62$). Three couples completed two sessions, seven couples completed three sessions, and 19 couples completed all four sessions. As described in more detail below, we accounted for the variability in week of assessment by using week of pregnancy (e.g., week 12, 13) as our measure of time in subsequent analyses, rather than session number (e.g., session 1, 2).

Couple members came to the laboratory together for each session. Sessions were conducted on the same day of the week at the same time (as possible) for each couple to control for diurnal and day-to-day variations in hormone levels. Because hormone levels are most stable in the afternoon to evening hours (Schultheiss and Stanton, 2009), all couples were tested between 12:30 h and 18:30 h. We also controlled for time of day in our analyses. Informed consent was obtained during the initial session and participants were told that they could withdraw from the study at any time without penalty. During each session, participants provided two saliva samples to assess hormone levels–the first after a 20-minute adaptation period and the second 20 minutes later–to increase measurement reliability. Participants also completed several questionnaires (e.g., assessing personality and relationship quality) that are not considered here (We did not find any reliable associations between participants' personality traits or prenatal relationship quality and changes in hormones during the prenatal period. Thus, we do not discuss these variables further in the current report).

Salivary hormones: collection and assessment

Participants were asked to refrain from eating, drinking (except for water), smoking, or brushing their teeth

for 1 h before the beginning of each session. After rinsing their mouths with water, participants used polypropylene tubes to provide two 7.5 mL saliva samples during each of the in-laboratory sessions. Samples were frozen in our laboratory until further processing in the University of Michigan Core Assay Facility. Testosterone, cortisol, and progesterone were assayed by radioimmunoassay (RIA), using commercially available kits from Siemens; estradiol was assayed by enzyme-linked immunosorbent assay (EIA), using commercially available kits from Salimetrics, Inc.

For testosterone, the inter-assay coefficient of variation (CV) was 5.26% and 14.97% at high and low levels, respectively; the intra-assay CV was 9.86%. Analytical sensitivity (B_0-2 SD) for testosterone was 1.14 pg/mL. The inter-assay CV for cortisol was 14.23% and 5.01% at high and low levels, respectively; the intra-assay CV was 7.31%. Analytical sensitivity (B_0-2 SD) for cortisol was 0.09 ng/mL. The inter-assay CV for estradiol was 14.69% and 14.39% at high and low levels, respectively; the intra-assay CV was 4.60%. Analytical sensitivity (B_0-2 SD) for estradiol was 0.10 μg/dL. The inter-assay CV for progesterone was 8.68% and 5.32% for high and low levels, respectively; the intra-assay CV was 13.43%. Analytical sensitivity (B_0-2 SD) for progesterone was 6.08 pg/mL. Samples were assayed in duplicate, and the average of duplicates was taken.

Hormone values were averaged for each participant and session for each of the four hormones; correlations between the two samples ranged from 0.92 to 0.98. Two participants (one female, one male) were missing data for one time point each for progesterone due to insufficient sample volume. Average hormone values were inspected for outliers, separately by gender and session. To maximize the use of all available data, hormone values that were larger than three standard deviations above the mean for each gender and session were replaced with values corresponding to three standard deviations above the mean for that particular variable (i.e., Winsorized; Reifman and Keyton, 2010; see also Edelstein et al., 2014, for a similar approach). Eleven values were replaced using this approach (1.3% of the total 822 samples): two for testosterone (both male), three for cortisol (all male), four for estradiol (two female, two male), and two for progesterone (one male, one female). Distributions of estradiol and progesterone were highly skewed (skewness and kurtosis values > 2.0), so these variables were log-transformed before analyses. Except as noted below, results were virtually identical when the raw hormone values were used instead of the Winsorized values and when analyses were conducted using untransformed estradiol and progesterone values.

