A physiological response to fatherhood: Testosterone and cortisol decrease, and estradiol increases in men becoming fathers

by

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SCHOOL OF GRADUATE STUDIES AND RESEARCH

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ABSTRACT

Progress is being made toward understanding neuroendocrine pathways involved in mammalian social behaviour. Parental behaviour is an important component of social behavioural repertoires. To date, mammalian research has focused on parturient female rodents and sheep. Males that naturally show paternal behaviour are an attractive research model because their physiological responses to fatherhood are not simultaneously confounded by pregnancy and lactation. Recent evidence suggests that animal fathers that naturally provide parental care experience hormonal changes before and after the birth. The only published study of hormones in men becoming fathers also found that hormone concentrations responded to fatherhood. This thesis reports results from the first longitudinal endocrine study of men becoming fathers. 'Dads' provided saliva samples from recruitment through to 3 months postpartum. Control men provided age, circannual, and time-of-day comparable saliva samples. After controlling for circannual and circadian effects, 'dads' had lower testosterone, lower cortisol, and a higher proportion of samples with detectable estradiol than control men. Within 'dads', the proportion of estradiol samples that were detectable increased from the month before the birth to the month after. In each of 13 'dads' providing frequent saliva samples, testosterone was low during, and for the week after, the birth. In this highly motivated population of Canadian volunteers attending prenatal classes as couples, men experience hormonal changes associated with fatherhood. Hormones are involved in priming and elicitation of maternal behaviour. The hormones changing in men becoming fathers have

known roles in maternal behaviour. Thus, in men, hormonal changes might also alter thresholds for the expression of paternal responses. If so, this study supports the hypothesis that there is a physiological response associated with involved fatherhood. Future research should examine the mechanisms which elicit hormonal changes within men becoming fathers and the functional role of the hormonal changes in men.

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DEDICATION

For George Berg, Hilda Berg and Gary Berg

Because of them, I understand the grace and fragility of life

TABLE OF CONTENTS

CO-AUTHORSHIP DECLARATION

All experiments were completed, and data generated by S. J. Berg. All analyses of data, preparation of tables and figures, and writing of manuscripts were supervised by Dr. K. E. Wynne-Edwards.

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

B	cortisol, (11 β,17,21-trihydroxy-4-pregnene-3,20-dione)
E_2	17-β estradiol, (1,3,5(10)–estratriene–3,17β–diol)
EB	E_2 benzoate
MPOA	medial preoptic area of the hypothalmus
P_4	progesterone, (4-pregnene-3,20-dione)
PRL	prolactin
RIA	radio-immunoassay
T_5	testosterone, (17 β–hydroxy-4-androsten-3-one)

CHAPTER 1: General Introduction

Progress is being made toward understanding neuroendocrine pathways involved in mammalian social behaviour. Parental behaviour is an important component of social behavioural repertoires (Carter, 1998;Young, Nilsen, Waymire, MacGregor, and Insel, 1999). To date, mammalian research has focused on parturient female rodents and sheep (Fabre-Nys and Martin, 1991;Grosvenor, 1967). Males that naturally show paternal behaviour are an attractive research model because their physiological responses to fatherhood are not simultaneously confounded by pregnancy and lactation. Recent evidence suggests that animal fathers that naturally provide parental care experience hormonal changes before and after the birth (Reburn and Wynne-Edwards, 1999;Ziegler, Wegner, and Snowdon, 1996). The only published study of hormones in men becoming fathers also found that hormone concentrations responded to fatherhood (Storey, Walsh, Quinton, and Wynne-Edwards, 2000). This thesis reports results from the first longitudinal endocrine study of men becoming fathers.

Human fathers are more involved in care of their offspring than most other mammalian fathers. They spend almost 1/3 of the time that mothers spend on childcare (Lamb, Pleck, Charnov, and Levine, 1985). Their degree of parental involvement is influenced by individual skills, support and motivation (Lamb, Pleck, Charnov, and Levine, 1985). In women, early contact with the newborn contributes to the attachment process (Anisfeld and Lipper, 1983;Prodromidis, Field, Arendt, Singer, Yando, and Bendell, 1995). In men, early newborn contact might also be important in their

attachment process (Bowen and Miller, 1980;Bowen and Miller, 1980;Chapman, 1991;Novak, 1990). Some men also experience symptoms of Couvade in which expectant fathers report physical symptoms of pregnancy (i.e. nausea, anxiety, headaches, reduced libido) (Clinton, 1986;Conner and Denson, 1990;Klein, 1991). However, little is known of the internal neural and endocrine mechanisms influencing male parental involvement.

In women, affiliative behaviour toward infants can emerge at any stage of development and is evoked by a wide range of sensory stimuli (tactile, olfactory and visual) (Uvnas-Moberg, 1996). Hormones are also involved in priming and elicitation of maternal behaviour in women and change at times that are important for the expression of maternal behaviour (Fleming, Ruble, Krieger, and Wong, 1997). Specifically, the onset and maintenance of appropriate maternal behaviour is influenced by steroid hormones including cortisol (B), testosterone (T_5) and estradiol (E_2) (Fahrbach and Pfaff, 1986;Fleming and Corter, 1988;Fleming, Ruble, Krieger, and Wong, 1997;Rosenblatt, Mayer, and Giordano, 1988).

In women, corticosteroid concentrations are positively correlated with social affiliation and mother-infant bonding (Fleming, Ruble, Krieger, and Wong, 1997;Leon, 1992). Increased olfactory acuity is positively correlated to corticosteroid concentrations in women during bonding to their newborn (Schaala and Marlierb, 1998). Throughout pregnancy, B increases until a peak at birth where a rapid decline occurs postpartum (Fleming and Corter, 1988). Patterns of B and correlated responses in other female mammals are not available.

In women, T5 increases throughout pregnancy, peaks at birth and decreases rapidly postpartum (Fleming and Corter, 1988). Women's T5 during late pregnancy can reach mean T5 levels in men (Dabbs, de La Rue, and Williams, 1990). New mother's feelings about pregnancy have been negatively correlated with circulating levels of T5 (Fleming and Corter, 1988) but female T5 has not been extensively studied. The peak and rapid decrease of women's T5 patterns have no known effects. It has been established that female mice experience T5 increases postpartum that facilitates maternal aggression (Ghiraldi, Plonsky, and Svare, 1993).

In female mammals, E2 during pregnancy primes maternal behaviour (Numan, 1974). In women, E2 is positively correlated with responsiveness to offspring (Fleming, 1990). Estradiol and progesterone (P4) are commonly known as the 'pregnancy hormones'. Female pigtail macaques increase their rate of interaction with infants in the last weeks of pregnancy in correspondence with an increase in plasma levels of E2 and P4 (Maestripieri and Zehr, 1998). In women, E2 and P4 increase from the first trimester to a peak at birth where a rapid decrease occurs postpartum (Fleming and Corter, 1988).

In men, we have good reason to hypothesize that similar hormonal patterns apply. All of the DNA in females and males is shared - except for a few genes on the Y-chromosome. Thus, all of the code which builds the maternal brain is present in males. The sexes are differentiated physiologically and behaviourally by patterns of gene activation (Arnold, 1996). During embryonic development, hormonal cascades act to differentiate brain, gonadal and secondary sexual structures which are later activated hormonally at times such as puberty or seasonal breeding (George and Wilson, 1994).

The elicitation of paternal behaviour should therefore be possible via the activation of pre-existing maternal neural and endocrine pathways. Evidence supporting this hypothesis is available for neonatally castrated male rats that show maternal behaviour when treated with maternal pregnancy hormones (E_2 and P_4) (Rosenblatt and Ceus, 1998;Rosenblatt, Hazelwood, and Poole, 1996;Sturgis and Bridges, 1997). When E_2- and P_4-treated, castrated male rats were injected with E_2 benzoate (EB), short-latency maternal behaviour was stimulated in males as it is in females (Rosenblatt, Hazelwood, and Poole, 1996). When adult male rats were implanted with E_2 and P_4, and infused with N-Methyl-DL-Aspartic acid, a decline in maternal male behaviour resulted, indicating neural substrates controlling parental behaviour in male and female rats are similar (Sturgis and Bridges, 1997). Using adult gonadectomized male rats primed with E_2 and P_4, E_2 implants in the media preoptic region of the hypothalmus (MPOA) yield shorter latencies for maternal behaviour in males indicating the MPOA mediates estrogen stimulation of maternal behaviour in males as it does in females (Rosenblatt and Ceus, 1998).

Unfortunately, the rat is probably not the ideal animal model because male rats are not normally paternal in the wild. Male rats initially attack or cannibalize neonates (Jakubowski and Terkel, 1985) but after several days of continuous laboratory exposure to pups, become 'sensitized' and show parental behaviours (i.e. licking, retrieval, crouching over pups) (Brown and Douglas, 1991;Rosenblatt, 1967;Rosenblatt and Siegel, 1981). Those changes are not correlated with hormone concentrations or affected by

castration. More appropriate models would be mammals which show paternal behaviour naturally in the wild.

Using such animal models, evidence for males activating neuroendocrine pathways that are similar to females is minimal but consistent (Wynne-Edwards and Reburn, 2000). Of particular relevance for research directed at understanding the biological basis for human behaviour, are recently documented hormonal correlates of paternal behaviour seen in naturally biparental non-human primate species (Dixson and George, 1982;Ziegler, Wegner, Carlson, Lazaro-Perea, and Snowdon, 2000;Ziegler, Wegner, and Snowdon, 1996). The first evidence of hormonal change in a biparental male mammal was shown in common marmosets (*Callithrix jacchus*) that carry their twin offspring on their back. Plasma prolactin (PRL) was five times higher in males with infants than in males without infants (Dixson and George, 1982). Elevated PRL has been associated with infant care-taking in common marmosets and cotton-top tamarins (*Saguinus oedipus*) (Dixson and George, 1982;Ziegler, Wegner, and Snowdon, 1996). In the socially monogamous male cotton-top tamarin, extensive parental care is provided shortly after the birth of his offspring. In tamarin fathers, PRL is higher postpartum than in other males and correlates with the number of previous births a male has experienced (Ziegler, Wegner, Carlson, Lazaro-Perea, and Snowdon, 2000;Ziegler, Wegner, and Snowdon, 1996).

In contrast, other studies of PRL in male rhesus monkeys indicate that PRL elevations reflect acute stress (Puri, Puri, and Anand Kumar, 1981;Quadri, Pierson, and Spies, 1978). In tamarins, if PRL is released in response to stress rather than parental care, both

PRL and B should increase following birth and remain elevated through the first weeks postpartum while the tamarin family adjusts. As mentioned, PRL is elevated in tamarins postpartum (Ziegler, Wegner, Carlson, Lazaro-Perea, and Snowdon, 2000;Ziegler, Wegner, and Snowdon, 1996) during which time, B is significantly lower in experienced tamarin fathers and mean B levels are in the same basal range reported for females (Ziegler, Wegner, and Snowdon, 1996). In experienced tamarin fathers, cortisol levels did not coincide with PRL levels during birth and fatherhood suggesting that different neural pathways are probably involved in PRL increase and B secretion during parental care compared to acute stress situations (Ziegler, Wegner, and Snowdon, 1996).

Mating and parenting occur simultaneously in cotton-top tamarins. In contrast, breeding and parenting are separated in time in seasonally breeding bird species. In biparental birds, PRL and T5 have a reciprocal relationship. In birds at the end of breeding season, T5 decreases, parental behaviour is expressed, and PRL increases (Hegner and Wingfield, 1987;Logan and Wingfield, 1995;Schoech, Ketterson, Nolan, Sharp, and Buntin, 1998). Father tamarins show parental behaviour for young immediately following birth (Snowdon, 1996) while females ovulate and conceive within 13-29 days postpartum (Ziegler, Wegner, Carlson, Lazaro-Perea, and Snowdon, 2000). Since tamarin males show immediate parenting behaviour upon birth and elevated PRL before birth, no change in PRL and therefore no reciprocal T5 change was expected (Ziegler, Wegner, Carlson, Lazaro-Perea, and Snowdon, 2000). However, experienced male cotton-top tamarins showed an increase in T5 during the first 5 days postpartum. The most significant T5 changes occurred in males whose mates ovulated during the 15

days following birth (Ziegler, Wegner, Carlson, Lazaro-Perea, and Snowdon, 2000). A T5 mating response occurs in cotton-top tamarins but unlike biparental birds, tamarins mate throughout the parenting time period. Male tamarin T5 levels might therefore increase during the first 15 days postpartum as a reflection of mating patterns, in contrast to a postpartum T5 suppression seen in other biparental mammals such as rodents (Reburn and Wynne-Edwards, 1999:Ziegler, Wegner, Carlson, Lazaro-Perea, and Snowdon, 2000).

In rodents where males show paternal care, there is similar evidence for hormonal influences on male parental behaviour. In male biparental hamsters (*Phodopus campbelli*), B was reduced after the establishment of a pairbond, and increased prior to birth. This increase possibly sensitized them to stimuli for olfactory imprinting on the impending litter (Reburn and Wynne-Edwards, 1999).

A T5 reduction at the onset of paternal behaviour occurs in biparental hamsters (Reburn and Wynne-Edwards, 1999) and Mongolian gerbils (*Meriones unguiculatus*) (Brown, Murdoch, Murphy, and Moger, 1995) after T5 is elevated at the end of pregnancy, facilitating mating as early as the day of birth. Recent work with Mongolian gerbils shows that manipulation of T5 affects paternal behaviour response. Elevation of T5 in Mongolian gerbils eliminates paternal behaviour while a reduction of T5 elicits paternal behaviour (Clark and Galef, 1999). In other rodent species where paternal care is not extensive, males typically exhibit a T5 reduction at birth which is involved in the suppression of infanticidal aggression (Brown, 1986;Perrigo, Belvin, and Vom Saal, 1991).

Estradiol has not been studied in male rodents or other male biparental mammals.

Thus, available evidence broadly supports a role for steroid hormones in paternal behavioural responsiveness.

A recent study in men has also identified hormonal changes in men becoming fathers and noted that these changes affect the same hormones known to influence women (Fleming, Ruble, Krieger, and Wong, 1997;Storey, Walsh, Quinton, and Wynne-Edwards, 2000). The preliminary study of men becoming fathers measured serum hormone concentrations and responses to infant stimuli within couples (Storey, Walsh, Quinton, and Wynne-Edwards, 2000). Two blood samples were collected from each couple by venipuncture during a home visit by the researchers before or after the birth of their child. The first blood sample was considered to be 'baseline' and the second blood sample, which followed the infant stimuli was considered to be 'responses to those stimuli'. The difference between the two concentrations was the 'situational reactivity' for that hormone. The infant stimuli consisted of auditory (tape-recorded cries from the neonatal unit), visual (a video of a mother nursing a newborn) and olfactory (video watching, while the man held a doll wrapped in a soiled receiving blanket from the neonatal unit) signals (Storey, Walsh, Quinton, and Wynne-Edwards, 2000).

Results showed changes in B among time stages for both men and women. For men and women, the highest B and PRL concentrations occurred in the last 3 weeks of pregnancy. In the situational reactivity test, there was a significant decrease from sample 1 to sample 2 for men and women for B and PRL. Both B and PRL increase in some stressful situations in humans (Aun, McIntosh, Lee, and Egdahl, 1984;Quadri, Pierson,

and Spies, 1978;Rotton, Dubitsky, Milov, White, and Cherie Clark, 1997). Men with higher baseline levels of B had greater situational reactivity and B decreases were greater between samples in the last 3 weeks of pregnancy (Storey, Walsh, Quinton, and Wynne-Edwards, 2000).

In the steroids, women's E2 showed the largest changes among time stages of any of the hormones. Women's E2 was significantly elevated during the last 3 weeks of pregnancy and dropped to undetectable concentrations after delivery of the placenta. Women were not measured for T5. Likewise, men were not measured for E2 (Storey, Walsh, Quinton, and Wynne-Edwards, 2000).

