Original Article

Changes in Testosterone, Cortisol, and Estradiol Levels in Men Becoming Fathers

SANDRA J. BERG, MSc, AND KATHERINE E. WYNNE-EDWARDS, PhD

- ***Objective*: To quantify longitudinally steroid hormone (testosterone, cortisol, and estradiol) concentrations in men becoming fathers for the first time ("dads").**
- ***Subjects and Methods*: Volunteer study subjects were recruited from first-trimester prenatal classes in Kingston, Ontario, in February 1999. Twenty-three dads provided saliva samples from recruitment through 3 months after the birth of their children. Fourteen men who were not fathers were recruited from the general population to serve as age-matched controls for season and time of day. Estradiol, testosterone, and cortisol levels were quantified.**
- ***Results*: After controlling for effects of time of day and season, dads had lower mean ± SE testosterone (6.5±0.7 vs 10.0±0.9 ng/dL; *P*<.005) and cortisol (morning values, 0.30±0.05 vs 0.53±0.05 μg/dL; *P*<.005) concentrations, a higher proportion of samples with detectable estradiol concentrations (68% [308/454] vs 57% [87/154]; *P*=.01), and higher estradiol concentrations in those detectable samples (3.81±0.09 pg/mL [13 dads] vs 3.26±0.11 pg/mL [9 controls]; *P*<.002) than did control men. Within 10 individual dads with frequent samples before and after the birth, the percentage of samples with detectable estradiol was lower during the month before the birth than during the month after (51% vs 71%; *P*=.02), and cortisol concentration was increased in the week before the birth (to a mean of 0.16 μg/dL). In each of 13 dads providing frequent samples, testosterone concentration and variance were low immediately after the birth (no change from previous levels in 5, decrease after prebirth increase in 3, and decrease relative to all other times in 5).**
- ***Conclusions*: In this population of Canadian volunteers attending prenatal classes, expectant fathers had lower testosterone and cortisol levels and a higher proportion of samples with detectable estradiol concentrations than control subjects. Individual patterns of testosterone variance relative to the birth and estradiol and cortisol concentrations immediately before the birth may be worthy of further investigation. The physiologic importance of these hormonal changes, if any, is not known. However, they are hormones known to influence maternal behavior.**

Mayo Clin Proc. 2001;76:582-592

The hormonal changes of pregnancy, birth, and early lactation are well known to facilitate the expression of maternal behavior in women, nonhuman primates, and other mammals.[1,2] Recent laboratory studies have suggested that natural fathers of the animal kingdom may also use hormonal changes to facilitate paternal behavior[3-7] and may be activating some of the same neuroendocrine circuits as females.[8]

In April 2000, the first study of hormonal changes in men becoming fathers was published.[9] Canadian couples were recruited from prenatal classes and visited at home either before or after the birth. Blood was sampled twice. During the 30 minutes between samples, the man held a soft doll wrapped in a soiled receiving blanket from the neonatal nursery while the couple listened to unconsoled baby cries recorded in the neonatal unit and watched a video clip about breast-feeding a newborn. Each adult then completed a brief questionnaire inquiring about male pregnancy symptoms[10] and emotional responses to the stimuli. Prolactin and cortisol concentrations were higher in men sampled during the final 3 weeks of pregnancy than in men sampled earlier in the pregnancy. Testosterone concentrations were lower in men sampled in the 3 weeks after the birth than in men sampled before the birth. The amplitude of within-individual hormonal changes between the 2 samples (called the "situational reactivity") also varied relative to the birth: just before the birth, the cortisol level decreased between samples, whereas just after the birth, the testosterone level increased between the 2 samples.

The hormones that were changing were the same ones implicated in animal studies of maternal and paternal behavior, and the psychometric measures correlated those hormones with paternal responsiveness.[9] As such, they suggested that men exposed to appropriate stimuli might experience a muted version of the endocrine changes of pregnancy.[9]

From the Department of Biology, Queen's University, Kingston, Ontario.

This work was supported by the Natural Sciences and Engineering Research Council of Canada (106386-99), the Advisory Research Committee of Queen's University, and Childbirth Kingston. It formed part of the MSc degree requirements for Ms Berg. Preliminary results were presented as a poster at the Endocrine Society annual meeting, Toronto, Ontario, June 21-24, 2000.

Address reprint requests and correspondence to Katherine E. Wynne-Edwards, PhD, Department of Biology, Queen's University, Kingston, Ontario, Canada K7L 3N6 (e-mail: wynneedw@biology.queensu.ca).

The present study was designed to test critically those results by improving statistical power, incorporating needed controls, and quantifying estradiol, the female sex steroid. Specifically, this study design differs from previously conducted studies by (1) collecting longitudinal information on men throughout the transition from the middle of their partners' pregnancy to early fatherhood ("dads"); (2) providing a control group of men matched for age, season, and time of day[11]; (3) reducing sampling stress by having both dads and controls collect saliva samples at home; and (4) quantifying estradiol concentrations.

SUBJECTS AND METHODS

Study and Control Subjects

Starting in February 1999, 45 subjects (age range, 23-43 years; median, 33 years) and their pregnant partners (age range, 23-37 years; median, 32 years) were recruited from first-trimester prenatal classes run by Childbirth Kingston in Kingston, Ontario. The population of these prenatal classes is a highly self-selected group in which women are accompanied by their partners, and couples pay for the classes (as opposed to free classes offered through the local health unit). Recruitment success and retention of study subjects were high. Approximately 50% of couples in classes were recruited. After recruitment, 34 (76%) of the 45 couples completed the study. Reasons for departure from the study ranged from premature delivery of twins to discomfort about having forgotten to collect samples. The experimental design was chosen a priori to maximize subject compliance over a longitudinal study that would span 10 to 12 months. Thus, subjects chose the time of day for sampling. Of the 34 couples, 23 were consistent about labeling samples by sex, date, and time of day and were included in hormone analyses.

Control subjects were included in the design (1) to calibrate the magnitude of expected circadian patterns in hormone concentration; (2) to rule out the possibility that temporal patterns in expectant fathers would be an artifact of the cohort moving relatively synchronously through seasons; and (3) to detect differences between expectant fathers and controls that preceded recruitment. Fourteen control subjects (age range, 22-46 years; median, 34 years; 7 living in a heterosexual partnership and 7 not "pair-bonded") were recruited from the Kingston community. Control subjects had never been fathers and were age and activity matched to the dads.

Recruitment, informed consent, and questionnaire procedures were approved by the Queen's University Research Ethics Board as Biol-009-98.

Sample Collection

Men and women were asked to collect approximately 10 mL of saliva on a weekly basis from recruitment until 3 months after the birth of their child. Only samples from male subjects were considered in these analyses. Saliva was chosen because (1) collection is noninvasive and therefore does not evoke apprehension in the way that venipuncture can; (2) the need for a trained health professional at the time of sampling is avoided; (3) the biologically active, unbound fraction of steroid hormones, including testosterone, estradiol, and cortisol, can easily be assayed from saliva[12]; (4) samples can be stored at home-freezer temperatures without degradation of the hormones[13]; and (5) saliva has a lower biohazard than blood.