Overview of statistical analyses

The Statistical Package for the Social Sciences (SPSS, version 21) was used to conduct all analyses. Our data has a multilevel structure: participants were assessed repeatedly over time and are nested within dyads, which means that individual observations cannot be treated as independent. To account for this multilevel structure and to model the interdependence of individuals within dyads, we computed dyadic growth curve models using multilevel modeling (MLM) procedures established for dyadic data analysis with repeated measures (i.e., SPSS Mixed; Kenny et al., 2006). Multilevel models are statistical techniques based on the linear mixed model, and they have several advantages over more traditional repeated measures analyses (see Kristjansson et al., 2007). For instance, in traditional repeated-measures ANOVA, only individuals with complete data (e.g., observations at each of several time points) would be included in estimates of change. Moreover, it is not possible to account for differences in the spacing of assessments between participants using traditional ANOVA. Multilevel models, in contrast, can accommodate missing data and observations that are unevenly spaced (e.g., assessments that occur at different weeks for different participants); thus, they provide more powerful and reliable estimates of changes over time. In addition, ANOVA models estimate only between-person differences (e.g., average changes in testosterone over time) and treat within-person differences (e.g., individual differences in the magnitude of change) as sources of error. Multilevel models use all of the available data to estimate changes both at the group-level and at the individual-level, treating the latter as meaningful as opposed to as error. Dyadic growth curve models are an extension of multilevel models that provide estimates of change over time while accounting for the statistical dependence of related individuals (e.g., couples; Kashy and Donnellan, 2008).

Following recommended procedures, participant gender was contrast coded (-1 = male, 1 = female). As described above, we used week of pregnancy, rather than session number, as our repeated measure of time. Using this metric, the estimate (slope) for time corresponds to the extent to which a particular hormone changes over time (by week). We centered the time variable at the study midpoint (week 24 of pregnancy); thus, values for the intercept in each model correspond to an individual's average hormone levels at the study midpoint. A significant slope for a particular variable indicates that change over time in that variable is significantly different from zero; as in a traditional regression analysis, a significant intercept for a particular variable indicates that average levels of that variable are significantly different from zero (which is not particularly informative in our case, but may be in others).

We used the two-intercept model, a derivation of the dyadic growth curve model, to estimate separate trajectories for men and women (Kashy and Donnellan, 2008). This approach allowed us to calculate within-dyad correlations in both hormones (covariances between couple members' intercepts) and changes in hormones (covariances between couple members' slopes). Both intercepts and slopes were treated as random (i.e., allowed to vary across individuals); however, preliminary analyses revealed very little variability in men's slopes, which limited our ability to test for correlated changes within couples. Thus, these covariances were set to zero and we report only within-couple correlations in intercepts. In addition, to limit the number of parameters in the model, and because there was limited evidence of intercept-slope correlations, these covariances were also constrained to zero.

RESULTS

Prenatal changes in hormones in women and men

Descriptive statistics for each hormone are presented in Table 1 by gender and session. These data are presented

TABLE 1. Descriptive statistics for hormones by gender and time point

	Time 1 ($n = 23$) *M* (SD)	Time 2 ($n = 27$) *M* (SD)	Time 3 ($n = 28$) *M* (SD)	Time 4 ($n = 25$) *M* (SD)
Week of pregnancy	12.78 (1.95)	21.15 (1.73)	28.71 (1.59)	36.28 (1.16)
Women				
Testosterone (pg/mL)	9.89 (4.80)	16.25 (7.74)	23.47 (11.79)	54.15 (24.30)
Cortisol (ng/dL)	1.04 (0.35)	1.47 (0.75)	2.03 (0.81)	2.52 (1.16)
Estradiol (ug/dL)	6.69 (2.59)	20.64 (8.96)	36.15 (14.60)	80.96 (42.84)
Progesterone (pg/mL)	229.76 (57.15)	402.19[a] (125.69)	710.25 (286.59)	1328.15 (579.68)
Men				
Testosterone (pg/mL)	50.23 (11.25)	49.79 (16.54)	48.45 (14.32)	47.62 (17.09)
Cortisol (ng/dL)	1.10 (0.70)	1.08 (0.75)	0.92 (0.48)	1.20 (0.91)
Estradiol (ug/dL)	2.34 (0.72)	2.24 (0.74)	2.25 (0.83)	2.13 (0.87)
Progesterone (pg/mL)	10.21 (4.61)	11.32 (6.99)	10.14 (5.95)	10.59[b] (6.12)

[a]$n = 26$; [b]$n = 24$.

for broad descriptive purposes only; it is important to note that our analyses of change account for week of measurement, rather than aggregating data for each session.