In men, T5 levels responded to fatherhood. During the first 3 weeks of fatherhood men's T5 was lower than the last 3 weeks of pregnancy. Between the two blood samples, T5 recovered to higher concentrations, suggesting that the changes in T5 in men were changes in reactivity, not basal changes in secretion (Storey, Walsh, Quinton, and Wynne-Edwards, 2000).

Hormonal changes in men might facilitate the expression of involved paternal behaviour. This does not imply that hormone changes are either necessary or sufficient to produce human paternal behaviour. Responses to hormones typically depend upon reproductive condition, social status and context. For example, in women, hormonal effects might act on feelings of nurturance and influence mothers' feelings of well-being since mothers that show less of an E2 decline from pregnancy to early postpartum report the highest feelings of attachment to their infants (Fleming, Ruble, Krieger, and Wong, 1997). Hormones can modulate womens' responses and feelings to infants but are not

directly related to increasing attachment feelings across pregnancy (Fleming, Ruble, Krieger, and Wong, 1997). Hormones can increase or decrease the probability and intensity of a behaviour shown in response to a standard stimulus by altering thresholds (Clark and Galef, 1999;Sapolsky, 1986). Hormones don't elicit parental behaviour. Even in rodents, behaviour can be elicited in the absence of hormones (Sapolsky, 1986;Wingfield, Jacobs, and Hillgarth, 1997).

Given this caveat that hormone changes will not 'cause' changes in men's behaviour, this thesis was designed to critically test the results of the earlier study of men becoming fathers. Specifically it was designed to quantify individual variability, improve statistical power, incorporate needed controls, and quantify the 'female sex steroid' E2, in men.

This thesis will therefore test the hypothesis that concentrations of the steroid hormones estradiol, testosterone and cortisol will change within individual men as they become fathers for the first time.

Specifically:

1/. E2 concentration will be low in new fathers; and

2/. T5 concentration will be low in new fathers; and

3/. B concentration increases will anticipate the birth.

These predictions are based upon a) best available data in non-human primates and rodents that naturally express paternal behaviour, b) the previous study in men (Storey, Walsh, Quinton, and Wynne-Edwards, 2000), and c) the assumption that natural selection will preferentially activate existing maternal neuroendocrine circuits when paternal behaviour is adaptive for male mammals.

If this study confirms robust patterns of hormonal change in men becoming fathers, then the biological basis for involved fatherhood will be established as a viable field of scientific inquiry.

CHAPTER 2: Testosterone and Cortisol Decrease, and Estradiol Increases, in Men Becoming Fathers

Abstract

Recent evidence suggests that animal fathers that naturally provide parental care experience hormonal changes before and after the birth. We report results from the first longitudinal endocrine study of men becoming fathers. 'Dads' (N =33) provided saliva samples from recruitment through to 3 months after the birth. Control men (N=14) provided age, season, and time-of-day comparable saliva samples. A subset of 13 'dads' also collected samples daily in the weeks before and after the birth. After controlling for effects of time of day and season, 'dads' had lower testosterone, lower cortisol, and a higher proportion of samples with detectable estradiol than control men. Within 'dads', the proportion of estradiol samples that were detectable also increased from the month before the birth to the month after. In each of 13 'dads' providing frequent samples, testosterone was low during, and for the week after, the birth. However, individuals differed in testosterone concentration and variation. For five men the low testosterone after the birth was no change from previous concentrations, for three men it was a decrease following a pre-birth increase, and for five men it was a decrease relative to all other times. Thus, in this highly motivated population of Canadian volunteers attending prenatal classes as couples, men experience hormonal changes associated with

fatherhood. As the hormones involved have known roles in maternal behaviour, the biological basis for becoming a 'dad' is worthy of further endocrinological investigation.

Introduction

We have long known that the hormonal changes of pregnancy, birth, and early lactation facilitate the expression of maternal behaviour in women, non-human primates, and other mammals (Fleming, Ruble, Krieger, and Wong, 1997;Numan, 1994). Recent laboratory studies have suggested that natural fathers of the animal kingdom may also use hormonal changes to facilitate paternal behaviour (Brown, Murdoch, Murphy, and Moger, 1995;Dixson and George, 1982;Gubernick and Nelson, 1989;Jones, Reburn, and Wynne-Edwards, submitted;Reburn and Wynne-Edwards, 1999;Ziegler, Wegner, and Snowdon, 1996), and may be activating the same neuroendocrine circuits as females (Wynne-Edwards and Reburn, 2000).

In April 2000, the first study of hormone changes in men becoming fathers was published (Storey, Walsh, Quinton, and Wynne-Edwards, 2000). Canadian couples were recruited from pre-natal 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 (Elwood and Mason, 1994) and emotional responses to the stimuli. Prolactin and cortisol concentrations were higher in men sampled during the

final three 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 two samples (called the 'situational reactivity') also varied relative to the birth: just before the birth, cortisol decreased between samples whereas just after the birth, testosterone increased between the two samples.

The hormones that were changing were the same ones implicated in animal studies of maternal and paternal behaviour, and the psychometric measures identified correlations between those hormones and paternal responsiveness (Storey, Walsh, Quinton, and Wynne-Edwards, 2000). As such, they suggested that men exposed to appropriate stimuli might experience a muted version of the endocrine changes of pregnancy.

The present study was designed to critically test those results by improving statistical power, incorporating needed controls, and quantifying the 'female sex steroid', estradiol. Specifically, these data differ from previously available results by: a) collecting longitudinal information on individuals throughout the transition from mid-pregnancy to early fatherhood, b) providing a control group matched for age, season and time of day, c) reducing sampling stress by having men collect saliva samples at home, and d) adding quantification of estradiol in men becoming fathers.

Experimental Subjects

Starting in February 1999, 45 men (ages 23-43, median = 33) and their pregnant partners (ages 23-37, median = 32) were recruited from first trimester prenatal classes in

Kingston, Canada run by Childbirth Kingston. The population is a highly self-selected group in which women are accompanied by the father and couples pay for the classes (as opposed to free classes through the local Health Unit). Only samples from men are considered in these analyses. Recruitment success and retention was high, with approximately 50% of couples in classes were recruited. After recruitment, 76% of couples completed the study. Reasons for departure ranged from premature delivery of twins to discomfort about having forgotten to collect samples. An additional 14 men who had never been fathers were age and activity-matched to the 'expectant' group and served as both baselines for comparison and controls for time-of-day and seasonal changes in hormone concentrations. The 'dads' and control men that completed the study were well-matched for age ($t_{47} = 0.05$, $P = 0.96$). All subjects were Caucasian. Recruitment, informed consent, and questionnaire procedures were approved by the Queen's University Research Ethics Board as Biol-009-98.

Materials and Methods

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. Saliva was chosen because a) it is non-invasive and therefore does not evoke apprehension the way that venipuncture can, b) it avoids the need for a trained health professional at the time of sampling, c) the biologically active, unbound fraction of steroid hormones including

testosterone, estradiol, and cortisol can easily be assayed from saliva (Dabbs, 1990b), d) samples can be stored at home freezer temperatures without degradation of the hormones (Dabbs, 1991), and e) saliva is a low biohazard relative to blood.

Couples were informed about the importance of choosing a consistent time of day and were provided with labeled clean glass vials and sugar-free chewing gum to stimulate saliva flow (Dabbs, 1991). Each couple was phoned on a weekly basis to remind them to collect their samples.

A sub-sample of 13 'dads' agreed to increase their sampling frequency during the last 3 weeks and first two weeks after the birth. Control men (N= 14; ages 22-46, median = 34) were recruited from the Kingston community and encouraged to provide samples on a similar pattern. Samples were collected and immediately frozen at home. Every few weeks, we retrieved those samples, thawed them, centrifuged them to remove mucous and solids, and then stored them at -20°C until they were assayed for hormone content.

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

Hormone determinations

Testosterone and cortisol were quantified using commercially available ^{125}I kits with specific guidelines validated for salivary determinations (Total Testosterone (= free testosterone because determinations were based on saliva) and Cortisol, Coat-a-Count, Diagnostic Products Corporation, Inter-Medico, Markham, ON). Estradiol was

determined via a ^{3}H radioimmunoassay in routine use in this laboratory. Samples (1.0 ml) and standards (diluted to 1.0 ml in dH2O) were extracted into two volumes of anesthesia-grade diethyl ether (AnalaR, BDH Inc., Toronto, CA) and dried before the competitive binding assay. Reactants were 2000 dpm ^{3}H estradiol (Dupont, NET 317 2,4,6,7 –^{3}H(N), Lot 2775-017) and GDN #244 anti-estradiol-6-BSA (G. D. Niswender, Colorado State University). Standards and calibration pools were assayed in duplicate. Triplicate determination of a pool of female saliva was used as a control on the linear portion of the binding curve and yielded an intra-assay variability of 8.9% and an interassay variability of 15.3%. The lower limit of estradiol assay sensitivity was arbitrarily set to 2.15 pg/ml of saliva, corresponding to the second of 11 estradiol calibration standards (= 88% binding). Estradiol concentrations in men (608 determinations in 13 assays, maximum = 17.5 pg/ml, median = 2.82 pg/ml) were often below that threshold. Data were therefore transformed into nominal results and each sample for each man was scored as estradiol 'undetectable' or 'detectable'.

Statistical analyses

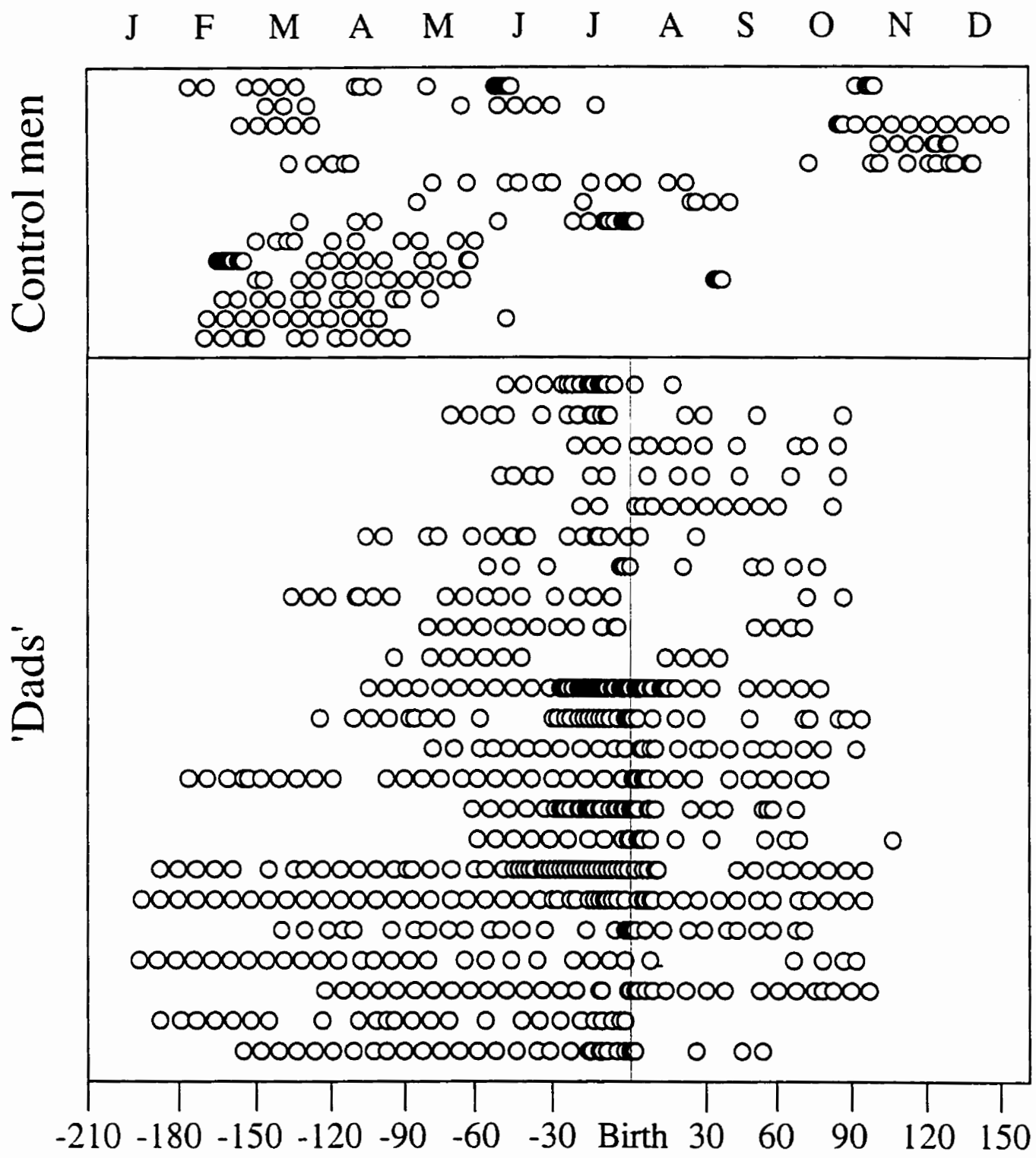
Results are expressed as mean ± standard error. All analyses were conducted using JMP (SAS Institute, Cary, N.C.) running on a Macintosh computer. Statistical comparisons were two-tailed and applied a critical alpha of 0.05. For analysis of variance, the overall F statistic and significance are shown. Subsequent mention of post hoc differences between groups is based on Tukey-Kramer with alpha = 0.05. 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

The temporal distribution of available samples is shown schematically in Figure 1. Control results are used to define patterns of hormone change during the day and across seasons. Following those analyses, hormone results for control men and 'dads' are compared, including investigation of individual patterns of variance.

Figure 1. Schematic representation of the temporal distribution of samples collected for each 'dad' (relative to the birth) and each control man (relative to Julian date)


J F M A M J J A S O N D
Control men
'Dads'
-210 -180 -150 -120 -90 -60 -30 Birth 30 60 90 120 150

Time of day effects

Subjects were allowed to choose the time of day for providing samples. This resulted in some substantive differences in sample collection frequency (Table 1). Data for 14 individual control men are shown in Appendix B. Control men were most likely to choose morning, with 153 of 228 samples (13 men) providing samples in that interval (67%) whereas 'dads' were most likely to choose late evening with 368 of 669 samples (15 men) providing samples in that interval (55%). Thus, comparisons between control men and 'dads' (below) consider time of day as a variable and focus on morning versus late evening.

