HORMONAL INDICATORS OF PATERNAL CARE IN HUMANS: A LONGITUDINAL STUDY OF FIRST-TIME PARENTS

CENTRE FOR NEWFOUNDLAND STUDIES

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HORMONAL INDICATORS OF PATERNAL CARE IN HUMANS: A LONGITUDINAL STUDY OF FIRST-TIME PARENTS

by

© Krista M. Delahunty

A thesis submitted to the

School of Graduate Studies

in partial fulfilment of the

requirements for the degree of

Master of Science

Cognitive and Behavioural Ecology Programme

Memorial University of Newfoundland

October 2003

St. John's Newfoundland

Abstract

Men and women expecting their first child were tested within two weeks and two months before and after birth. A control group of couples were tested twice, at four weeks apart. Baseline finger stick blood samples were collected. At all stages except the early prenatal, an infant stimuli test was administered and a second blood sample was taken 30-minutes later. For the prenatal test, couples held a doll, listened to a tape of infant cries, and watched a video. Postnatally, the father held his baby and the mother held the doll with no other stimuli presented. Blood spot prolactin, cortisol, and testosterone levels were measured. Men in the pregnant group had changes in baseline prolactin, with highest levels two weeks after the birth. Fathers had short-term decreases in prolactin during the early postnatal stage. Prolactin levels in control men did not change. Pregnant women's baseline prolactin levels changed over time, with highest levels in the two weeks before the birth. During the late prenatal stage, pregnant women had short-term increases in prolactin levels. No baseline or short-term changes in free testosterone or cortisol levels were found for men in either group. Pregnant women had changes in baseline cortisol levels, with a peak at the late prenatal stage, but no short-term changes. Cortisol levels were correlated between pregnant women and men, but not between control couples. Men and women who reported difficulty with parenting had higher cortisol levels at some stages. Fathers who reported 'concern' prenatally in response to baby cries had short-term increases in prolactin levels during the early postnatal stage, and decreases in testosterone levels during the late postnatal stage. The birth of a child can cause significant longitudinal hormonal changes in men. Fathers'

prenatal responses to infant stimuli may be predictive of certain patterns of hormonal change after the birth of their babies.

Acknowledgements

I am forever grateful for the guidance, wisdom, and insight of my supervisor, Dr. Anne Storey. Throughout each stage of this project, Anne has been always ready to offer whatever she could, from financial support to many hours of her time driving around and visiting couples and babies with me. Thanks, Anne, for all you have done for me.

Donald McKay, my co-supervisor, has provided much guidance and insight on the laboratory portion of this project. Don has also been a wonderful source of support and wisdom over the years.

Additional thanks to my committee members, Anne, Don, and Dr. Charles Malsbury, for helpful feedback and comments, and for being so generous with your time.

Carla Baker accompanied me on many of the home visits to the couples in this study. Thanks, Carla, for giving up so many of your evenings, often on short notice. Having you along for company made those evenings a little more fun, and always interesting! Thanks, also, for your help in the lab.

Thanks to Marion Counsel-Dawe, who graciously allowed us into her evening prenatal classes and was always accommodating with her time. Kathy McKay accompanied us to each prenatal class and took finger stick samples from the couples. Kathy also taught me everything I know about taking blood, and often made time in her day to patiently take blood samples when I needed them.

Prolactin blood spot samples were analyzed in the Hormone Assay Laboratory of the Health Sciences Centre. Thanks to Dr. Edward Randell, and to Rowena Walsh for

making time to do those assays. Dr. Sudesh Vasdev and his staff helped with some of the early blood spot work, and allowed me to count many assays on their gamma-counter.

I have spent many hours in the lab with Mackenzie, Sabina, Carla, Diana, and Sarah. I couldn't have asked for better lab mates! Thanks to Diana for help with many aspects of this project. Though you came in at the end of it all, you have made an invaluable and important contribution.

Throughout the years, my family has been a constant source of encouragement, love, and support. Mom and Dad, thanks for everything you have done to help me along the way. Stephen and Laura have often provided a sympathetic ear, and you all have been great company in the time I worked from home. My extended family has also been very supportive. Special thanks to Kevin for use of his laptop throughout the writing phase of the thesis.

Finally, none of this would have been possible without the participation of both the pregnant and control couples in this study. You have all been wonderful, allowing us into your homes repeatedly, often when it was often less than convenient! Thanks to all of you.

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Chapter 1: Introduction and Overview

1.1: Background of study

The role that hormones play in the expression of parental care behaviours has been studied in many different animals ranging from birds, rodents, primates, and, more recently, humans. Earlier work focused upon the maternal contribution and hormones, but paternal care has also been investigated, albeit in some species more than others. The following is a review of hormone studies related to parenting in these species, with a focus on paternal care and its relation to prolactin, cortisol, and testosterone levels.

1.1.1: Review of avian studies

The role of hormones in paternal care has been well investigated in birds, and varies depending upon the species' reproductive strategy. Biparental care with social monogamy is the evolutionary primitive pattern in class Aves, occurring in over 90% of the species (Lack, 1968). Unlike mammals, where the male's physiological contribution is restricted due to an inability to lactate, male birds have the potential to participate in all aspects of parenting with the exception of oviposition.

1.1.1.1: Prolactin in birds

Clear patterns of hormonal change usually occur throughout the breeding season for biparental male birds, though much variation is seen between species and individuals (for a review, see Buntin, 1996). Prolactin has been well studied in both sexes and across many species, though less of an absolute pattern is evident in males. Generally, prolactin

levels are low in male birds during the pre-laying period, and they begin to increase once parental behaviours are required around the time of incubation (Schradin & Anzenberger, 1999). Usually, prolactin levels begin to decline in precocial species after the chick is hatched, but this decline does not begin in altricial species until after the brooding period ends (Lormee, Jouventin, Chastel, Mauget, 1999).

The effect of prolactin on parental behaviour has also been studied experimentally by artificially increasing levels through exogenous administration, although most studies of this nature have involved only the female. In domestic laying turkeys *(Meleagris gallopavo)*, incubation has been induced by injecting prolactin intracranially (Youngren, Halawani, Silsby, Phillips, 1991), and the onset and maintenance of incubation in ovariectomized turkeys has been shown to only occur after prolactin injection following a period of sex steroid priming (El Halawani, Silsby, Behnke, Fehrer, 1986). Female willow ptarmigan *(Lagopus l. lagopus)* infused with ovine prolactin increase distractive defence displays, require more threat to flush from the brood, and flush shorter distances from the nest (Pederson, 1989). One of the few studies involving male birds showed that ring doves *(Streptopelia risoria)* of both sexes sitting on infertile eggs prolong their incubation period and take longer to lay their second clutch after being injected with ovine prolactin (Lea, Vowels, Dick, 1986). Both male and female ring doves had an increased frequency and incidence of feeding in response to foster squabs of different ages after subcutaneous infusion of ovine prolactin. Additionally, intracerebroventricular (ICV) injections of prolactin increased the incidence of feeding behaviour, parental

feeding invitations, and crouching or sitting on the nest (Buntin, Becker, Ruzycki, 1991). Results of experiments such as these suggest that prolactin can act directly on the brain

In many temperate species, environmental factors such as photoperiod may also influence prolactin secretion. European starlings *(Sturnus vulgaris)* are the best known example of prolactin release being induced by increasing daylight. Dawson and Goldsmith (1985) measured prolactin levels in starlings at different stages throughout the breeding season by leaving some nests undisturbed (normal seasonal breeding), and progressively destroying others to either induce laying and incubating later and later in the season, or to prevent it altogether. They found that regardless of the birds' reproductive cycle activities, prolactin levels increased as daylength increased, even in non-breeding birds. However, the presence of eggs in the nest of breeders stimulated prolactin release more than the increasing daylight could account for. This suggests that the main factor influencing prolactin secretion in starlings is photoperiod, but that breeding behaviour can also modulate these levels.

In other species, photoperiod plays less of a role in inducing prolactin changes. For free-living white-winged crossbill males, seasonal food availability is the main factor that influences the initiation of breeding. However, decreasing photoperiod in autumn is related to decreasing prolactin levels and subsequent moulting, which prevents breeding at that time regardless of food availability (Deviche & Sharp, 2001).

Other environmental factors such as stimuli from the nest, egg, and chick can also be important factors for prolactin release, depending upon the species. Studies of ring doves deprived of their nests (Lea et *al.*, 1986) and Cape gannets (*Sula capensis)*

deprived of their egg (Hall, 1986), show that both males and females have rapid drops in prolactin levels. In incubating bantam hens, adoption of chicks prevents the drop in prolactin concentrations seen in other hens that have their nests and chicks removed (Sharp, Macnamee, Sterling, Lea, & Pederson, 1988).

Stimuli from the chick can increase parental prolactin levels, as shown in ring doves that had nests removed and then foster squabs presented at days 3, 8, and 12 of incubation (Lea et *al.*, 1986). Only males incubating on the 12th day showed an increase in prolactin levels after 90 minutes, so the timing of the introduction of young determines whether or not prolactin levels will increase.

These types of external stimuli do not appear to influence the pattern of prolactin release in some other species, especially in many pelagic seabird species. Adélie penguins have lengthy foraging bouts at sea while their partners incubate the eggs or chicks, but prolactin concentrations do not change from the time of first returning from an 8-11 day foraging trip to 1-3 days after resuming incubation (Vleck, Ross, Vleck, Bucher, 2000). Similarly, prolactin levels do not fluctuate in male or female King penguins during chick feeding bouts or between foraging bouts at sea (Garcia, Jouventin, Mauget, 1996). Emperor penguins also share parenting duties; females brood and males incubate. One compelling study of Emperor penguins' hormonal state during breeding was conducted in a year of particularly harsh weather, which resulted in total nest failure. Despite this absence of incubating stimuli, prolactin levels in both male and female breeders remained high (Lormee, Jouventin, Chastel, Mauget, 1999). Pelagic seabirds, particularly penguins, may be relatively unique in having prolonged prolactin

maintenance. This may be due to the unusually long periods of time they spend away from the breeding site foraging at sea. Prolactin levels remaining elevated in the absence of external stimuli may be a mechanism that has evolved in order to facilitate this breeding strategy.

Less well understood, and more variable by species, is how previous breeding experience interacts with prolactin to affect parental behaviour in birds. Wang & Buntin (1999) studied this by repeatedly presenting non-breeding female ring doves with hungry foster chicks over several days. They found that experienced females displayed a higher incidence of crop milk regurgitation, defensive behaviour, and nest attendance. The female ring doves were then injected with ovine prolactin, which resulted in increased numbers of parental behaviours for both inexperienced and experienced females. However, though the inexperienced group displayed these behaviours at a rate comparable to the control group of normally breeding experienced birds, the prolactin-injected females with previous breeding experience displayed over eight times more regurgitation acts than inexperienced females. This suggests that prolactin does induce parental behaviour in female ring doves, but that this effect is also enhanced by previous breeding experience. More work is required in this area, on a wider range of species, and especially with regard to the effect of experience in males.

In some cooperatively breeding species, prolactin responses occur in breeders and others that help with chick care. In Florida scrub-jay nest helpers, prolactin levels increase even before the chicks hatch, though at lower levels than the breeders (Schoech, Mumme, Wingfield, 1996). Furthermore, increased prolactin levels are correlated with

more food delivery to the nest and a higher number of nest visits. In the alloparental red-cockaded woodpecker, male non-breeding helpers have prolactin levels comparable to those of male and female breeders, and show a pattern of increasing prolactin levels from pre-breeding through to incubation (Khan, McNabb, Walters, Sharp, 2001).

The rare cases of polyandrous avian species, where males do most or all of the incubating and caring for the young, provide an interesting comparison to the biparental norm. Wilson's phalaropes *(Phalaropus tricolor)* have exclusively male parental care, and males show an increase in prolactin levels by the time of incubation and a drop after hatching (Oring, Fivizzani, Colwell, El Halawani, 1988). Males also have significantly higher prolactin levels than females do at the laying stage. In another species with exclusive male parenting, the red-necked phalarope *(Phalaropus lobatus)*, incubating males have higher prolactin levels than non-incubating males and females (Gratto-Trevor, Oring, Fivizzani, El Halawani, Cooke, 1990). Polyandrous spotted sandpiper *(Actitis macularia)* males show a trend towards higher prolactin levels than the females through the breeding season, though this tendency is only statistically significant during the incubation stage (Oring, Fivizzani, Halawani, Goldsmith, 1986).

Though prolactin is almost always associated with incubating and, less importantly, parental care activities, there are a few cases where the behaviours occur in the absence of this general pattern. Ring doves do not experience any increase in prolactin levels until day 4 or 5 of a 14-day incubation cycle, but still initiate and maintain the behaviour in the absence of elevated prolactin levels (Lea *et al.*, 1986). In

pied flycatchers, prolactin levels decrease after around 3 days post-hatch, but parents continue to care for the hatchlings for another 9 days (Silverin & Goldsmith, 1990).

1.1.1.2 : Testosterone in birds

Generally, testosterone levels in male birds follows the opposite pattern of prolactin. Usually testosterone levels are high during the courtship, but these levels drop before incubation and caring for chicks, at the same time that prolactin levels are rising (Feder, Storey, Goodwin, Reboulleau, Silver, 1977; Ball, 1991). Increased levels of testosterone are associated with mating activities such as singing and aggression (Ketterson & Nolan, 1992), which are counterproductive to parenting. In species such as the song sparrow *(Melospiza melodia)* which renest after loss of the first brood, testosterone levels rise again in response to the second mating effort (Feder et *al.*, 1977; Wingfield & Goldsmith, 1990). Usually polygynous males maintain consistently high levels of testosterone throughout the breeding season, whereas monogamous males have an initial, brief peak (Wada, Shimizu, Kobayashi, Yatani, Sandaiji, Ishikawa, Takemure, 1999).

Male birds implanted with testosterone-filled Silastic® tubing after the courtship period decrease their paternal care behaviours. Hegner & Wingfield (1987) showed that male house sparrows *(Passer domesticus)* with testosterone implants fed their young less frequently and engaged in more competition with other males, and as a result had lower breeding success than controls. They also implanted some males with the antiandrogen flutamide, which resulted in higher feeding rates for those males as compared to control

males. Testosterone-implanted male dark-eyed juncos have been shown to have more frequent singing, have a larger home range size, and feed their nestlings less frequently (Ketterson & Nolan, 1992). Spotted sandpiper males, the predominant parental care providers for that species, show marked behavioural effects after implantation with testosterone. During the incubation period, a higher proportion of implanted males desert their clutches and show reduced incubation constancy (Oring, Fivizzani, El Halawani, 1989). Male song sparrows implanted with testosterone have also been shown to be more aggressive (Wingfield, 1984).

Seasonal changes in testosterone release are controlled by photoperiod to some degree for most species, but for other species it is less important (Dawson, King, Bentley, Ball, 2001). Male brown-headed cowbirds *(Molothrus ater)* housed under long daylight conditions have elevated testosterone levels (Dufty & Wingfield, 1986). A recent study of wild canaries *(Serinus canaria)* showed that in one abnormal year, testosterone levels increased six weeks earlier than usual, independent of photoperiod. This was related to heavy rainfalls, which caused breeding conditions to become favourable (Leitner, Van't Hof, Gaur, 2003). Domestic male turkeys reared in either a short-day and a long-day photoperiod had increases in testosterone levels upon sexual maturation, but the magnitude of increase was larger in the long-day group. Furthermore, once turkeys in the short-day group were switched to a long-day schedule, further testosterone increases were observed (Yang, Long, Inpanbutr, Bacon, 1998). Taken together, these results suggest that, though photoperiod modulates testosterone release in some species, other mechanisms are often involved. Dawson, King, Bentley, and Ball (2001) state that in

most non-tropical bird species with a limited breeding season, photoperiod is the main controlling factor in the seasonal variation of hormones, but that "in tropical or opportunistic breeders endogenous circannual rhythmicity may be most important".

Though stimuli from the nest, egg, and chicks can be important factors influencing prolactin release, stimuli from partners and potential male rivals are most important in modulating testosterone release. Similar to males of many species, male ring doves show rapid increases in testosterone levels after pairing with a receptive female (Feder et al., 1977). The elevated plasma levels of male brown-headed cowbirds as a result of exposure to long daylight are further enhanced, occur sooner, and last longer when males are housed with females (Dufty & Wingfield, 1986). In free-living polygynous male red-winged blackbirds, high testosterone levels are associated with nesting in high density areas, having more mates and nests per territory, and greater fledgling success (Beletsky, Orians, Wingfield, 1992). These higher testosterone levels in dense colonies may be reflecting an interaction between an increased number of male rivals and more potential female partners.

Male song sparrows with territories next to testosterone-implanted males do not have the normal seasonal decrease in testosterone levels observed in males with territories next to controls (Wingfield, 1984). This increase in response to interactions with more aggressive male neighbours highlights how stimuli from competing males are important for testosterone release and maintenance for some species. Wingfield (1985) obtained similar results in male song sparrows by removing males that occupied a territory and measuring testosterone levels in the replacement males and male

neighbours, which were both interacting to establish new territory boundaries. Though replacement males had elevated testosterone levels, even further elevations were seen in the male neighbours, suggesting that defending a territorial boundary against a new unfamiliar male can stimulate a rise in testosterone levels. This testosterone response to male-male interactions during breeding activities in some species is the basis for the "challenge hypothesis" (Wingfield, Hegner, Dufty, Ball, 1990).

Though testosterone is often required for early breeding behaviours, previous experience can enhance the expression of these behaviours in some species. Reproductively experienced ring doves show higher levels of nest-oriented courtship behaviours than age-matched inexperienced males, even when their testosterone levels are the same (Cheng, Klint, Johnson, 2000). Studies of other species, such as the red-winged blackbird, have found no relationship between breeding experience and testosterone levels (Beletsky et al., 1992).

In co-operatively breeding species, it is typical for helpers at the nest to have lower testosterone levels than breeders. This has been shown in white-browned sparrow weavers, *Plocepasser mahali* (Wingfield, Hegner, Lewis, 1992), and in Florida scrub-jays (Schoech, Mumme, Moore, 1991).

In polyandrous mating systems, where males provide parental care and females participate in intra-sexual competition, the typical "male" testosterone pattern might be expected to apply to females and not to breeding males. Indeed, earlier studies on northern and Wilson's phalaropes showed a higher concentration of testosterone in female ovarian tissue than in male testicular tissue (Hohn & Cheng, 1967& Hohn, 1970;

cited in Rissman & Wingfield, 1984). However, tissue concentrations do not necessarily reflect the physiologically active, circulating hormone levels. Accordingly, though female spotted sandpipers are "sex-role reversed", they are not reversed in their patterns of testosterone secretion. They have been found to have circulating testosterone levels comparable to those of nesting males, but much lower than for non-nesting males (Rissman & Wingfield, 1984). A later study confirmed these findings, and reported that spotted sandpiper males had higher levels of testosterone than females prior to incubation, after which levels did not differ between the sexes due to a sharp drop in male testosterone levels. The same pattern of declining testosterone levels in males after incubation behaviour begins has also been documented in the polyandrous Wilson's phalarope (Oring et *al.*, 1988).

1.1.2: Review of biparental rodent studies

Biparental care in rodents is rare, as typically the female solely cares for her young. However, about 6% of rodent genera include biparental species (Gubernick & Nelson, 1989), including California mice (*Peromyscus californicus)*, Mongolian gerbils *(Meriones unguiculatus)*, Djungarian hamsters *(Phodopus campbelli)*, and prairie voles *(Microtus ochrogaster)* (Gubernick & Nelson, 1989; Brown, Murdoch, Murphy, Moger, 1995; Jones & Wynne-Edwards, 2000; Wang, Liu, Young, Insel, 2000). The hormonal changes underlying breeding and parenting, though not as well studied as with birds, have become an increasingly important topic among scientists studying these biparental species.

The earliest studies of rodent paternal behaviour involved common laboratory species such as rats and mice, which are not naturally paternal. The negative results often obtained from such species led to the belief that most male rodents have nonexistent or limited hormonal and behavioural responses to pups and pregnant mates. For example, male Long-Evans hooded rats housed with pups did not show prolactin increases, and were less responsive to pups than previous studies of females (Brown & Moger, 1983; Tate-Ostroff & Bridges, 1985). Though male rats are not naturally paternal, exposure to pups can sometimes induce parental care, but not reliably, and with longer latencies than females for those that do respond (Schradin & Anzenberger, 1999).