To examine changes over time, we conducted separate multilevel models predicting changes in each hormone. We tested the linear effects of time and included time of day as a covariate. Results from these analyses, reported as unstandardized regression weights, are presented in Table 2. Consistent with prior research, we found that expectant mothers showed large prenatal increases in testosterone, cortisol, estradiol, and progesterone. Also, as predicted, expectant fathers showed a significant prenatal decline in testosterone; however, contrary to our expectations, there were no significant changes in men's cortisol, $P = 0.96$. Men's estradiol also showed a significant prenatal decline, but there were no significant changes in men's progesterone, $P = 0.28$ (As shown in Table 2, when untransformed values were used in the multi-level analyses, prenatal declines in men's estradiol were in the same direction but did not reach statistical significance, $P = 0.17$). Our findings were virtually identical when we statistically controlled for initial body mass index (BMI), average BMI across sessions, or participants' age; in the interest of parsimony, we report our findings without these covariates.

In sum, our findings for women replicate previous research on expectant mothers' prenatal hormone changes. Our findings for men support the hypothesis that testosterone declines prenatally in advance of fatherhood, but our findings differ from previous research with respect to prenatal changes in cortisol and estradiol.

Within-couple correlations in hormones

Results from our multilevel models also provide information about within-couple correlations in average hormone levels (i.e., covariances between intercepts, which reflect hormone values at the study midpoint). There was significant inter-individual variability in the intercepts for all four hormones, for both men and women, all P's < 0.05. Moreover, this variability was significantly correlated within-couples for cortisol, $r = 0.64$, $P < 0.05$, and progesterone, $r = 0.52$, $P < 0.05$. Intercepts were not significantly correlated within-couples for testosterone, $r = 0.21$, $P = 0.47$, or estradiol, $r = 0.13$, $P = 0.62$. In sum, all within-couple correlations were positive, but only couples' mean levels of cortisol and progesterone were significantly intercorrelated at the study midpoint.

To further examine within-couple correlations, we also correlated partners' hormone levels separately at each time point, statistically controlling for time of day. As shown in Table 3, all but two of the 20 correlations were positive, but only 5 reached or approached statistical significance. Correlations between *average* hormone levels across the study time points, also shown in Table 3, were consistent with our analyses of within-couple correlations in intercepts: mean levels of cortisol and progesterone were significantly correlated within couples. Within-couple correlations of average testosterone levels were

TABLE 2. Parameter estimates from dyadic growth curve models predicting hormone changes

	Women ($n = 29$)		Men ($n = 29$)	
Testosterone (pg/mL)	*b* (SE)	*b* (SE) Untransformed	*b* (SE)	*b* (SE) Untransformed
Intercept	23.90** (1.79)		49.76** (2.31)	
Slope	1.78** (0.21)		−0.25* (0.11)	
Cortisol (ng/dL)				
Intercept	1.73** (0.10)		1.07** (0.10)	
Slope	0.06** (0.01)		−0.0002 (0.01)	
Estradiol (μg/dL)				
Intercept	3.11** (0.05)	34.18** (2.46)	0.76** (0.05)	2.26** (0.12)
Slope	0.10** (0.004)	2.95** (0.33)	−0.01* (0.003)	−0.01 (0.01)
Progesterone (pg/mL)				
Intercept	6.21** (0.05)	620.67** (21.31)	2.23** (0.10)	11.29** (8.35)
Slope	0.07** (0.004)	45.29** (2.98)	−0.01 (0.005)	−0.07 (2.09)

Results are presented as unstandardized regression weights. Time of assessment was centered at the study midpoint (week 24 of pregnancy). All analyses include time of day as a covariate. Results for raw (untransformed) values are presented for variables that were log-transformed.
*$P < 0.05$, **$P < 0.01$.

TABLE 3. Within-couple correlations in hormones by time point

	Time 1 ($n = 23$)	Time 2 ($n = 27$)	Time 3 ($n = 28$)	Time 4 ($n = 25$)	Average across time points ($n = 29$)
Testosterone	−0.04	0.21	0.27	0.44*	0.32^{+}
Cortisol	−0.01	0.33	0.49**	0.24	0.40*
Estradiol	0.14	0.17	0.12	0.03	−0.03
Progesterone	0.21	0.40*[a]	0.44*	0.39[b+]	0.62**

Partial correlations controlling for time of day; values for estradiol and progesterone were log-transformed before analysis.
[a]$n = 26$ couples; [b]$n = 24$ couples; $^{+}$ $P \leq 0.10$, *$P \leq 0.05$, **$P < 0.01$.

marginally significant, and within-couple correlations of average estradiol levels were not statistically significant. Thus, our findings provide some evidence for interdependence between couple members' hormone levels, but suggest that this interdependence may be stronger for some hormones than others.