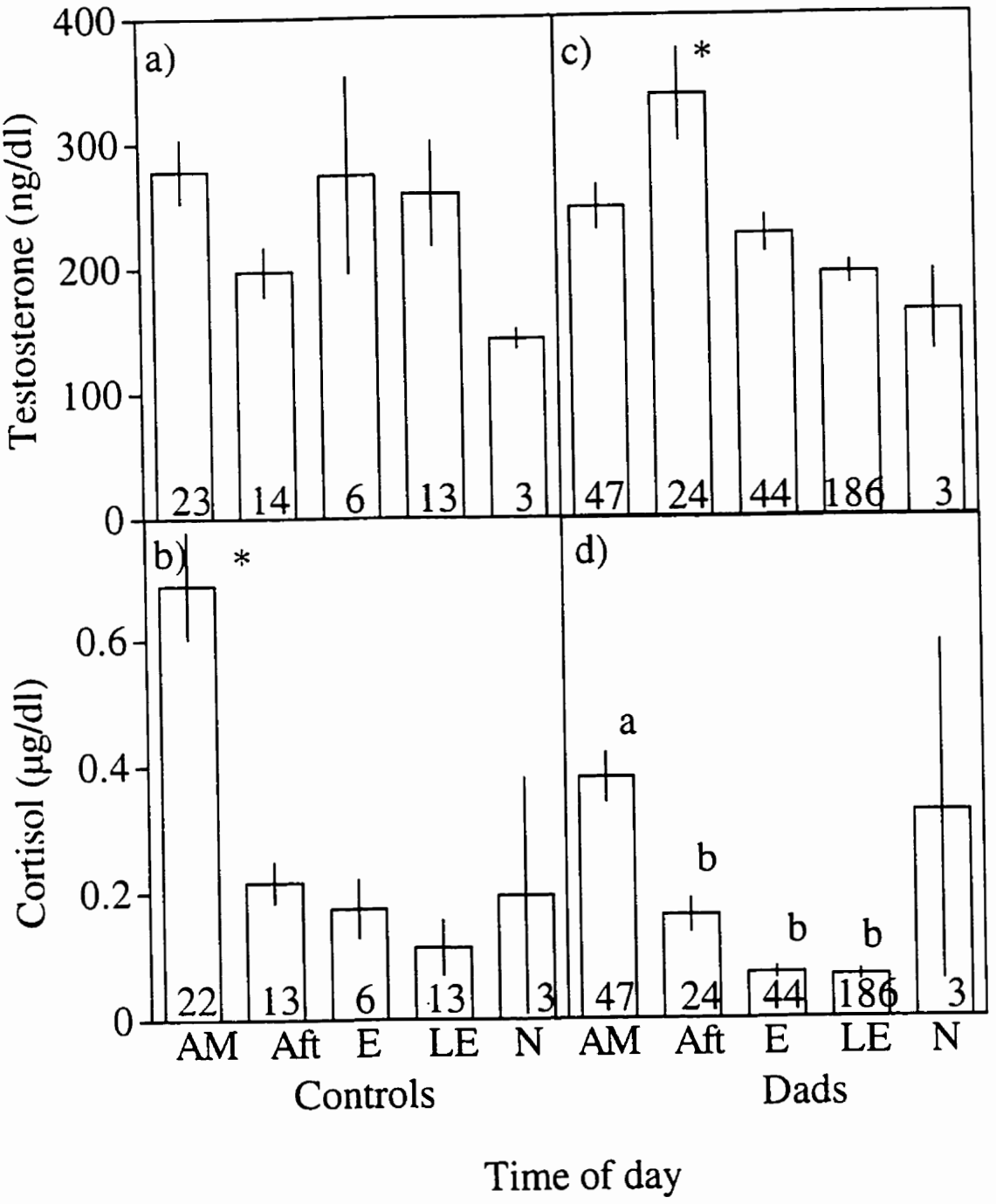
Table 1. Time-of-day distribution of samples.

	Time	Night	Morning	Afternoon	Evening	Late Evening	Unknown	Total
	Hours	0:01 – 06:30	06:31 - 11:00	11:01 - 16:00	16:01 – 19:00	19:01 – 0:00		
Control	# samples	5	153	21	32	17	0	228
	% samples	2.2	67.1	9.2	14.0	7.5		100
	# men	3	13	7	7	5		14
'Dad'	# samples	14	97	51	100	368	39	669
	% samples	2.1	14.5	7.6	15.0	55.0	5.8	100
	# men	9	12	10	14	15	2	23

Six control men were asked to provide samples at different times of day (night = 000 – 0630h, morning = 0630-1100 h, afternoon = 1100-1600h, evening = 1600 – 1900 h, late evening = 1900 – 2400 h) over a 3-6 day time span, resulting in 59 samples (range 9-13 per individual) with between 2 and 6 men providing samples in each time category (those samples are indicated as open circles in Figures 9-11, Appendix B). Testosterone did not vary systematically across the time categories ($F_{4,58} = 1.52$, $P = 0.21$; Figure 2a) although morning testosterone was 88 ng/dl higher than late evening in the five control men with samples in both intervals (paired $t_4 = 4.56$, $P = 0.01$). A morning elevation in testosterone has been seen in previous studies (Dabbs, 1990b;Marrama, Carani, Baraghini, Volpe, Zini, Celani, and Montanini, 1982). As expected (Hucklebridge, Clow, Abeyguneratne, Huezo-Diaz, and Evans, 1999), there was a significant elevation in cortisol during the morning relative to all other times ($F_{4,57} = 12.09$, $P < 0.0001$; Figure 2b). For the five men with morning and late evening samples, the magnitude of the difference was 0.48 µg/dl, from 0.09 to 0.57 µg/dl (paired t4 = 3.70, P = 0.02). The probability of detecting estradiol in a sample was also affected by the time of day (Pearson Chi-square (df = 4) = 13.80, $P < 0.008$). Similar to patterns in women (Patrick, Challis, Natale, and Richardson, 1979;Reck, Noss, and Breckwoldt, 1979), estradiol was significantly lower during the afternoon than during the morning with 11 of 16 samples (5 of 6 men) readily quantified in the morning but only 2 of 13 samples (1 of 5 men) quantifiable in the afternoon (Pearson Chi-square (df =1) = 8.26, $P < 0.005$). Morning and late evening estradiol results did not differ ($P = 0.56$).

A total of 18 'Dads' who were not shift workers, excluding all samples collected during labour and delivery or after the birth (when circadian patterns were expected to be disrupted) yielded 304 samples for distribution across the same time-of-day bins as control men (Figures 2c and 2d). Time of day affected testosterone concentration ($F_{4,303}$ = 8.22, $P < 0.001$; Figure 2c). Posthoc Tukey-Kramer HSD testing ($P < 0.05$) revealed that testosterone concentrations were significantly higher in the afternoon than at all other times. Time of day also affected cortisol concentration ($F_{4,303} = 48.077$, $P < 0.0001$; Figure 2d). Posthoc Tukey-Kramer HSD testing ($P < 0.05$) revealed that morning cortisol concentrations were significantly higher than afternoon, evening and late evening.

Figure 2. Mean ± SE a) testosterone concentration and b) cortisol concentration for the 59 samples from six control men who deliberately collected samples at different times of day over a 3-6 day period, and c) testosterone concentration and d) cortisol concentration for the 304 samples from 18 'dads' who are not shift workers, across the same time-of-day bins. Sample sizes are indicated for each time-of-day and hormone. For cortisol, the asterisk indicates that morning samples were significantly higher than all other times. For testosterone, the asterisk indicates that afternoon samples were significantly higher than all other times. Times of day with different lower case letters were significantly different with Tukey-Kramer posthoc testing.


a)
c)
b)
d)
*
*
a
b
b
b
400
300
200
100
0
0.6
0.4
0.2
0
Testosterone (ng/dl)
Cortisol (µg/dl)
23
14
6
13
3
47
24
44
186
3
22
13
6
13
3
47
24
44
186
3
AM
Aft
E
LE
N
AM
Aft
E
LE
N
Controls
Dads
Time of day

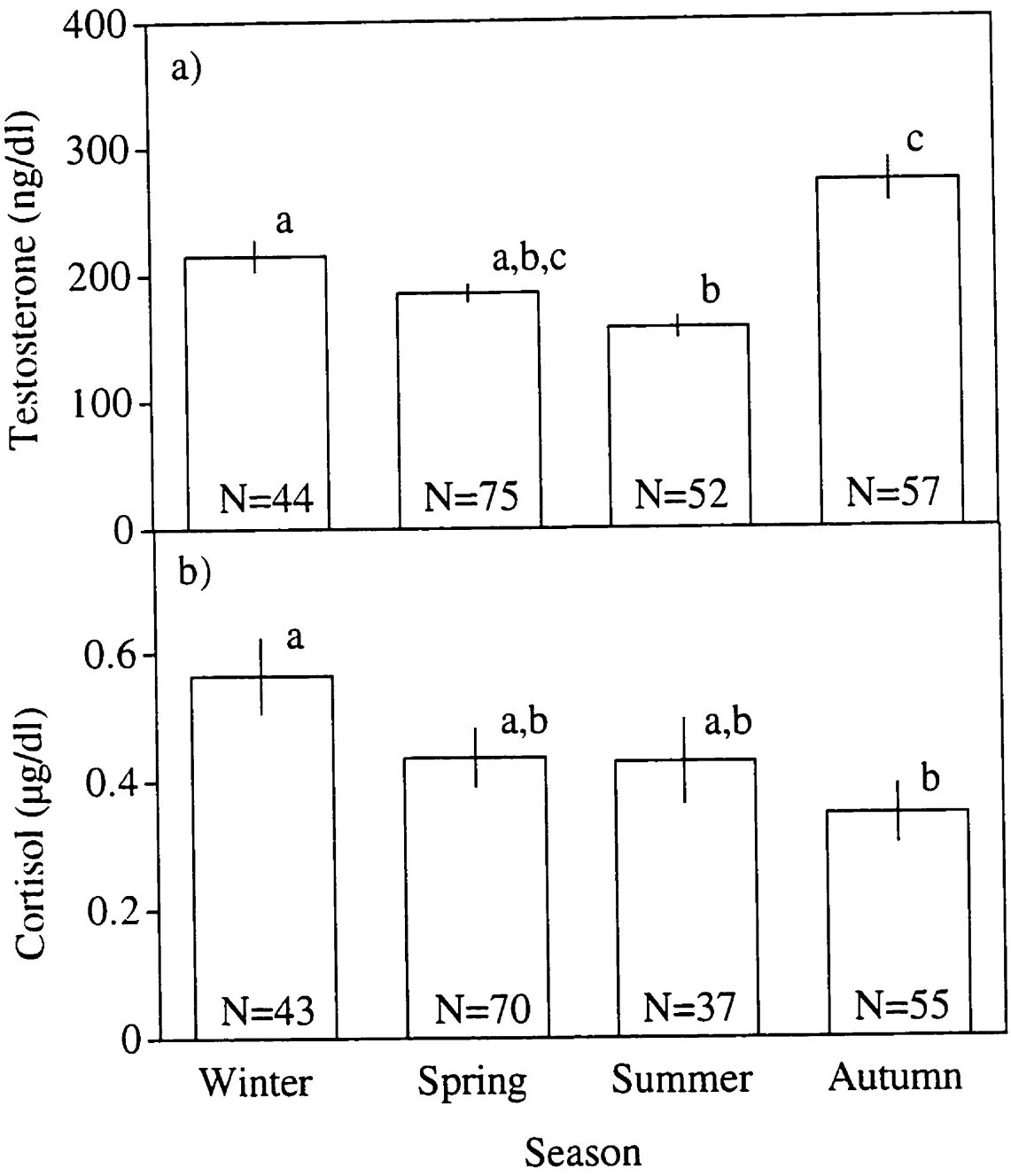
Seasonal effects

Samples were similarly distributed across seasons in control men and 'dads' (Table 2). Testosterone concentration in samples from control men was influenced by season ($F_{3,227} = 19.58$, $P < 0.0001$; Figure 3a). There was a significant increase in salivary testosterone during the autumn (September 22-December 31) and a decrease in testosterone during the summer (June 22 – September 21). Results were similar when each man only contributed a single value per season to the analysis ($F_{3,33} = 4.64$, $P = 0.01$) with summer testosterone significantly lower than autumn testosterone. Seasonal patterns of testosterone variation with autumn peaks have been reported previously (Dabbs, 1990a;Reinberg, Lagoguey, Chauffournier, and Cesselin, 1975), although other studies find other seasonal peaks (Cousins, Yen, Meis, Halberg, and Brink, 1986;Smals, Kloppenborg, and Benraad, 1976). When all control cortisol samples were considered, there was also an effect of season ($F_{3,205} = 2.79$, $P < 0.05$; Figure 3b) with winter cortisol concentrations higher than autumn concentrations. When the statistical power was reduced to a single value for each man in any season, the effect of season was not significant ($F_{3,30} = 1.07$, $P = 0.38$). There was no evidence for a seasonal pattern of variation in estradiol detection (N= 140, Pearson Chi-square (3) = 4.43, $P = 0.22$).

Table 2. Seasonal distribution of samples.

	Season	Winter	Spring	Summer	Autumn	Total
	Julian Dates	1 – 82	83 – 172	173 – 265	266 – 365	
Control	# samples	44	75	52	57	228
	% samples	19.3	32.9	22.8	25.0	
	# men	10	13	6	4	
'Dad'	# samples	97	314	176	82	669
	% samples	14.5	46.9	26.3	12.3	
	# men	17	21	22	6	

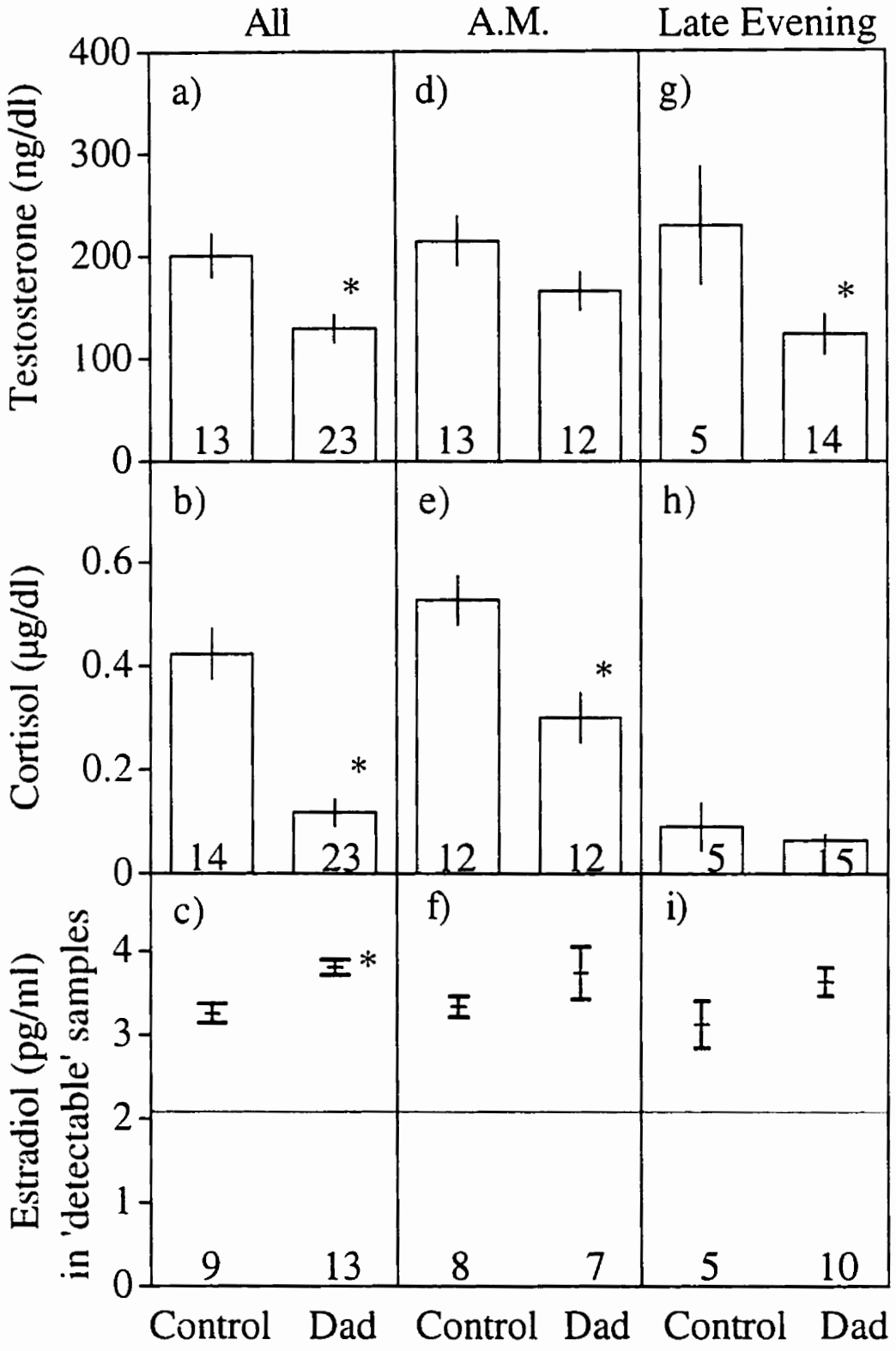
Figure 3. Mean ± SE a) testosterone concentration and b) cortisol concentration for the samples from control men relative to season. Sample sizes are indicated for each season and hormone. Seasons with different lower case letters were significantly different with Tukey-Kramer posthoc testing.


a)
400
300
200
100
0
Testosterone (ng/dl)
a
a,b,c
b
c
N=44
N=75
N=52
N=57
b)
0.6
0.4
0.2
0
Cortisol (µg/dl)
a
a,b
a,b
b
N=43
N=70
N=37
N=55
Winter
Spring
Summer
Autumn
Season

Differences between Controls and 'Dads'

Testosterone concentration was significantly lower in the overall sample of 23 'dads' than in the 14 control men (t_{35} = 3.0, P = 0.005: Figure 4a). Time of day was a potentially important confounding variable in these comparisons because testosterone was higher in the morning than the late evening in control men and that difference was in the same direction as the sampling bias and the difference between control men and 'dads'. However, different times of day did not explain the observed difference between control men and 'dads'. In a two-way ANOVA with control men versus 'dads' and morning versus late evening as class variables, there was an effect of fatherhood ($F_{1,43}$ = 9.86, $P < 0.005$) but no effect of the two sampling times (P = 0.85) and no interaction (P = 0.47). In morning samples (Figure 4d), testosterone was higher in control men (t_{22} = 2.09, P = 0.05) by approximately 65 ng/dl and in late evening samples (Figure 4g), testosterone was higher in control men (t_{17} = 2.28, P = 0.04) by approximately 100 ng/dl. As expected based on results with control men, testosterone in 'dads' was elevated in autumn ($F_{3,636}$ = 10.12, $P < 0.0001$). However, the effect was not seen when each man contributed a single value for each season ($F_{3,61}$ = 0.49, P = 0.69).