Biparental rodent species may the best place to start studying which proximate mechanisms are important to parental processes. Males that share parental duties likely are experiencing more pronounced hormonal and behavioural changes.

1.1.2.1: Paternal behaviour in biparental rodents

Many studies have correlated paternal behaviours with factors such as previous breeding experience, photoperiod, and stimuli from the young without measuring any circulating hormone levels. Though the main focus of the current work is the relationship of hormones to parental behaviours, there are many insights to be gained from the behavioural studies.

Most biparental rodent species seem to share a common pattern with regard to paternal care benefiting overall reproductive success. Djungarian hamster fathers actually assist the female during the delivery of pups, performing a range of behaviours

usually seen only from the female (Jones & Wynne-Edwards, 2000). The presence of these fathers is also important to pup growth, an effect achieved not through direct paternal care, but by indirectly affecting the mother's physiology through alleviation of her hyperthermia (Walton & Wynne-Edwards, 1998). Though chemical cues from the female mate have been found to be important in eliciting paternal behaviours in some other rodent species, such is not the case for Djungarian hamsters. Jones & Wynne-Edwards (2001) separated males from their mates shortly after copulation, and did not return them until just before the birth, with no effect on paternal behaviour in males. The males still helped with the birth, cared for pups, and retrieved pups in a displacement experiment, which suggests that contact with the female or even the sight, smell, or sound of her is not important for Djungarian hamster males to behave paternally.

The California mouse is sexually and socially monogamous in the wild, and males provide extensive parental care. The importance of males to pup survival in this species was clearly demonstrated in a field experiment involving male removal, which resulted in significantly lower offspring survival in the father-absent group (Gubernick & Teferi, 2000). In a laboratory experiment where California mice parents ran on a wheel in order to obtain food, the presence of the male resulted in four times as many pups reared than the female could rear alone (Cantoni & Brown, 1997).

After having young of their own, California mouse males behave parentally toward foster pups, but virgin males do not typically express parental behaviours (Gubernick & Alberts, 1987). Most males of this species require chemical stimuli from their partners to remain paternal postnatally, and stimuli from the pups seems to be of

little importance, at least for the first three days postpartum (Gubernick & Alberts, 1989). In a follow-up study, maintenance of paternal care was found to be a result of chemical cues specifically from the female partner, with excreta from her being sufficient to maintain the paternal behaviours (Gubernick, 1990). There are individual differences, however, as about one-third of males display paternal behaviours within a day of copulation, which persist through the pregnancy even in the absence of the female (Gubernick, Schneider, Jeannotte, 1994).

Male Mongolian gerbils spend much of their time in physical contact with their pups. Paternal behaviours such as crouching over and licking pups are evident throughout the whole rearing period (Brown et *al.*, 1995). Alloparental behaviour as a juvenile seems to be important to later parenting effort for Mongolian gerbils, as juvenile males that are exposed to sibling neonates produce their first litter sooner than inexperienced males, and pups of the experienced males gain weight faster and open their eyes sooner. The inexperienced males seem to "catch up" in terms of parenting quality after rearing a single litter, as these differences are no longer significant by the time the second litter is born (Salo & French, 1989).

Prairie vole males contribute to parental care both directly, by spending much of their time in the nest with pups, thus aiding in thermoregulation, and also indirectly by building and maintaining the nest (Oliveras & Novak, 1986). Like Mongolian gerbils, juvenile prairie voles are alloparental towards pups of their parents' subsequent litters. Alloparental behaviour is significantly enhanced, especially in male juveniles, when the

father is present along with the mother during rearing. Development is also faster in pups reared by both parents (Wang & Novak, 1994).

Though meadow voles (*Microtus pennsylvanicus)* are primarily polygamous and show less paternal care than prairie voles, males nest together with females and pups in the fall and winter (Madison, FitzGerald, McShea, 1984). The female regulates access to the pups, preventing unfamiliar individuals from entering the nest (Storey, Bradbury, Joyce, 1994). Male contact with pups helps to increase paternal responsiveness and decrease aggression (Storey & Joyce, 1995). Chemical cues from the female partner and pups serve to abolish male aggression toward pups, but actual physical contact with the pups is required for most males to show increased paternal behaviour (Storey & Walsh, 1994). Male meadow voles housed under short daylight conditions, which simulate the nesting fall and winter months, were more paternal towards their own pups than those housed in long daylight conditions (Parker & Lee, 2001).

1.1.2.2: Prolactin in biparental rodents

As with birds, prolactin does play an important role in the expression of parental behaviours for female rodents, and for males of biparental species. However, contrary to the apparent pattern seen in birds, the mechanism by which prolactin exerts its action may differ between the sexes in mammalian species (Lonstein & DeVries, 2000).

California mice fathers have increased prolactin levels two days postpartum that are comparable to maternal levels, and are significantly higher than levels for both the 'expectant fathers' at ten days prior to birth and virgin males. Tests with pups show that

80% of fathers behave paternally, as opposed to only 19% of virgin males (Gubernick & Nelson, 1989). These results are consistent with the hypothesis that prolactin is related to paternal behaviour in the California mouse, and that exposure to, or birth of, the pups may be an important factor for prolactin release.

Mongolian gerbils show similar prolactin level changes after the birth of their young. Brown et *al.* (1995) measured prolactin levels in mated males before and after the birth of their pups, and found that there was a gradual increase that became statistically significant by the 20th day postpartum. It seems that stimuli from the young or, less likely, from the mother, facilitates prolactin release and paternal behaviours in this case.

The Djungarian hamster *(Phodopus campbelli)* is biparental, whereas the closely related Siberian hamster *(Phodopus sungorus)* is primarily maternal. Reburn and Wynne-Edwards (1999) compared the prolactin profiles for these two species, and found that biparental male Djungarian hamsters experience prolactin level increases somewhere between the third and fifth days postpartum. The increased prolactin levels at this time are found only in the biparental species, a similar pattern to the postnatal increases found in Mongolian gerbils and California mice. In some species, prolactin may work prenatally as with female mammals, priming the male for the birth of his young. Administration of bromocriptine, a dopamine agonist that suppresses pituitary prolactin release, to Djungarian hamster males three days before the birth of their pups suppressed paternal behaviours when they were later tested on the third day after birth. In contrast, administration of bromocriptine from the day of birth to the third day postnatal, the day of testing, did not affect paternal behaviours (Jones, 2000 in Wynne-Edwards, 2001).

1.1.2.3: Testosterone in biparental rodents

Generally, less work has been done on testosterone than prolactin in biparental rodent species. However, it seems that testosterone's role is quite different than in birds. It has been well established that the increases in aggression and mating effort, and the decreases in incubating and chick provisioning activities that are related to testosterone levels in avian species are counterproductive to paternal care. For biparental rodents, however, emerging evidence suggests a positive correlation between testosterone and parental behaviour.

The best-studied biparental rodent species with regard to testosterone and paternal behaviour is the California mouse. A study by Gubernick & Nelson (1989) comparing fathers with 'expectant' fathers and virgin males found no difference in testosterone levels between groups and relatively low concentrations overall. More recently, Trainor & Marler (2001) have demonstrated that testosterone seems to be necessary for paternal behaviour in California mice. They found that reproductively experienced males showed a marked reduction in paternal behaviours after they were castrated, as opposed to both sham-operated males and castrated males that had testosterone replacement. A follow-up study (Trainor & Marler, 2002) confirmed this result, and demonstrated that the paternal behaviours occur in the presence of elevated testosterone levels via the aromatisation of testosterone to estrogen in California mice.

Testosterone also seems to be important for the expression of paternal behaviour in prairie voles. Castrated male prairie voles show reduced paternal responsiveness, which is prevented by testosterone treatment (Wang & De Vries, 1993). Male prairie

voles castrated on the day of birth show a significant reduction in paternal behaviours as adults, which suggests that postnatal exposure to androgens are important for normal development of these behaviours (Lonstein, Rood, De Vries, 2002). In the same study, females that were administered testosterone as adults also showed more parental behaviours than untreated females did. This pattern of testosterone facilitating the expression of paternal behaviour in prairie voles was not found by Roberts, Zullo, Gustafson, and Carter (1996), who showed that when male prairie voles were injected with testosterone at postnatal day 6, their alloparental behaviour was disrupted when later tested at 24 and 42 days of age. In the same study, prenatal administration of testosterone had no effect on alloparental behaviour in males.

The only study to measure circulating male Djungarian hamster testosterone levels with respect to their mate's pregnancy found that levels increased steadily through the gestation period and dropped off dramatically by the first day after the birth (Reburn & Wynne-Edwards, 1999). An unexplained jump in testosterone levels occurred by the fifth day of lactation, with levels equivalent to those found during late pregnancy.

Male Mongolian gerbils housed in monogamous pairs showed a steady increase in testosterone levels from mating up to the time of birth of their pups, after which levels dropped off and remained low (Brown et *al.*, 1995). In castrated male Mongolian gerbils, paternal behaviours were more frequent than for intact and sham-operated males (Clark & Galef, 1999). As with females, castrated males also preferred displaced pups in a choice test between these pups and nest sites, whereas intact and sham-operated males preferred the nest. These patterns are consistent with the generalized pattern found for

birds, as well as with the results of some previously mentioned California mouse studies (Trainor & Marler, 2001; 2002).

1.1.2.4: Glucocorticoids in biparental rodents

Three studies have examined the role of glucocorticoids in the paternal behaviour of biparental rodents. Both Djungarian and Siberian hamster males show a drop in cortisol concentrations of over 50% after pairing with females (Reburn & Wynne-Edwards, 1999). The biparental Djungarian hamsters showed an increase in cortisol levels on the 17th day of gestation, a change that was not seen in the less paternal species. The authors hypothesize that this rise in the paternal species may function in bonding with pups that are born approximately two days later.

Roberts et *al.* (1996) administered corticosterone to prairie voles at postnatal days 1 to 6, and found no effect on alloparental behaviour for male prairie voles tested at postnatal days 24 or 42, but noted a significant reduction in females. Castro & Matt (1997) reported that after introduction of a male intruder, cortisol levels of Siberian hamsters were significantly elevated in the males residing with a female or a family as compared to solitary-living males. The paired males also displayed more aggressive behaviours towards the intruder than solitary males did.

1.1.2.5: Other hormones in biparental rodents

Vasopressin and oxytocin have also emerged as having a major role in rodent parental care, and most work in this area has been conducted at the cellular and molecular level on voles. Vasopressin appears to be more important for the expression of male parental behaviour and pair-bond formation. The pattern and distribution of vasopressin receptors in the vole brain differs from one species to the other, and is thought to be related to the differential mating strategies of each. Vasopressin administration has been shown to induce parental care and pair bonding in prairie voles, whereas vasopressin 'blockers' reduce these behaviours (for a review, see Wang, Young, De Vries, Insel, 1998). Oxytocin, however, appears to be more important for the expression of female parental behaviour and pair bonding. Central administration of oxytocin increases these behaviours, whereas oxytocin antagonists reduce these effects (for a review, see Carter, De Vries, Taymans, Roberts, Williams, Getz, 1997).

1.1.3: Review of nonhuman primate studies

Hormonal changes associated with infant care in male primates have also been studied, mainly in three co-operatively breeding species from the family Callitrichidae, common marmosets *(Callithrix jacchus)*, cotton-top tamarins *(Saguinus oedipus)*, and black tufted-ear marmosets *(Callithrix kuhlii)*. These species are primarily monogamous, produce twin infants, and the males provide extensive parental care both as helpers and as parents (for a complete review, see Ziegler, 2000).

1.1.3.1: Paternal behaviour in nonhuman primates

Callitrichid species are often co-operative breeders, which appears to be important for parental quality since experience gained as a juvenile helper may facilitate later parental efforts. Tardif, Richter, and Carson (1984) reported that inexperienced cotton-top tamarin and common marmoset parents have higher infant mortality rates than experienced parents. Their study showed that for the cotton-top tamarins, infant survival depended upon the mother being experienced, but not the father.

1.1.3.2: Prolactin in nonhuman primates

The study of hormones and paternal care in primates is a relatively new area, and much work remains to be done. There have been only a handful of studies conducted on this topic, and prolactin is the hormone most commonly measured. Generally, there seems to be a strong association between prolactin levels and paternal care for the two callitrichid species studied to date, the common marmoset and the cotton-top tamarin.

1.1.3.2.1: Prolactin in common marmosets

Male common marmosets paired with females and infants have been shown to have significantly higher serum prolactin levels than males living with non-pregnant or pregnant females only (Dixon & George, 1982). Prolactin levels in males were also higher on days when they had been carrying infants immediately before sampling than days when they were not. These higher levels may be due to either physical contact with

the infant eliciting increases in prolactin release, or the higher prolactin levels stimulating some of the males to engage in carrying behaviour.

The role of carrying was further investigated by measuring prolactin levels in common marmoset fathers and male helpers (Mota & Sousa, 2000). Though there were no pre to post-birth changes for fathers or helpers, those helpers that carried infants had higher prolactin levels than non-carrying helpers, and higher levels than prior to the birth. Fathers that carried infants also had higher levels than they did before birth, but these levels were not significantly higher than those of fathers not carrying.

Bromocriptine, which is a dopamine-receptor agonist, reduces secretion of prolactin (Schradin & Anzenberger, 1999). In a recent study of unpaired captive common marmosets, bromocriptine was administered to six females and two males who had no prior infant experience (Roberts, Jenkins, Lawler Jr., Wegner, Newman, 2001). Though all individuals consistently retrieved infants in a test before bromocriptine administration, half failed to do so after the treatment, when their serum prolactin levels had dropped to undetectable levels. Furthermore, those four treated marmosets that continued to retrieve took significantly longer to do so than when treated with the vehicle only, and they carried for significantly shorter periods of time. It seems that prolactin is necessary for normal parental behaviours to occur, and a deficiency in prolactin significantly impairs expression of these behaviours.

1.1.3.2.2: Prolactin in cotton-top tamarins

Parentally experienced cotton-top tamarin males living in family groups had higher urinary prolactin levels than parentally inexperienced males during the postpartum period, as well as higher levels than their eldest sons (Ziegler, Wegner, Snowdon, 1996). A different pattern was noted for first-time fathers, with a steady increase from the time of pairing with a female through to two weeks after the birth, and a significant difference from preconception to postpartum levels. Ziegler & Snowdon (1997) found that at two weeks postnatal, cotton-top tamarin fathers had higher levels of prolactin than either males paired to females or those living in a cage next to a female. The fathers, however, did not have significantly higher levels than their oldest sons living with the group. First-time fathers had significantly lower prolactin levels than those that had multiple births.

Parenting experience strongly influences prolactin patterns in male cotton-top tamarins, as prolactin levels were shown to be correlated to the number of births a male had previously experienced, regardless of age (Ziegler, Wegner, Carlson, Lazaro-Perea, Snowdon, 2000). Fathers' prolactin levels did not change over the period around birth, nor did they differ between fathers whose infants died and those fathers with surviving infants. Experienced males have higher prolactin levels during their partner's pregnancy, peaking at the third month of gestation, which is halfway through the pregnancy (Ziegler & Snowdon, 2000). Prolactin levels were also correlated to the number of infants surviving from previous births, with earlier elevations corresponding to a greater number of surviving infants.

1.1.3.3: Testosterone in nonhuman primates

Less is known about the relationship of testosterone to prolactin in nonhuman primate species, as only a few studies have been conducted on this topic. To date, however, the only callitrichid species that has been reported to have any significant relationships of testosterone to paternal or alloparental behaviours is the black tufted-ear marmoset.

1.1.3.3.1: Testosterone in common marmosets

In their landmark study of paternal care and hormones in common marmosets, Dixon & George (1982) reported no significant differences in serum testosterone levels between males paired with females and infants and those paired with either pregnant females or non-pregnant females. There was, however, a non-significant trend towards lower levels in the males with infants relative to the males without infants. Contrary to the bird pattern, there were no significant correlations between rising levels of prolactin and any decline in testosterone levels for these males.

1.1.3.3.2: Testosterone in cotton-top tamarins

Ziegler et *al.* (2000) measured urinary testosterone levels in captive cotton-top tamarin males for 15 days before and after their partners gave birth, but found no difference in levels of fathers with and without infants. There was also no difference in testosterone levels for fathers who lost infants and those that did not, and no correlation was found between amount of time spent carrying and urinary testosterone levels.

Additionally, no inverse relationship existed between urinary prolactin and testosterone levels for these males. The only significant result for testosterone was related to timing of ovulation for their mates; when their partner's ovulation occurred in the first 15 days after birth of the young, urinary testosterone levels were higher in the males throughout these 15 days postpartum. Overall, these results suggest that stimuli from infants do not influence testosterone levels in cotton-top tamarins, but the reproductive state of the female partner does.

In order to investigate whether any testosterone changes occur earlier than the sampling period for the previous study, Ziegler & Snowdon (2000) measured urinary testosterone levels throughout the whole six-month gestation period. Testosterone levels increased steadily through each month of pregnancy, with significant elevations occurring from the third until the fifth month, after which a drop was seen in the last month before birth. The peak month did not correlate with the number of surviving previous infants, as it had for prolactin. Again, no inverse relationship between prolactin and testosterone levels was found.

1.1.3.3.3: Testosterone in black tufted-ear marmosets

Only one laboratory has investigated the relationship of testosterone levels and parenting in the black tufted-ear marmoset. Nunes, Fite, French (2000) reported that urinary testosterone concentrations of fathers dropped to their lowest levels by three to four weeks after birth of the infant, which coincides with the peak of infant carrying behaviour. Testosterone levels then increased significantly in the two-week period

following this. Experienced males that had previously cared for four or more litters had significantly lower urinary testosterone levels than inexperienced males with no or one previous litter. No differences in urinary testosterone levels were observed between fathers whose young died at birth versus those whose young survived to weaning.

This finding was later supported by Nunes, Fite, Patera, French (2001) who found that urinary testosterone levels were lower among black tufted-ear males that carried infants at high rates when compared to those that carried at lower rates. Infant carrying and testosterone concentrations were also negatively correlated during the period of infant care for males. Experience played a role in testosterone levels, as they decreased in males between their first and second litters. The percent decrease of testosterone levels after the birth was also greater after the second litter was born, with the largest proportional decrease seen by 3-4 weeks postnatally, a time when males do the most carrying.

1.1.3.4: Cortisol in nonhuman primates

To date, only two studies have been published relating cortisol to nonhuman primate parenting. As is the case with testosterone, it seems that there may be a different pattern of cortisol level changes between cotton-top tamarins and black tufted ear marmosets during the period surrounding birth of their young.

Ziegler & Snowdon (2000) measured urinary cortisol levels in experienced and inexperienced male cotton-top tamarins throughout their partners' pregnancy and the postpartum period. They reported that levels barely changed at all through the whole

sampling period for both inexperienced and experienced males, and there was no difference in cortisol concentrations between both groups.

Nunes et *al.* (2001), however, reported significant changes in urinary cortisol concentrations for male black-tufted ear marmosets following the birth of their young. Levels were higher in males that carried infants at low rates, especially for the first 1-2 weeks postpartum. Thereafter, urinary cortisol levels declined to levels comparable to those of males that carried at high rates. Accordingly, there was a negative correlation between urinary cortisol levels and infant carrying rates during the first six weeks after birth. Though previous parental experience affected testosterone levels, there was no difference in cortisol levels from one litter to the next. When the percentage of change in cortisol levels were analyzed, there was a significant interaction between time and litter, with increasing levels after the birth of the first litter and decreasing levels immediately after the birth of the second.

1.1.4: Review of human studies

1.1.4.1: Paternal behaviour in humans

The behaviours and rituals human fathers engage in around the time of their partner's pregnancy and birth of their child has been studied across many cultures, and anthropological explanations abound in the literature (Broude, 1988). From these reports, the medical definition of a 'couvade syndrome' arose, referring to "physical or psychosomatic symptoms experienced by men in industrialized cultures during their partner's pregnancies" (Elwood & Mason, 1994). Elwood & Mason (1994) redefined

couvade in a biological sense by saying it "may be better viewed as a ritualized expression of underlying biological changes that function to bring about a state of heightened paternal responsiveness … triggered by the partner's pregnancy".

Recently, this definition of couvade has been applied to the hormonal changes human males may experience around the time of the birth of their infant. Along with various physiological measures, emotional responses of new fathers have been recorded to provide insight into how physical changes may be expressed.