Subjects were informed about the importance of choosing a consistent time of day for sample collection and were provided with labeled, clean glass vials and sugar-free chewing gum to stimulate saliva flow.[13] All subjects were reminded by telephone on a weekly basis to collect their samples.

A subsample of 13 dads increased their sampling frequency during the last 3 weeks before and the first 3 weeks after the birth. Samples were collected and immediately frozen at home. Every few weeks we retrieved the samples, thawed them, centrifuged them to remove mucus and solids, and then stored them at –20°C until they were assayed for hormone content.

Except for a recruitment questionnaire inquiring about subjects' age, date of birth, shift work, exercise level, and smoking status, no attempt was made to obtain quantitative psychometric measures of parental expectations or experiences.

Hormone Determinations

Testosterone and cortisol were quantified with use of commercially available radioactive iodine kits (Coat-a-Count, Diagnostic Products Corporation, Inter-Medico, Markham, Ontario) by following the specific technical instructions from the manufacturer for quantitative salivary determinations. In both cases, the antibody would recognize both bound and unbound steroid, but the determinations were of free steroid concentration because the bound steroid fraction is excluded from saliva.[12,13] Three internal serum controls provided by the commercial supplier were diluted and stored as frozen aliquots for use in each assay. Results for those serum controls were exactly as expected (Table 1), and results for saliva samples were similar to previously published salivary steroid concentrations from other laboratories.[12,14]

Estradiol was determined via a ^{3}H radioimmunoassay in routine use in our laboratory.[15] Samples (1.0 mL) and standards (diluted to 1.0 mL in dH_2O) were extracted into 2 volumes of anesthesia-grade diethyl ether (AnalaR, BDH Inc, Toronto, Ontario), separated by freezing, and dried before the competitive binding assay. Reactants were 2000

Table 1. **Internal Control Results (Mean ± SD) for Testosterone and Cortisol Assays***

		Testosterone					Cortisol				
Internal control	No. of assays	Expected (ng/dL)	Measured (ng/dL)	Binding (%)	Intra-assay CV (%)	Inter-assay CV (%)	Expected (μg/dL)	Measured (μg/dL)	Binding (%)	Intra-assay CV (%)	Inter-assay CV (%)
CON 4	19	70±11	73±12	89	15.9	16.3	4±0.4	3.5±1.0	68	3.1	28.5
CON 5	20	353±22	347±54	69	10.8	15.4	10.5±0.8	10.6±1.7	46	3.3	16.0
CON 6	20	683±40	669±57	57	7.4	8.5	27.1±2.2	27.5±3.8	28	3.4	14.0

*CV = coefficient of variation.

dpm ^{3}H estradiol (Dupont Canada, Mississauga, Ontario, NET 317 2,4,6,7-^{3}H(N), lot 2775-017) and GDN 244 anti-estradiol-6-BSA (supplied by G. D. Niswender, Colorado State University, Fort Collins). Although this assay detects both bound and unbound estradiol, saliva contains only unbound estradiol so these results must be considered free estradiol concentrations. Standards (1.1-500 pg/mL) were assayed in duplicate. No attempt was made to estimate recovery after extraction because the standards were simultaneously extracted in each assay. Triplicate estradiol determination of a pool of first-trimester female saliva (mean ± SE, 55.8±4.9 pg/mL; intra-assay variance, 15.4%; interassay variance, 26.8%; 22% binding) was used as a control and yielded results similar to previously published salivary estradiol concentrations from other laboratories.[16] A second pool of male saliva was also assayed in duplicate as an internal control for the low end of the sensitivity range (mean ± SE, 3.0±0.4 pg/mL; intra-assay variance, 22.9%; interassay variance, 26.6%; 81% binding). The lower limit of assay sensitivity was arbitrarily set to 2.15 pg/mL of saliva, corresponding to the second of 11 calibration standards (equal to 88% binding). Repeated determinations of the male saliva pool fell below this threshold in 9% of samples.

In spite of the fact that all men's saliva samples fell near the operational limit of the assay (608 determinations in 13 assays, maximum, 17.5 pg/mL; median, 2.82 pg/mL), where the intra- and interassay variance are increased, we are confident that the results reported here are robust. First, care was taken to analyze all samples from each subject in the same assay run and to ensure that each assay run contained both control subjects and dads. Second, for statistical analyses, data were transformed into nominal results, and each sample for each man was scored as having undetectable or detectable estradiol. Third, a total of 76 men's samples were assayed in duplicate (r^2=0.37; P<.001), and in spite of the median's falling so close to the imposed lower limit, only 29% of those duplicates placed 1 sample above the threshold and the other below. Thus, although between-assay variance was a potential factor in whether a sample had detectable estradiol concentrations, there was no possibility for a systematic difference between assay runs to yield a difference within a subject or a difference between control subjects and dads.

Statistical Analyses

Results are expressed as mean ± SE except where specifically noted. All analyses were conducted with use of JMP software (SAS Institute, Cary, NC) running on a Macintosh computer. Statistical comparisons were 2-tailed and applied a critical α of .05. For analysis of variance (ANOVA), the overall F statistic and significance are shown. Subsequent mention of post hoc differences between groups is based on the Tukey-Kramer test with α=.05. To optimize statistical power, subsets of the samples were used for different analyses. In each case, the assumed level of statistical independence (sample or individual) and the resulting sample size are indicated.

RESULTS

First, control results were used to define patterns of hormonal change during the day and across seasons to ensure that results for dads were not an artifact of synchrony between season and date relative to the birth. Following those analyses, appropriate hormone levels for control men and dads were compared. Those results were then followed by within-individual comparisons of the month before and the month after the birth, reporting of individual patterns of variance in testosterone concentrations within men and, finally, reporting of hormone patterns during labor and delivery. As an aid to the interpretation of these data, the temporal distribution of available samples is shown schematically in Figure 1. Scatterplots of hormone determinations for each of the 14 control men and the 13 dads with high sampling compliance are archived at http://biology.queensu.ca/~wynneedw/dads.pdf and http://biology.queensu.ca/~wynneedw/controls.pdf. The dads and controls who completed the study were well matched for age (t_{47}=0.05, P=.96). All subjects were white.

Time of Day Effects in Control Subjects

At recruitment, all subjects chose the time of day for sampling to be convenient for their schedule and to maxi-

mize compliance. This resulted in substantive differences in sample frequency that were not predicted a priori (Table 2). Control men tended to choose morning, with 153 (67.1%) of 228 samples (from 13 of 14 men) collected in that interval, whereas dads tended to choose late evening, with 368 (55.0%) of 669 samples (from 15 of 23 men, excluding labor and delivery) collected in that interval. Thus, time of day was retained as a variable throughout analyses.