DISCUSSION

The current study represents the most extensive investigation to date of prenatal hormone changes in expectant couples. Expectant mothers' hormone changes have been well-documented (Fleming et al., 1997); however, much less is known about such changes among expectant fathers. Consistent with prior research (Fleming et al., 1997; Makieva et al., 2014), we found that women showed large prenatal increases in testosterone, cortisol, estradiol, and progesterone. Expectant fathers showed declines in testosterone and estradiol throughout the prenatal period, but we found no evidence for prenatal changes in men's cortisol or progesterone.

Our findings regarding men's testosterone are consistent with cross-sectional research indicating that fathers have lower testosterone than men without children (Gray et al., 2006; Perini et al., 2012). They are also consistent with longitudinal studies documenting pre- to postnatal declines in men's testosterone (Berg and Wynne-Edwards, 2001; Gettler et al., 2011). Pre- to postnatal changes are thought to reflect shifts in new fathers' focus toward caregiving and nurturant behavior (Gettler et al., 2011; van Anders et al., 2011). Postpartum declines in testosterone may also reflect other changes in the lives of new fathers, such as disruptions in sleep patterns (Rosenblatt et al., 1996), declines in sexual activity (Gettler et al., 2013), or simply the presence of an infant (van Anders et al., 2012). Some of the same environmental factors might be associated with prenatal changes in expectant fathers' hormones; for instance expectant fathers report declines in sexual activity even before the birth of their child (Bogren, 1991). The psychological, emotional, and behavioral changes that accompany first-time parenthood (Genesoni and Tallandini, 2009) might also lead to anticipatory changes in men's hormones.

Importantly, to our knowledge, our study is the first to document men's hormone changes prenatally. In one longitudinal study of new fathers, Perini et al. (2012) compared the testosterone levels of expectant fathers with men in committed relationships who did not have children. Men were assessed at two time points: 1 month before and 2 to 3 months following childbirth (for those expecting a child). At both time points, expectant/new fathers had lower testosterone than men without children, suggesting that men's testosterone may begin to decline before the arrival of the baby. Moreover, men did not show a significant decline in testosterone after becoming fathers, again suggesting that changes related to fatherhood may have begun earlier. Nevertheless, because Perini et al. (2012) included only one prenatal assessment, their findings cannot speak to prenatal *changes*. That expectant fathers had lower testosterone prenatally than men without children suggests men who desire or intend to have children might have lower testosterone levels than those who do not. For instance, there is some evidence that women who report greater "reproductive ambition" (e.g., liking children, possessing maternal characteristics) have lower endogenous testosterone levels (Deady et al., 2006); however, it is not clear whether such associations would also be observed among men. It is also worth noting that Gettler et al. (2011) found that single men who ultimately became partnered fathers had *higher* testosterone levels 4 years earlier compared with those who did not become fathers. These findings suggest that changes associated with pair-bonding and/or fatherhood contribute to the lower testosterone levels of fathers versus non-fathers, as opposed to pre-existing characteristics that might lower testosterone among men who intend to become fathers. Unfortunately, our study cannot speak to exactly when partnered men's testosterone declines in advance of fatherhood; these changes could occur soon after partnering or even before conception. However, our findings contribute to this literature by demonstrating that hormone changes associated with fatherhood may occur before birth and do not necessarily depend on the presence of a child.