In a recent study of the emotional responses that new fathers experience, those who listened to a tape of infant hunger and pain cries reported that they felt more sympathetic and alert than fathers who did not listen to the tape and control non fathers who did (Fleming, Corter, Stallings, Steiner, 2002). Both experienced and inexperienced fathers reported a greater 'need to respond' to the pain than to the hunger cries. Only the experienced fathers showed a non-significant trend towards a greater 'need to respond' report after listening to the tape than inexperienced fathers and non-fathers did. When fathers with the greatest amount of experience in holding and diapering infants were considered, they reported a significantly 'greater need to respond' to the infant pain cry only.

1.1.4.2: Prolactin in humans

The endocrine profile of women through pregnancy and around the time of birth has been previously established (review, Corter & Fleming, 1995), but until recently no work had been conducted on fathers. The first study to measure hormonal changes in

new fathers reported significant changes in the men's prolactin levels throughout their partner's pregnancy (Storey, Walsh, Quinton, Wynne-Edwards, 2000). The men in the study had higher prolactin levels two weeks before birth than men tested earlier in the pregnancy. Storey et *al.* (2000) also confirmed the previously found prolactin pattern for women, with levels increasing prenatally, reaching a peak right before birth, and then dropping postnatally. Thus, both the expecting man and woman have similar patterns in that the peak for both occurs prior to birth.

Storey et *al.* (2000) conducted an additional test of short-term hormonal reactivity to infant cues. After participants had baseline venipuncture samples taken, they were given a doll to hold which was wrapped in a receiving blanket that had recently been worn by an infant. Postnatally, fathers held their newborn while the mothers held the doll. Couples then listened to a 6-minute audiotape of hunger and pain cries, followed by a 5-minute video of an infant nursing for the first time after birth. At 30-minutes after the first sample, a second venipuncture sample was taken in order to measure the hormonal changes which occurred in response to the infant stimuli. For both men and women at all stages, prolactin levels were significantly lower after the 30-minute reactivity test than before. This result is contrary to the established patterns of parenting stimuli causing increases in prolactin levels for male bird, rodent, and primate species. However, it has been suggested that the novelty of researchers visiting the couple's home to sample blood and present infant stimuli may induce hormonal changes normally seen in other parental animals in response to a 'challenge', with levels returning to normal by 30 minutes after the initial sample (Wynne-Edwards, 2001; see also 'challenge hypothesis' proposed by

Wingfield et *al.*, 1990). It is possible that interaction with stressors may result in the consistent decreases in prolactin levels for the 30-minute period, and that this decrease in prolactin concentrations may not be in response to the infant stimuli.

Storey et *al.* (2000) also reported that men who felt concern upon hearing the baby cries had higher prolactin levels for the first "baseline" sample than those who did not report concern, and prolactin levels were higher in men with two or more 'pregnancy symptoms' when compared to those with fewer than two symptoms. Women whose partners reported two or more 'pregnancy symptoms' also had higher prolactin levels than women with partners that did not. These results correspond with the general model for birds, some rodents, and primates, where parenting responses are associated with higher levels of prolactin.

Fleming et *al.* (2002) reported that experienced fathers who had listened to a tape of infant hunger and pain cries had a larger percentage increase in prolactin levels than either inexperienced fathers who also heard the cries tape, or all fathers who listened to hissing or buzzing noise for the control condition. Fathers with more experience showed a non-significant trend towards having higher prolactin levels, and those fathers with higher prolactin levels reported significantly greater alertness in response to hunger-type infant cries.

1.1.4.3: Testosterone in humans

Storey et *al.* (2000) reported an overall change in men's serum testosterone levels throughout the two prenatal and two postnatal stages of their partner's pregnancy, with a

significant drop in levels from around the last three weeks of pregnancy to within the first three weeks postnatally. For the men tested within the first three weeks of their infant's birth, testosterone levels increased in the 30-minute period where fathers held their own newborn and were exposed to infant cries and a video of a newborn nursing. There were no significant changes in testosterone reactivity seen for fathers tested at two prenatal and one other postnatal stage. When all groups were combined, men who felt more concerned in response to the baby cries showed significant decreases in testosterone levels.

Berg & Wynne-Edwards (2001) reported that fathers' evening salivary testosterone concentrations, measured weekly from the first trimester through to three months postnatally, were lower than in non-fathers once the time of day and seasonal effects had been controlled. There was no difference between fathers' and non-fathers' morning testosterone levels, and no clear pattern of testosterone change over time was found for the fathers around the time of birth.

Berg & Wynne-Edwards (2002) published results from 9 of their original 45 couples that had good compliance, were expecting their first child, and continued to breastfeed through the postnatal sampling period. They found that this subset of fathers had a significant drop in salivary testosterone levels from the last week before birth to the first week afterwards. There were, however, no within couple correlations for testosterone levels.

When tested in the hospital within a short period of time after the birth of their babies, fathers who listened to infant hunger and pain cries showed higher levels of

salivary testosterone and a larger percentage increase than fathers who did not hear the cries (Fleming et *al.*, 2002). This result is consistent with those of Storey et al. (2000) at the early postnatal stage, where serum testosterone levels in new fathers increased following presentation of infant stimuli, including cries.

Fleming et *al.* (2002) found that both fathers and non-father controls with lower levels of salivary testosterone scored higher on a self-report measure of feelings of 'sympathy' and/or 'need to respond' to the infant cries when compared to men with high levels of salivary testosterone. This corresponds to a similar result of low testosterone levels in men reporting 'concern' in response to baby cries (Storey et *al.*, 2000). Fathers in the Fleming et *al.* (2002) study with more experience caring for infants also had lower salivary testosterone levels, as did the combined group of fathers when compared to control men.

Gray, Kahlenberg, Barrett, Lipson, Ellison (2002) measured morning and evening salivary testosterone levels in unmarried men without children and in married men with and without children. Since testosterone concentrations decline with age, they controlled for possible effects of age by using the residuals of a regression of age versus testosterone levels. Married men with and without children had lower salivary testosterone levels in the evening, but not in the morning, than unmarried men. Testosterone levels were negatively correlated with the amount of time the men had spent with their spouse in their last day off work, as well as with scores on a spousal investment scale.

1.1.4.4: Cortisol in humans

More is known of the relationship between parenting and cortisol in mothers than in fathers, as men have only recently begun to be included in studies of parenting. Though many of the studies discussed here involve only the mother, they are included because the implication that cortisol may facilitate parent-infant 'bonding' may also apply to fathers.

1.1.4.4.1: Cortisol in human mothers

The first study of hormones and their relation to maternal responsiveness (Fleming, Steiner, Anderson, 1987) found that high levels of plasma cortisol in new mothers at 3 or 4 days postnatal were associated with behaviours that involved attention directed towards the infant, but not direct care activities. Further work by Fleming, Steiner, Corter (1997) showed that first-time mothers, but not mulitparous mothers, who reported their own newborn's body odours to be more attractive had higher salivary cortisol levels than those who reported their newborn's odours to be less attractive. Mothers with high cortisol concentrations also performed better on a recognition task involving their own infant's body odours.

A recent study involving mothers at 1 to 2 days postpartum reported interesting differences between salivary cortisol levels based on parity, as well as interactions between cortisol and emotional responses to infant cries (Stallings, Fleming, Corter, Worthman, Steiner, 2001). First-time mothers had higher baseline salivary cortisol levels than multiparous mothers, and when compared to a control group of nonparous women,

new mothers also had higher mean salivary cortisol levels. In a 40-minute test involving exposure to infant cries, the pattern of change in salivary cortisol levels differed depending upon parity. Experienced mothers had consistently low levels, whereas new inexperienced mothers had initially high levels that dropped over time but still remained higher than those of experienced mothers. Experienced mothers, but not first-time mothers, with higher baseline salivary cortisol levels reported more sympathy in response to infant hunger cries. Mothers best able to discriminate between hunger and pain-type cries, as judged by the difference in sympathy reports for each cry, had higher baseline cortisol levels. Though their baseline levels were higher, high-sympathy mothers showed a pattern of cortisol decline over time that differed from the consistently low levels seen in the low-sympathy mothers. Though the non-cry control group did not show any of the cortisol level changes that the new mothers receiving the cry stimuli did, a significant association between heart rate and cortisol levels did occur in both groups.

1.1.4.4.2: Cortisol in human fathers

Storey et *al.* (2000) found that cortisol levels followed the same pattern for both men and women across the prenatal and postnatal period, and the highest baseline levels were seen within the three weeks before birth. Cortisol concentrations decreased overall for both men and women within the 30-minute test involving infant cues, with a significant post-hoc decrease for the men within the last three weeks before birth. It has been hypothesized that the second sample at this visit may be more indicative of normal 'baseline' levels, and that the first sample, which was taken as baseline, may actually be

reflecting increased reactivity in response to the researcher's visit in the weeks before birth (Storey et *al.*, 2000; Wynne-Edwards, 2001).

Storey et *al.* (2000) also found that women whose partners reported two or more 'couvade' symptoms had higher baseline cortisol levels. Similarly, men partnered to women who reported 'concern' in response to the baby cries had a larger decrease in cortisol levels over the 30-minute 'reactivity' test. Men and women's cortisol levels were positively correlated at the two prenatal tests, and at two months postnatal but not within the three weeks postnatal group.

Berg & Wynne-Edwards (2001) sampled salivary cortisol levels once weekly in men from the early stages of their partner's pregnancies through to 3 months postnatal. They reported that for morning samples, where salivary cortisol levels were significantly higher for all men, control men had higher mean levels of cortisol than fathers. There was no difference between the two groups for late evening samples. Similar to the results from Storey et *al.* (2000), cortisol levels were highest in the week before the birth and lowest in the week following it.

Berg & Wynne-Edwards (2002) later published results from a subset of 9 couples of the original 45 that had participated in their earlier study (Berg & Wynne-Edwards, 2000). They reported a significant positive correlation for salivary cortisol levels between men and women prenatally, and confirmed the previous report of an increase in salivary cortisol concentrations for men in the week before birth.

1.1.4.4.5: Other steroid hormones

Though estrogen and progesterone are not reviewed in detail here, they also play a role in parental behaviours, especially for females. The general pattern for estrogen in birds is similar to that for testosterone. Estrogen levels are high during the prelaying and laying periods, and decline as incubation begins and prolactin levels increase (Ball, 1991). In female galliform birds, incubation cannot be induced without an initial period of priming with estrogen followed with progesterone, and only then can prolactin sustain nesting (for a review, see Buntin, 1996). Buntin (1996) also states that "progesterone is a more effective agent than prolactin in inducing incubation behavior in intact, nonbreeding doves". In mammals, both estrogen and progesterone are high during pregnancy (Corter & Fleming, 1995). These high levels of estradiol stimulate prolactin release in females (Ziegler, 2000).

Storey et *al.* (2000) measured serum estradiol in human mothers before and after the birth of their first child, and confirmed previous findings of increasingly elevated estradiol levels in the time leading up to the birth and sharp declines postnatally. Since few most of the interesting results of that study were related to cortisol, prolactin, and testosterone, the focus of the current work is on these hormones only.

Berg & Wynne-Edwards (2001) measured salivary estradiol in men around the time of birth of their child, and found that they had a higher proportion of samples with detectable estradiol concentrations than control men. For those men that had samples with measurable salivary estradiol levels, fathers had higher estradiol concentrations than control men.

1.2: Purpose of the current study

Four goals have been established for the current study, the first of which is to study the long-term hormonal changes within individual couples, but especially in men, from the early stages of their partner's pregnancies into the months following birth.

Secondly, short-term hormonal changes in response to infant stimuli will be measured, with particular attention to the differences in magnitude of response by stage of pregnancy.

A third purpose of the study is to correlate the baseline hormonal levels, as well as magnitude of change in the short-term test, for individual couples throughout the pre and postnatal period. One previous study has reported correlations between women's prolactin and cortisol levels and both the men's baseline cortisol and its magnitude of change in the men (Storey et *al.*, 2000). One other study (Berg & Wynne-Edwards, 2002) reported salivary cortisol correlations between partners before birth, but found no within-couple correlations for testosterone or estradiol levels.

A fourth goal is to establish a reliable method of using blood spot sampling in place of either serum or saliva collection methods to measure prolactin, cortisol, and testosterone levels.

1.3: Significance of the study

One important benefit of the current study design is the use of blood spot sampling over either serum or venipuncture. Serum is traditionally obtained from venipuncture, which is more invasive than either saliva or finger sticks for blood spot

sampling. Obtaining serum in this fashion often requires the use of a trained technician. Handling and storage of the blood require methods that are not as robust as those for dried blood spots or saliva. Long-term storage of serum is often not an option, depending upon the hormone of interest. Repeated, frequent sampling is not as easy as with other methods, and some subjects may react rapidly with hormonal changes if they are apprehensive about the procedure. Cortisol concentrations, in particular, can change within minutes (Kanaley, Weltman, Pieper, Weltman, Hartman, 2001), and this can potentially confound the results of a study.

Saliva sampling is less invasive and easier to collect, especially repeatedly and frequently, and without the need for a trained technician. Though the use of this method has greatly increased in recent years, there are several drawbacks to consider. Of greatest importance is the fact that saliva contains only the unbound, free portion of hormones, and does not represent the total circulating hormone levels (Worthman & Stallings, 1997). The reduced total levels may present problems in measuring those hormones that are physiologically available in low levels, especially with hormones that are present in low levels for a particular sex (estradiol in prepubescent boys, Shirtcliff, Granger, Schwartz, Curran, Booth, Overman, 2000; estradiol in men, Berg & Wynne-Edwards, 2001). Saliva is frequently contaminated with blood, minuscule amounts of which can artificially increase the results of an assay (Worthman & Stallings, 1997). Furthermore, Shirtcliff, Granger, Schwartz, Curran (2001) recently reported that the cotton used in collection of saliva for the most popular method can produce artificially elevated results for some hormones (testosterone, DHEA, progesterone, estradiol), and lowered results for

others (IgA). They also found that the samples collected using the cotton lowered the correlation between DHEA serum and saliva levels. Other studies have also found a disappointing correlation between serum and saliva estradiol (Shirtcliff et *al.*, 2000) and testosterone (Shirtcliff, Granger, Likos, 2002).

Blood spot sampling through a finger-stick, though not as non-invasive as saliva sampling, is minimally invasive and can be done repeatedly with little discomfort. As with saliva, blood spots can be collected without the need for a trained technician, and are easy to store and transport. Dried blood spot samples are more robust than serum when stored for the long-term. Perhaps the most important advantage of using blood spots is their excellent correlation to serum values (estradiol in men and women, Shirtcliff et *al.*, 2000; LH and FSH in women, Worthman & Stallings, 1994; estradiol, progesterone, and testosterone in men and women, Shirtcliff et *al.*, 2001; testosterone in men and women, Shirtcliff et *al.*, 2002).

Since people may be more inclined to think of venipuncture blood sampling as a negative or fearful experience than finger stick sampling (Givens, Oberle, Lander, 1993), it is expected that more people from each prenatal class would volunteer for the new finger stick procedure than volunteered for the original study. This is also expected to provide a more diverse or statistically 'normal' population than those that volunteered for the earlier work.

A second benefit of the current study is the use of a longitudinal, within-subjects design. This allows for the study of individual variation within individual couples, with an emphasis on the men, from the early stages of their partner's pregnancy into the

months following birth. The first study to examine in depth the relationship of prolactin, cortisol, and testosterone levels in new mothers and fathers employed a between-subjects design (Storey et *al.*, 2000). Couples were recruited from prenatal classes and assigned into one of four stages, early or late prenatal and early or late postnatal. They were sampled when the timing of the pregnancy and birth was appropriate to their assignment. Though this was advantageous in controlling for possible confounding effects due to habituation to repeated blood sampling, it did not allow for following through of individual couples. The current use of a within-subjects design has the added advantage of being more statistically sensitive than a between-subjects design because of the fact that individual differences are controlled for (Keppel, 1991).

Third, the additional value of the short-term test of situational reactivity to infant stimuli is important. Examining whether short-term hormonal reactivity patterns differ by stage of pregnancy can provide insight into the physiological 'readiness' at different times relative to the birth. The increases or decreases in hormone levels in response to relevant stimuli can provide information about the physiological state of an individual that cannot be seen by looking at absolute levels. In general, short-term human hormonal reactivity with respect to infant stimuli in new parents has only been studied in three previous publications (Storey et *al.*, 2000; Stallings et *al.*, 2001; Fleming et *al.*, 2002), and only one of which was conducted at different times throughout the pre and postnatal period (Storey et *al.*, 2000).

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Co-authorship Statement

Under the guidance of my supervisor, Dr. Anne Storey, I was responsible for the design and planning of the research outlined in this thesis. I collected all the blood samples, with the exception of some samples at the prenatal class that were collected in my presence. I attended each prenatal class and home visit along with another researcher. I completed all of the hormonal analysis under the guidance of Dr. Donald McKay. I was responsible for all data analysis and writing all of the chapters. My committee members, Dr. Anne Storey, Dr. Donald McKay, and Dr. Charles Malsbury provided comments on each chapter, which I incorporated before submission of the thesis. Dr. Anne Storey is a co-author on Chapters 2 and 3, as she provided much support, including financial, help with home visits, and doing the initial work in this area.

Chapter 2 – Abstract

Only three previous studies have investigated the hormonal changes that men may experience around the time of birth of a child. In the current study, men and women expecting their first child were recruited from prenatal classes, and later visited at two prenatal and two postnatal times; within two weeks and two months of birth. A control group of married or co-habitating couples that were not expecting a baby and had no children were visited twice, four weeks apart. Researchers visited couples' homes during evening hours and collected finger stick blood samples. Couples completed questionnaires that included ratings of pregnancy symptoms, infant difficulty ratings, and parenting difficulty ratings. Blood spot AutoDELFIA prolactin and radioimmunoassay cortisol levels were quantified for both men and women, and blood spot free testosterone radioimmunoassay levels were measured in men. Men in the pregnant group had significant long-term changes in baseline prolactin levels, with highest levels within two weeks after the birth. Control non-pregnant men did not have any changes in prolactin levels between the two visits. Pregnant women's prolactin levels also changed over time, with highest levels in the two weeks before the birth. No significant baseline changes in free testosterone or cortisol levels were found for men in the pregnant group. Pregnant women had significant cortisol level changes, with highest levels occurring at the last stage before the birth. Cortisol levels were correlated between men and women in the pregnant group, but not for men and women in the control group. When fathers reported parenting to be more difficult than they expected, both they and their partners had higher cortisol levels at the early postnatal stage. When mothers reported parenting to be more

difficult than they expected, they had higher cortisol levels at all stages except the late prenatal, and their partners had higher cortisol levels at the early prenatal and early postnatal stages. Women rating parenting to be more difficult than they expected also had lower prolactin levels at the late postnatal stage than other women. The longitudinal changes in men's baseline prolactin levels, as well as the differences in cortisol levels based upon parenting difficulty ratings, show that the birth of a child can cause significant hormonal changes in men.

Chapter 2 - Baseline hormonal changes in men and women before and after the birth of their first child

2.1: Introduction

Although the hormonal changes that women undergo before, during, and after pregnancy have been well documented (Corter & Fleming, 1995), much less is known of the physiological changes that their male partners experience. To date, only three studies have reported long-term hormonal data for fathers.

The first study to report significant hormonal changes in men before, around, and after the birth of their first child was only recently published (Storey, Walsh, Quinton, Wynne-Edwards, 2000). Using a between-subjects design, researchers collected baseline venipuncture samples from couples at either early or late prenatal, or early or late postnatal dates. Prolactin and cortisol levels were highest in the men and women tested within two weeks before the birth. Testosterone concentrations were lowest in men sampled within three weeks after the birth.

Individuals in the Storey et *al.* (2000) study also reported the pregnancy symptoms they had experienced. Men who reported two or more pregnancy symptoms had higher baseline prolactin levels than men with fewer symptoms. Additionally, serum cortisol concentrations were positively correlated within couples at both prenatal visits and at the late postnatal visit.

Berg & Wynne-Edwards (2001) collected weekly saliva samples from men starting from their partner's first trimester and continued postnatally for three months.

After controlling for time of day and season, fathers had lower morning salivary testosterone levels than control men who were not fathers. There was no difference between groups for the evening samples, nor was there any change in testosterone levels over time. Similarly, cortisol concentrations were higher in control men than in the fathers for morning, but not evening, salivary samples. Salivary cortisol levels in the fathers were highest in the week before birth and lowest in the week after.

Berg & Wynne-Edwards (2002) published the results of a subset from their original population, using 9 of the 45 couples that had the best compliance, continued breastfeeding, and first pregnancies. From the last week before birth to the first week after, fathers had a significant drop in salivary testosterone levels. Though there were no correlations within the couples for testosterone levels, salivary cortisol levels were positively correlated within the couples prenatally.