Six control men were specifically asked to provide samples at different times of day (night = 0001-0630; morning = 0631-1100; afternoon = 1101-1600; evening = 1601-1900; late evening = 1901-2400) over a 3- to 6-day time span, resulting in 59 samples (range, 9-13 samples per individual) with between 2 and 6 men providing samples in each time category. Although there was a strong individual effect on testosterone concentration ($F_{5,58}$=28.7, P<.001), testosterone levels did not vary across the time categories (range, 7.1-13.9 ng/dL; $F_{4,58}$=1.52, P=.21; Figure 2, top), and there was no significant interaction between time of day and individual ($F_{11,58}$=1.0, P=.47). Thus, there was no evidence that the different biases in sampling time would contribute to differences between controls and dads.[12]

As expected,[14] time of day affected cortisol concentration ($F_{4,57}$=12.09, P<.001; Figure 2, bottom) with a significant post hoc elevation in the cortisol level during the morning (0.69±0.08 μg/dL) relative to all other times (range, 0.11-0.22 μg/dL). There was no evidence for differences between individuals ($F_{5,57}$=1.5, P=.22) or an interaction between individual and time of day ($F_{11,20}$=2.1, P=.11). Thus, the bias toward morning samples in controls and toward late evening samples from dads was expected to contribute to a higher cortisol concentration in the controls.

The probability of detecting estradiol in a sample was also affected by the time of day (Pearson χ^2 [df=4] = 13.80, P<.008). Similar to patterns in women,[17,18] the estradiol level was significantly lower during the afternoon than during the morning, with 11 of 16 samples (from 5 of 6 controls) readily quantified in the morning, but only 2 of 13 samples (from 1 of 5 controls) were quantifiable in the afternoon (Pearson χ^2 [df=1] = 8.26, P<.005). For the purpose of these analyses, however, estradiol patterns during the morning and late evening were the most critical. Morning and late evening estradiol results did not differ in either the proportion of samples detectable (25/32 morning and 5/13 late evening; P=.29) or the estradiol concentration in detectable samples (3.7±0.3 and 3.2±0.3 pg/mL; P=.30). Thus, only cortisol had a predicted difference between controls and dads based on the time of day when samples were collected.

Seasonal Effects in Control Subjects

Samples were collected in every season for both controls and dads (Table 3). Dads typically participated for long enough to provide samples in at least 3 seasons. Seasonal information for individual controls is shown by day of the year (month) in Figure 1. Those data were analyzed for effects of season that might influence results in dads progressing as a cohort through the seasons.

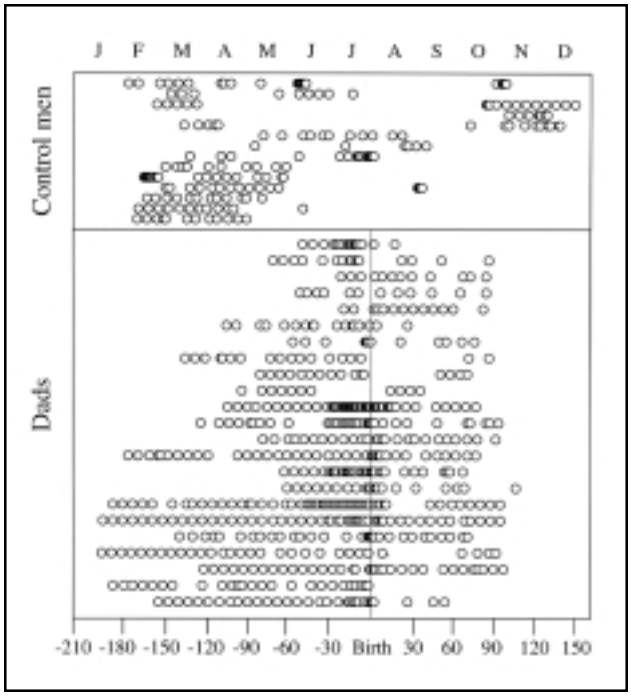

Figure 1. Schematic representation of the temporal distribution of samples collected for each dad (relative to the birth) and each control (relative to day of the year [month]). The lower 13 rows of data are from the 13 dads with frequent samples used in some analyses.

In a model with the individual control as the matching variable, testosterone concentration was influenced by season ($F_{3,227}$=3.4, P<.02; Figure 3, top) with a significant post hoc increase during the autumn (September 22-December 31; increase to 13.6±0.8 ng/dL) and a decrease during the summer (June 22-September 21; decrease to 7.9±0.41 ng/dL). However, the effect of the individual ($F_{13,227}$=13.1, P<.001) and the interaction of the individual with season ($F_{16,227}$=2.7, P<.001) were larger than the underlying effect of season. Time of day was not significant as a matching variable in the analysis (P=.48). Seasonal patterns of testosterone variation with autumn peaks have been reported previously,[19,20] although other studies have reported other seasonal peaks.[21,22]

For cortisol, there was also an effect of season ($F_{3,205}$=2.79, P=0.4; Figure 3, bottom) with post hoc differences between high winter concentrations (0.56±0.06 μg/dL) and lower autumn concentrations (0.34±0.05 μg/dL). However, when time of day was included as a matching

Table 2. **Distribution of Samples by Time of Day Collected**

Subjects	Night (0001-0630)	Morning (0631-1100)	Afternoon (1101-1600)	Evening (1601-1900)	Late evening (1901-2400)	Unknown
Controls*						
No. (%) of samples	5 (2.2)	153 (67.1)	21 (9.2)	32 (14.0)	17 (7.5)	0 (0.0)
No. of men	3	13	7	7	5	…
Dads†						
No. (%) of samples	14 (2.1)	97 (14.5)	51 (7.6)	100 (15.0)	368 (55.0)	39 (5.8)
No. of men	9	12	10	14	15	2

*Fourteen men contributed 228 samples.
†Twenty-three men contributed 669 samples.

variable in the analysis, season was not a significant variable (P=.25), and time of day was significant (P<.001). Thus, differences in the seasonal distribution of samples between controls and dads would not contribute to differences in cortisol concentration. There was no evidence for a seasonal pattern of variation in estradiol detection (in 140 samples, Pearson χ^2 [df=3] = 4.43, P=.22). Thus, season but not time of day altered testosterone in controls, time of day but not season altered cortisol in controls, and estradiol differences in dads were restricted to times of day with low sampling frequencies.

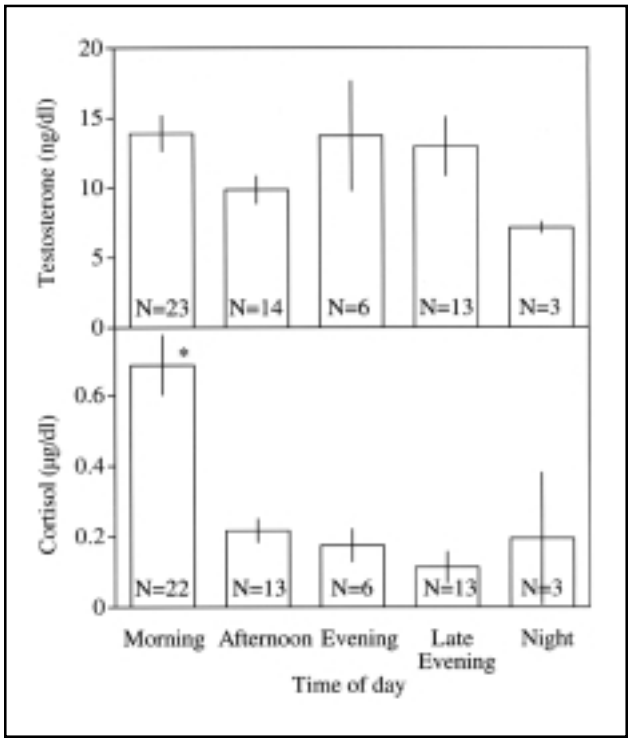

Figure 2. Mean testosterone and cortisol concentrations for samples from 6 control subjects who collected samples several times per day over a 3- to 6-day interval. Sample sizes are indicated for each time of day and hormone. For cortisol, the asterisk indicates that values determined from morning samples were significantly higher than all other times. Error bars indicate SEs.