Figure 4. Mean ± SE All a) testosterone concentration and b) cortisol concentration and c) 'detectable' estradiol for control men versus 'Dads', d-f) morning samples only for control men versus 'Dads' for all three hormones and g-i) Late Evening samples only for control men versus 'Dads' for all three hormones. All samples for each man were reduced to an average for him so that the independent sample size is the number of men followed, not the number of samples assayed. Asterisks indicate significant differences.


All
A.M.
Late Evening
Testosterone (ng/dl)
400
300
200
100
0
a)
d)
g)
13
23
13
12
5
14
*
Cortisol (µg/dl)
0.6
0.4
0.2
0
b)
e)
h)
14
23
12
12
5
15
*
Estradiol (pg/ml) in 'detectable' samples
4
3
2
1
0
c)
f)
i)
9
13
8
7
5
10
Control
Dad
Control
Dad
Control
Dad

Cortisol concentration was significantly lower in 'dads' than in control men (t_{34} = 6.05 P = 0.0001; Figure 4b). In a two-way ANOVA with control men versus 'dads' and morning versus late evening as class variables, cortisol was higher in control men ($F_{1,43}$ = 10.99, P = 0.002) and, as expected, morning was also higher than late evening ($F_{1,43}$ = 56.36, P = 0.0001) with a significant interaction term ($F_{1,43}$ = 6.86, P < 0.02). In morning samples, cortisol was approximately 25 μg/dl higher in control men (Figure 4e) (t_{21} = 3.65, P < 0.002). In late evening samples (Figure 4h), there was no difference (t_{18} = 0.86, P = 0.40). The morning elevation of cortisol remained following the birth of the child. In samples from 'dads' that were obtained after the birth, when sleep disruption could alter circadian secretion patterns, the two-way ANOVA yielded the same pattern of variation. Thus, the sampling bias towards morning samples from control men contributes to the magnitude of the difference in Figure 4b, but does not explain it. In the morning, before the sleep-disruption of fatherhood, cortisol is higher in control men than 'dads' (t_{20} = 3.01, P < 0.01). Like control men, there was no evidence that season altered concentrations of cortisol across all samples ($F_{3,655}$ = 1.54, P = 0.20), with only one value per man ($F_{3,66}$ = 0.55, P = 0.65), or when cortisol determinations after the birth were excluded ($F_{3,30}$ = 0.77, P = 0.52).

Only men with high sampling frequencies were assayed for estradiol, resulting in a range of 12-25 determinations for 9 controls and 19-59 determinations for each of 13 'dads'. Overall, estradiol was detected in a significantly higher proportion of the 454 'dad' than the 154 'control' samples (Pearson Chi-square (df = 1) = 6.51, P < 0.01). In the samples where estradiol was reliably detected, concentrations were also higher in

'dads' than controls (t_{393} = -3.16 $P < 0.002$). This pattern was the same when the 'detectable' samples were reduced to an average estradiol concentration for each man (t_{20} = -3.75 P = 0.001; Figure 4c). However, comparison of the proportion of each man's samples which were detectable did not identify a significant difference between controls and 'dads' (t_{20} = -0.88 P = 0.39). Differential representation of time of day in control men and 'dads' was not expected to influence these results because morning and late evening estradiol results were similar in controls. Restriction of time-of-day to morning and late evening in control men and dads confirmed no significant difference between the groups (Figures 4f and 4i). Morning estradiol in both groups was similar (t_{13} = -1.266 P = 0.2277), as was estradiol when restricted to late evening (t_{13} = -1.640 P = 0.1250). In contrast to control men, estradiol was elevated during spring ($F_{3,453}$ = 3.78, P = 0.01). However that effect is explained below by changes in estradiol relative to the birth.

Thus, after controlling for time of day and season changes in control hormone samples, 'dads' had lower testosterone concentrations, lower cortisol concentrations and higher estradiol concentrations than control men.

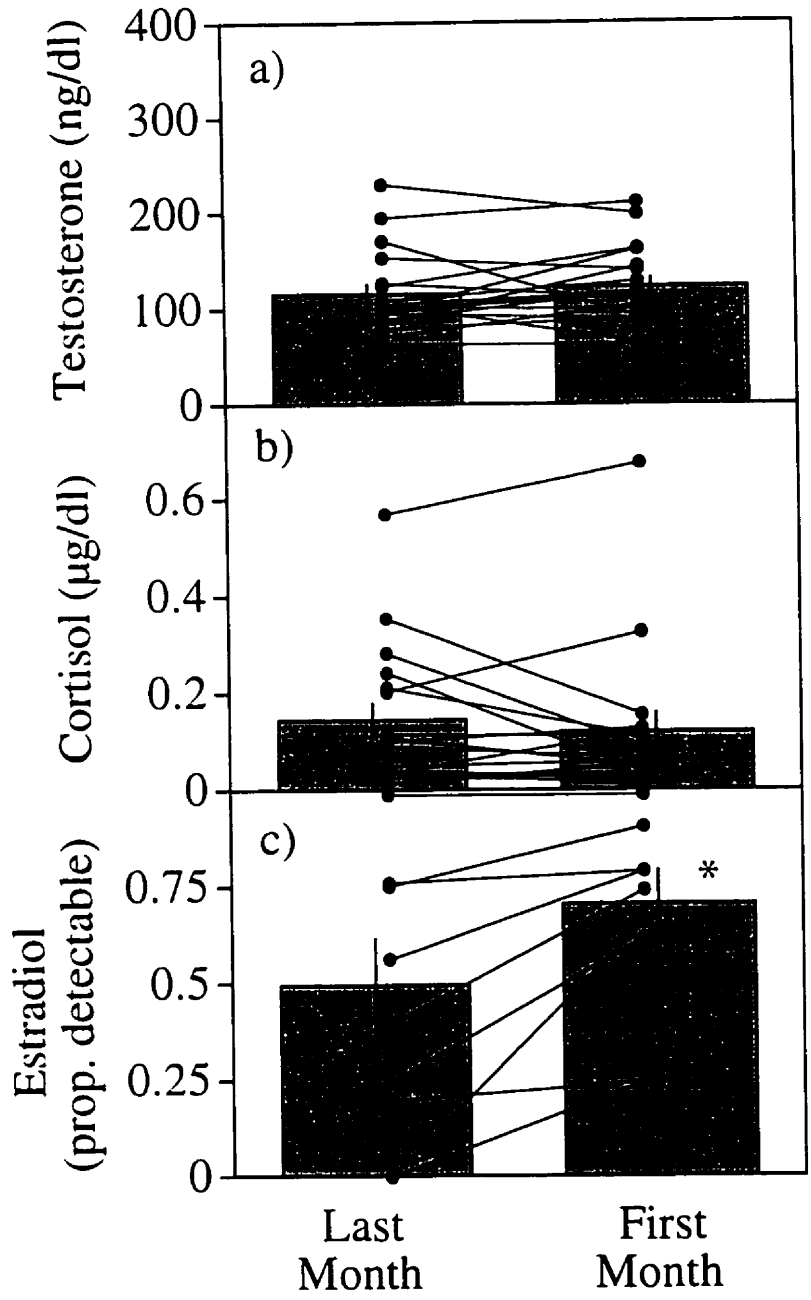
Changes within 'dads'

Samples were retrospectively assigned to one of nine stages of pregnancy based on the actual date of the birth. Those stages were 'first trimester' =-200 to -181 d (8 samples from 4 men), 'second trimester' = -180 to -88 d (98 samples from 13 men); 'third trimester' = -87 to -31 d (139 samples from 21 men); 'last month' = -30 to -9 d (120 samples from 21 men); 'last week' = -8 to -1 d (69 samples from 20 men); 'birth' = -1 to

1d (29 samples from 9 men), 'first week' = 2 to 8 d (50 samples from 17 men); 'first month' = 9 to 30 d (52 samples from 19 men); and 'established' =31 to 110 d (104 samples from 20 men)

Eleven men had samples in each of 'third trimester', 'last month', 'last week', 'first week', 'first month' and 'established' intervals. These 'dads' were uniform relative to annual cycles. All but one had spring or summer births. In a two-way ANOVA for testosterone with stage and couple as class variables, there was a significant effect of stage ($F_{5,358} = 2.78$, $P = 0.02$) and couple ($F_{10,358} = 16.70$, $P < 0.0001$) as well as a strong interaction ($F_{50, 358} = 2.76$, $P < 0.0001$). Restricting the comparison to the nineteen men which had samples in the month preceding the birth ('last month' + 'last week') and the comparable interval after the birth ('first week + 'first month') did not clarify the pattern. Within those men, there was no evidence for a systematic change in testosterone concentration (Paired $t_{18} = 0.89$, $P = 0.39$; Figure 5a). To clarify the significant effect of couple and the interaction term, patterns of testosterone variance within individual men are considered below.

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 ± SE a) testosterone, b) cortisol, and c) proportion of estradiol samples detectable are shown as histograms. Symbols and lines connect mean values for individuals across the two intervals. The asterisk indicates the significant increase in the proportion of estradiol samples detectable from before until after the birth. Patterns of testosterone variance are explored further in Figure 6.


a)
b)
c)
Testosterone (ng/dl)
Cortisol (µg/dl)
Estradiol
(prop. detectable)
400
300
200
100
0
0.6
0.4
0.2
0
0.75
0.5
0.25
0
*
Last
Month
First
Month

In a two-way ANOVA for cortisol with stage and couple as class variables, there was no effect of stage ($F_{5,356} = 1.69$, $P = 0.14$), a significant effect of couple ($F_{10,356} = 10.75$, $P < 0.0001$) and no interaction ($F_{50,356} = 1.25$, $P = 0.13$). Eighteen men had samples in the month preceding the birth ('last month' + 'last week') and the comparable interval after the birth ('first week + 'first month'). Within those men, there was no evidence for a systematic change in cortisol concentration (Paired $t_{17} = 1.11$, $P = 0.28$; Figure 5b).

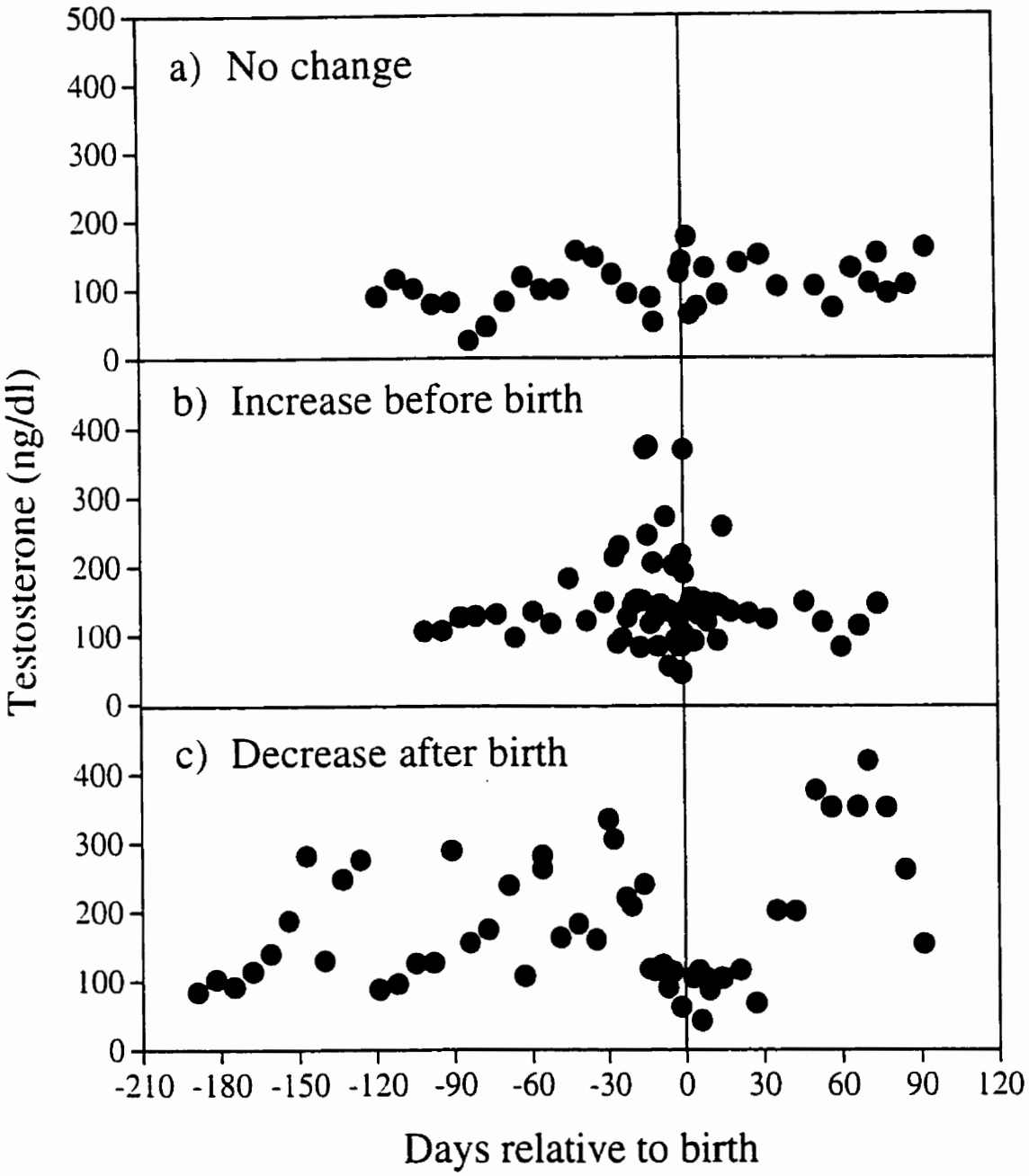
In a two-way ANOVA for estradiol with stage and couple as class variables, there was a significant effect of stage ($F_{5,324} = 2.56$, $P = 0.03$) and a significant effect of couple ($F_{10,324} = 6.35$, $P < 0.0001$) but no interaction ($F_{50,324} = 1.14$, $P = 0.25$). The proportion of estradiol samples that were detectable did not change across the six stages ($F_{5,66} = 0.93$, $P = 0.47$) because small sample sizes in the 'last week' and 'first week' stages gave low confidence values for the proportion of samples detectable. Ten men had at least 5 samples in the month preceding the birth ('last month' + 'last week') and the comparable interval after the birth ('first week + 'first month'). For those men, the proportion of samples with detectable estradiol concentrations increased significantly from before until after the birth (Paired $t_9 = 3.12$, $P = 0.01$; Figure 5c).