Though Gray, Kahlenberg, Barrett, Lipson, Ellison (2002) did not measure testosterone levels in men around the time of birth, their work is highly relevant to the study of human paternal care. After controlling for age, they found that married men, both with and without children, had lower evening salivary testosterone levels than unmarried men. There was, however, no difference between the two groups for morning testosterone levels.

In summary, the aforementioned studies have found similar results for cortisol. Cortisol concentrations in serum or saliva are greatest in fathers during the time leading up to the birth (Storey et *al.*, 2000; Berg & Wynne-Edwards 2001 & 2002). A drop in testosterone concentrations around the time of birth has been shown in two studies

(Storey et *al.*, 2000; Berg & Wynne-Edwards, 2002). Prolactin levels increased prenatally in the original study, peaking in the last three weeks before birth (Storey et *al.*, 2000). Less is known about long-term changes in prolactin, and no studies have been published to confirm or refute the original findings of Storey et *al.* (2000).

The current study uses a within-subjects design to measure long-term hormonal changes within individual couples, with a focus on the men before and after the birth of their first child. Correlations in baseline levels of hormones within individual couples are examined, and reliable methods of measuring blood spot samples are established. Based upon the previous work, it is hypothesized that prolactin levels will increase prenatally in men, reaching a peak before birth. Testosterone concentrations are hypothesized to drop postnatally, with this change occurring right after birth.

Cortisol levels are known to increase with stress. Storey et *al.* (2000) used venipuncture to collect blood samples, and found an increase in cortisol concentrations leading up to birth. As the finger stick method may have less of an effect on adrenal responsiveness, the cortisol levels may be lower than those reported in studies that use venipuncture. Additionally, the within-subjects design used here may produce a decrease in the first sample response at each test due to habituation. However, Berg & Wynne-Edwards (2001; 2002) used a non-invasive and repeated saliva sampling technique, and found that salivary cortisol levels were highest in the week before birth and lowest in the week after. So an increase in cortisol levels before the birth for men in the current study is hypothesized.

Additionally, Berg & Wynne-Edwards (2001) found that control men had higher morning salivary cortisol and testosterone levels than fathers. However, samples in the current study are taken late in the evening when cortisol levels did not differ, and so it is reasonable to expect that no difference will be found between the control and 'pregnant' groups of men. Though Gray et *al.* (2002) reported that married men had lower evening testosterone levels, both the experimental and the control groups in the current study contain men that are married or co-habitating, so the difference between married and non-married men cannot be tested. Based upon all the previously mentioned work, men and women are expected to have positively correlated cortisol levels, at least at the prenatal stages.

New mothers have been shown to rate infant odours as more attractive than women who have not had children (Fleming, Corter, Franks, Surbey, Schneider, Steiner, 1993). Additionally, higher levels of cortisol in new mothers has been linked to finding their own baby odours to be more attractive, and to better recognizing odour from their own infants (Fleming, Steiner, Corter, 1997). Women and men in the current study are asked to rate how attractive they find infant odours to be, and these responses are compared with their cortisol levels. Those that rate odour attractiveness the highest are hypothesized to show correspondingly higher cortisol levels.

Reported perception of infant difficulty with respect to the average baby has been shown to be an important factor in transition to parenthood for fathers. When new fathers had partners who were experiencing postpartum depression, the men were more likely to rate their infant as worse than average in terms of difficulty. Those that rated their infant

as worse were also more likely to have reported significant stress, especially at work, and also reported that they had more parental responsibilities (Zelkowitz & Milet, 1997). Based upon this work, it is hypothesized that those fathers who rate their infant as more difficult than average may have higher cortisol levels due to increased stress.

Blood spots are collected in the current study as a minimally invasive method of sampling hormones. The venipuncture procedure traditionally used to obtain serum is invasive, requires a trained technician, and can be perceived as a fearful procedure, thus deterring potential participants from a study. Additionally, the serum is not as robust to long-term storage as are the dried blood spots (Worthman & Stallings, 1997). Saliva sampling is a non-invasive alternative, and saliva can be collected frequently and repeatedly without requiring a technician. Saliva can also be easily stored, and is more robust to long-term storage than serum (Worthman & Stallings, 1997). However, there are a few drawbacks to using saliva sampling, one being that only the free unbound portion of circulating hormones are available, and not the total levels (Worthman & Stallings, 1997). Saliva can be contaminated with blood, which even in small amounts can artificially increase the results of an assay (Worthman & Stallings, 1997). Another consideration is the cotton used to collect saliva, which can skew the results higher or lower than they should be, depending upon the hormone (Shirtcliff, Granger, Schwartz, Curran, 2001). Some studies have reported poor correlations between serum and saliva estradiol (Shirtcliff, Granger, Schwartz, Curran, Booth, Overman, 2000) and testosterone (Shirtcliff, Granger, Likos, 2002), which makes the values obtained hard to relate to studies that have used serum or serum equivalents. Blood spot sampling is used here

because it is minimally invasive, does not require a technician, samples can be stored for longer than for serum, and results have good correlation to serum values (Shirtcliff et *al.*, 2000; Worthman & Stallings, 1994; Shirtcliff et *al.*, 2001; Shirtcliff et *al.*, 2002).

2.2: Methods

2.2.1: Data collection

2.2.1.1: Subjects

Twenty-two couples expecting their first child were recruited from evening prenatal classes occurring at the Women's Health Centre in the Health Sciences Centre, St. John's, Newfoundland. The prenatal instructor allowed the experimenters to give out a written summary, and to talk about the purpose and methods of the experiment to the expectant couples. Couples who wished to participate in the study filled out contact information on a sign-up sheet. Only couples who did not experience any extended periods of separation (i.e. due to work or travel) were included in the study, and to ensure this was the case, couples were asked at each visit if any separation had occurred since the last time they had been surveyed.

The mean age for the pregnant group was 30.6 years for female participants (median: 31 years, range 21-36 years), and 32.1 years for males (median: 33 years, range 20-50 years). All couples participated through to the end of the study, with the exception of one couple who moved outside the study area after the birth of their child. Of the 21 couples who provided postnatal data, all initially breastfed their babies, and at postnatal

month two only four women were no longer breastfeeding. None of the participants had children prior to the current pregnancy.

An additional group of seven non-pregnant control couples was recruited from St. John's, Newfoundland and the immediate surrounding areas. Each couple was either married or co-habitating, and none had ever had children. The mean age of women in this group was 26 years (median: 28 years, range: 20-29 years) and for men the mean was 29 years (median: 29 years, range: 24-36 years).

2.2.1.2: Prenatal class

The initial sampling occurred immediately at the end of a prenatal class, with all of the volunteers tested on one evening in the prenatal classroom. After receiving an explanation of the procedure, couples were asked to sign consent forms approved by the Human Investigation Committee at Memorial University of Newfoundland. Initially, individuals completed a short questionnaire, which asked the baby's due date, whether the expected child was to be their first, and whether any extended period of separation during the pregnancy were anticipated. They also reported which pregnancy symptoms both they and their partners had experienced since the pregnancy began by choosing from a checklist that included anxiety, depression, weight gain, weight loss, irritability, nausea, fatigue, indigestion, happiness, increase in appetite, decrease in appetite, and muscle aches.

Capillary blood spot samples were then obtained, first from the woman, then from the man. Blood was collected by pricking a finger with a single-use sterile capillary

blood sampling device (Vacutainer blue Genie Lancet, Becton-Dickinson catalogue number 366582) wiping the first bit of exudate with cotton gauze, and allowing the drop to reach maximal size (~50μL) before letting it naturally drop onto circles pre-marked on a standardized paper blood collection card (Sigma Diagnostics Inc., catalogue number 160-C). The card was dried overnight at room temperature and then placed inside individual plastic bags and frozen at -20 °C in a container with desiccant. Immediately after the blood sample had been obtained, the couples rated on a five-point scale how stressful they perceived the finger stick to be, with 1 being 'no anxiety' about the procedure, and 5 being 'very high anxiety'.

2.2.1.3: Home visits

Two researchers conducted a late prenatal and two postnatal visits for all couples in the participant's homes. As some couples were recruited at 'early-bird' prenatal classes, and others at later stages of pregnancy, results for the early prenatal visit (two months before the time of birth) include only 8 of the 22 couples.

For these eight couples with results in the early prenatal stage, data were obtained in one of two ways. Four of the couples were recruited at the early stages of pregnancy, and home visits were scheduled for around 8 weeks before the due date. Upon the arrival of a pair of researchers, the consent forms were first signed by both participants. Then a single finger stick blood sample was taken from first the woman and then the man, after which they filled out the checklist of pregnancy symptoms for themselves and their partners, as well as a rating of how stressful the finger stick was for each person. Four

other couples were sampled at the prenatal class at around 8 weeks prior to the birth of their baby, so the finger sticks, pregnancy symptom checklists, and stress ratings from this initial period were used as an early prenatal sample. For all eight couples combined at this stage, the samples were obtained an average of 62 days before birth (median: 59, range of 56-72).

The late prenatal visit was scheduled for sometime within two weeks prior to the woman's due date. Five couples had their babies early and no data were obtained from them for this stage of pregnancy. Three couples had their babies later than expected, and though visits were conducted two weeks before the due date, the samples that were obtained were at an average of three weeks prior to the birth. Subsequently, these were excluded from analysis for the late prenatal stage. For the remaining fourteen couples in this stage, home visits were conducted an average of 8.23 days prior to birth of the infant.

Efforts were made to schedule early postnatal visits at each couple's home for within two weeks after the baby was born. Sometimes the new mother was not feeling well enough for experimenters to visit so soon after the birth, and these visits occurred a few days later than planned. The mean number of days after the birth for the early postnatal home visit was 16 days (n = 21, median: 15, range of 13-22 days).

Late postnatal visits were scheduled for two months after birth. The mean number of days from birth for the late postnatal home visits was 61 days (n = 21, median: 59, range of 49-106 days). One couple left town after the first postnatal visit, and did not return until postnatal week 15. The late postnatal visit was still carried out at this time,

and results were included in the analysis. The range of days from birth for the late postnatal visit is 49-70 days for all others excluding this latter couple.

At each of the home visits, the same procedure was used to obtain the baseline sample to study the long-term hormonal changes. A further procedure was carried out and a second sample was then obtained in order to measure short-term hormonal reactivity, which is discussed in the next chapter.

All home visits were conducted between 1600 h and 2000 h, and efforts were made to schedule individual couples for the same time at each testing stage. As diurnal variations occur for each of the three hormones of interest, sampling occurred at the same time of day to minimize potential confounding effects of time. Cortisol levels reach a daily peak before waking in the morning, and gradually decline to lowest levels in the evening (Kanaley, Weltman, Pieper, Weltman, Hartman, 2001). Prolactin levels reach their maximum during sleep and decline when awake (Franks, 1979). Testosterone levels in men reach their peak in the early morning (Miyatake, Morimoto, Oishi, Hanasaka, Sugita, Iijima, Teshima, Hishikawa, Yamamura, 1980). As all three hormones peak near early morning hours and are not rapidly changing during the evening hours, the time chosen to sample the participants is advantageous in further minimizing extraneous variation.

For each visit, a pair of researchers arrived together at the couple's homes. The consent forms were signed, and the baseline finger stick blood samples were taken, always in the same order: first from the woman and then from the man. After the short-term test and second sample were done, couples completed different questionnaires,

which varied by stage. For the late prenatal visit, they filled out the checklist of pregnancy symptoms for both themselves and their partners, as well as the 5-point anxiety scale rating of the finger stick procedure. They each filled out questionnaires that asked about their expectations of functional adjustment to having a newborn (how much of the household chores, baby care, etc. they expect to perform). The questionnaires also asked about how the couples felt about becoming parents, and how the pregnancy has influenced conversation and thought. The couples were also asked to rate their previous experience with infants, changes in the quality of the relationship with their partner, and if any periods of separation were expected or had occurred.

At the early postnatal home visit, there was no questionnaire. For the late postnatal questionnaire, each person rated themselves and their partners on the amount of time they spent in childcare duties, play, and household chores, if the adjustment was more or less difficult than expected, and if the quality of the couple's relationship had changed (become better or worse). They also rated their responsiveness to infants since the birth. Couples were asked whether the mother was breastfeeding, and whether the father had the opportunity to bottle-feed, and, if so, how often. Finally, they again rated how stressful the finger-stick sampling was perceived to be.

For the control non-pregnant group, two home visits were conducted approximately one month apart. These served as a control for the late prenatal and early postnatal visits to the pregnant group, which was where the significant hormonal changes had been seen previously (Storey *et al.*, 2000). Both visits were conducted at the same time of the evening, as with the experimental group, in order to control for diurnal

variation in hormone levels. At each visit, consent forms were signed, and finger stick blood samples were taken from the woman and then the man. As with the pregnant group, a second sample was obtained in order to test short-term hormonal reactivity (to be discussed in Chapter 3). After this second sample, couples were asked whether they had experienced in the past three months any of the same checklist of pregnancy symptoms as had been completed by the pregnant couples.

2.2.2: Laboratory methods

For measurement of hormones, blood spot samples as an alternative method to serum sampling has been used. No commercial kits, however, are available for cortisol, free testosterone, or prolactin blood spot measurement. Other researchers have modified the serum assay kits to measure blood spot samples (prolactin: Worthman & Stallings, 1997; Stallings, Worthman, Panter-Brick, Coates, 1996; Bassett, Gross, Eastman, 1986; total testosterone: Shirtcliff, Reavis, Overman, Granger, 2001; Worthman & Stallings, 1997; cortisol: Kraiem, Kahana, Elias, Ghersin, Sheinfeld, 1980; Worthman & Stallings, 1997, LH and FSH: Worthman & Stallings, 1994).

All samples were assayed in duplicate and the two resultant values were averaged. In order to eliminate inter-assay variation in quantifying the results for individual couple's samples, all initial samples for each couple were assayed in the same run. To monitor inter-assay variation, the same set of controls were measured for each hormone assayed (low and high volunteered samples for prolactin; low, medium, and high commercially-available controls for cortisol and testosterone). For each of the three

hormones, pilot work was conducted using a separate population of volunteer's blood samples assayed for serum and blood spot values in order to determine the linear regression formula for later conversions.

2.2.2.1: Prolactin analysis

Samples were assayed using a time-resolved fluoroimmunoassay AutoDELFIA™ Prolactin kit (Perkin Elmer Wallac Canada, catalogue number BO18-301). Prolactin assays were performed at the Hormone Assay laboratory in the Health Science Centre in St. John's, Newfoundland. An AutoDELFIA™ machine with a software protocol customized for this study was used to measure blood spot prolactin concentrations (Perkin Elmer Wallac Canada). The same low and high control samples from two volunteers were used in each assay to monitor inter-assay variability.

Prior to analysis of blood spot samples from the study participants, initial work was carried out to establish the linear relationship between traditionally reported serum prolactin levels and the blood-spot values. A separate subset of eleven subjects (n = 5 males, n = 6 females) voluntarily gave both venipuncture (Becton Dickinson, Vacutainer 5-mL yellow-top with SST gel and clot activator) and finger-stick samples, which were taken near simultaneously. Blood spots were analyzed following the procedure below, and serum prolactin levels were measured in duplicate following the normal procedure outlined by the kit manufacturer.

Values for both serum (SE) and blood spot (BS) prolactin for this subset of volunteers were highly correlated in a linear regression ($R^2 = 0.977$), which produced a

predictive equation to convert blood spot to serum, $SE_{PRL} = 0.1928 (BS_{PRL}) - 1.2835$ (Fig.2.1). All subsequent samples were measured using the blood spot method described below, and this regression equation was employed to convert prolactin blood spot results to the more traditionally used serum values reported hereafter.

Blood spot prolactin levels were measured using the following modification of the same kit. In preparation for the assay, 50 μL of kit standards (consisting of 0, 0.25, 2.5, 25, 125, and 250 ng/mL) were pipetted onto standardized blood collection cards and allowed to dry overnight. Disks measuring 1/8th inch in diameter were cut in duplicate for both the standards and blood samples. Single disks were placed in the appropriate anti-prolactin microtitration wells along with 200 μL of assay buffer. The plate was placed in the AutoDELFIA machine, which carried out the subsequent steps automatically. The contents of the wells were shaken, and then the plate was incubated overnight at 4° C. Following this, the well contents were further shaken for one hour at room temperature, the disks were aspirated from solution, and the wells were washed twice with wash solution. 200 μL of anti-prolactin europium tracer (diluted 1:75) was added, wells were incubated a further 90 minutes, and then washed six times. 200 μL of enhancement solution were added to the wells, which were shaken for 5 minutes, and fluorescence was measured. From this, a standard curve was produced and prolactin concentrations of the unknown samples were automatically determined using the custom software.

For the prolactin blood spot assay, the intra-assay coefficient of variation was 8.4% for the high control and 6.4% for the low control. The inter-assay coefficient of

variation was 8.8% for the high control and 7.0% for the low control. In cases where the coefficient of variation between the two samples exceeded 10%, a third sample was re-assayed and the closest two of the three were then averaged. This was the case for 25% of the original samples. The range of prolactin converted serum equivalent values for women was 4.82 ng/mL to 316.7 ng/mL, and for men the range was 2.01 ng/mL to 28.76 ng/mL.

2.2.2.2: Free testosterone analysis

Free testosterone radioimmunoassays were performed on male samples in duplicate at the Division of Basic Medical Sciences, Memorial University of Newfoundland. Low, medium, and high multivalent controls (CON6 Mulitvalent Control Module, DPC) were included in each free testosterone assay to measure inter-assay variation.

The relationship between serum and blood spot free testosterone values were initially established by obtaining serum (Vacutainer 5-mL yellow-top with SST gel and clot activator, Becton Dickinson) and whole blood (Vacutainer 5-mL green top with sodium heparin) through venipuncture from 14 volunteers (n = 10 men, n = 4 women). An additional 10 samples were obtained artificially by adding exogenous testosterone (Sigma, catalogue number T-1500) to a pool of whole blood obtained from one male to ensure the upper end of the range was represented.

The whole blood was gently swirled in a disposable Petri dish to ensure the red blood cells didn't settle, while 50μL samples were pipetted onto blood collection cards

and dried overnight. The serum was assayed in duplicate for free testosterone using a coated tube radioimmunoassay kit (Coat-A-Count Free Testosterone RIA, DPC), employing the normal method outlined in the kit instructions. Blood spots were assayed for free testosterone using the method later described below.

The values obtained for both serum and blood spot free testosterone were highly correlated in a linear regression ($R^2 = 0.88$), which allowed for conversion of blood spot free testosterone results to serum using the formula $SE_T = 0.5218\ (BS_T) - 1.4341$ (Fig.2.2). All free testosterone samples for this study were measured using the blood spot method, and values were converted to and reported as serum.

Blood spot free testosterone values were obtained using the following modification of the same kit. First, 25 μL of the liquid standards which are supplied with the kits were pipetted onto the blood collection cards and dried overnight. Using a manual hole puncher (NNHP8, DPC) disks measuring 1/8th-inch in diameter were cut in duplicate for both the standard and control samples. Two disks were added to the appropriate testosterone coated assay tubes along with 1.0 mL of ^{125}I-labelled testosterone solution. These were covered, gently swirled using a multi-tube vortexer (SMI Multi-Tube Vortexer), and incubated at 27 °C overnight for 18 hours. About halfway through the incubation period, when the blood had been thoroughly soaked from the collection cards, tubes were again gently swirled. The contents of each assay tube, except for the total count tubes, were decanted and discarded. The edge of each tube was blotted against absorbent gauze to remove residual liquid, and then left to drain in an inverted position for approximately 30 minutes. Finally, the edge of each tube was again

blotted on gauze before being counted for one minute in an automatic gamma counter (LKB Wallac Gammamaster 1277). A standard curve was produced, and based on this curve free testosterone concentrations were automatically calculated using a spline function algorithm (RiaCalc Software).