Comparisons Between Control Subjects and Dads

Testosterone concentration was significantly lower in the overall sample of 23 dads than in the 14 controls (t_{35}=3.0, P=.005; Figure 4, a). Different times of day did not explain the observed difference between controls and dads. In a 2-way ANOVA with controls vs dads and morning vs late evening as class variables, there was an effect of fatherhood ($F_{1,43}$=9.86, P<.005) but no effect of the 2 sampling times (P=.85) and no interaction (P=.47). In morning samples, findings in controls and dads did not differ (t_{22}=1.69, P=.12; Figure 4, b). In late evening samples, testosterone was higher in controls (11.5±29 and 6.2±1.0 ng/mL; t_{17}=2.28, P=.04; Figure 4, c). As expected based on results with controls, the testosterone level in dads was elevated during autumn ($F_{3,636}$=10.12, P<.001). When both season and controls vs dads were combined in a matching fit, both season (P<.001) and controls vs dads (P<.001) remained significant contributors to the testosterone results. Thus, testosterone concentration was higher in controls than in dads.

Cortisol concentration was significantly lower in dads than in controls (t_{34}=6.05, P<.001; Figure 4, d). In a 2-way ANOVA with controls vs dads and morning vs late evening as class variables, the cortisol level was higher in controls ($F_{1,43}$=10.99, P=.002), and as expected, the morning level was also higher than the late evening level ($F_{1,43}$=56.36, P<.001) with a significant interaction term ($F_{1,43}$=6.86, P=.01). In morning samples, the cortisol level was higher in controls (0.53±0.05 vs 0.30±0.05 µg/dL; t_{21}=3.65, P<.002; Figure 4, e). In late evening samples, there was no difference (0.09±0.05 and 0.06±0.01 µg/dL; t_{18}=0.86, P=.40; Figure 4, f).

To rule out the possibility that sleep-wake patterns of the infant might cause changes in the circadian timing of the postawakening cortisol surge in dads, the analysis was

Table 3. **Distribution of Samples by Time of Year Collected***

Subjects	Winter (1-82)	Spring (83-172)	Summer (173-265)	Autumn (266-365)
Controls†				
No. (%) of samples	44 (19.3)	75 (32.9)	52 (22.8)	57 (25.0)
No. of men	10	13	6	4
Dads‡				
No. (%) of samples	97 (14.5)	314 (46.9)	176 (26.3)	82 (12.3)
No. of men	17	21	22	6

*Numbers accompanying season indicate ordinal day of the year.
†Fourteen men contributed 228 samples.
‡Twenty-three men contributed 669 samples.

repeated after excluding samples obtained after the birth. In samples collected before the birth, the cortisol level remained higher in controls than in dads (t_{20}=3.01, P=.007). As in the control subjects, there was no evidence that season altered concentrations of cortisol across all samples ($F_{3,655}$=1.54, P=.20), even when cortisol determinations after the birth were excluded ($F_{3,30}$=0.77, P=.52).

Only men with high sampling frequencies were assayed for estradiol, resulting in a range of 12 to 25 determinations for each of 9 control men and 19 to 59 determinations for each of 13 dads. Overall, estradiol was detected in a significantly higher proportion of dad samples (68% [308/454]) than control samples (57% [87/154]) (Pearson χ^2 [df=1] = 6.51, P=.01). In samples in which estradiol was detected reliably, concentrations were also higher in dads than in controls (t_{393}=–3.16, P<.002). When the samples with detectable estradiol were reduced to an average estradiol concentration for each man, estradiol concentrations remained higher in the 13 dads than in the 9 controls (3.81±0.09 vs 3.26±0.11 pg/mL; t_{20}=–3.75; P<.002; Figure 4, g). The 2 groups were not significantly different in morning (Figure 4, h) or late evening samples (Figure 4, i). The proportion of each man's samples with detectable estradiol closely matched the overall proportions (0.67±0.07 vs 0.57±0.08), but the difference was not significant (t_{20}=–0.88, P=.39). Assuming the observed mean and variance estimates, a sample size 5 times larger would have been needed to detect the difference with α set at .05 (power analysis). In contrast to control men, the estradiol level was elevated during spring ($F_{3,453}$=3.78, P=.01) in dads; however, that effect is explained by changes in estradiol concentration relative to the birth.

Thus, after controlling for time of day and seasonal changes in hormone samples from control subjects, each hormone differed in the 2 groups of men. Compared with the controls, dads had lower testosterone and cortisol concentrations, a higher proportion of samples with detectable estradiol concentrations, and higher estradiol concentrations in samples in which it was detectable.

Changes Within Dads

Samples were retrospectively assigned to 1 of 9 stages of pregnancy based on the actual date of the birth. Those stages were first trimester, –200 to –181 days (8 samples from 4 men); second trimester, –180 to –88 days (98 samples from 13 men); third trimester, –87 to –31 days (139 samples from 21 men); last month, –30 to –9 days (120 samples from 21 men); last week, –8 to –1 days (69 samples from 20 men); birth, 0 to 1 day (29 samples from 9 men); first week, 2 to 8 days (50 samples from 17 men);

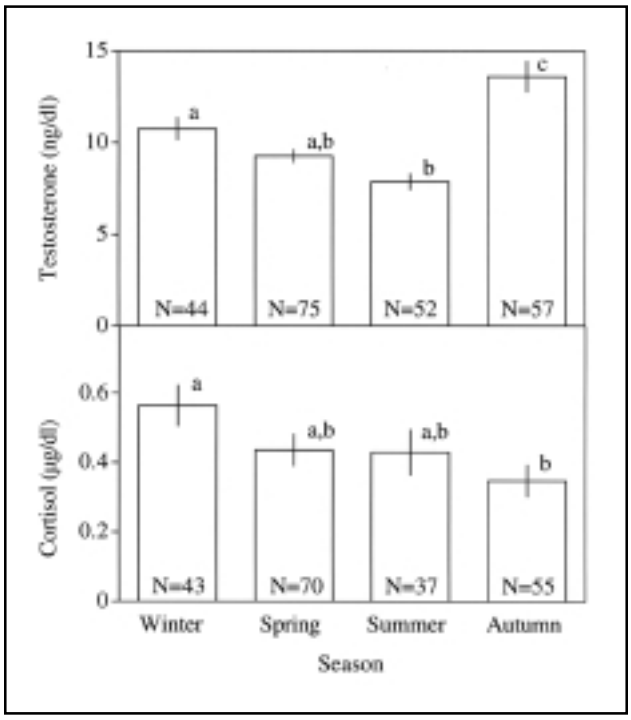

Figure 3. Mean testosterone and cortisol concentrations during winter, spring, summer, and fall for samples from 14 control subjects. Sample sizes are indicated for each season and hormone. Seasons that share a lowercase letter were not significantly different with post hoc testing. Error bars indicate SEs.