Patterns of Testosterone Variance

The significant effects of couple and interaction resulted from at least three underlying patterns of testosterone variation relative to the birth. Data for 13 individual 'Dads' are shown in Appendix C. Assignment into these variance categories was based on all samples from each man (those samples are indicated as closed circles in Figures

12-14, Appendix C)Thirteen 'dads' with 25-69 samples were considered for this analysis. Five 'dads' (38.5%) had stable, low testosterone concentrations throughout the study (TYPE I) (Figure 6a). They averaged 88 ± 4 ng/dl testosterone (N = 5) with a range of 73 to 97 ng/dl and an average standard deviation of 35 ng/dl (range 39 to 56 ng/dl, N = 5). The next group of three 'dads' (23%) had a significant increase in testosterone before the birth (TYPE II) (Figure 6b). They averaged 111 ± 16 ng/dl testosterone (range 80 to 129 ng/dl, N = 3) with larger testosterone standard deviations (68 ± 8 ng/dl; range 52 to 77 ng/dl, $t_6 = 2.47$, $P < 0.05$) and a significant increase (20.0 ± 3.2 ng/dl increase) in testosterone when comparing the last 30 days before the birth ('last month' plus 'last week') to all other samples (Paired $t_2 = 6.24$, $P < 0.03$). The final group of five 'dads' (38.5%) had low testosterone immediately after the birth (TYPE III) (Figure 6c). Those five 'dads' had higher average testosterone (141 ± 10 ng/dl, range 92 to 186, N = 5) than the 'no change' group ($t_8 = 2.73$, $P < 0.03$) but not the 'testosterone increase' group ($t_6 = 1.05$, P = 0.33). Similarly, these five 'dads' had almost twice the standard deviation (84 ng/dl, range 52 to 105 ng/dl) of the 'no change' group ($t_8 = 3.20$, $P = 0.01$) but were not significantly different from the 'testosterone increase' group ($t_6 = 1.03$, $P = 0.33$). Notably, in these 'dads' with high testosterone concentrations and high testosterone variances, testosterone concentration decreased by an average of 45.5 ± 14.8 ng/dl during the interval including 'birth' and the 'first week' relative to all other samples (Paired $t_4 = 3.09$, $P < 0.04$).

Figure 6. Examples of the three patterns of testosterone variation around the birth that are discussed in the text. Each panel includes all testosterone determinations for one 'dad'. In panel a, testosterone is stable, low, and unaffected by the birth. In panel b, testosterone increases before the birth but is otherwise stable and low. In panel c, testosterone is higher and variable before and after the birth, but low and invariant around the birth.

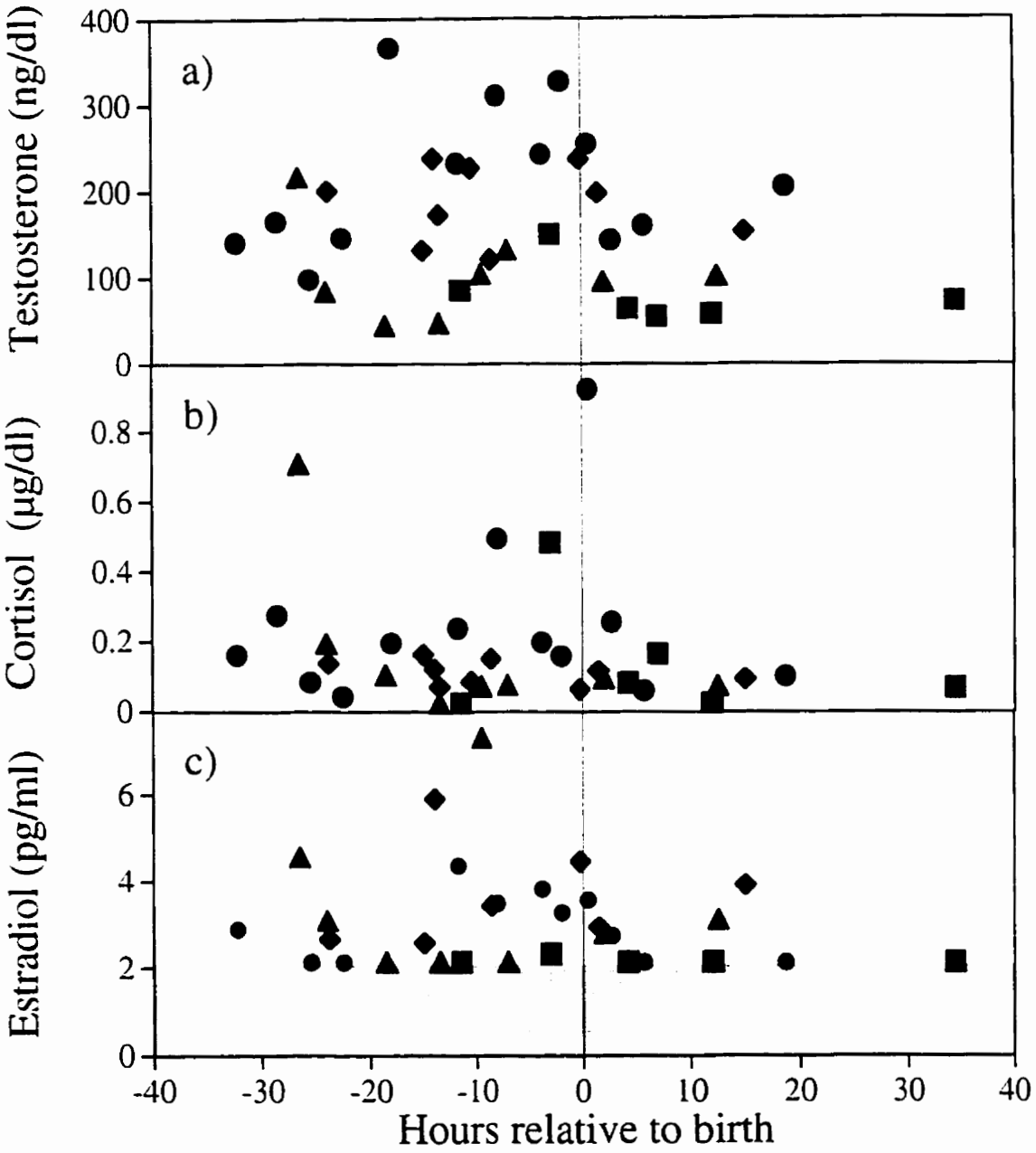

a) No change
b) Increase before birth
c) Decrease after birth
Testosterone (ng/dl)
Days relative to birth
500
400
300
200
100
0
-210
-180
-150
-120
-90
-60
-30
0
30
60
90
120

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

Endocrinology surrounding the birth

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

Figure 7. a) testosterone, b) cortisol and c) estradiol concentrations for the four men (different symbols) providing at least 6 samples during the peri-birth interval. Estradiol determinations which fell below the detection limit of the assay (2.15 pg/ml) are shown at the limit.


a)
b)
c)
Testosterone (ng/dl)
Cortisol (µg/dl)
Estradiol (pg/ml)
400
300
200
100
0
0.8
0.6
0.4
0.2
0
6
4
2
0
-40
-30
-20
-10
0
10
20
30
40
Hours relative to birth

Discussion

After controlling for hormonal changes associated with the season and time of day when samples were collected, 'dads' had lower testosterone concentrations, lower cortisol concentrations, and a higher proportion of samples with detectable estradiol concentrations, than control men.

Estradiol is an important hormonal component of mammalian maternal behaviour in women (Fleming, 1990;Fleming, O'Day, and Kraemer, 1999;Fleming, Ruble, Krieger, and Wong, 1997), non-human primates (Pryce, 1996), and other mammals (González-Mariscal, 1996;Numan and Sheehan, 1997;Rosenblatt, Olufowobi, and Siegel, 1998;Siegel and Rosenblatt, 1975). In addition to peripheral effects, estradiol is active in the central nervous system (Rosenblatt, Wagner, and Morrell, 1994). We also know that those neuroendocrine circuits can be activated in males. Although the ancestors of laboratory rats are not paternal (Takahashi and Blanchard, 1982), exogenous estradiol applied to the medial preoptic area of neonatally castrated male rats elicits maternal behaviour from them (Rosenblatt and Ceus, 1998;Rosenblatt, Hazelwood, and Poole, 1996). Unfortunately, no animal research has described estradiol changes in naturally paternal male mammals. Nevertheless, although the estradiol concentrations in men becoming fathers are very low compared to pregnant women, these men becoming fathers were exposed to more estradiol than control men and that exposure increased following the birth of their child. This estradiol could be sufficient to activate pathways

in the paternal brain that facilitate parental bonding to the infant (Wynne-Edwards and Reburn, 2000).

There was a clear reduction in testosterone concentration from control men to 'dads' and all 'dads' had low testosterone concentrations during the week including and immediately following the birth. Testosterone decreases have also been reported in new fathers of species with extensive paternal care. The pattern has been shown for Mongolian gerbils (*Meriones unguiculatus*), California mice (*Peromyscus californicus*), and Djungarian hamsters (*Phodopus campbelli*) (Brown, Murdoch, Murphy, and Moger, 1995;Gubernick and Nelson, 1989;Reburn and Wynne-Edwards, 1999) as well as cotton-top tamarins (*Saguinus oedipus*) and common marmosets (*Callithrix jacchus*) (Dixson and George, 1982;Ziegler, Wegner, and Snowdon, 1996). It also replicates the results of the original study (Storey, Walsh, Quinton, and Wynne-Edwards, 2000) in which testosterone was reduced in the three weeks following the birth. Pregnant women also have high testosterone concentrations before the birth which decline rapidly after the birth (Fleming, Ruble, Krieger, and Wong, 1997).

Patterns of individual variation in testosterone secretion made this result more difficult to detect. Almost half of these 'dads' had low, stable testosterone concentrations which were not altered by the progress of the pregnancy, the birth process, or the circadian disruption which we assume accompanied returning home with their new child. The remaining men were split between two other distinct patterns of variation. In one group the men had stable, low testosterone concentrations until the birth approached but had a pronounced, approximately 30 d long, increase in testosterone before the birth. In

those 'dads', testosterone declined at the birth and reverted to the initial, stable profile. "Dads' in the final group were 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. In the absence of psychometric assessments, the potential for these patterns of testosterone to vary with other aspects of the personality or experiences of these 'dads' are not known.

The functional role of low testosterone immediately after the birth is not known. In avian species, reduced testosterone is a prerequisite for a behavioural shift to parental care (Schoech, Ketterson, Nolan, Sharp, and Buntin, 1998;Wingfield, Hegner, Dufty, and Ball, 1990). The mechanism for the decrease in testosterone after the birth is also not known although altered patterns of activity and behaviour may have contributed. For example, decreased coital frequency or time away from work to care for the pregnant wife and new child may have reduced testosterone concentrations and patterns of variation. Whatever the 'reasons' for the decrease in testosterone immediately after the birth, that decrease should alter neuroendocrine responsiveness, and thus may be involved in the emotional and behavioural responses of new fathers.

In addition to the well-established early morning increase in cortisol (Schmidt-Reinwald, Pruessner, Hellhammer, Federenko, Rohleder, Schürmeyer, and Kirschbaum, 1999), there was a robust decrease in cortisol concentrations from control men to 'dads'. There is evidence from animal studies for 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 (Carter, De Vries, Taymans, Roberts, Williams, and Getz, 1997;Castro and Matt, 1997;Reburn and Wynne-Edwards, 2000;Ziegler, Wegner, and Snowdon, 1996), and is implicated in social affiliation and pairbond formation (Carter, 1998) as well as mother-infant bonding (Fleming, Ruble, Krieger, and Wong, 1997;Leon, 1992).

However, there was no evidence that cortisol changes anticipated or responded to the birth of the child. This result differed from previous animal studies and results in men. In naturally biparental Djungarian hamsters, but not in closely related Siberian hamsters (*Phodopus sungorus*), cortisol is increased before the birth, but not the day of the birth (Reburn and Wynne-Edwards, 1999). In male cotton-top tamarins, cortisol also increases in the weeks surrounding the birth (Ziegler, Wegner, and Snowdon, 1996). Similarly, cortisol concentration was elevated in men sampled during the three weeks preceding the birth in the earlier study (Storey, Walsh, Quinton, and Wynne-Edwards, 2000).

Methodological differences between the studies probably contributed to this difference. 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 two concentrations was the 'situational reactivity' for that hormone. However, in the last three weeks before the birth, the 'reaction' of cortisol was to decrease back to previous concentrations over the 30 min interval (Storey, Walsh,

Quinton, and Wynne-Edwards, 2000). Thus, it is probable that the situational reactivity measured was an enhanced cortisol response to the stressor of the home visit rather than an increase in resting cortisol concentrations. In retrospect, this result is not surprising. Hormone-behaviour relationships are dynamic and we should expect responses to key biological events, like impending fatherhood, to involve changes in reactivity and hormone dynamics as well as general alterations in concentration.

We do not anticipate a strict hormone-behaviour relationship in men. Instead, these results highlight the extent of individual variability in hormone concentrations and patterns of variation and the value of longitudinal sampling to clarify that variation. However, these results, and the previous study (Storey, Walsh, Quinton, and Wynne-Edwards, 2000), do clearly indicate that men experience hormonal changes as they become fathers for the first time. These changes, which involve hormones known or implicated in mammalian maternal behaviour, may subtly alter hormone receptor expression, sensitivity to infant stimuli, or reactivity to social stimuli, in ways that enhance the psychosocial experience of becoming a father. The mechanism, required stimuli, and functional implications of these hormone changes remain to be investigated.

CHAPTER 3: General Discussion

These data support the hypothesis that concentrations of the steroid hormones estradiol, testosterone and cortisol change within some individual men as they become fathers for the first time. Men becoming fathers underwent hormonal change during pregnancy. Some of these changes were similar to those seen in pregnant women (Fleming, Ruble, Krieger, and Wong, 1997).

Specific prediction 1/. E2 concentration will be low in new fathers

Although E2 has not been measured in males of any biparental species, men's E2 was predicted to be low at the onset of fatherhood. This prediction was based on the assumption that existing maternal neuroendocrine circuits will most likely be activated through natural selection in situations where paternal behaviour is adaptive for male mammals. In pregnant women, E2 levels peak at parturition and decline rapidly after birth (Fleming, 1990;Fleming and Corter, 1988;Storey, Walsh, Quinton, and Wynne-Edwards, 2000). In female mammals, E2 primes maternal behaviour (Numan, 1974) and is correlated with responsiveness to offspring in women (Fleming, 1990), pigtail macaques (Maestripieri and Zehr, 1998), and rats (Rosenblatt, Mayer, and Giordano, 1988;Rosenblatt, Olufowobi, and Siegel, 1998).

Men's E2 did not follow pregnant women's patterns of a peak at birth followed by a rapid postpartum decline (Berg and Wynne-Edwards, in prep). Within individual men, E2 increased significantly from the month before to the month after the birth. Thus, the

pattern of secretion of E2 in men does not parallel changes in women and cannot be considered homologous.