The lower limit of sensitivity for the free testosterone blood spot assay was 0.4 pg/mL, calculated by taking two times the standard deviation from the mean counts per minute of 20 replicates of the zero standard. Recovery, reported as a linear regression between the concentration of testosterone added versus the concentration measured was $R^2 = 0.992$ ($Y_{Measured} = 0.4833\ X_{Added} + 13.741$). Additivity, reported as a linear regression between the concentration measured and number of disks added was $R^2 = 0.993$, ($Y_{Concentration} = 23.852\ X_{\#Disks} - 3.9077$). Intra-assay variation was 8.17%, and inter-assay variation was 12.5% for the low control, 17.9% for the medium control, and 10.6% for the high control. The values were very close for individual duplicates, with only 15% of samples having duplicates over 15% CV. As the quantity of blood samples was limited and duplicates were closer in value than for the other two assays, none were re-assayed for free testosterone in order to have enough blood spots to repeat the analyses for cortisol and prolactin, if necessary. The range of free testosterone converted serum equivalent values for men was 4.07 pg/mL to 31.05 pg/mL.

2.2.2.3: Cortisol analysis

Cortisol assays were performed in duplicate at the Division of Basic Medical Sciences, Memorial University of Newfoundland, with all samples for each couple again

included in the same run. Low, medium, and high multivalent controls (CON6 Mulitvalent Control Module, DPC) were also included in each cortisol assay to monitor inter-assay variation.

As with the other hormones, a regression formula was obtained by assaying volunteers blood samples (n = 4 men, n = 9 women) for both serum and blood spot cortisol levels. The serum cortisol radioimmunoassay was performed following the instructions outlined in the kit (Coat-A-Count Cortisol RIA, DPC), and the blood spot assay used a modification of this kit, described in detail later. The serum and blood spot cortisol values for these volunteers were highly correlated in a linear regression (R^2 = 0.92), which produced a predictive equation to convert blood spot to serum, SE_{CORT} = 0.0302 (BS_{CORT}) (Fig.2.3). The trend line for this conversion had the y-intercept set at zero to ensure the very low evening cortisol values would be included; otherwise these low values would convert to negative numbers.

In preparation for the blood spot assay, the standards supplied with the kit were pipetted (25 μL) onto the blood collection cards and dried overnight. From these standard cards, one 1/8th inch diameter disk was punched per assay tube for the standard and non-specific binding tubes. From the unknown and control cards, a approximately $7/16^{th}$ inch diameter (exactly 0.1503 $inches^2$ in area) filter paper disk was cut using a custom-made punch fashioned from hollowed out and sharpened high carbon steel that was pressed against a Plexiglas cutting surface. Each filter paper disk was cut exactly in half using a custom-made jig and a utility knife, and both halves were used to obtain duplicate samples. Before adding the halves to the appropriate assay tubes, they were

again cut into four smaller pieces in order to enable better soaking and thus provide closer duplicate values (data not shown). Finally, 1.0 mL of ^{125}I-labelled cortisol was added to each assay tube, after which the incubation, decanting, and counting was as outlined above for the free testosterone assay.

The lower limit of sensitivity for the cortisol blood spots assayed using this method was 3.7μg/dL. Recovery, reported as a linear regression between the concentration of cortisol added versus the concentration measured was $R^2 = 0.999$ ($Y_{Measured} = 21.346\ X_{Added} + 528.64$). Additivity, reported as a linear regression between the concentration measured and number of quarter 7/16th inch disks added was $R^2 = 0.912$, ($Y_{Concentration} = 155.32\ X_{\#Disks} - 99.458$). Intra-assay variation was 3.9%. Where the maximum error for duplicates exceeded 5%, a single sample was re-assayed and the closest two of the three were averaged to obtain a more reliable result. In some cases there was not enough blood to re-assay the sample, and no third value was obtained. The maximum error exceeded 5% for 93 of the 336 cortisol samples assayed (28%), 95% of which were re-assayed for the third sample (88 out of the 93).

One problem emerged with the cortisol assay that did not occur using the same method for free testosterone. Each assay produced cortisol values in very different ranges, as evidenced by the high inter-assay variations (70%CV for the high control and 48%CV for the low control). Though the free testosterone assay also used a coated-tube method, there are two possible reasons why the extreme variation seen between each cortisol assay may be related to this. First, compared to the free testosterone assay, more than 12 times the amount of filter paper was necessary for each cortisol assay tube

(0.1520 inches^2 in area for a cortisol assay, versus 0.01227 inches^2 in area for a free testosterone assay). Second, the semi-circles for the cortisol assay were cut in four pie-shaped pieces, as opposed to the smaller round disks used in the free testosterone assay. Both factors may have contributed to possible 'scouring' of the antibody coating on the walls of the cortisol assay tubes. This did not occur uniformly, however, which may be because of extreme sensitivity to slight variations in vortexing time or speed, or to some other unknown factor.

As there was so much variation between assays, it was necessary to normalize the data, using the multivalent controls as a mathematical 'yardstick'. The published serum values for the low, medium, and high controls were 4, 11.8, and 28 μg/dL, respectively. Accordingly, blood spot results for these controls in each of the five assays were individually correlated (Fig. 2.4), producing a regression equation and a R^2 value to convert blood spots to serum for each assay (Table 2.1).

One further problem was encountered with the cortisol assay method, namely the size of the disks used. The 7/16th inch puncher was used because the samples obtained from participants were always taken at the later hours of the evening, when cortisol levels are at their lowest for the whole day. Thus, many of the samples yielded undetectable results when assayed using smaller diameter disks in pilot work. The larger disk was required in order to have high enough levels of cortisol in solution to produce values within the range of the standard curve. This compensation worked well for the majority of samples, but some droplets of dried blood on the filter paper disks were not of sufficient diameter to be assayed using the normal method. As males generally have

higher hematocrit values, and thus their blood droplets diffuse less once dropped on the filter paper, this was more of a problem for the men in the study. Additionally, some men also tended to have more calloused skin, making it harder to prick with the lancets. This also caused the droplets of blood to be smaller in diameter than those of most women.

For those blood samples with smaller than 7/16th inch diameters, smaller disks were cut and the values obtained from the assay were converted to serum using the multivalent controls in the same manner as for the primary method. As pilot work had been done on different sized disks, information was available to aid in converting the blood spot values to serum.

One 5/16th inch disk, though equivalent in area to the semi-circles described earlier (which are half of a 7/16th inch disk), resulted in only half the concentration of a semi-circle when assayed. Therefore, the results for these 5/16th inch disks were doubled to obtain the equivalent value for a semi-circle. Six and a quarter 1/8th inch disks are roughly equal in area to both the semi-circle and the 5/16th inch disk. Again, the concentrations measured in assay were not proportional to this, with a semi-circle resulting in 1.46 times the concentration of six and a quarter 1/8th inch disks. Therefore, the results of the 1/8th inch assays were multiplied by this factor to obtain the equivalent value for a semi-circle.

Once both these sizes were converted to the appropriate semi-circle equivalent, these values were then converted using the original blood spot to serum formula (Fig.2.3). The main method of 7/16th inch halves was used to assay 72.8% of the 423 total blood spot samples (including the third values for samples that required re-

assaying), while the 5/16th inch method was used on 22.9%, and the 1/8th inch method was used on only 4.3%. After all of the blood spot cortisol values were converted in one of these three ways, the range of serum equivalent values for men was 0.78 μg/dL to 18.44 μg/dL, and for women the range was 0.88 μg/dL to 36.08 μg/dL.

2.2.3: Statistical methods

Repeated measures ANOVAs were performed on baseline prolactin, testosterone, and cortisol levels for the men, and baseline prolactin and cortisol levels for the women using SPSS 10.0 for Windows. All further tests were carried out on SPSS 11.0 for Windows. Post-hoc analyses of change from one stage to the next were done using paired t-tests. The relationship of baseline hormone levels between the men and women, as well as within individuals, was investigated using Pearson two-tailed bivariate correlations. The hormone levels for experimental and control groups were compared (late prenatal to first control visit, early postnatal to second control visit) using one-way ANOVAs. The change in hormone levels from one control visit to the next was tested with two-tailed paired t-tests. The self-reported pregnancy symptoms for the experimental and control groups were compared with one-way ANOVAs. The 'couvade' versus 'non-couvade' comparison was done using two-tailed independent t-tests. Infant difficulty and parenting difficulty ratings, as well as responses to the baby cries tape were evaluated using one-way ANOVAs. Correlations between the self-reported anxiety levels in response to the finger stick procedure and the hormone baseline levels were

done using Spearman bivariate correlations. All tests were computed with a critical value of alpha set at 0.05.

2.3: Results

Though there were 21 couples involved in the study, data for all four stages through the pre and postnatal period were obtained for only five of them. This was mainly due to the lack of subjects in the early prenatal group ($n = 8$) and three of these couples either missed the late prenatal visit due to an early birth, or had their data for that visit excluded because its timing was too early once they had a late birth. For any cases when data for all couples were included instead of just these five couples, it is stated in that specific analysis.

2.3.1: Prolactin

A repeated-measures ANOVA showed that the men had significant overall changes in baseline prolactin levels through the four stages measured ($F(3,12) = 5.677$, $p = 0.012$, Fig.2.5), as did the women ($F(3,12) = 16.782$, $p = 0.000$, Fig.2.6).

For men, post-hoc paired t-tests showed that prolactin levels (Fig.2.5) in the early prenatal stage were lower than both postnatal stages (early postnatal: $t(4) = -3.870$, $p = 0.009$; late postnatal: $t(4) = -2.498$, $p = 0.034$), and also showed a non-significant trend towards being lower than the late prenatal stage ($t(4) = -1.890$, $p = 0.066$). Men's prolactin levels in the late prenatal stage were significantly lower than the early postnatal stage ($t(4) = -2.148$, $p = 0.049$), but not the late postnatal ($t(4) = -0.710$, $p = 0.517$), and

there was no difference in men's prolactin levels between the two postnatal stages ($t(4) = 1.427$, $p = 0.227$).

Women's prolactin levels (Fig.2.6) at the early prenatal stage differed from both postnatal stages (early postnatal $t(4) = 2.128$, $p = 0.05$; late postnatal $t(4) = 4.930$, $p = 0.008$), but was not significantly different from the late prenatal stage ($t(4) = -1.458$, $p = 0.110$). The late prenatal prolactin levels were higher than both postnatal stages (early postnatal: $t(4) = 4.391$, $p = 0.006$; late postnatal: $t(4) = 5.886$, $p = 0.002$), and there were significant decrease in prolactin levels between the two postnatal stages for women ($t(4) = 2.809$, $p = 0.024$).

A 2 (repeated factor: late prenatal to early postnatal prolactin levels) by 2 (between factor: experimental or control group) ANOVA was carried out for the women and the men. There was a significant difference between the women's experimental and control groups ($F(1,20) = 40.05$, $p = 0.000$). There was, however, no significant effect of women's change in prolactin levels from the prenatal to the postnatal period ($F(1,20) = 0.33$, $p = 0.572$), and no significant interaction between group and women's prolactin levels ($F(1,20) = 0.79$, $p = 0.385$). There was also no difference between men's experimental and control groups ($F(1,21) = 0.37$, $p = 0.548$), no difference in men's change in prolactin levels from the prenatal to the postnatal period ($F(1,21) = 0.04$, $p = 0.848$), and no significant interaction between group and men's prolactin levels ($F(1,21) = 0.50$, $p = 0.487$).

The pregnant women had significantly higher prolactin levels at the late prenatal visit than the control group of women did at their first visit ($F(1,19) = 79.517$, $p = 0.000$,

mean prolactin for pregnant women: 147.08 ng/mL, SE = 10.12, n = 14, mean prolactin for control women at first visit: 16.84 ng/mL, SE = 2.64, n = 7). The new mothers also had higher prolactin levels at the early postnatal visit than the control group of women at the second visit ($F(1,25) = 16.447$, $p = 0.000$, mean prolactin level for new mothers: 118.42 ng/mL, SE = 14.03, n = 20, mean prolactin level for control women at second visit: 20.84 ng/mL, SE = 2.65, n = 7).

Men in the late prenatal pregnant group did not have any significant differences in prolactin levels from control men at the first visit ($F = 0.983$, $p = 0.332$, mean prolactin for expecting men: 9.11 ng/mL, n = 18, mean prolactin for control men: 11.18 ng/mL, n = 7). New fathers in the early postnatal pregnant group did not have any differences in prolactin levels than control men at the second visit ($F = 0.016$, $p = 0.901$, mean prolactin for new fathers: 10.57 ng/mL, n = 21, mean prolactin for control men: 10.83 ng/mL, n = 7).

2.3.2: Free testosterone

No statistically significant change was found for men's baseline free testosterone levels across the four stages (Fig.2.7; $F(3,12) = 0.620$, $p = 0.615$). There was no change over time for men in the control group (paired $t(6) = -0.053$, $p = 0.959$, mean testosterone for visit 1: 15.14 pg/mL, SE = 2.59 pg/mL, mean testosterone for visit 2: 15.28 pg/mL, SE = 3.66 pg/mL, n = 7).

A 2 (repeated factor: late prenatal to early postnatal free testosterone levels) by 2 (between factor: experimental or control group) ANOVA was carried out for the men.

There was no difference between men's experimental and control groups ($F(1,21) = 0.3.39$, $p = 0.080$), no difference in men's change in free testosterone levels from the prenatal to the postnatal period ($F(1,21) = 0.06$, $p = 0.802$), and no significant interaction between group and men's free testosterone levels ($F(1,21) = 0.16$, $p = 0.695$).

There was no difference in testosterone levels between 'expectant' fathers in the late prenatal visit and men in the first control visit ($F(1,20) = 2.609$, $p = 0.122$, mean testosterone for 'expectant' fathers: 11.45 pg/mL, SE = 0.81, n = 15, mean testosterone for control men at first visit: 15.14 pg/mL, SE = 2.59, n = 7). There was a statistically non-significant trend towards control men in the second visit having higher baseline testosterone levels than the new fathers in the early postnatal visit ($F(1,26) = 3.477$, $p = 0.074$, mean testosterone for new fathers: 10.68 pg/mL, SE = 0.80, n = 21, mean testosterone for control men at second visit: 15.28 pg/mL, SE = 3.66, n = 7).

2.3.3: Cortisol

Men did not show significant changes in cortisol levels through their partners' pregnancies and postnatal periods ($F(3,12) = 1.109$, $p = 0.384$, Fig.2.8). Women's cortisol levels changed during and after their pregnancies, as had been previously documented ($F(3,12) = 12.148$, $p = 0.001$, Fig.2.9). Post-hoc paired t-tests on the difference between stages showed an decrease from the late prenatal to early postnatal stage for women ($t(12) = 4.240$, $p = 0.001$), and a further decrease in women's cortisol between the early postnatal stage to the late postnatal stage ($t(19) = 2.878$, $p = 0.010$). Women's early prenatal cortisol levels were higher than levels at both postnatal stages

(early postnatal stage: $t(7) = 3.776$, $p = 0.007$, late postnatal stage: $t(7) = 7.351$, $p = 0.000$), and their late prenatal cortisol levels were higher than those at the late postnatal visit ($t(13) = 5.759$, $p = 0.000$).

A 2 (repeated factor: late prenatal to early postnatal cortisol levels) by 2 (between factor: experimental or control group) ANOVA was carried out for the men and the women. There was a significant difference in the women's cortisol levels from the prenatal to the postnatal period ($F(1,18) = 9.874$, $p = 0.006$), and women in the experimental group had higher cortisol levels than women in the control group ($F(1,18) = 8.216$, $p = 0.010$). Men's cortisol levels did not differ from the prenatal to the postnatal period ($F(1,20) = 0.746$, $p = 0.398$), and there was no difference between cortisol levels for men in the pregnant and control groups ($F(1,20) = 0.005$, $p = 0.947$).

Pregnant women at the late prenatal stage had significantly higher cortisol levels than the control women at the first visit ($F(1,20) = 11.027$, $p = 0.003$, mean cortisol for pregnant women: 36.53 μg/dL, SE = 4.34, n = 15, mean cortisol for control women at first visit: 14.64 μg/dL, SE = 2.12, n = 7). New mothers at the early postnatal stage did not have any significant differences in cortisol levels than the control women at the second visit ($F(1,25) = 0.655$, $p = 0.426$, mean cortisol for pregnant women: 15.31 μg/dL, SE = 0.1.10, n = 20, mean cortisol for control women at second visit: 13.61 μg/dL, SE = 1.65, n = 7). There was no difference over time for the control women (paired $t(6) = 0.474$, $p = 0.652$, mean cortisol for women at visit one: 14.64 μg/dL, SE = 2.12, visit two: 13.61 μg/dL, SE = 1.65, n = 7).

There were no differences in cortisol levels between men for the late prenatal group and control men at the first visit ($F(1,21) = 0.000$, $p = 0.988$), and no difference in cortisol levels between men for the early postnatal group and control men at the second visit ($F(1,26) = 0.061$, $p = 0.806$). Cortisol levels did not differ over time for men in the control group (paired $t(6) = 0.775$, $p = 0.468$, mean cortisol for men at visit one: 13.45 μg/dL, SE = 2.79, visit two: 12.61 μg/dL, SE = 3.03, n = 7).

2.3.4: Within-couple correlations

Correlations of baseline hormones within couples were determined for each stage of testing. These analyses used all couples with data at each stage, and not just the five couples used in the repeated measures analysis.

The only statistically significant correlations among the level of any of the three male hormones and any of the two female hormones were for cortisol. Cortisol levels between couples were correlated at all of the four stages (early prenatal: $R^2 = 0.966$, $p = 0.000$, late prenatal: $R^2 = 0.536$, $p = 0.039$, early postnatal: $R^2 = 0.802$, $p = 0.000$, late postnatal: $R^2 = 0.447$, $p = 0.042$).

In contrast, the cortisol levels for the control group men and women were not correlated at either visit (visit one: $R^2 = 0.487$, $p = 0.268$, visit two: $R^2 = 0.120$, $p = 0.797$).

The correlations for the pregnant group may be artefact of the problem encountered with the cortisol assay. Since there was such high inter-assay variability, and all the samples for one couple were assayed in the same run, the results for individual

couples would be in the same range (i.e., all either higher or lower) because of the assay itself and not because a true correlation exists. However, the cortisol data were normalized based upon the same controls used in each assay, and the lack of a correlation for the control couples supports the idea that a real relationship may exist. Caution must be used, however, when interpreting these results due to the assay difficulties.

There was no statistically significant relationship between the self-reported anxiety levels in response to the finger stick procedure and the cortisol baseline levels for either men or women in any group or at any stage measured.

2.3.5: Pregnancy symptoms in men and women

Storey et *al.* (2000) classified men who reported two or more pregnancy symptoms for themselves as having 'couvade' syndrome. In the current study, 76% of men at the late prenatal stage self-reported two or more of these symptoms (13 of the 17 men with the appropriate timing for late prenatal visit). Furthermore, 53% of the men at this stage reported three or more pregnancy symptoms (9 of the 17 men). As there are insufficient numbers of 'non-couvade' men ($n = 4$) to compare groups using the two-symptom criteria, a report of three symptoms were used as a cut-off point instead. The 'couvade' men ($n = 9$) were not significantly different than the 'non-couvade' men ($n = 8$) in baseline prolactin ($t(15) = -1.483$, $p = 0.159$), cortisol ($t(15) = 0.520$, $p = 0.613$), or testosterone levels ($t(15) = 0.488$, $p = 0.633$), nor for their partner's baseline prolactin ($t(15) = -1.429$, $p = 0.174$) or cortisol levels ($t(15) = 1.081$, $p = 0.301$).

The number of symptoms that men in the experimental group reported at the late prenatal visit was not different from the number that control men at the first visit reported ($F(1,22) = 0.381$, $p = 0.544$; mean number of symptoms for control men: 3.43, SE = 0.95, mean number of symptoms for 'expecting' men: 2.88, SE = 0.42). Similarly, the two groups of women did not report a significant differences in the number of their partner's pregnancy symptoms ($F(1,22) = 0.908$, $p = 0.351$; mean number of symptoms as reported by control men's partners: 2.71, SE = 0.84, reported by experimental men's partners: 3.76, SE = 0.62).

As expected, pregnant women at the last prenatal stage ($n = 17$) reported a significantly greater number of pregnancy symptoms than the control women ($F(1,22) = 28.622$, $p = 0.000$; mean number of symptoms for pregnant women: 7.71, SE = 0.55, mean number of symptoms for control women: 2.71, SE = 0.52). Additionally, 'expecting' men reported more pregnancy symptoms for their pregnant partners than control men ($F(1,22) = 13.086$, $p = 0.002$; mean number of symptoms as reported by experimental men for their partners: 6.94, SE = 0.48, mean number reported by control men for their partners: 3.57, SE = 0.87). The number of pregnancy symptoms reported by women for themselves was positively correlated with the number reported for them by their partners ($R^2 = 0.495$, $p = 0.036$). There was no correlation between the number of symptoms that men self-reported and the number that their partner's reported for them ($R^2 = 0.050$, $p = 0.433$), nor between the number self-reported for both men and women ($R^2 = 0.261$, $p = 0.184$), with the women reporting a higher number of symptoms for both themselves and for their partners than the men.