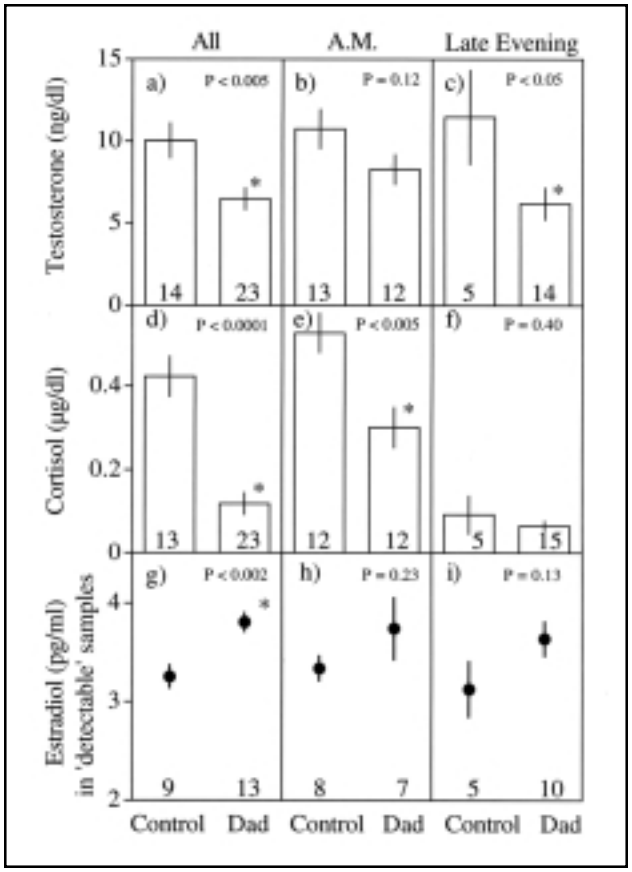

Figure 4. Mean testosterone concentration of dads and controls in all samples (a), samples collected in the morning (b), and samples collected in the late evening (c). Mean cortisol concentration in all samples (d), samples collected in the morning (e), and samples collected in the late evening (f). Mean estradiol concentration for all detectable (≥2.15 pg/mL) samples (g), detectable samples collected in the morning (h), and detectable samples collected in the late evening (i). All samples for each subject were reduced to an average so that the independent sample size indicated on each panel is the number of subjects, not the number of samples assayed. For each panel, the relevant *P* value is shown. Asterisks indicate significant differences. Error bars indicate SEs.

first month, 9 to 30 days (52 samples from 19 men); and established, 31 to 110 days (104 samples from 20 men).

When testosterone concentration was compared across these 9 stages (with subject as a nested variable), there was a significant effect of the model ($F_{139,636}$=7.50, P<.001), the nested effect (F_{117}=2.18, P<.001), and the dad (F_{22}=18.39, P<.001). Dads were not uniformly distributed over the 9 intervals; therefore, the analysis was repeated with a subset of 6 stages (third trimester, last month, last week, first week, first month, and established) including only the 11 dads with samples in each (all had spring or summer births). As seen in the complete data set, the significant effect of the model ($F_{64,353}$=7.09, P<.001), the nested effect (F_{54}=3.10, P<.001), and the dad (F_{10}=15.82, P<.001) all remained. Samples in the first week after the birth had the lowest testosterone concentration (35 samples from 11 dads; 5.86±0.42 ng/dL), and samples from established fathers (>30 days after birth) had the highest testosterone concentrations (71 samples from 11 subjects; 7.99±0.56 ng/dL). However, the difference was not significant in a post hoc paired *t* test (mean difference = 1.77 ng/dL; paired t_9=1.63, P=.14). Similarly, when samples in the month preceding the birth (last month + last week) and the comparable interval after the birth (first week + first month) were combined, there was no evidence for a transition at the birth (19 dads; paired t_{18}=0.89, P=.39; Figure 5, top). Post hoc analysis of patterns of variance in testosterone concentration within dads are discussed below.

When cortisol concentration was subjected to the same analysis with 9 stages (with subject as a nested variable), there was a significant effect of the model ($F_{141,654}$=4.32, P<.001) and the dad (F_{22}=16.02, P<.001) but not the nested effect (F_{119}=1.20, P=.09). As for testosterone, the analysis was also repeated with the subset of 6 stages (third trimester, last month, last week, first week, first month, and established) including only the 10 dads with samples in each. The significant effect of the model ($F_{59,335}$=3.27, P<.001) and the dad (F_9=10.88, P<.001) remained and were joined by a significant effect of the nested variable (F_{50}=1.42, P=.04). Samples in the last week before the birth had the highest cortisol concentrations (48 samples from 10 subjects; 0.16±0.03 µg/dL), and samples from the first month had the lowest cortisol concentrations (32 samples in 10 subjects; 0.06±.0.01 µg/dL). That difference was significant in a post hoc paired *t* test (mean difference = 0.08 µg/dL; paired t_9=2.82, P=.02). When samples in the month preceding the birth (last month + last week) and the comparable interval after the birth (first week + first month) were combined, there was no evidence for a transition at the birth (18 subjects; paired t_{17}=1.38; P=.19; Figure 5, middle).

As in previous analyses, comparison of estradiol levels was complicated by the large number of samples below the detection limit of the assay. Over the 6 stages (third trimester, last month, last week, first week, first month, and established) including the 10 dads with samples in each, the last week had the lowest percentage of samples with detectable estradiol (51% [21/41]) compared with mid pregnancy (74% [55/74]), the first week (71% [24/34]), the first month (73% [19/26]), and the established (72% [46/64]) categories. Detectable estradiol in late pregnancy was intermediate (64% [42/66]). When compared with the overall percentage of samples with detectable estradiol for dads

(68% [308/454]), this was a significantly reduced percentage of samples with detectable estradiol during the last week before the birth (Pearson χ^2 [df=1] = 4.66, P=.03). For the 10 dads with at least 5 samples in the month before the birth and the month after the birth, the proportion of samples with detectable estradiol was significantly lower before the birth (0.51±0.12) than after the birth (0.71±0.9; paired t_9=2.8, P=.02; Figure 5, bottom). This difference could not be attributed to a shift in the assay because the concentration in samples with detectable estradiol did not change from the month before the birth (3.49±0.28 pg/mL) to the month after the birth (3.47±0.22 pg/mL) for the 8 dads with detectable estradiol in both intervals (paired t_7=0.06, P=.95).

Patterns of Testosterone Variance

The significant effects of subject and interaction resulted from at least 3 underlying patterns of testosterone variation relative to the birth. Thirteen dads with 25 to 69 samples each were considered for this analysis. Five (38.5%) had stable, low testosterone concentrations throughout the study (5.1±0.2 ng/dL; Figure 6, top). The next group of 3 dads (23.0%) also maintained low testosterone concentrations (6.5±1.3 ng/dL) but had a significant increase in testosterone before the birth (paired t_2=6.24, P=.02; Figure 6, middle). The final group of 5 dads (38.5%) had low testosterone immediately after the birth (Figure 6, bottom). Those 5 dads had higher average testosterone (8.5±1.2 ng/dL) than the group with no change (t_8=2.73, P=.02) and almost twice the SD (t_8=3.20, P=.01). In these dads with high testosterone concentrations and high testosterone variances, testosterone concentration was reduced by 2.2±0.7 ng/dL during the interval including birth and the first week relative to all other samples (paired t_4=3.09, P=.04).