Prior research also provides evidence for prenatal increases in men's cortisol, although such changes have been evident primarily in the very last days before birth (Berg and Wynne-Edwards, 2001; Storey et al., 2000). Thus, we may not have detected cortisol changes because not all of the men in our sample were assessed in such close proximity to the delivery. Or, perhaps we did not detect cortisol changes because of some unique characteristics of our sample, which was relatively educated, primarily Caucasian, and somewhat older than the average age of first-time parents (Martin et al., 2013). It is also important to note that all couples in our sample were living together, and the vast majority were engaged or married, indicating that our sample is not representative of most first-time parents (Martin et al., 2013). Thus, although our sample characteristics are similar to those of previous studies (Berg and Wynne-Edwards, 2001; Storey et al., 2000), our findings should be considered in light of the homogeneity of our sample, which may have limited individual differences in mean hormone levels as well as hormone changes. The relatively small size of our sample may also have limited our ability to detect very small changes in cortisol. Future research should examine expectant fathers' cortisol changes in

larger, more diverse samples to better understand the generalizability of our findings.

In addition, in one prior study of nine expectant fathers, a larger percentage of men showed detectable levels of estradiol following versus before the birth of their child, potentially indicating increases in estradiol during this period among some fathers (Berg and Wynne-Edwards, 2001; but see Berg and Wynne-Edwards, 2002). However, in the current study, we found significant prenatal *declines* in men's estradiol. Perhaps men's estradiol declines preemptively during the prenatal period but then increases postnatally. Prenatal declines in men's estradiol could also reflect the fact that, in men, estradiol is aromatized from circulating testosterone (Jones and Lopez, 2014); thus, men's estradiol levels may decline in tandem with testosterone. Lower levels of estradiol also appear to facilitate the expression of paternal care in some animal species (Cushing et al., 2004). For instance, in male prairie voles, a species characterized by social monogamy and biparental care, increases in estradiol inhibit prosocial behavior (Cushing et al., 2008). Thus, preemptive declines in estradiol could facilitate paternal care. Given that the men in Berg and Wynne-Edwards' (2001) study were sampled only twice, once pre- and once postnatally, and our study did not include a postnatal hormone assessment, it will be important for future research to examine whether the patterns we observed continue into the postpartum period.

Further, to our knowledge, the current study is the first to examine progesterone among expectant fathers. In fact, very little is known about the role of progesterone in paternal behavior, particularly in humans (Wynne-Edwards and Reburn, 2000). Thus, our findings contribute important new information about potential changes (or lack thereof) in men's progesterone during the prenatal period. Given that social connection can increase men's progesterone (Schultheiss et al., 2004), it is possible that new fathers' progesterone would increase pre- to postnatally. Progesterone may also be higher among new fathers compared with men without children. In the one study to examine progesterone among human fathers, Gettler et al. (2013) found that men who reported more positive emotion after interacting with their toddlers had higher progesterone levels throughout the interaction. Thus, men who find expect to find parenting more rewarding might be more likely to show progesterone changes in advance of fatherhood.

Taken together, our findings for testosterone and estradiol are consistent with the idea that the same hormones may be involved in maternal and paternal care (Wynne-Edwards, 2001). The prenatal changes that we observed in men's hormones were relatively small, especially in comparison with those observed among women; however, our effect sizes are comparable with those reported in the few published studies of short-term longitudinal changes in new fathers' hormone levels (e.g., d's ≈ 0.50 for within-person changes; Berg and Wynne-Edwards, 2001). Nevertheless, an important limitation of our study is that we did not include a comparison group of non-expectant couples, which would have allowed us to isolate hormone changes that occur as a function of fatherhood specifically from those that occur as a function of the passage of time (e.g., due to increasing age or relationship length). Although the hormones that we measured generally show very good longitudinal stability (Shi et al., 2013), there is cross-sectional evidence for age-related declines (Leifke et al., 2000). Berg and Wynne-Edwards (2001) did not report longitudinal changes in hormones among non-fathers and Storey et al. (2000) did not include a comparison group of non-expectant couples. However, it is worth noting that Perini et al. (2012) did not find significant longitudinal changes in fathers or non-fathers' testosterone over a three-month period. Moreover, in a large population-based longitudinal study, men's average levels of testosterone did not show significant annual declines (Shi et al., 2013). Thus, it is not clear that the effects of aging would be apparent over a period of several months, but we cannot rule out this possibility. A more direct test of the hypothesis that impending fatherhood causes men's hormone changes necessitates a comparison with changes in men who are not fathers.