2.3.6: Self-reported questionnaire ratings

The 21 couples tested at the late postnatal stage rated how attractive they found baby smells to be since the birth of their own child, ranging from 'much more attractive', 'a little more attractive', or 'not really'. The baseline hormone levels for individuals who found baby smells to be 'much more' attractive were compared against those reporting 'a little more' or 'not really' using one-way ANOVAs. There were no differences for any hormone levels, at any of the four stages, for either the mothers or the fathers.

At the late postnatal visit, parents were also asked to rate their infant difficulty in terms of other babies they had known, choosing from 'easier', 'average', or 'more difficult'. Again, the baseline hormone levels of parents reporting their baby to be more difficult were compared against those of parents who reported their newborn to be 'average' or 'easier' combined together. Only three fathers rated their infants as 'more difficult' than other babies, and at the late postnatal visit they had higher prolactin levels ($F(1,19) = 9.630$, $p = 0.006$, mean prolactin for 'infant difficult' fathers: 17.20 ng/mL, SE = 2.74, mean prolactin for 'infant not difficult' fathers: 9.69 ng/mL, SE = 0.89) and higher testosterone levels than other fathers ($F(1,19) = 17.027$, $p = 0.001$, mean testosterone for 'infant difficult' fathers: 16.68 pg/mL, SE = 0.99, mean for 'infant not difficult' fathers: 10.20 pg/mL, SE = 0.61). Only one woman out of the 21 sampled at the late postnatal stage rated their own infant as more difficult than others, so no parallel comparison was made for the women.

As some parents rated their infant's difficulty levels differently than their partner did, further tests were conducted to investigate whether hormone levels were related to a

differential perception of the infant. When the father rated his own infant to be more difficult than the mother did at the late postnatal visit (n = 4 versus n = 13 couples reporting the same rating), there were no differences in any hormone levels for either parent. There was also no difference in hormone levels for either parent at any stage when the father rated his own infant to be less difficult than the mother did (n = 4 versus n = 13 couples reporting the same rating).

At the late postnatal visit, parents were also asked to rate on a 5-point scale how difficult they were finding parenting to be, ranging from 'much less' and 'somewhat less' to 'what I expected' to 'somewhat more' and 'much more'. The baseline hormone levels for those who responded 'somewhat' or 'more difficult' were collectively compared in a one-way ANOVA against those who responded 'much less', 'somewhat less', or 'what I expected' combined.

When the fathers reported parenting to be more difficult than expected at this late postnatal visit, both they and their partners had higher cortisol levels at the early postnatal stage (Table 2.2). There was also a statistically non-significant trend for fathers who reported parenting to be more difficult to have higher testosterone at the first postnatal visit ($F(1,19) = 3.340$, $p = 0.083$, mean testosterone for 'difficult' group: 13.53 pg/mL, SE = 2.12, n = 4, mean testosterone for 'not difficult' group: 10.02 pg/mL, SE = 0.80, n = 17), and to have higher prolactin levels at the late postnatal visit ($F(1,19) = 3.250$, $p = 0.087$, mean prolactin for 'difficult' group: 14.34 ng/mL, SE = 2.78, n = 4, mean prolactin for 'not difficult' group: 9.93 ng/mL, SE = 1.01, n = 17).

When the same comparison of parenting difficulty was made using the mother's self-rating, women's cortisol levels were found to be higher at all stages (Table 2.2 and Figure 10), except at the late prenatal, where it approached significance ($F(1,12) = 3.569$, $p = 0.083$, mean cortisol for 'difficult' group: 19.76 μg/dL, SE = 3.98, n = 7, mean cortisol for 'not difficult' group: 10.63 μg/dL, SE = 2.74, n = 7). The partners of women who reported difficulty with parenting had higher cortisol levels at the early prenatal and early postnatal stages (Table 2.2). Additionally, women who rated parenting to be more difficult also had lower prolactin levels at the late postnatal stage than the other women (Table 2.2).

2.4: Discussion

In the current study, prolactin levels increased in men beginning at the prenatal period, and peaked at the early postnatal stage. The only other study of prolactin change in men used a between-subjects design, and found from that prolactin concentrations weres highest in the men tested at the late prenatal visit (Storey et *al.*, 2000). One possible explanation for why this peak occurs later here is related to the timing of visits. Couples in the current study were visited at an average of 16 days postpartum for the early postnatal stage (but at 17.8 days for the five couples with repeated samples at all stages), which may be closer to the time of birth than for the original study. Storey et *al.* (2000) defined the early postnatal visit as within three weeks after the birth, and if visits were conducted closer to the three-week mark the prolactin levels may have already peaked in men and begun to decline. Although the women's prolactin levels here

followed the same pattern as previous studies (Corter & Fleming, 1995), the men's did not (Storey et *al.*, 2000).

The current experiment also included a control group, where no change in prolactin concentrations over time was noted. This strengthens the hypothesis that that these types of changes occur only in men around the time of birth of their baby, and not in all men. However, there was no difference in levels of prolactin between control and 'pregnant' groups of men, just a significant difference in the pattern over time for 'pregnant' men. This underscores the importance of looking at the long-term fluctuations in studies like this, and not just focusing on a single sampling event.

Men's baseline free testosterone levels did not change over the prenatal and postnatal period of their partners' pregnancy. Considering that a drop in testosterone concentrations after the birth has been the most consistently found pattern in the two previous populations studied (Storey et *al.*, 2000; Berg & Wynne-Edwards, 2002), this was not expected. However, Berg & Wynne-Edwards (2001) did not report any effect of testosterone levels changing over time in their original sample of 45 men, and an effect was only detected in a later subset of 9 men (Berg & Wynne-Edwards, 2002). Additionally, the drop in testosterone levels that they reported occurred from the last week before birth to the first week after, which is closer to the time of birth than the samples obtained in the current study. Perhaps if larger numbers of men were included with repeated data in this study, thus increasing statistical power, an effect of changing testosterone levels across stages may actually have been seen.

The statistically non-significant trend of control men having higher testosterone levels at their second visit than new fathers at the early postnatal visit hints at possible differences that may not have been significant due to the low power. Additionally, there was a postnatal drop in testosterone levels for men in the pregnant group that could not be investigated further die to the lack of an overall effect of testosterone levels. However, this does suggest that if a larger number of men were measured, and subsequently statistical power increased, the predicted difference in testosterone levels may be seen.

The lack of change in men's serum cortisol levels over time was not unexpected, given that a less invasive sampling technique was used than in the first report of men's cortisol concentrations peaking late in the prenatal stage (Storey et *al.*, 2000). Though a non-invasive saliva sampling technique was used for the other population of men with reported cortisol changes (Berg & Wynne-Edwards, 2001; 2002), the peak was at a week before birth with a drop in the week following birth. Again, this is closer to the time of birth than samples for this study. The most important factor affecting the absence of a cortisol effect in this study for men may be the loss of sensitivity due to difficulties experienced with the quantifying the cortisol in the modified blood spot assay.

Though the cortisol levels were positively correlated between experimental couples as predicted, this cannot be confidently stated as a reliable result because of the problems experienced with the cortisol assay. As all samples for individual couples were assayed together, the fact that some assays had a consistently higher or lower range of values may have contributed to the positive correlation. This should have been minimized by normalizing the data, so the correlation cannot be completely discounted,

but should be regarded with caution. The control couples did not have positively correlated cortisol levels at either stage measured, which suggests that it is not only some aspect of living together (i.e., sleep/wake schedules, experiencing major events together, emotional closeness) causing cortisol levels of pregnant couples to change together. Rather, some aspect of the pregnancy and parenthood appear to be causing the cortisol levels of the pregnant couples to change together. However, due to the assay difficulties, this can only represent an avenue for further research to explore, and to either discount or prove that the correlation really occurs.

The fact that experimental and control men did not report a significantly different number of pregnancy symptoms raises the question of whether these self-reports are actually related to the pregnancy at all. This is furthered by the results of the comparison between men who reported more symptoms versus those who reported few, with no difference in any hormone levels found. It may be more accurate to simply call them 'physiological' symptoms than it is to say 'pregnancy' symptoms. It appears that the men in the current study self-reported more symptoms than the men in the original study (Storey et *al.*, 2000), which may imply that the population of volunteers differ in some underlying way. What that difference may be is unknown, as both populations were recruited in the same manner, from the same type of prenatal classes and in the same city. However, the current study required more of a longitudinal commitment from volunteers than those that participated in a single visit for the between-subjects design employed by Storey et *al.* (2000), which may have selected for a sample of men that were already more interested in the pregnancy and the issue of paternal involvement.

The absence of a link between men and women's rating of how attractive they found baby smells to be and their cortisol levels was unexpected. Couples were asked to rate this at the late postnatal visit, which was performed at around two months postnatal. Unfortunately, this may have been too late to detect any effect, as earlier studies that have reported links between cortisol levels and high ratings of infant odour attractiveness were conducted in the days after birth, when bonding is initially occurring. Additionally, it would have been interesting to test parents' preference for their own infants odours in a choice test as was done by Fleming et *al.* (1997), and to see if the control group rated these smells any differently than the experimental group did.

At the late postnatal visit, fathers who rated their infants as more difficult than average had higher prolactin and testosterone levels than other fathers at that stage. It would be hypothesized that higher testosterone concentrations, which may be counterproductive to parenting effort, would occur in men who perceive their own infant to be difficult. However, the fact that prolactin levels are also higher in these men does not follow the predicted pattern for testosterone and prolactin, which often have an inverse relationship, at least in birds. Usually as testosterone levels drop, prolactin levels rise, both events coinciding with some aspect of incubation, hatching, or chick care in birds (for a review, see Buntin, 1996). However, some biparental rodents actually have the opposite occur, displaying testosterone levels that increase as a paternal behaviour is performed (California mouse: Trainor & Marler, 2001, 2002; prairie vole: Wang & De Vries, 1993; Lonstein, Rood, De Vries, 2002; Mongolian gerbil: Clark & Galef, 1999). Additionally, no inverse relationship between prolactin and testosterone levels has been

found in most nonhuman primates (common marmoset: Dixon & George, 1982; cotton-top tamarin: Ziegler, Wegner, Carlson, Lazaro-Perea, Snowdon, 2000; Ziegler & Snowdon, 2000, but see black tufted-ear marmoset: Nunes, Fite, French, 2000; Nunes, Fite, Patera, French, 2001). It may be that prolactin levels are elevated in men who reported their own infant to be more difficult than average because the men are spending more time hearing their babies cry and holding them, due to their difficult nature. Two previous findings indicate that this may, in fact, be the case. First, Storey et *al.* (2000) reported that men tested within three weeks after their baby was born showed increases in testosterone concentrations after holding their newborn for 30 minutes in an infant stimuli test that included a tape of baby cries. Secondly, Fleming et *al.* (2002) reported that fathers who listened to a tape of baby cries had a greater short-term percent increase in testosterone levels than fathers who did not hear the tape.

Fathers who rated parenting as more difficult than they expected it to be had hormonal differences at the early postnatal stage from the other fathers. Cortisol levels were higher for those men and their partners at this stage, which suggests that elevated cortisol levels within the weeks after birth may be an indicator of some aspect of postnatal coping ability. Fathers who rated parenting to be more difficult than they had expected also showed a statistically non-significant trend towards having higher testosterone and prolactin levels at the early postnatal visit than other fathers. These two hormone concentrations were also elevated at the last postnatal visit for fathers reporting their infant to be more difficult than the average baby, which may reflect increased attempts to comfort these difficult babies. We considered the fact that men who rated

their infant as difficult may also have rated parenting as difficult as well, thus the increases in testosterone and prolactin levels in both cases may simply be due to the same individuals occurring for both. However, of the three men reporting their infant to be difficult, only one reported parenting to be more difficult than expected, so these seem to be separate phenomena.

Women reporting higher parenting difficulty ratings had higher cortisol levels at all stages except the late prenatal, where the difference approached statistical significance. Additionally, their male partners had higher cortisol levels at the early prenatal and early postnatal stages. Cortisol levels, especially in women, appear to be an indicator of difficulties with parenting, though it is impossible to discern which precedes the other. The elevated prenatal cortisol levels in parents who report difficulty with parenting postnatally may be reflective of anxiety even before the baby arrives. Women who reported difficulty with parenting also had lower prolactin levels at the late postnatal stage when compared to the other women, which may be a result of an interaction with the elevated cortisol levels. Alternatively, the reduced prolactin levels may be a hormonal indicator of reduced feelings of nurturance or bonding.

Further research is necessary to examine these hormonal patterns more closely, especially in men. Not only are the baseline changes over time important to consider, but also the relationship of hormones at each stage to emotional and behavioural measures. The hormone changes as related to the self-report of infant difficulty and parenting difficulty need to be further pursued, perhaps on a finer scale than the rating system used here.

The blood spot measurement of cortisol in this study had many unforeseen difficulties due to the lower circulating levels late in the evening, which necessitated modification to the procedure. This resulted in unforeseen technical problems, as well as concerns about using more of the sample than prior planning had allowed for. Nonetheless, the use of finger stick sampling for cortisol should not be abandoned, but rather, refined. The easiest way to measure this adrenal hormone non-invasively is with saliva sampling, but careful consideration must be given to ensure that the collection method does not interfere with the results, as is possible with the most common methods. Since this study commenced, newer methods of collecting small amounts of serum from finger sticks have become available, which would be ideal for future work. The BD Microtainer™ tubes (Becton, Dickinson and Company) that have recently become available allow for collection following the normal procedure used in this study, but instead of the blood dropping onto filter paper collection cards, it flows directly into the Microtainer™ tube. Since these are available with the same additives and coloured-top system as the traditional, larger Vacutainer™ tubes, serum can be obtained as usual. In this way, a finger stick technique can still be used without sacrificing accuracy, and without encountering the pitfalls of other techniques. Though low circulating levels of cortisol in the evening was the source of technical problems, this method allows for future sampling to continue at this time of day. Levels of cortisol are relatively stable in the evening, and since reactivity to infant stimuli (discussed in chapter 3) is an additional focus of this work, these low levels may allow for the reactivity to be studied better than higher morning levels would.

The low number of couples with repeated data at all stages was a problem in terms of statistical power for this study. As changes occur so close to the time of birth, visits should ideally be conducted as close to within two weeks of the due date as possible. However, problems arise when an early birth occurs and the late prenatal visit is missed, or when a late birth occurs and the data for the late prenatal visit has to be excluded as it has become too far away from the time of birth. A possibility for future research is to retest overdue couples closer to the birth in order to obtain a usable sample for the late prenatal stage. The full visit would not have to be repeated, just a single blood sample from each person. The other stage where a low number of volunteers was a problem was at the early prenatal stage. A larger number of couples attended prenatal classes during the later stages of pregnancy, so some were recruited too late to obtain samples for this early stage. Moreover, the couples in the early prenatal classes had a lower volunteer rate, further hindering recruitment for this early prenatal stage. Future studies employing a within-subjects design may try to recruit couples earlier in their pregnancy through additional avenues, such as posting signs in the offices of doctors who specialize in obstetrics or taking out advertisements in local newspapers. Early recruitment is imperative to obtain more early prenatal samples, and larger overall numbers are also required in order to offset the possible loss of data from subjects before the time of birth due to early or late births.

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Table 2.1 – Regression equations used to normalize cortisol blood spot data, based on the multivalent control values for each individual assay (also see Figure 1)

	Assay 1	Assay 2	Assay 3	Assay 4	Assay 5
Date	22-May-02	5-June-02	14-June-02	22-June-02	19-Aug-02
Low control blood spot (ug/dL)	98.7	92.6	159	33.3	21.14
Medium control blood spot (ug/dL)	189.2	141.4	257	73	53.54
High control blood spot (ug/dL)	360	336	442	165.8	127.75
Regression equation	y = 0.0922x - 5.3203	y = 0.0924x – 3.3206	y = 0.0852x – 9.757	y = 0.1800x – 1.723	y = 0.2240x – 0.5115
R^2 value	0.9994	0.9823	0.9994	0.9992	0.9995

Table 2.2 – Men and women's difference in cortisol levels and their parenting difficulty ratings (only statistically significant results shown)

	Was parenting more difficult than the father expected?		
	YES	**NO**	**F-test and p-value**
Early postnatal	His cortisol:16.81 μg/dL SE = 0.25 N = 4	His cortisol: 10.82 μg/dL SE = 1.23 N = 17	F (1,19) = 4.977, p = 0.038
	Partner's cortisol: 9.40 μg/dL SE = 0.73 N = 4	Partner's cortisol: 5.09 μg/dL SE = 0.68 N = 16	F(1,18) = 9.109, p = 0.007
	Was parenting more difficult than mother expected?		
	YES	**NO**	**F-test and p-value**
Early prenatal	Her cortisol: 15.93 μg/dL, SE = 3.77, N = 4	Her cortisol: 5.50 μg/dL, SE = 1.77, N = 4	F(1,6) = 6.257, p = 0.046
	Partner's cortisol: 8.00 μg/dL, SE = 1.57, N = 4	Partner's cortisol: 1.69 μg/dL, SE = 0.47, N = 4	F(1,6) = 14.845, p = 0.008
Early postnatal	Her cortisol: 7.91 μg/dL, SE = 0.81, N = 9	Her cortisol: 4.36 μg/dL, SE = 0.77, N = 11	F(1,18) = 9.882, p = 0.006
	Partner's cortisol: 14.98 μg/dL, SE = 1.58, N = 10	Partner's cortisol: 9.21 μg/dL, SE = 1.21, N = 11	F(1,19) = 8.623, p = 0.0008
Late postnatal	Her cortisol: 6.03 μg/dL, SE = 0.88, N = 10	Her cortisol: 3.33 μg/dL, SE = 0.62, N = 11	F(1,19) = 6.446, p = 0.020
	Her prolactin: 39.69 ng/mL, SE = 11.46, N = 10	Her prolactin: 84.93 ng/mL, SE = 16.64, N = 11	F(1,19) = 4.820, p = 0.041

CHAPTER 2 – Figures

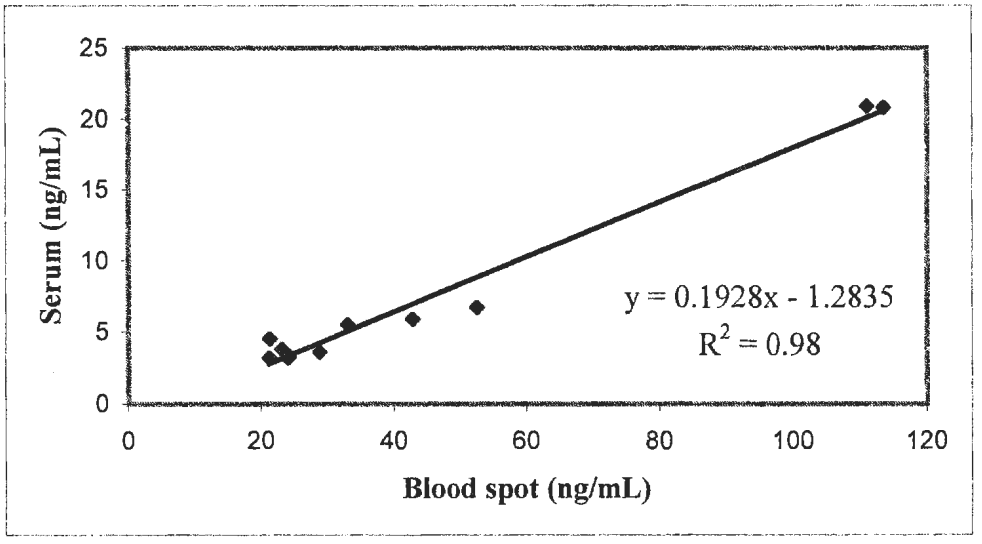

Figure 2.1 – The linear relationship between serum and blood spot prolactin, based on blood samples from an outside subset of volunteers (n=5 men, n=6 women).