Thus, each of the 13 men with frequent samples had low testosterone immediately after the birth, but for 5 men, that low concentration was no change from previous concentrations, for 3 men, it was a decrease following a prebirth increase, and for 5 men, it was a decrease relative to all other times.

Endocrinology Surrounding the Birth

A total of 47 samples from 11 dads were collected on the day before, the day of, or the day after the birth of their first child. Four of those dads provided between 6 and 14 samples over that interval and also provided the exact time of birth. In 3 cases, the birth was by unplanned cesarean delivery; in the other case, the birth was within 3 hours of arrival at the hospital. There were no consistent patterns of change in testosterone, cortisol, or estradiol levels over the birth interval (Figure 7).

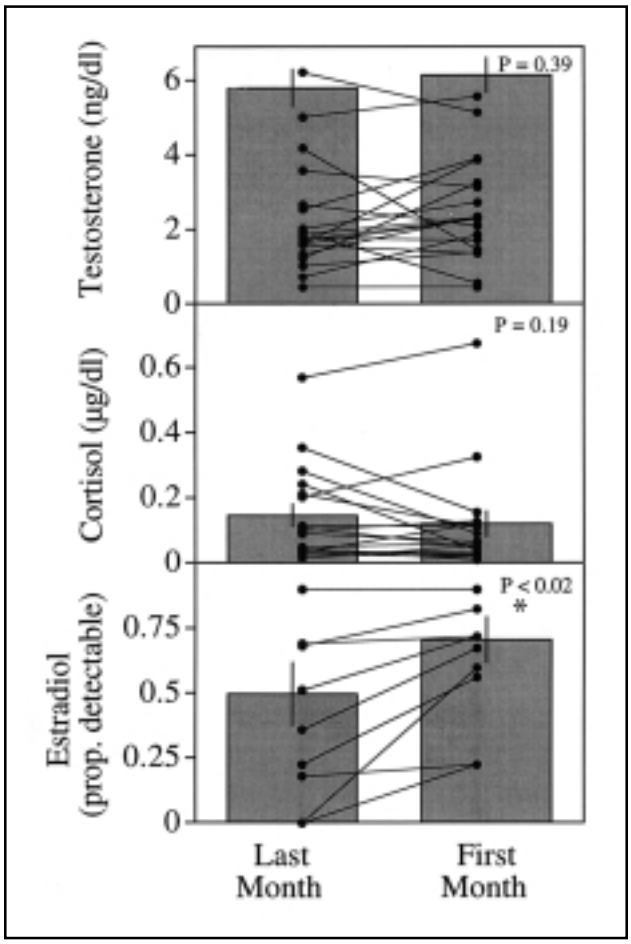

Figure 5. For dads with samples in the last month preceding the birth (last month + last week) and the first month after the birth (first week + first month), the mean testosterone and cortisol concentrations and the proportion of samples with detectable estradiol are shown as histograms. Error bars indicate SEs. Lines connect mean values for individuals across the 2 intervals. The asterisk indicates a significant increase in the proportion of samples with detectable estradiol from before until after the birth (paired test). Patterns of testosterone variance are explored further in Figure 6.

DISCUSSION

After controlling for hormonal changes associated with the season and time of day when samples were collected, dads had lower testosterone and cortisol concentrations and a higher proportion of samples with detectable estradiol concentrations than control subjects. Results also suggest that patterns of testosterone variance within dads, a possible decrease in the estradiol levels in dads just before the birth, and an increase in the cortisol levels during the week before the birth are worthy of further investigation.

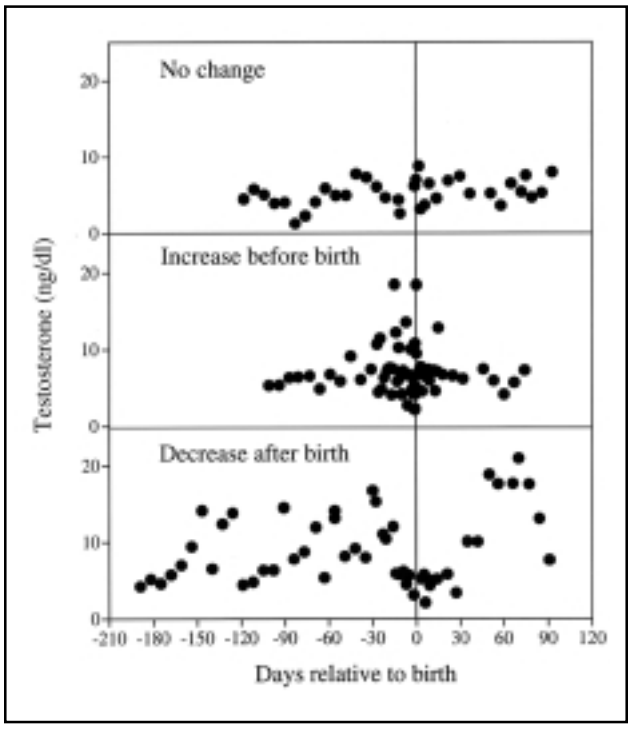

Figure 6. Examples of the 3 patterns of testosterone variation around the birth that are discussed in the text. Each panel includes all testosterone determinations for 1 dad. Top, Testosterone is stable, low, and unaffected by the birth. Middle, Testosterone increases before the birth but is otherwise stable and low. Bottom, Testosterone is higher and more variable before and after the birth but low and invariant around the birth. Equivalent representations for each dad and each control are available at http://biology.queensu.ca/~wynneedw/dads.pdf and http://biology.queensu.ca/~wynneedw/controls.pdf.

Estradiol

The finding that estradiol was detected in a larger proportion of samples from dads than from controls is novel. Men becoming fathers were exposed to more estradiol than control men, and that exposure increased after the birth of their child. Estradiol is an important hormonal component of mammalian maternal behavior in women,[1,23,24] nonhuman primates,[25] and other mammals,[26-29] but no animal research has yet described estradiol changes in naturally paternal male mammals. Thus, an increase in estradiol concentration could influence paternal behavior via neurocircuits homologous to maternal behavior.[8] Within the expectant fathers, there was also a significant decrease in the proportion of samples in which estradiol was detectable during the last week (and last month) before the birth. Thus, estradiol was more readily detected in expectant fathers than in control subjects, but the dynamic response of estradiol relative to the birth was a prenatal decrease.

Estradiol concentrations were near the operational limit of the assay technique. Thus, many samples were not reliably quantified, and intrinsic measurement errors necessitated the use of cautious statistical approaches to analysis. Nevertheless, the statistical findings of increased estradiol in dads relative to controls and the decreased proportion of samples with detectable estradiol within men from before relative to after the birth were measured within single assays that yielded repeatable results. Potentially confounding effects of time of day and/or season of the year could not explain the estradiol results. Additional studies applying more sensitive assay techniques to obtain more quantitative estradiol concentrations are needed to confirm the relationship in men. In addition, since free estradiol is typically a small percentage of total estradiol, studies of plasma or urine would greatly expand the inferences that could be drawn. Studies of nonhuman mammals are also needed to explore the generality of the relationship and to form the basis for experimental studies of the physiologic importance of estradiol in fathers.