Specific prediction 2/. T5 concentration will be low in new fathers

Men's T5 was predicted to be low at the onset of fatherhood. Men becoming fathers had low T5 during the week including and following the birth of his first child. Similarly, T5 decreases have been reported in new fathers of species with extensive paternal care (i.e. biparental hamsters, Mongolian gerbils, cotton-top tamarins, and common marmosets (Clark and Galef, 1999;Dixson and George, 1982;Reburn and Wynne-Edwards, 1999;Ziegler, Wegner, and Snowdon, 1996)). The previous study of men also reported that T5 was decreased in the first 3 weeks of fatherhood (Storey, Walsh, Quinton, and Wynne-Edwards, 2000).

Like men, pregnant women experience a postpartum T5 decrease (Fleming, Ruble, Krieger, and Wong, 1997). Thus, data for T5 supported the prediction that paternal males will activate existing 'maternal' neuroendocrine circuits (where the maternal plan is assumed to be the template) when paternal behaviour is adaptive for male mammals. However, individual males differed in patterns of T5 variation and that result was not homologous with women.

Patterns of individual T5 variation in men

Almost 50% of the 'dads' had low, stable T5 concentrations which were not altered by the progress of the pregnancy, the birth process, or the circadian disruption which we assume accompanied men returning home with their new child. The remaining men were

split between two other distinct patterns of variation. One group of men had stable, low T5 concentrations until the birth approached but had a pronounced (approximately 1 month long) increase in T5 before birth. In those men, T5 reverted to the initial, stable profile at the birth. Men in the final group were characterized by high, variable T5 concentrations throughout the pregnancy and after the child was 3 weeks old. However, during and immediately after the birth that variance was suppressed, in spite of increased sampling intensity. In the absence of psychometric assessments, the potential for these patterns of T5 to vary with other aspects of the personality or experiences of these men is not known.

The functional role of low T5 immediately after the birth is not known but in avian species, reduced T5 is a prerequisite for a behavioural shift to parental care (Schoech, Ketterson, Nolan, Sharp, and Buntin, 1998;Wingfield, Hegner, Dufty, and Ball, 1990). The mechanism for the decrease in T5 after the birth is also not known although altered patterns of activity and behaviour might have contributed. For example, sleep deprivation might (Cortes-Gallegos, Castaneda, Alonso, Sojo, Carranco, Cervantes, and Parra, 1983) or might not (Miyatake, Morimoto, Oishi, Hanasaki, Sigita, Iijima, Teshima, Hishikawa, and Yamamura, 1980) effect a change in T5. There exists a circadian pattern in free T5 (Plymate, Tenover, and Bremner, 1989) so sleep deprivation might alter T5 by disrupting activity patterns (Campbell, Walker, Riad-Fahmy, Wilson, and Griffiths, 1982). Lifestyle changes including decreased coital frequency (Dabbs and Mohammed, 1992;Knussmann, Christiansen, and Couwenbergs, 1986) or a hormonal response to time away from work (Christiansen, Knussmann, and Couwenbergs, 1985) might have

reduced T5 concentrations and patterns of variation. Whatever the 'reasons' for the T5 decrease immediately following birth, that decrease should alter neuroendocrine responsiveness, and thus may be involved in the emotional and behavioural responses of new fathers.

Specific prediction 3/. B concentration increases will anticipate the birth

Men's B concentration was predicted to increase before the birth. Men becoming fathers showed no significant B increase or response to the birth of the child. This result differed from previous results in animal studies. In biparental hamsters, B is increased before the birth (Reburn and Wynne-Edwards, 1999) and in male cotton-top tamarins, B increases in the weeks surrounding the birth (Ziegler, Wegner, and Snowdon, 1996). My results differ from the original study of men becoming fathers where B concentration was elevated in men sampled during the three weeks preceding the birth (Storey, Walsh, Quinton, and Wynne-Edwards, 2000).

Pregnant women experience a B increase before birth (Fleming and Corter, 1988). Men becoming fathers did not experience the similar B increase. Thus, the pattern of secretion of B in men does not parallel changes in women and cannot be considered homologous.

Differing results between human studies might have been due to methodological differences.

Methodological differences

Methodological differences between my study and the original study of men include: the collection of longitudinal information, and sampling stress reduction due to collection of saliva samples by the couples in the comfort of their home. In the original study, blood was collected by venipuncture during a home visit by the researchers. The design of the original study considered the first blood sample to be 'baseline' and the second blood sample, which followed infant stimuli, to be responses to those stimuli. The difference between the two concentrations was the 'situational reactivity' for that hormone. In the last three weeks before the birth, the 'reaction' of B was to decrease back to previous concentrations over the 30 minute interval (Storey, Walsh, Quinton, and Wynne-Edwards, 2000). Thus, it is probable that the situational reactivity measured was an enhanced B response to the stressor of the home visit rather than an increase in resting B concentrations. Hormone-behaviour relationships are dynamic. We should expect responses to key biological events, like impending fatherhood, to involve changes in reactivity and hormone dynamics as well as general alterations in concentration (Chapter 2).

Thus, these data provide no evidence supporting of homology in hormone secretions of men and women. This might result from the disassociation of pregnancy for paternal responses in men. If so, then these data might usefully illuminate effects in women. On the other hand, these data are also consistent with different, as opposed to homologous, neuroendocrine circuits for men and women.

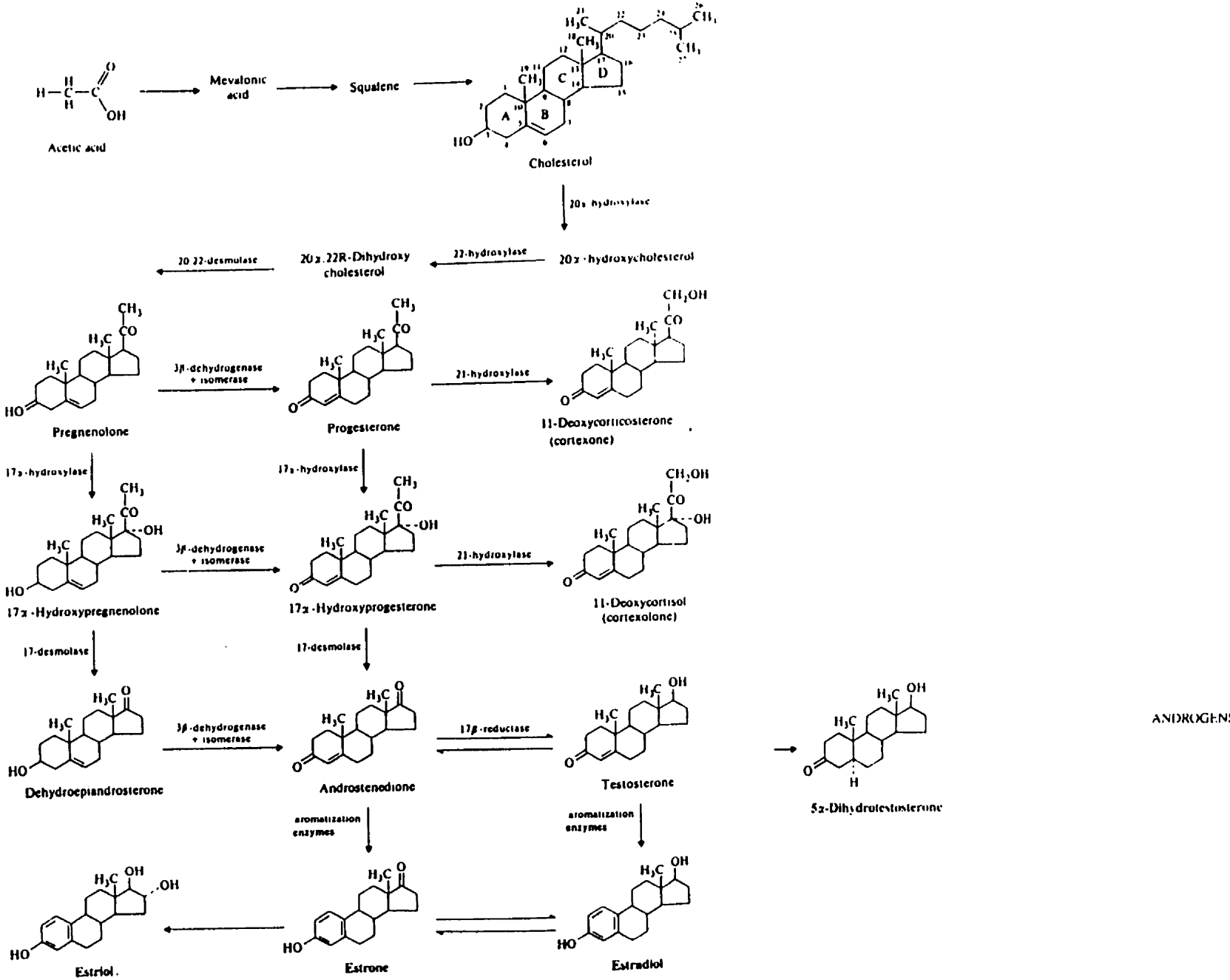
Other possibilities also exist. Biochemical pathways for steroid synthesis make multiple, biologically active steroids. Androgens are the substrate for the aromatase enzyme that makes estrogens. Thus, peripheral steroid secretions might reflect substrate changes and not hormone concentrations and actions at neural sites.

Aromatization of T5 into E2

The pattern of a significant increase in E2 from the month before to the month after the birth in men, is probably due to several factors of which aromatization of T5 into E2 is a strong candidate. The E2 increase postpartum in men is coincident with a T5 decrease.

Cytochrome P450 aromatase is the enzyme responsible for the aromatization of T5 into E2 (Figure 8). Steroids are chemical compounds derived from cholesterol. Enzymes mediate the conversion of one steroid hormone into another. The presence of aromatase is necessary to render cells responsive to that hormone. Thus, cells with E2 receptors may respond to T5 if the cells contain aromatase activity. Males actually possess the ability to produce estrogen via aromatization of T5 into E2. Moreover, aromatase activity is greater in male than in female rats (Rhoda, Corbier, and Roffi, 1984;Roselli, Horton, and Resko, 1985;Steimer and Hutchison, 1990), and can be influenced by external factors such as photoperiod and socio-sexual stimuli (Hutchison, 1993;Hutchison, Hutchison, Steimer, Steel, Powers, Walker, Herbert, and Hastings, 1991).

Figure 8. Interrelationships and formation of the steroid hormones as an illustration of the aromatization of testosterone to estradiol (Bentley, 1998).


Acetic acid
Mevalonic acid
Squalene
Cholesterol
20α-hydroxylase
20α-hydroxycholesterol
22-hydroxylase
20α,22R-Dihydroxy cholesterol
20,22-desmolase
Pregnenolone
3β-dehydrogenase + isomerase
Progesterone
21-hydroxylase
11-Deoxycorticosterone (cortexone)
17α-hydroxylase
17α-Hydroxypregnenolone
17α-Hydroxyprogesterone
11-Deoxycortisol (cortexolone)
17-desmolase
Dehydroepiandrosterone
Androstenedione
17β-reductase
Testosterone
5α-Dihydrotestosterone
ANDROGENS (C19)
aromatization enzymes
Estriol
Estrone
Estradiol

Aromatization might be important in the interpretation of hormonal changes in men during pregnancy when men's T5 decreases postpartum and E2 is increasing. The functional role of low T5 immediately after the birth is unknown. The role of increasing E2 in men postpartum is also unknown. It is known that the aromatase mechanism is important in organizational and activational effects of androgens on sexual dimorphism of sexual behaviour in males (Freeman and Rissman, 1996;Robbins, 1996;Vom Saal, 1983). The aromatization of T5 to estrogens, presumably in the MPOA (Rosenblatt and Ceus, 1998) might be an important component in the onset of paternal behaviour in species where maternal behaviour has been shown to have a strong hormonal influence. Thus, although these results do not support homology between men and women in peripheral endocrinology, homologous neuroendocrine circuitry remains possible.

Mechanism for hormonal change?

The mechanism responsible for hormonal change in men becoming fathers is not known (Wynne-Edwards, 2001). Maternal behavior can be elicited by a variety of sensory cues and these cues that elicit maternal responses vary among species. In many mammals, pheromonal communication (olfaction) is a major form of sensory communication by which conspecifics influence each other's behaviour and physiology (Wabinga, Parkin, Wabwire-Mangen, and Nambooze, 2000). The existence of vomeronasal sensory input (VNS) in adult humans has only recently been established and results suggest that human VNS is involved in pheromone detection (Moran, Monti-Bloch, Stensaas, and Berliner, 1995). Pheromonal communication between partners is an

obvious candidate for hormonal correlations within couples because pheromones can influence endocrine status in humans (Stern and McClintock, 1998;Weller, Weller, and Avinir, 1995).

In the socially monogamous cotton-top tamarin fathers, PRL was higher postpartum than in other males (Ziegler, Wegner, Carlson, Lazaro-Perea, and Snowdon, 2000;Ziegler, Wegner, and Snowdon, 1996). The most significant tamarin T5 changes occurred in males whose mates ovulated during the 15 days following birth, suggesting that unlike female tamarins, males do not show hormonal changes in response to infants (Ziegler, Wegner, Carlson, Lazaro-Perea, and Snowdon, 2000). Male tamarins might be stimulated physiologically by chemical cues from a female's prepartum hormones (Ziegler, Wegner, and Snowdon, 1996). In men and women, Storey *et al.* (Storey, Walsh, Quinton, and Wynne-Edwards, 2000) found positive correlations within couples of PRL and change in B, suggesting that men's hormonal change was more strongly correlated with the concentrations of the same hormone in their partner, than timing relative to the birth. Hormonal correlation within couples suggested that communication within the couples might be related to the physiological changes that men experience. Future studies in men becoming fathers should examine steroid hormone correlations within couples as a mechanism responsible for hormonal change in men.

Summary

In this highly motivated population of Canadian volunteers attending prenatal classes as couples, men experience hormonal changes associated with fatherhood. Hormones are

involved in priming and elicitation of maternal behaviour. The hormones changing in men becoming fathers have known roles in maternal behaviour. Thus, in men, hormonal changes might also alter thresholds for the expression of paternal responses. If so, this study supports the hypothesis that there is a physiological response associated with involved fatherhood.

A clear understanding of the biological basis for paternal behaviour by men will have high value in western society. Based on detailed cross-cultural surveys, human paternal care is most pronounced when couple intimacy is high and social contact is prolonged within couples (Barry and Paxson, 1971;Broude, 1983;Whiting and Whiting, 1975). North American societies fall amongst the 40% of human cultures with moderate to high levels of paternal contact between men and their children (Barry and Paxson, 1971). That paternal care can have a large impact on offspring survival (Hewlett, 1988;Hurtado and Hill, 1992;Lamb, Pleck, Charnov, and Levine, 1985). Unfortunately, high divorce rate means that couple intimacy often involves a man who is not the biological father of children in the household. If men experience hormonal changes before and after the birth of their child which facilitate the activation of neuroendocrine pathways which in turn facilitate appropriate parental behviour, then an understanding of the behavioural endocrinology of fatherhood may eventually improve familial harmony in those households.

This research contributes to our understanding of the hormonal variables contributing to involved fatherhood. Human relationships are complex and subtly influenced in ways that are poorly understood. Adoptive parents are not denied the bonds of love simply

because they lack the pregnancy and birthing experience. Nevertheless, the brain will be rendered more receptive to appropriate stimuli through the actions of hormones. The hormonal changes of impending parenthood might facilitate neuronal changes that profoundly affect the relationships of parents and their children. My results add to the body of research on paternal care and contribute to overturning the accepted paradigm that hormones are not involved in male parental behaviour. Modern western civilizations provide opportunities for men to be active participants in the birth process, for men to take paternity leave to care for an infant, and for men to publicly acknowledge the joys of fatherhood. My study suggests that a sound biological basis for those experiences will be found. By exploring new ground in the emerging disciplinary integration of behaviour, neuroendocrinology and evolutionary biology, studies such as this one might eventually improve mental health, enhance positive social relationships and further illuminate the extraordinary human experience of parenthood.