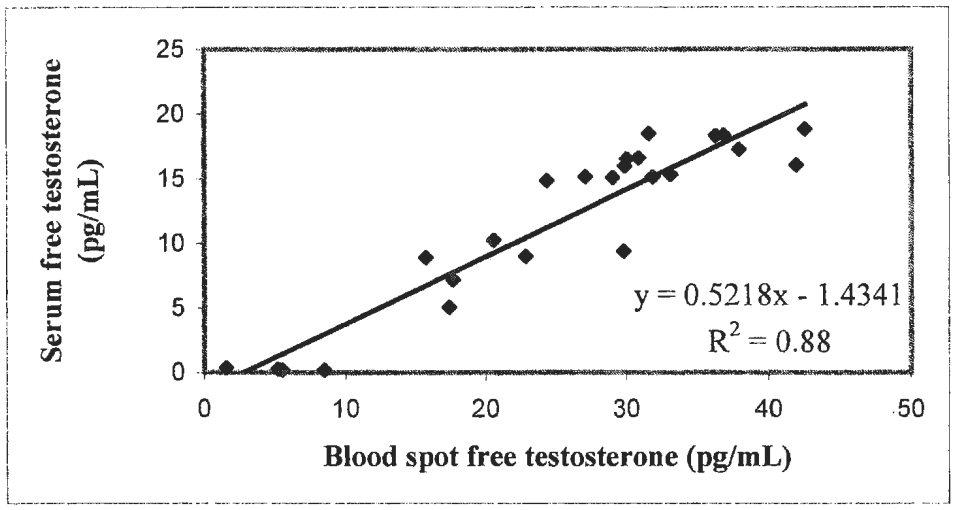

Figure 2.2 – The relationship between serum and blood spot free testosterone, based on blood samples from an outside subset of volunteers (n=10 men, n=4 women), as well as samples from one male that had exogenous testosterone added (n=10). The four lowest points are for women's values.

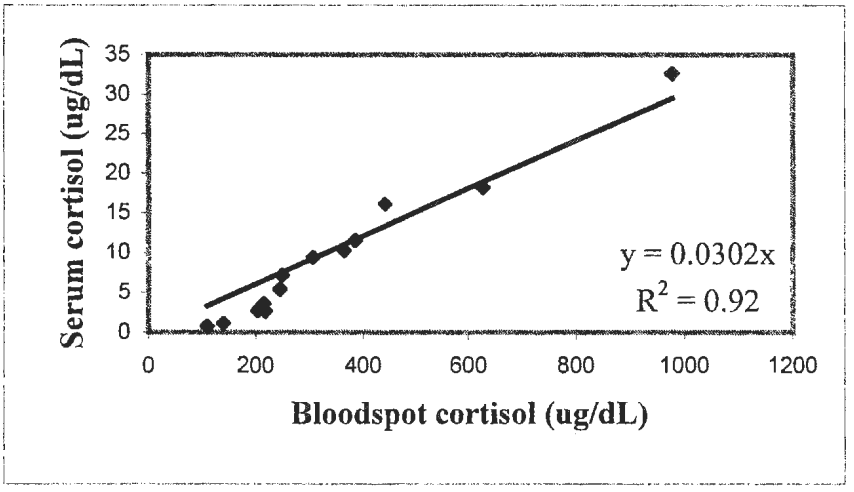

Figure 2.3 – The relationship between serum and blood spot cortisol, based on blood samples from an outside subset of volunteers (n=4 men, n=9 women).

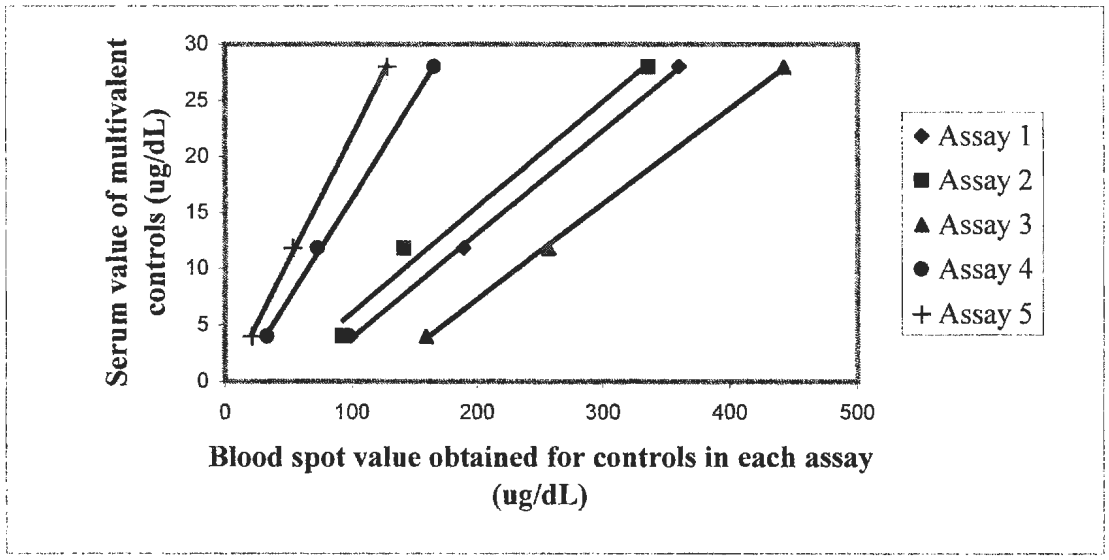

Figure 2.4 – The relationship of low, medium, and high multivalent serum cortisol controls to the blood spot values obtained for them in each cortisol assay (see Table 2.1 for regression equations for each trend line, used to normalize the cortisol data).

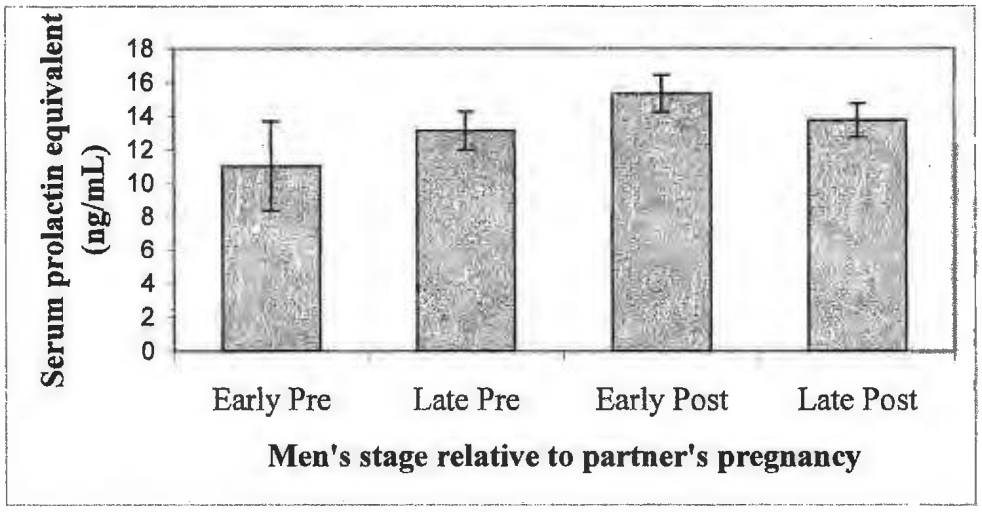

Figure 2.5 – Men's serum prolactin levels (ng/mL) through the pre and postnatal stages of their partner's pregnancy (n = 5). Prolactin levels changed significantly across stages ($F(3,12) = 5.677$, $p = 0.012$). Early prenatal prolactin levels were lower than at both postnatal stages, and early postnatal levels were higher than at the late prenatal stage. Error bars represent standard error.

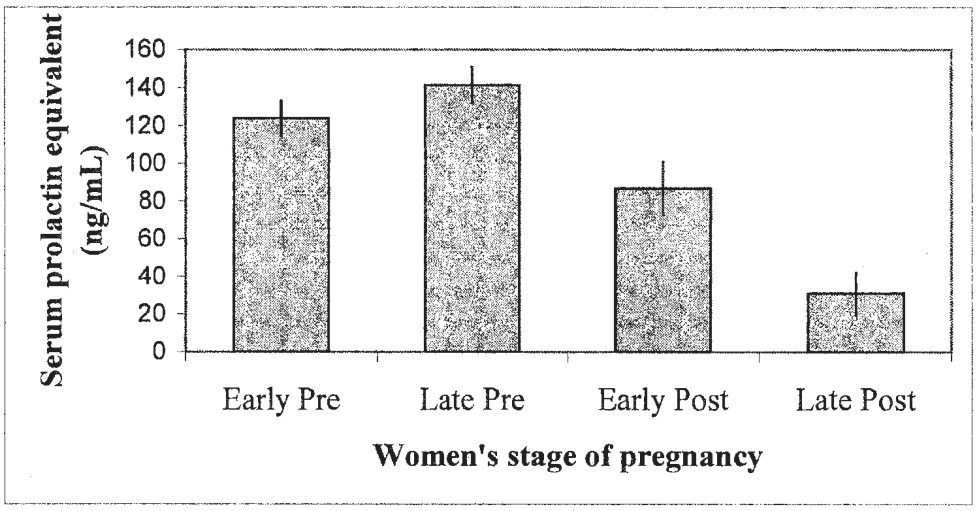

Figure 2.6 – Women's serum prolactin levels (ng/mL) through the pre and postnatal stages of their pregnancy (n = 5). Prolactin levels changed significantly across stage ($F(3,12) = 16.782$, $p = 0.000$). Early prenatal levels were lower than those for both the early postnatal and late postnatal stages, late prenatal prolactin levels were higher than those at both the early and late postnatal stages, and the two postnatal stages also differed from each other. Error bars represent standard error.

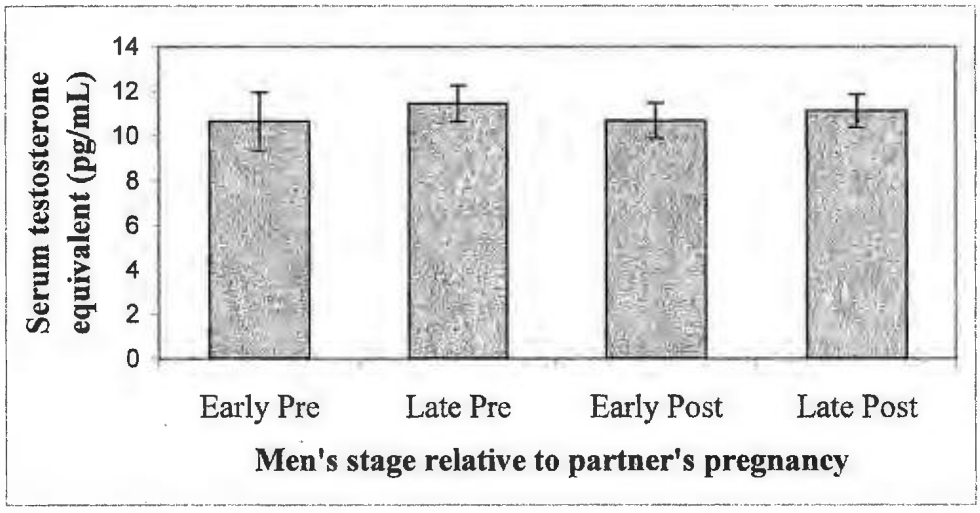

Figure 2.7 - Men's serum testosterone levels (pg/mL) through the pre and postnatal stages of their partner's pregnancy (n = 5). There was no significant difference in testosterone levels across the stages ($F(3,12) = 0.620$, $p = 0.615$). Error bars represent standard error.

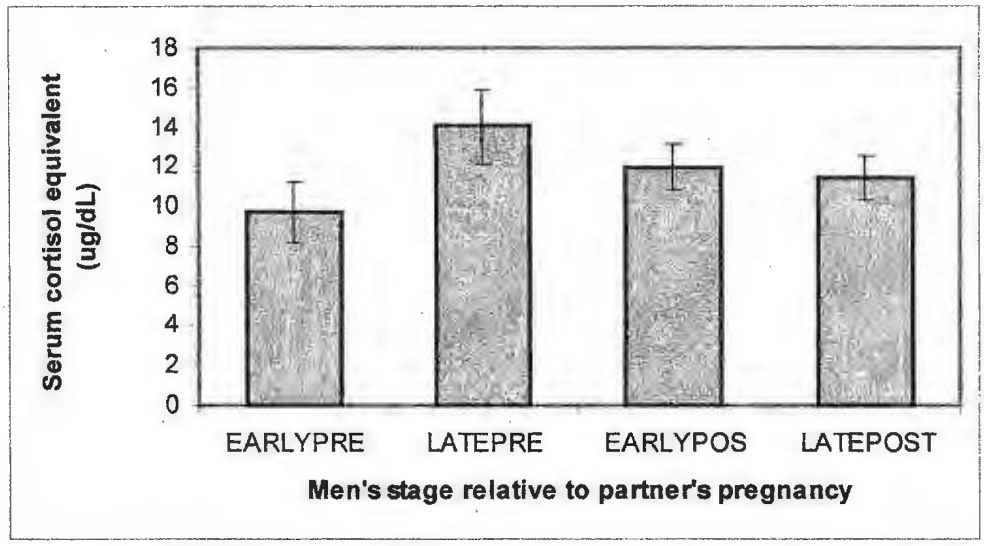

Figure 2.8 - Men's serum cortisol levels (ug/dL) through the pre and postnatal stages of their partner's pregnancy (n = 5). There was no significant difference in cortisol levels across the stages ($F(3,12) = 1.109$, $p = 0.384$). Error bars represent standard error.

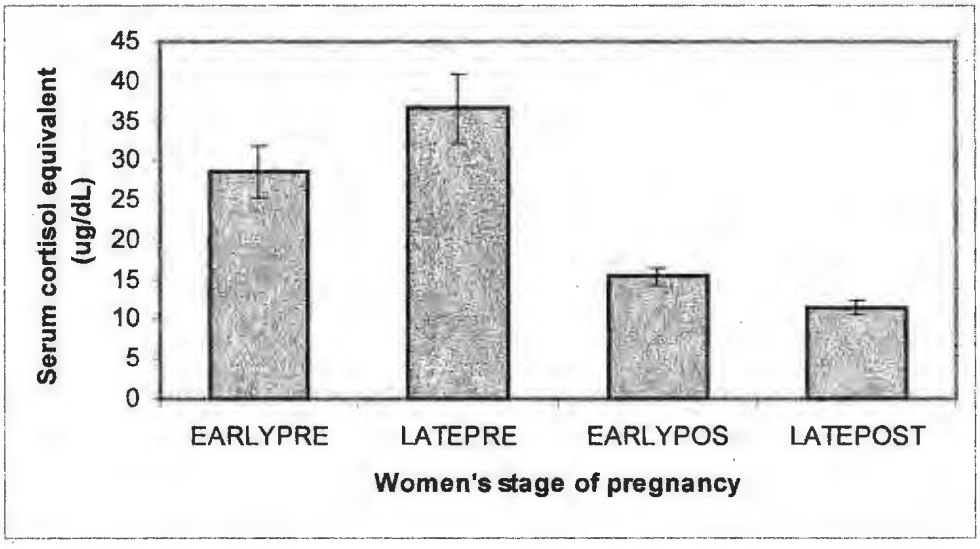

Figure 2.9 - Women's serum cortisol levels (ug/dL) through the pre and postnatal stages of their pregnancy (n = 5). Cortisol levels differed significantly across the stages ($F(3,12) = 12.148$, $p = 0.001$). Early prenatal levels were lower than at the late prenatal stage, and higher than at the late postnatal stage. Late prenatal levels were higher than both the early and late postnatal levels. Error bars represent standard error.

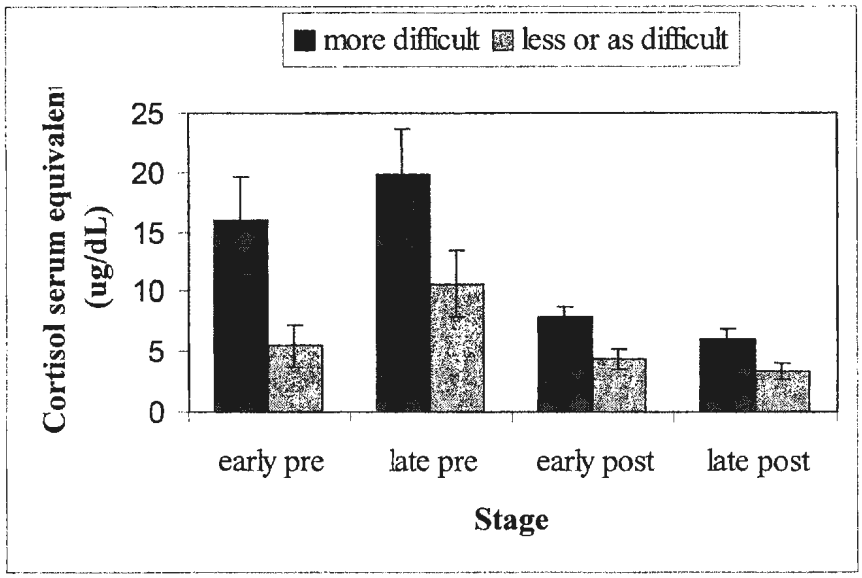

Figure 2.10 – Cortisol levels (ug/dL) of women who rated parenting to be more difficult than they expected versus those who rated parenting to be less or as difficult than they expected at the late postnatal visit. At each stage except the late prenatal, the difference is significant ($p < 0.05$). Error bars represent standard error.

Chapter 3 – Abstract

Two studies have reported short-term hormonal changes in men in response to infant stimuli around the time of birth of their child. In the current study, couples expecting their first child were recruited from prenatal classes, and later visited three times; within two weeks before, and within two weeks and two months after the birth. A control group of married or co-habitating couples that were not expecting a baby and had no children were visited twice, at four weeks apart. Researchers visited couples at home during evening hours and collected baseline finger stick blood samples, administered an infant stimuli test, and took a second fingerstick sample 30-minutes after the first. For the prenatal infant stimuli test, couples held a soft-bodied doll, listened to a tape of infant hunger and pain cries, watched a video of an infant breastfeeding and of men discussing becoming a parent. Postnatally, the father held his baby and the mother held the doll, with no other stimuli presented between blood samples. Couples completed questionnaires that included ratings of pregnancy symptoms, infant difficulty ratings, and parenting difficulty ratings. Prolactin and cortisol levels for men and women, and free testosterone levels for men were quantified. During the late prenatal stage, pregnant women had significant short-term increases in prolactin levels. New fathers had significant short-term decreases in prolactin during the early postnatal stage. Control men and women did not show any short-term changes in prolactin. There were no short-term changes in testosterone for men, or cortisol for men and women, in either the pregnant or the control group. Fathers who reported 'concern' in response to baby cries at the late prenatal test had increases in prolactin levels during the early postnatal stage

and decreases in testosterone during the late postnatal stage. Fathers who reported 'excitement' in response to the cries tape had increases in prolactin levels during the late postnatal stage. Fathers' prenatal responses to infant stimuli may be predictive of certain patterns of hormonal change after the birth of their babies.

Chapter 3 – Short-term hormonal situational reactivity to an infant stimuli test for men and women before and after the birth of their first child

3.1: Introduction

Two studies have looked at short-term changes in men's hormone levels in response to infant stimuli around the time of the birth of their child. In the first study, couples held a doll wrapped in a receiving blanket recently worn by a baby (men held their own babies postnatally), listened to a tape of baby cries, and then watched a video of a newborn nursing in the 30-minute interval between two venipuncture samples (Storey, Walsh, Quinton, Wynne-Edwards, 2000). For both men and women at all stages, serum prolactin and cortisol levels significantly decreased by the second sample. Men tested during the three weeks prior to the birth had the largest decrease in serum cortisol levels over the 30-minute test. Men tested within the first three weeks after their infant's birth had testosterone levels increase during the short-term test. No other changes in hormone reactivity were seen in the men tested at any of the other stages. When data from all stages were combined, men who felt more concerned in response to the baby cries showed a significant decrease in testosterone levels.

This decrease in prolactin levels for men and women at all stages is opposite to the pattern observed in parental male bird, rodent, and primate species. This disparity may be due to the invasive venipuncture sampling or the between-subjects design used in the Storey et *al.* (2000) study. The novelty of researchers visiting the couple's home to sample blood and conduct a test may have induced hormonal changes normally seen in other parental animals in response to a 'challenge', with levels returning to normal by 30

minutes after the initial sample (Wynne-Edwards, 2001; see also 'challenge hypothesis' proposed by Wingfield et *al.*, 1990).

Following this reasoning, the researchers have suggested that the second cortisol sample at the late prenatal visit may be more indicative of normal 'baseline' levels, and that the first sample, which was assumed to be baseline, may actually reflect increased reactivity in response to the researcher's visit in the weeks before birth (Storey et *al.*, 2000; Wynne-Edwards, 2001). Hormonal reactivity, then, as well as baseline levels, may change with the time period relative to birth.