Testosterone

The testosterone concentration was significantly lower in dads compared with controls. There was no alternative explanation for this difference that could be attributable to age or seasonal, temporal, or demographic variables. Half the control subjects were in pair-bonded relationships, and pair-bonded status did not appear to affect testosterone concentration within control subjects. In the absence of additional psychometric and behavioral information, the proximate stimuli underlying the difference are unknown. For example, it might have resulted from altered coital frequency, sleep-wake patterns, or other differences in environmental variables.[30] Nevertheless, in the absence of a proximate explanation for the lower testosterone, it remains true that the brains of dads were bathed in lower testosterone concentrations than the brains of controls. Thus, the testosterone decrease between controls and dads might alter neuroendocrine responsiveness and thereby be involved in the emotional and behavioral responses of new fathers.[31]

Within individual dads no consistent pattern of change relative to the birth was evident. Almost half the dads had low, stable testosterone concentrations that were not altered by the progress of the pregnancy, the birth process, or the circadian disruption that we assume accompanied returning home with their new child. The remaining men were split between 2 other distinct patterns of variation. In 1 group the men had stable, low testosterone concentrations until the birth approached but had a pronounced, approximately 30-day-long increase in testosterone before the birth. In those dads, testosterone declined at the birth

and reverted to the initial stable profile. The final group was characterized by high, variable testosterone concentrations throughout the pregnancy and after the child was a few weeks old. However, during and immediately after the birth, that variance was suppressed in spite of increased sampling intensity. Sample sizes were insufficient to draw conclusions about the reliability of these categories or their relative abundance in the subject population. Additional studies are clearly needed to explore individual patterns of testosterone variance with improved statistical power and psychometric assessment that might identify correlates of the patterns.

Nevertheless, the testosterone pattern of all men becoming fathers did share a common feature. During the interval immediately after the birth, testosterone concentration was stable and low in all men. This is consistent with the earlier finding of reduced testosterone concentrations in men sampled during the first 3 weeks after the birth.[9] Testosterone decreases have been reported in new fathers of nonhuman species with extensive paternal care, including Mongolian gerbils (*Meriones unguiculatus*), California mice (*Peromyscus californicus*), Djungarian hamsters (*Phodopus campbelli*),[3-5] cotton-top tamarins (*Saguinus oedipus*), and common marmosets (*Callithrix jacchus*).[6,7]

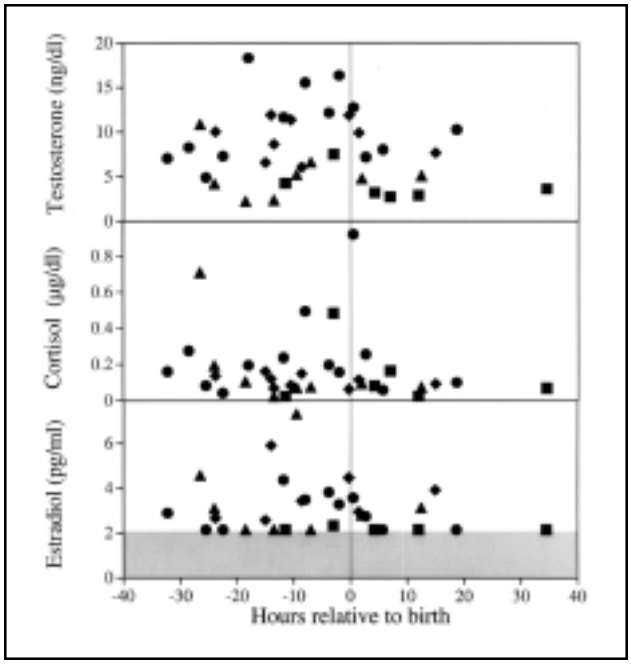

Figure 7. Testosterone, cortisol, and estradiol concentrations for the 4 subjects (each has a different symbol) who provided at least 6 samples during labor, delivery, and recovery. Estradiol determinations that were less than the detection limit of the assay (shaded area <2.15 pg/mL) are shown at that limit.

Cortisol

In addition to the well-established early morning increase in cortisol,[32] cortisol concentration was significantly lower in dads compared with controls. Animal studies have shown a decrease in glucocorticoid concentrations with pair-bond formation and social stability. Male-female pair formation reduces male corticosteroid concentrations in cotton-top tamarins, prairie voles (*Microtus ochrogaster*), and Djungarian hamsters[7,33-35] and is implicated in social affiliation and pair-bond formation[36] as well as mother-infant bonding.[1,37]

The only evidence that cortisol changes anticipated or responded to the birth of the child was an increase in the final week before the birth that was not present during the last month before the birth. This result was consistent with the possibility of apprehension preceding the birth and with previous findings in animals and in men. In naturally biparental Djungarian hamsters but not in closely related Siberian hamsters (*Phodopus sungorus*), cortisol is increased before the birth but not on the day of the birth.[3] In male cotton-top tamarins, cortisol also increases in the weeks surrounding the birth.[7] Similarly, cortisol concentration was elevated in men sampled during the 3 weeks preceding the birth in the only other study of hormonal changes in men becoming fathers.[9]

Methodologic differences between these 2 studies might have contributed to the difference in duration of the cortisol elevation before the birth. These samples were saliva routinely collected at home, whereas the earlier study involved blood collected by venipuncture during a home visit by the researchers. The design of the earlier study considered the first blood sample to be baseline and the second blood sample, which followed the infant stimuli, to be responses to those stimuli. The difference between the 2 concentrations was the situational reactivity for that hormone. However, in the last 3 weeks before the birth, cortisol decreased to previous concentrations over the 30-minute interval.[9] Thus, it is possible that the situational reactivity measured in the previous study was an enhanced cortisol response to the stress of the home visit rather than an increase in resting cortisol concentrations. If so, then the same effect would not have been seen in this study.

Conclusions

Relative to control subjects, expectant fathers have lower testosterone and cortisol concentrations and more frequently detectable estradiol. These results confirm and expand on the results of the only previous study[9] and collectively suggest that expectant fathers reach the birth with elevated prolactin concentrations, reliably detectable estradiol concentrations, reactive cortisol dynamics,

and stable, low testosterone concentrations. The physiologic relevance, if any, of these hormone changes is not known.

These data neither establish a functional role for these hormones in the neuroendocrine experience of fatherhood nor identify candidate environmental, behavioral, or physiologic cues responsible for the changes. Instead, they suggest that individual differences, even within this apparently homogeneous group of highly motivated Canadian volunteers, are important considerations for future studies.