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Appendix A: RIA Protocol for E_2

BUFFERS AND SOLUTIONS

1. 0.1 M PBSG (phosphate buffered saline with gelatin)

$NaH_2PO_4:H_2O$	10.7 g
Na_2HPO_4	29.6 g
NaCl	18.0 g
Na Azide (NaN_3)	2.0 g
H_2O	2.0 L
pH	7.0
Knox Gelatin	2.0 g

1) Add salts to 1800 ml of distilled H_2O (Heat and stir).
2) Let dissolve.
3) pH to 7.0 using 1.0 M HCl or 1.0 M NaOH.
4) Add 200 ml of H_2O and gelatin. Heat slowly and stir until gelatin goes into solution.
5) Add gelatin solution to 1800 ml solution, cool and refrigerate at 4 degrees C.

2. Dextran-Charcoal Solution

Dextran	0.0625 g
Washed Charcoal	0.625 g
0.1 M PBSG	100 ml

1) Add Dextran to PBSG. Stir.
2) Allow Dextran to dissolve completely (1-2 minutes)
3) Add charcoal and stir for 5 minutes.
4) Keep refrigerated at 4 degrees C.

Note: To wash charcoal, fill a 1 L glass beaker with approximately 200 g of charcoal and 600 ml of distilled water. Stir for 1 hour (using a stir bar), and then allow to settle for 1 hour. Decant the liquid supernatant and repeat the procedure 5 - 6 times. Dry charcoal in oven. This entire wash - dry cycle should be repeated twice. This process takes several days.

PROTOCOL

1) Sample is not diluted in a 13 x 100 mm disposable glass culture tube.

Standards

- 10 standards are prepared.
- The E_2 concentrations in the 10 standards are such that each tube contains:

STD1 has 1.075 pg/tube; STD2 has 2.15 pg/tube; STD3 has 4.3 pg/tube; STD4 has 8.6 pg/tube; STD5 has 17.2 pg/tube; STD6 has 34.376 pg/tube; STD7 has 68.57 pg/tube; STD8 has 137.5 pg/tube; STD9 has 275 pg/tube; STD10 has 550 pg/tube;

- 5 µl of each E_2 standard goes into a test tube + 995 µl dH_2O
- 5 µl of standard 0 (100 % methanol) goes into tubes 'TB' (total binding), 'TC' (total counts) and 'N' (non-specific binding), to which 995 µl dH_2O is added.

Samples

- 1 ml of saliva into a 13 x 100 mm tube

2) 2 ml of anhydrous ethyl ether ($C_4H_{10}O$ = 74.12) (AnalaR, BDH Inc., Toronto, CA) is added to all samples. Samples are mixed with ether using a Scientific Product Deluxe vortex two at a time (duplicates) for approximately 15-20 seconds. Steroid hormones have a higher affinity for ether (steroids being lipophilic and hydrophobic) and will extract into it.

3) After vortexing, samples are allowed to settle for approximately 5 minutes to ensure complete separation of the ether and aqueous phases. The ether phase sets on top of the aqueous phase.

4) Samples are placed in a dry ice-ethanol bath so that the aqueous phase is frozen within 15-20 seconds (until an ice peak forms in the center of the tube). Tubes are carefully removed from the bath and the outside of the tube is dried with paper towel to prevent the ethanol from mixing with the sample when decanting. The ether phase is poured directly into previously labelled 12 x 75 mm disposable glass test tubes. The extracted aqueous phase is discarded.

5) The ether phase (in 12 x 75 mm test tubes) is evaporated to dryness in a vortex evaporator (Buchler Instruments) at 30°C at a speed of 7/10 of maximum. After evaporation, the hormone crystals will be on the bottom (and lower sides) of the test tube. Tubes are covered with aluminum foil and may then sit for up to an hour on the bench at room temperature, or placed in the fridge at 4°C overnight before preceeding with the final steps of the assay.

6) In a 20 ml glass scintillation vial, add 100 µl of Dupont, estradiol NET 317 2,4,6,7 -^{3}H(N), 1,2,6,7 - ^{3}H (Lot 2775-017) to 20 ml of 0.1 M PBSG. In another glass scintillation vial, add E2 antibody (GDN #244 anti-estradiol-6-BSA (G. D. Niswender, Colorado State University), to 20 ml of 0.1 M PBSG. This allows for the ^{3}H hormone to be bound.

7) To all test tubes add 100 µl of the ^{3}H E2/PBSG solution using the repeater pipette. Thereafter add 100 µl of the primary antibody solution to all tubes except 'TC' and 'N'. Add 100 µl 0.1 M PBSG to test tubes 'TC' and 'N'. All tubes now have a total volume of 200 µl. Tubes are vortexed by hand by shaking the racks. Samples are then incubated on the tabletop at room temperature for 1 hour, or overnight at 4°C before proceeding with the final steps of the assay.

8) Samples are placed in an ice bath for 20 minutes. 750 µl of dextran coated charcoal is added to each tube except 'TC' which receives 750 µl of 0.1 M PBSG. Samples are kept on ice for another 20 minutes. (Total volume is 950 µl per tube ~ 1 ml). The outside surface of the tubes are dried before placing them in the centrifuge.

9) Tubes are centrifuged at 1600 x g (clinical centrifuge) for 20 minutes at 4°C. The liquid fraction is decanted into 7 ml plastic scintillation vials. This is followed by the addition of 4 ml Scintiverse II (a toluene based counting solution; Fisher Chemicals).

10) The vials are capped and counted for 5 minutes in a beta spectrometer.

Appendix B: Hormone Concentration in Individual Control Men

Figure 9. Scatter plots for 14 individual control men for testosterone during the day and across seasons. Closed circles indicate time-of-day when sample collection took place (AM = morning; E = evening). Open circles indicate the 59 samples from six control men who collected samples at different times of day over a 3-6 day time period. The asterisk indicates a shift worker.

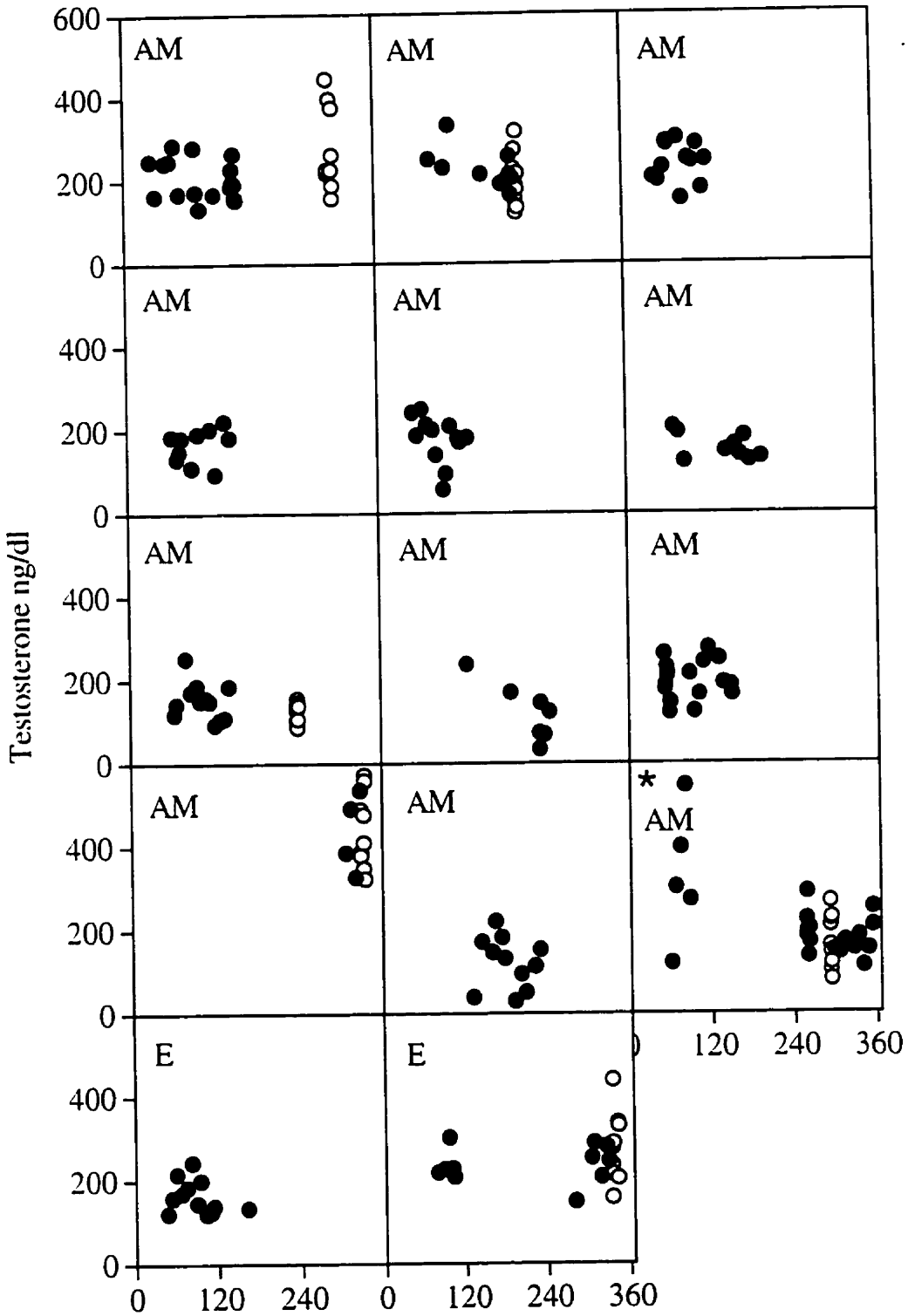

Testosterone ng/dl
600
400
200
0
AM
E
*
0
120
240
360

Figure 10: Scatter plots for 14 individual control men for cortisol during the day and across seasons. Closed circles indicate time-of-day when sample collection took place (AM = morning; E = evening). Open circles indicate the 59 samples from six control men who collected samples at different times of day over a 3-6 day time period. The asterisk indicates a shift worker. The individual control men are represented in the same positions for cortisol as that for testosterone.

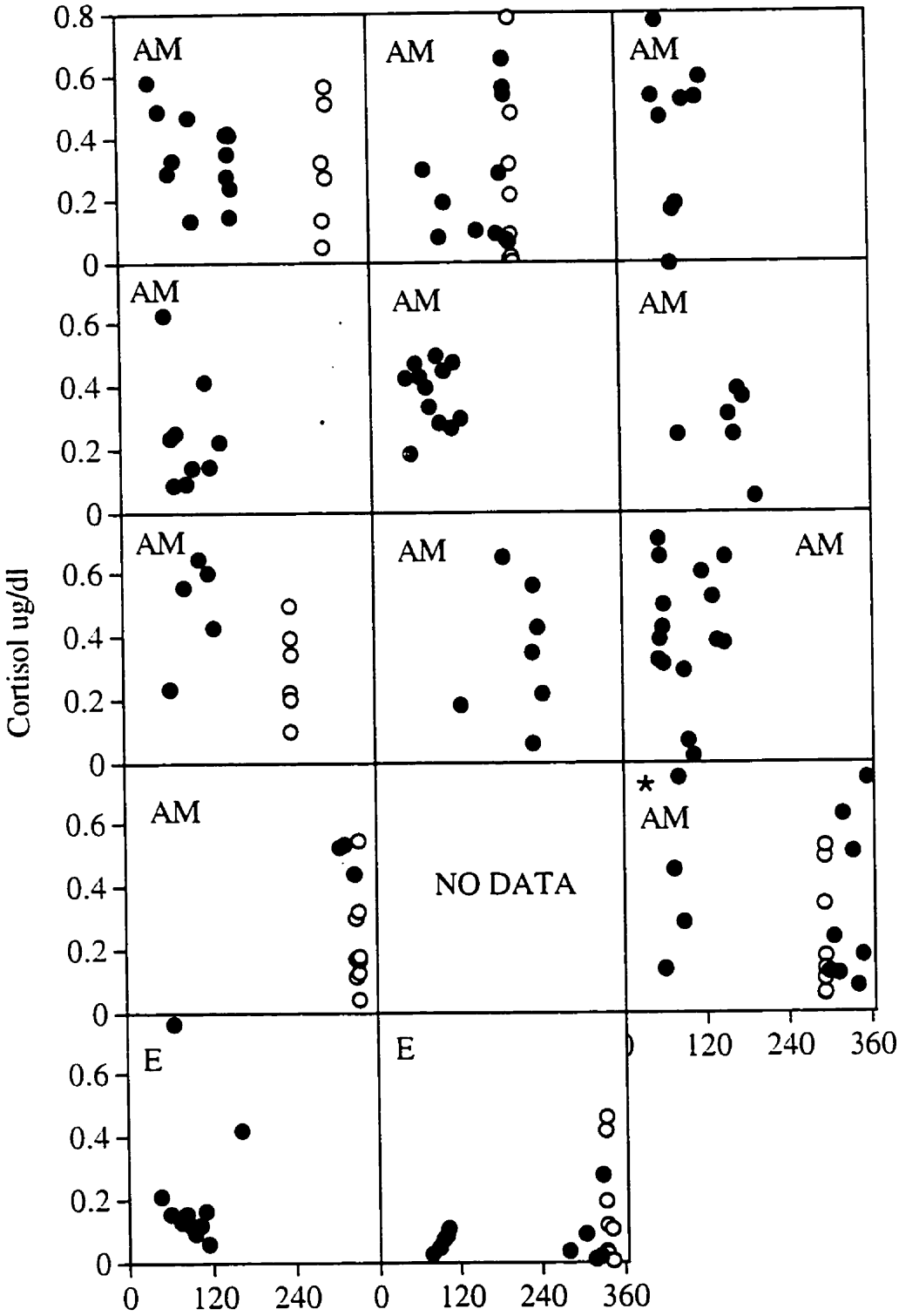
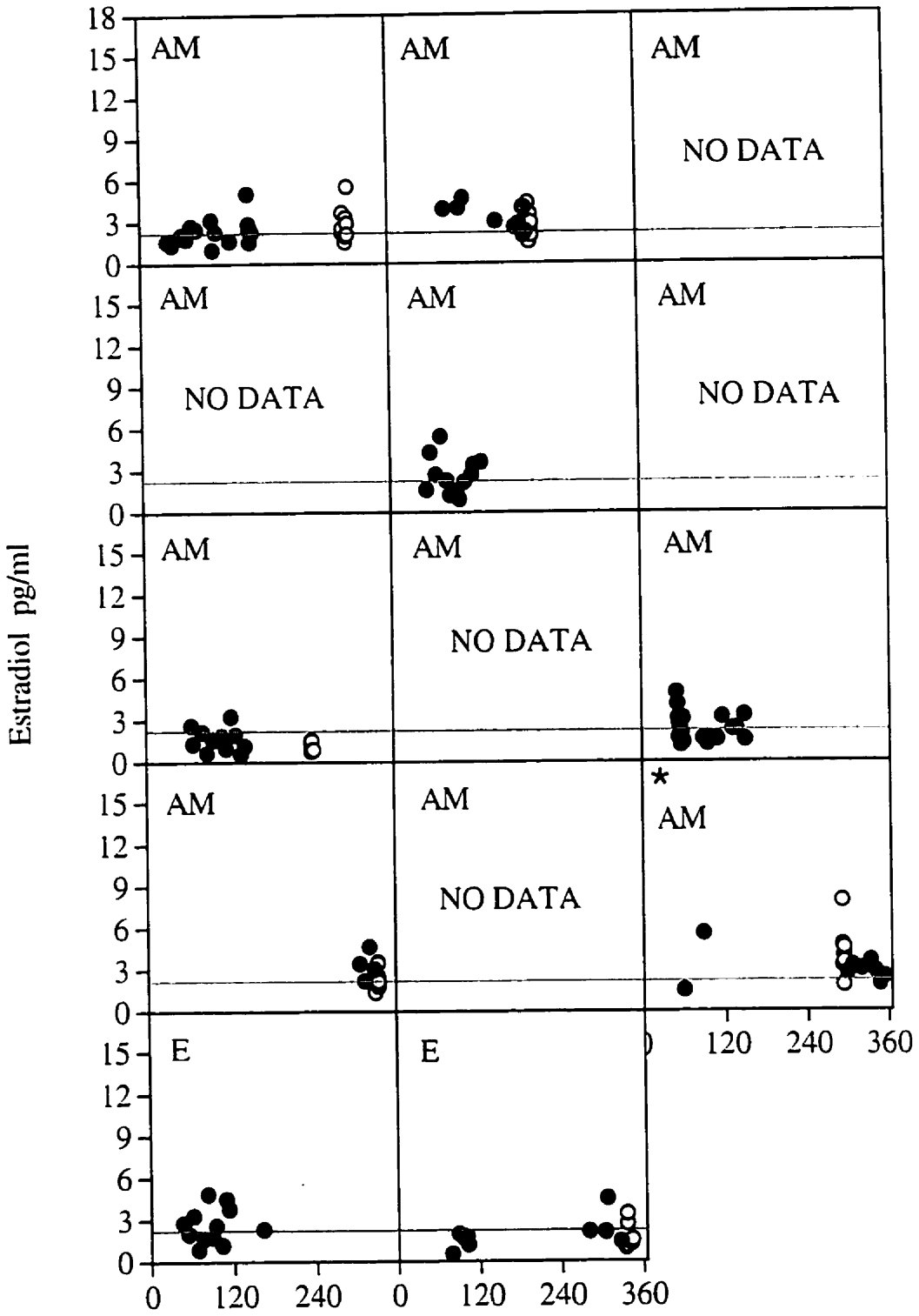