Moreover, because we did not assess new fathers' hormones before conception or postnatally, we cannot determine whether and how men's hormones change throughout the entire transition to parenthood, including as a result of pair-bonding. Longitudinal research suggests that men's testosterone declines both as a function of pair-bonding and of fatherhood (Gettler et al., 2011; Mazur and Michalek, 1998), so it is possible that the changes we observed reflect the enduring influences of pair-bonding on men's hormones, as opposed to impending fatherhood per se. It is also possible that hormone changes associated with fatherhood are larger or occur more rapidly pre- to postpartum as opposed to prenatally. These possibilities could be investigated with larger-scale longitudinal studies, such as those conducted over several decades as men transition from single to partnered status and become first-time fathers (Gettler et al., 2011).

The design of our study also allowed us to test whether hormone concentrations were correlated between partners. Some have argued that such correlations reflect the interdependence between partners and/or the complementarity of couple members' hormone changes as they prepare to become parents (Berg and Wynne-Edwards, 2001). In the current study, we found evidence for within-couple correlations in both cortisol and progesterone, reflected in the significant correlations between couple members' intercepts and average hormone levels. Cortisol and progesterone may be especially likely to show within-couple associations because of their respective links to stress (Wirth, 2011; Wirth et al., 2007), which may be shared between partners. We did not find evidence for significant within-couple correlations in average levels of testosterone or estradiol. Notably, testosterone and estradiol were the two hormones that showed significant changes in both men and women, and in opposite directions, which could have limited within-partner correlations. It is also possible that unmeasured individual differences—such as the extent to which men assimilate fatherhood into their self-concept or focus attention away from other reproductive opportunities—explain the magnitude of within-couple correlations in hormones. For instance, to the extent that men are not invested in their current relationship or in their identity as a father, one might expect smaller hormone changes as a function of fatherhood (Gray, 2003; Muller et al., 2009) and smaller within-couple correlations in hormones. Nevertheless, taken together, our findings suggest modest interdependence among couples, at least with respect to these neuroendocrine measures.

Unfortunately, because we found only limited evidence of hormone changes among men, and because there was so little inter-individual variability in men's rates of change, we were unable to examine the extent to which prenatal hormone *changes* were correlated within couples. Perhaps research with larger and/or more diverse samples would provide better estimates of within-couple correlations in prenatal hormone changes. It is also possible, however, that men's hormones simply show so few changes, at least prenatally, that within-couple correlations are severely restricted. Perhaps postpartum, with the presence of an infant and the many changes that accompany first-time parenthood, new parents' hormone levels and changes in hormones might become more similar. This intriguing possibility could be tested in future research by assessing a larger number of couples at more time points.

Another important direction for future research will be to examine the long-term implications of both parents' prenatal hormone levels and changes in hormones. Among expectant mothers, for instance, larger prenatal increases in testosterone and cortisol have been associated with poorer infant outcomes (Carlsen et al., 2006; Davis and Sandman, 2010). Perhaps, among expectant fathers, changes in testosterone and/or estradiol would predict other outcomes for themselves, their partners, and/or their children. For example, fathers who show larger prenatal declines in testosterone may subsequently be more engaged with their infants. In addition, both men and women are more satisfied with and committed to their relationships to the extent that they (and their partners) have lower testosterone (Edelstein et al., 2014; Hooper et al., 2011). Prenatal declines in men's testosterone might be associated with their own and/or their partners' postpartum relationship satisfaction. Further longitudinal research with multiple postpartum assessments can begin to address these important questions.

In sum, the current study represents the most extensive investigation to date of prenatal hormone changes in both expectant parents. We found evidence for large prenatal increases in testosterone, cortisol, estradiol, and progesterone among expectant mothers. We also found evidence for significant prenatal declines in testosterone and estradiol among expectant fathers; however, and despite some prior evidence for cortisol changes in expectant fathers, we did not find significant prenatal changes in men's cortisol or progesterone. Thus, our findings provide some support for the idea that similar neuroendocrine pathways support maternal and paternal behavior. There was also evidence for within-couple correlations in cortisol and progesterone, suggesting some physiological interdependence between partners. It will be important for future research to determine whether the changes that we observed in men's hormones reflect processes associated with fatherhood specifically or long-term pair-bonding more generally. Another important direction for future research will be to understand whether and how both partners' hormones and changes in hormones are associated with postpartum behavior and adjustment.

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