We do not anticipate a strict hormone-behavior relationship in men. Nevertheless, these changes, which involve hormones known or implicated in mammalian maternal behavior, may subtly alter hormone receptor expression, sensitivity to infant stimuli, or reactions to social stimuli in ways that enhance the psychosocial experience of becoming a father. In conjunction with ongoing animal studies of male mammals that naturally express paternal behavior, these data suggest that the neuroendocrinology of the paternal brain is worthy of more scientific attention.[8]

REFERENCES

1. Fleming AS, Ruble D, Krieger H, Wong PY. Hormonal and experiential correlates of maternal responsiveness during pregnancy and the puerperium in human mothers. *Horm Behav*. 1997;31:145-158.
2. Numan M. Maternal behavior. In: Knobil E, Neill JD, eds. *The Physiology of Reproduction*. 2nd ed. New York, NY: Raven Press; 1994:221-302.
3. Reburn CJ, Wynne-Edwards KE. Hormonal changes in males of a naturally biparental and a uniparental mammal. *Horm Behav*. 1999;35:163-176.
4. Brown RE, Murdoch T, Murphy PR, Moger WH. Hormonal responses of male gerbils to stimuli from their mate and pups. *Horm Behav*. 1995;29:474-491.
5. Gubernick DJ, Nelson RJ. Prolactin and paternal behavior in the biparental California mouse, *Peromyscus californicus*. *Horm Behav*. 1989;23:203-210.
6. Dixson AF, George L. Prolactin and parental behaviour in a male New World primate. *Nature*. 1982;299:551-553.
7. Ziegler TE, Wegner FH, Snowdon CT. Hormonal responses to parental and nonparental conditions in male cotton-top tamarins, *Saguinus oedipus*, a New World primate. *Horm Behav*. 1996;30: 287-297.
8. Wynne-Edwards KE, Reburn CJ. Behavioral endocrinology of mammalian fatherhood. *Trends Ecol Evol*. 2000;15:464-468.
9. Storey AE, Walsh CJ, Quinton RL, Wynne-Edwards KE. Hormonal correlates of paternal responsiveness in new and expectant fathers. *Evol Hum Behav*. 2000;21:79-95.
10. Elwood RW, Mason C. The couvade and the onset of paternal care: a biological perspective. *Ethol Sociobiol*. 1994;15:145-156.
11. Marrama P, Carani C, Baraghini GF, et al. Circadian rhythm of testosterone and prolactin in the ageing. *Maturitas*. 1982;4:131-138.
12. Dabbs JM Jr. Salivary testosterone measurements: reliability across hours, days, and weeks. *Physiol Behav*. 1990;48:83-86.
13. Dabbs JM Jr. Salivary testosterone measurements: collecting, storing, and mailing saliva samples. *Physiol Behav*. 1991;49:815-817.
14. Hucklebridge FH, Clow A, Abeyguneratne T, Huezo-Diaz P, Evans P. The awakening cortisol response and blood glucose levels. *Life Sci*. 1999;64:931-937.
15. McMillan HJ, Wynne-Edwards KE. Evolutionary change in the endocrinology of behavioral receptivity: divergent roles for progesterone and prolactin within the genus *Phodopus*. *Biol Reprod*. 1998;59:30-38.
16. Sufi SB, Donaldson A, Gandy SC, et al. Multicenter evaluation of assays for estradiol and progesterone in saliva. *Clin Chem*. 1985; 31:101-103.
17. Reck G, Noss U, Breckwoldt M. Circadian rhythm of free oestradiol in relation to plasma cortisol in late human pregnancy. *Acta Endocrinol (Copenh)*. 1979;90:519-524.
18. Patrick J, Challis J, Natale R, Richardson B. Circadian rhythms in maternal plasma cortisol, estrone, estradiol, and estriol at 34 to 35 weeks' gestation. *Am J Obstet Gynecol*. 1979;135:791-798.
19. Dabbs JM Jr. Age and seasonal variation in serum testosterone concentration among men. *Chronobiol Int*. 1990;7:245-249.
20. Reinberg A, Lagoguey M, Chauffournier JM, Cesselin F. Circannual and circadian rhythms in plasma testosterone in five healthy young Parisian males. *Acta Endocrinol (Copenh)*. 1975;80:732-734.
21. Smals AG, Kloppenborg PW, Benraad TJ. Circannual cycle in plasma testosterone levels in man. *J Clin Endocrinol Metab*. 1976; 42:979-982.
22. Cousins L, Yen SS, Meis P, Halberg F, Brink G. Circadian rhythm and diurnal excursion of plasma cortisol in diabetic pregnant women. *Am J Obstet Gynecol*. 1986;155:1176-1181.
23. Fleming AS. Hormonal and experiential correlates of maternal responsiveness in human mothers. In: Krasnegor NA, Bridges RS, eds. *Mammalian Parenting: Biochemical, Neurobiological, and Behavioral Determinants*. New York, NY: Oxford University Press; 1990:184-208.
24. Fleming AS, O'Day DH, Kraemer GW. Neurobiology of mother-infant interactions: experience and central nervous system plasticity across development and generations. *Neurosci Biobehav Rev*. 1999;23:673-685.
25. Pryce CR. Socialization, hormones, and the regulation of maternal behavior in nonhuman simian primates. In: Rosenblatt JS, Snowdon CT, eds. *Parental Care: Evolution, Mechanisms, and Adaptive Significance*. San Diego, Calif: Academic Press; 1996: 423-473.
26. Siegel HI, Rosenblatt JS. Estrogen-induced maternal behavior in hysterectomized-ovariectomized virgin rats. *Physiol Behav*. 1975; 14:465-471.
27. Rosenblatt JS, Olufowobi A, Siegel HI. Effects of pregnancy hormones on maternal responsiveness, responsiveness to estrogen stimulation of maternal behavior, and the lordosis response to estrogen stimulation. *Horm Behav*. 1998;33:104-114.
28. González-Mariscal G. Maternal behavior in rabbits. In: Rosenblatt JS, Snowdon CT, eds. *Parental Care: Evolution, Mechanisms, and Adaptive Significance*. San Diego, Calif: Academic Press; 1996: 333-360.
29. Numan M, Sheehan TP. Neuroanatomical circuitry for mammalian maternal behavior. *Ann N Y Acad Sci*. 1997;807:101-125.
30. Place NJ. Low testosterone in new fathers: comment from Place [letter]. *Trends Ecol Evol*. 2001;16:75-76.
31. Wynne-Edwards KE. Low testosterone in new fathers: reply from Wynne-Edwards [letter]. *Trends Ecol Evol*. 2001;16:76.
32. Schmidt-Reinwald A, Pruessner JC, Hellhammer DH, et al. The cortisol response to awakening in relation to different challenge tests and a 12-hour cortisol rhythm. *Life Sci*. 1999;64:1653-1660.
33. Carter CS, DeVries AC, Taymans SE, Roberts RL, Williams JR, Getz LL. Peptides, steroids, and pair bonding. *Ann N Y Acad Sci*. 1997;807:260-272.
34. Castro WLR, Matt KS. The importance of social condition in the hormonal and behavioral responses to an acute social stressor in the male Siberian dwarf hamster (*Phodopus sungorus*). *Horm Behav*. 1997;32:209-216.
35. Reburn CJ, Wynne-Edwards KE. Cortisol and prolactin concentrations during repeated blood sample collection from freely moving, mouse-sized mammals (*Phodopus* spp). *Comp Med*. 2000;50:184-198.
36. Carter CS. Neuroendocrine perspectives on social attachment and love. *Psychoneuroendocrinology*. 1998;23:779-818.
37. Leon M. The neurobiology of filial learning. *Annu Rev Psychol*. 1992;43:377-398.