Cortisol ug/dl
0.8
0.6
0.4
0.2
0
AM
E
NO DATA
*
0
120
240
360

Figure 11: Scatter plots for 14 individual control men for estradiol during the day and across seasons. Closed circles indicate time-of-day when sample collection took place (AM = morning; E = evening). Open circles indicate the 59 samples from six control men who collected samples at different times of day over a 3-6 day time period. The asterisk indicates a shift worker. The individual control men are represented in the same positions for estradiol as that for testosterone. The lower limit of estradiol assay sensitivity was arbitrarily set to 2.15 pg/ml of saliva. Estradiol concentrations in men were often below that threshold as indicated by a grey line.


Estradiol pg/ml
AM
NO DATA
E
0
3
6
9
12
15
18
120
240
360
*

Appendix C: Hormone Concentration in Individual 'Dads'

Figure 12. Scatter plots for 13 individual 'Dads' for testosterone. Closed circles indicate time-of-day when sample collection took place (AM = morning; E = evening; LE = late evening). Sampling is arranged relative to time of birth indicated by the vertical line at time zero.

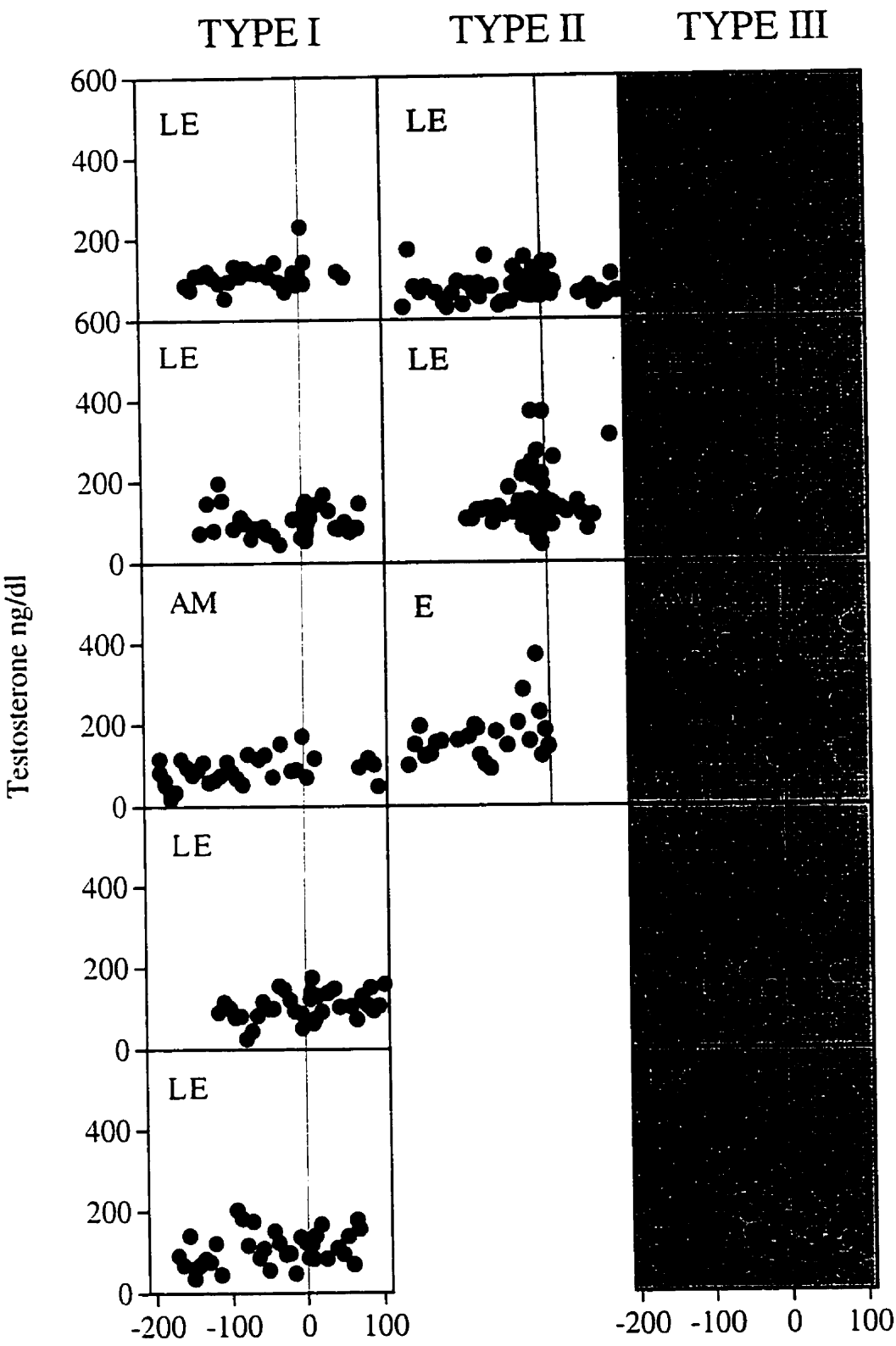

TYPE I
TYPE II
TYPE III
Testosterone ng/dl
600
400
200
0
LE
LE
LE
LE
AM
E
LE
LE
-200
-100
0
100

Figure 13: Scatter plots for 13 individual 'Dads' for cortisol. Closed circles indicate time-of-day when sample collection took place (AM = morning; E = evening; LE = late evening). Sampling is arranged relative to time of birth indicated by the vertical line at time zero. The individual 'Dads' are represented in the same positions for cortisol as that for testosterone.

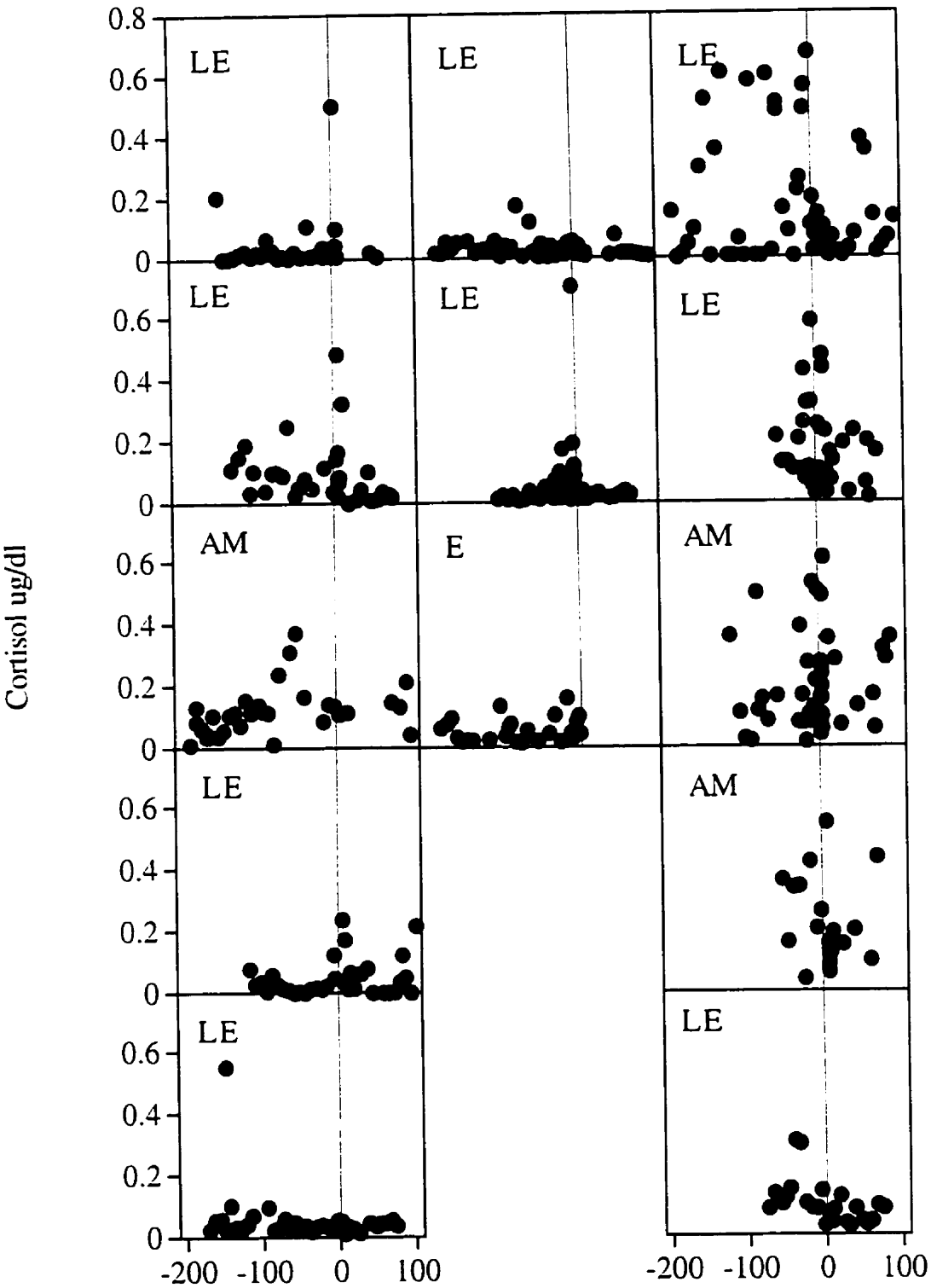
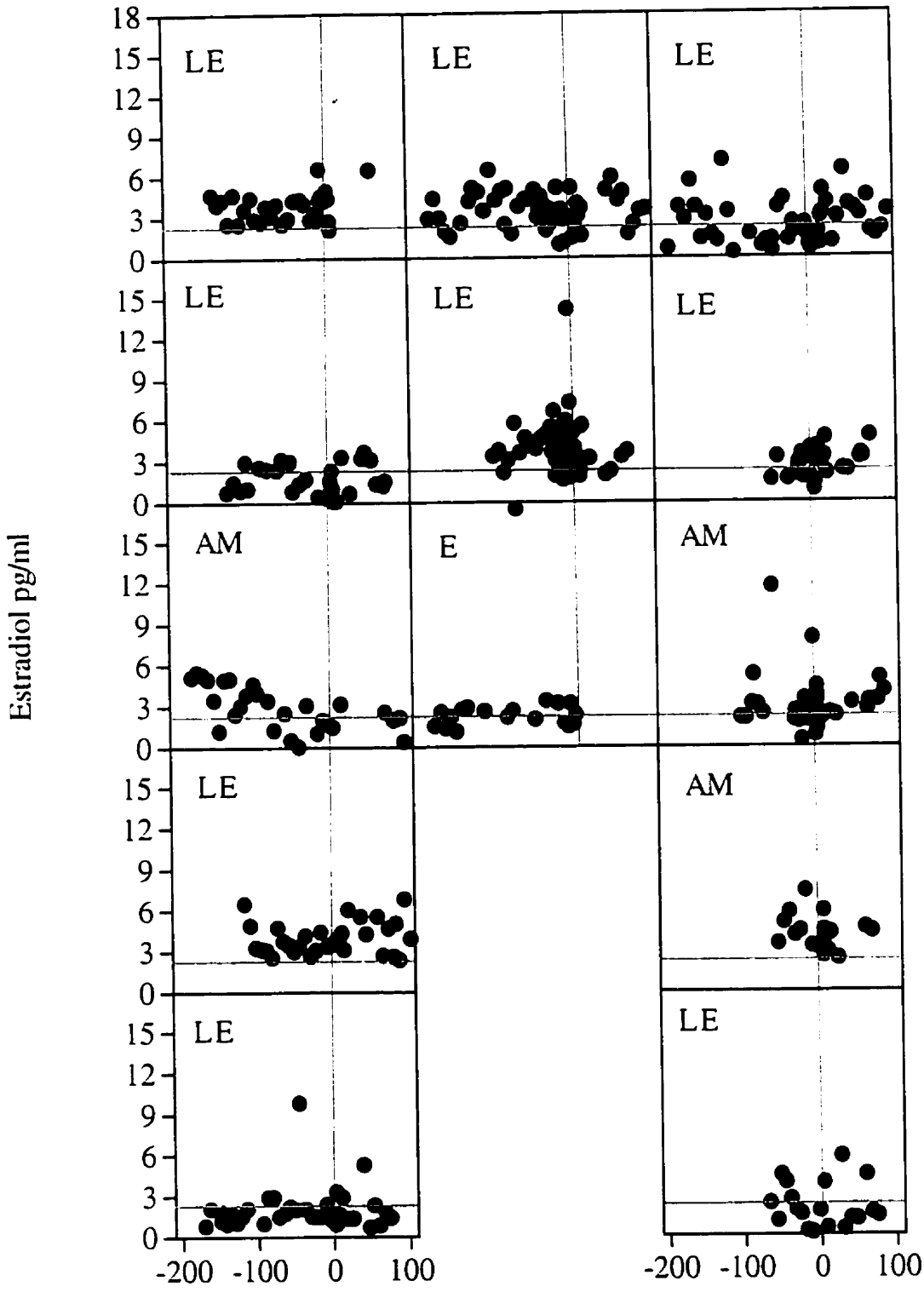

Cortisol ug/dl
0.8
0.6
0.4
0.2
0
LE
LE
LE
LE
LE
LE
AM
E
AM
LE
AM
LE
LE
-200
-100
0
100
-200
-100
0
100

Figure 14: Scatter plots for 13 individual 'Dads' for estradiol. Closed circles indicate time-of-day when sample collection took place (AM = morning; E = evening; LE = late evening). Sampling is arranged relative to time of birth indicated by the vertical line at time zero. The individual 'Dads' are represented in the same positions for estradiol as that for testosterone.


Estradiol pg/ml
LE
LE
LE
LE
LE
LE
AM
E
AM
LE
AM
LE
LE
0
3
6
9
12
15
18
-200
-100
0
100