The second study to measure father's short-term responses to infant stimuli found that parentally experienced men who listened to a tape of infant cries days after the birth of a new baby showed a greater percentage prolactin increase than both new first-time fathers who heard the same tape, and fathers who heard a control tape (Fleming, Corter, Stallings, Steiner, 2002). Those fathers with highest prolactin levels reported significantly more alertness in response to the hunger-type cries. Fathers who heard the tape of infant cries had higher salivary testosterone levels, and a larger percent increase in testosterone levels than fathers who did not hear the tape. When results from both the fathers and the non-father controls were combined, men with lower testosterone levels reported more 'sympathy' and/or 'need to respond' in response to the cries.

One other study of mothers found changes in salivary cortisol levels in response to infant cries (Stallings, Fleming, Corter, Worthman, Steiner, 2001). Mothers who reported greater sympathy in response to infant cries had higher baseline salivary cortisol

levels. These levels dropped over the testing period in 'higher sympathy' mothers, but the 'lower sympathy' mothers had levels that remained low and stable.

Changes in heart rate have been used as an indicator of physiological autonomic response to infant stimuli, (for a discussion, see Corter & Fleming, 1995). Corter and Fleming (1995) state that "accelerations are generally assumed to reflect the salience of the stimulus to the subject or a preparatory response to that stimulus; decelerations are in general thought to reflect attention". Stallings et *al.* (2001) reported that mothers who responded with greater sympathy to a recording of infant cries had higher heart rates. Fleming et *al.* (2002), however, found no differences in new fathers' heart rates based on either experience status or if they listened to recorded infant cries or not.

Maternal responses to infant odours have been linked to cortisol levels. Fleming, Corter, Franks, Surbey, Schneider, and Steiner (1993) reported that new mothers rate infant odours to be more attractive than do women who have not had children. Fleming, Steiner, and Corter (1997) showed that new first-time mothers with higher salivary cortisol levels reported their own infant's body odour to be more attractive than odours of unfamiliar infants. They also found that these mothers were better able to recognize their own infant's odours in a two-choice recognition task. A later study that included fathers, however, found no differences in their hormonal or self-reported responses to infant odours (Fleming et *al.*, 2002). Mothers' responses to infant odours did not differ in another study (Stallings et *al.*, 2001).

In consideration of the aforementioned studies, but respecting their different methodologies and the overall paucity of historical data, the following can be

hypothesized. Men may have short-term decreases in cortisol levels, especially at the stage before birth for men, a short-term increase in testosterone levels at the stage after birth, and overall decreases in prolactin levels at all stages combined. If it is true, however, that the second venipuncture sample in the Storey et *al.* (2000) study actually reflected the 'adrenal baseline', then the opposite may occur for cortisol and, possibly, for prolactin. Only experienced fathers in the Fleming et *al.* (2002) study had prolactin increases in response to baby cries, so first-time fathers in the current study may not show this increase. Fathers may have increased testosterone levels after listening to the baby cries tape, and men with lower testosterone levels may report more sympathy to the cries (Storey et *al.*, 2000; Fleming et *al.*, 2002). Though heart rate changes and a relationship between cortisol and infant odour attractiveness have been reported for mothers, it is not expected that men will show any of these changes in response to the infant stimuli based on the only available study (Fleming et *al.*, 2002).

3.2: Methods

3.2.1: Data collection

Details on recruitment of the participants, the sampling procedure at the prenatal class, and demographic information on the couples is described in Chapter 2. As the current chapter deals with short-term hormonal changes, only information relating to data collection of the two blood spot samples and the procedure for the infant stimuli test is presented here.

The pregnant group was divided into two subgroups; control (n = 6) and experimental (n = 16). The pregnant control was not given the infant stimuli test at the same stage as the pregnant experimental group in order to provide a way to compare the effect of the stimuli separate from the researcher's visit. The pregnant control group did not receive the stimuli test at the late prenatal stage, but did receive it at the late postnatal stage, which is opposite to the testing for the pregnant experimental group. However, many of the couples assigned to the pregnant control group had their baby earlier than expected. As a result, an insufficient number of these pregnant control couples were tested at the late prenatal stage, and no further analysis was conducted for their data, nor are they discussed further. The pregnant experimental group are hereafter referred to as the pregnant group.

3.2.1.1: Infant stimuli test

A short-term test involving the various infant stimuli was performed at the late prenatal stage for the pregnant group, and at the first home visit for the non-pregnant control group. This infant stimuli test began once the experimenter had fitted a Polar Coach heart rate monitor across the man's chest, which recorded his heart rate at each minute throughout the visit. Couples gave the initial finger stick blood samples, from the woman first and then from the man. They then sat in comfortable chairs, each holding a soft-bodied doll wrapped in a blanket, and listened to a 6-minute tape of infant hunger and pain cries that had been recorded in the neonatal unit of the Grace Hospital, St. John's, Newfoundland. This was followed by a 10-minute video consisting of scenes of a

newborn infant struggling to nurse for the first time, as well as a short video clip of a father attending his first child's birth. At 30-minutes from the first sample, a second finger stick blood sample was obtained from participants, in the same order as before. The only difference in the infant stimuli test for the postnatal visits was that the father held his newborn while the mother held the doll throughout the 30-minute period.

3.2.1.2: Home visits to pregnant couples

Two researchers conducted a late prenatal and two postnatal visits for all couples in the participants' homes. The late prenatal visit was scheduled for sometime within two weeks prior to the due date, and was the only time the infant stimuli test was conducted. Though 22 couples participated in the study, data from only 11 of them were included for the short-term test at the late prenatal stage. This reduction in numbers occurred because six couples were assigned to the pregnant control group, which is not further discussed. Of the remaining 16 couples, three were excluded at this late prenatal stage because they had their babies later than expected, and no data was obtained for two couples that had their babies earlier than anticipated.

Efforts were made to schedule early postnatal visits at each couple's home for within two weeks of the baby's birth. As was often the case in Caesarean section births (n = 7), when the new mother was not feeling well the visits were delayed until the earliest time she felt that experimenters could visit. The mean number of days after the birth for the early postnatal home visit was 16 days (n = 21, median: 15, range of 13-22 days). At this early postnatal stage, the couples did not receive the infant stimuli test; the

father simply held his newborn while the mother held the doll during the 30-minutes between blood samples.

Late postnatal visits were scheduled for when the newborn was around two months old. The mean number of days from birth for the late postnatal home visits was 61 days (n = 21, median: 59, range of 49-106 days). One couple left town after the first postnatal visit, and did not return until their child was 15 weeks old. The late postnatal visit with this family was still carried out at this time, and results were included in the analysis. The range of days from birth for the late postnatal visit is 49-70 days for all others excluding this latter couple. As with the early postnatal visit, the father held his baby and the mother held a doll, with no infant stimuli test given in the time between the two blood samples.

All home visits were conducted between 16:00 and 20:00, and efforts were made to schedule individual couples for the same time of day at each testing stage. As cortisol, prolactin, and testosterone peak near early morning hours and do not rapidly change during the evening hours, this sampling time also minimizes variation due to rapid diurnal fluctuations (Kanaley, Weltman, Pieper, Weltman, Hartman, 2001; Franks, 1979; Miyatake, Morimoto, Oishi, Hanasaka, Sugita, Iijima, Teshima, Hishikawa, Yamamura, 1980).

The same procedure was carried out for each visit, beginning with a pair of researchers arriving together at the couples' home. The consent forms were signed, and the father was fitted with a Polar Coach heart rate monitor. The baseline finger stick blood samples were taken, always in the same order: first from the woman and then from

the man, as described in Chapter 2. Couples then received the infant stimuli test if it was the late prenatal stage, and postnatally the father held his baby and the mother held the doll. At 30-minutes from the first blood sample, a second finger stick blood sample was obtained, again first the woman and then from the man. At the late prenatal and late postnatal stages, couples completed a questionnaire, but there was no questionnaire provided at the early postnatal stage.

The questionnaire for the late prenatal visit consisted of a checklist of pregnancy symptoms for both the individual and their partner, and included the following symptoms: anxiety, depression, weight gain, weight loss, irritability, nausea, fatigue, indigestion, happiness, increase in appetite, decrease in appetite, and muscle aches. Individuals were asked about how much of the household chores, baby care, etc. they expect to perform, how they felt about becoming parents, and how the pregnancy has influenced conversation and thought. The couples were also asked to rate their previous experience with infants, changes in the quality of the relationship with their partner, and if any periods of separation were expected or had occurred. Couples in the experimental condition were asked to check all appropriate responses they felt when they listened to and watched the baby cues, choosing from a list that included 'concerned, anxious, content, irritated, wanted to comfort baby, and other'. Finally, they completed a 5-point anxiety scale rating of how they perceived the finger stick procedure.

For the late postnatal visit, each person rated themselves and their partners on the amount of time they spent in childcare duties, play, and household chores, if the adjustment was more or less difficult than expected, and if the quality of the couple's

relationship had changed (become better or worse). They also rated their responsiveness to infants since the birth. Couples were asked if the mother was breast feeding, as well as if the father had the opportunity to bottle-feed, and, if so, how often. Those that received the infant stimuli test were asked to check the responses they felt. Finally, they again rated how stressful the finger-stick sampling was perceived to be.

3.2.1.3: Home visits to 'non-pregnant' control couples

Two home visits were conducted at the same time of the evening (between 16:00 and 20:00 hours) for the seven non-pregnant control couples. The visits were scheduled for approximately one month apart as a control for the late prenatal and early postnatal visits to the pregnant couples. The first visit to the non-pregnant couples was the same as the late prenatal test for the experimental group, and consisted of consent form signing, putting the heart rate monitor on the man, and taking an initial finger stick blood sample from the woman and then the man. Both of them were then given the infant stimuli test in the same manner as previously outlined, followed by a second blood sample taken at 30 minutes after the initial one. The couples were then asked to fill out the same checklist of pregnancy symptoms, but asked to report how many they had recurrently experienced in the last three months. They were also asked to complete the checklist of responses they experienced while listening to and watching the baby cues.

The second visit was done at about a month after the first, the same time interval apart as the late prenatal and early postnatal visits were for the pregnant group. This second visit was similar to the early postnatal visit, and consisted of consent form

signing, heart rate monitor outfitting, and an initial blood sample from the woman and then the man. The man and woman each held a doll for half an hour, and then the second finger prick blood samples were taken.

3.2.2: Laboratory methods

Blood spot samples were analysed in duplicate using commercially available serum kits. Prolactin was measured using a fluoroimmunoassay AutoDELFIA™ Prolactin kit (Perkin Elmer Wallac Canada, catalogue number BO18-301). Both cortisol and free testosterone were analysed using coated tube radioimmunoassay kits (Coat-A-Count Cortisol RIA and Coat-A-Count Free Testosterone RIA, DPC). Modifications of the normal serum procedure for each kit were implemented, and each hormonal assay was validated. Additionally, linear regression formulas were established to convert the blood spot values to traditional serum values. All details of the hormone assays are discussed in Chapter 2.

3.2.3: Statistical analysis

The change in each hormone from the first to the second finger stick blood sample was analyzed using two-tailed paired t-tests. Repeated-measures ANOVAs were used to test the magnitude of change over the three stages for each sex and group separately. When these were statistically significant, post hoc paired t-tests were carried out to test the change from one stage to the other. For the self-reported questionnaire responses, parents were grouped into one of two groups based upon their responses, and one-way

ANOVAs were used to test the differences in the magnitude of hormonal change. To compare the pregnant experimental late prenatal and early postnatal visits with the non-pregnant control visits one and two, respectively, 2 (repeated factor: magnitude of change in hormone level at the first versus the second visit) x 2 (between factor: pregnant experimental group versus non-pregnant control group) ANOVAs were carried out for each hormone and for each sex. Two-tailed Pearson product moment correlations were used to test the within-couple correlations in magnitude of hormonal change at each stage. Heterogeneity of variance was tested for in all one-way ANOVAs using the Brown-Forsythe test, and where heterogeneity of variance was significant, the Brown-Forsythe F-test values were reported (Keppel, 1991). This allows for testing between groups without the interference of heterogeneity of variance. Brown-Forsythe F-tests appear in the text with decimal places in the degrees of freedom for the denominator.

At the late postnatal visit, parents were also asked to rate their infant's difficulty in terms of other babies they had known, choosing from 'easier', 'average', or 'more difficult'. The change in hormone levels for parents reporting 'more difficult' was compared with those reporting the other possible responses combined. Parents were also asked to rate on a 5-point scale how difficult they were finding parenting to be, ranging from 'much less' and 'somewhat less' to 'what I expected' to 'somewhat more' and 'much more'. The change in hormone levels for those who responded 'somewhat' or 'more difficult' were combined and collectively compared in a one-way ANOVA against those who responded 'much less', 'somewhat less', or 'what I expected' combined. For both the infant and parenting difficulty ratings, parents who had higher and lower

difficulty ratings than their partners were compared in a one-way ANOVA to those with the same rating each.

3.3: Results

3.3.1: Prolactin short-term change

Men in the pregnant group received the infant stimuli test at the late prenatal stage, and control men received the test at their first visit. Neither group had a statistically significant change in prolactin levels during these times (Fig.3.1, Table 3.1).

Women exposed to the infant stimuli at the late prenatal test, however, did show a significant short-term increase in prolactin levels (paired $t(10) = 2.670$, $p = 0.024$, Fig.3.2, Table 3.1). Non-pregnant control women showed no change in prolactin levels during their first visit, when they were exposed to infant stimuli (Table 3.1).

At the early postnatal stage, where men held their own baby, their prolactin levels decreased significantly through the 30-minute testing period (paired $t(20) = -2.758$, $p = 0.012$, $n = 21$, Fig.3.1, Table 3.1). Women's early postnatal prolactin levels, however, did not change between the first to the second blood sample. The non-pregnant control men and women also did not have any significant changes in prolactin levels during their second visit, where they both held dolls during the 30 minutes between blood samples.

During the late postnatal visit, neither men nor women in the pregnant group showed any significant changes in prolactin levels through the 30-minute testing period.

Repeated measures ANOVAs on the magnitude of prolactin change over the three stages did not show any significant differences for either sex in the pregnant group (men:

$F(2,20) = 0.637$, $p = 0.539$, $n = 11$; women: $F(2,18) = 1.889$, $p = 0.180$, $n = 10$). The non-pregnant control men and women did not have any significant changes in the magnitude of prolactin levels from the first to the second visit (men: paired $t(6) = -0.300$, $p = 0.774$, $n = 7$; women: paired $t(6) = 0.201$, $p = 0.847$, $n = 7$).

A 2 (repeated factor: change in prolactin at the first and second visits) by 2 (between factor: pregnant versus non-pregnant groups) ANOVA was carried out for both the men and the women. No statistically significant difference in the magnitude of prolactin change was found from one visit to the next for women ($F(1,17) = 0.980$, $p = 0.336$) or for the men ($F(1,18) = 0.226$, $p = 0.640$).

There was no difference in the magnitude of prolactin change for men in the pregnant group during the late prenatal visit and control men during their first visit ($F(1,19) = 0.065$, $p = 0.802$), or for men in the pregnant group during the early postnatal visit and control men during their second visit ($F(1,21) = 0.001$, $p = 0.978$). The magnitude of increase in prolactin levels for pregnant women during the late prenatal visit approached statistically significant greater levels than the change for control women during their first visit ($F(1,19) = 4.060$, $p = 0.058$). There was no difference in the magnitude of prolactin change for women in the pregnant group during the early postnatal visit and control women during their second visit ($F(1,20) = 0.32$, $p = 0.415$).

3.3.2: Free testosterone short-term change

Men in both the pregnant group and non-pregnant group did not have any significant changes in magnitude of change of free testosterone levels between the two blood samples at any of the stages (Fig.3.3, Table 3.2).

Repeated measures ANOVAs on the magnitude of free testosterone change over the three stages did not show any significant differences for men in the pregnant group ($F(2,20) = 0.564$, $p = 0.577$, $n = 11$). There was also no significant difference in the magnitude of free testosterone change from the first to the second visit for the non-pregnant control men (paired $t(6) = -0.348$, $p = 0.740$, $n = 7$).

A 2 (repeated factor: change in free testosterone at the first and second visits) by 2 (between factor: pregnant versus non-pregnant groups) ANOVA was carried out for the men. There was no difference in the magnitude of free testosterone change from one visit to the next for men ($F(1,21) = 0.064$, $p = 0.802$).

There was no difference in the magnitude of free testosterone change for men in the pregnant group during the late prenatal visit and control men during their first visit ($F(1,19) = 0.184$, $p = 0.673$), or for men in the pregnant group during the early postnatal visit and control men during their second visit ($F(1,21) = 0.693$, $p = 0.415$).

3.3.3: Cortisol short-term change

At each of the three stages (late prenatal, early postnatal, and late postnatal), neither men nor women in the pregnant experimental group had any changes in cortisol levels from the first to the second sample (Fig. 3.4, Fig.3.5, Table 3.3). Likewise, both

sexes in the non-pregnant control group did not show any significant changes in cortisol levels during the first or second visit (Table 3.3).

Repeated measures ANOVAs on the magnitude of cortisol change over the three stages did not show any significant differences for either sex in the pregnant experimental group (men: $F(2,20) = 0.962$, $p = 0.394$, $n = 11$; women: $F(2,18) = 0.282$, $p = 0.758$, $n = 10$). There was no significant difference in the magnitude of cortisol change from the first to the second visit for either non-pregnant control men ($t(6) = 0.656$, $p = 0.536$) or women ($t(6) = 0.251$, $p = 0.810$).

A 2 (repeated factor: change in cortisol at the first and second visits) by 2 (between factor: pregnant experimental versus non-pregnant control groups) ANOVA was carried out for both the men and the women. There was no significant difference in the magnitude of change in cortisol levels from one visit to the next for women ($F(2,18) = 0.751$, $p = 0.486$) or for men ($F(2,20) = 0.655$, $p = 0.530$).

There was no difference in the magnitude of cortisol change for men in the pregnant group during the late prenatal visit and control men during their first visit ($F(1,19) = 0.811$, $p = 0.379$), or for men in the pregnant group during the early postnatal visit and control men during their second visit ($F(1,20) = 2.346$, $p = 0.141$). There was no difference in the magnitude of cortisol change for pregnant women during the late prenatal visit and control women during their first visit ($F(1,19) = 0.164$, $p = 0.690$), or for women in the pregnant group during the early postnatal visit and control women during their second visit ($F(1,19) = 0.135$, $p = 0.718$).

3.3.4: Change in hormone concentrations correlated at all stages

Correlations were determined for the magnitude of change (i.e. difference between the first to the second sample) for each hormone and at each stage for both men and women (Table 3.4). The magnitude of change in men's cortisol levels during the late postnatal stage was positively correlated to the change in men's cortisol levels during both the late prenatal and early postnatal stages, as well as negatively correlated to the change in cortisol levels for women during the late prenatal stage. The change in prolactin levels of men at the late prenatal stage were negatively correlated to their change in prolactin levels during the early postnatal stage. At the late prenatal stage, the change in testosterone levels for men was negatively correlated to the change in prolactin levels for women at the late postnatal stage. Women's change in cortisol levels during the late postnatal stage was positively correlated to their change in prolactin levels during the early postnatal stage.

There were no statistically significant correlations between non-pregnant control men and women's magnitude of change for any hormones at either of the two visits.

3.3.5: Pregnancy symptoms in men and women

Men who reported two or more pregnancy symptoms for themselves at the late prenatal stage were classified as 'couvade'. The magnitude of change of each hormone for the pregnant experimental 'couvade' men and their partners (n = 9) were compared to the 'non-couvade' men and their partners (n = 5) at the late prenatal stage. The groups differed significantly in the change in men's cortisol levels during the late prenatal stage

($F(1,9) = 6.257$, $p = 0.034$, 'couvade' men cortisol change: -1.31 μg/dL, SE = 1.29, n = 7; 'non-couvade' men cortisol change: 4.55 μg/dL, SE = 2.15, n = 4), the change in men's testosterone levels during the early postnatal stage ($F(1,14) = 6.631$, $p = 0.022$, 'couvade' men testosterone change: -0.32 pg/mL, SE = 0.42, n = 9; 'non-couvade' men testosterone change: 1.82 pg/mL, SE = 0.77, n = 7), and the change in their female partners' prolactin levels during the late prenatal stage ($F(1,9) = 7.387$, $p = 0.024$, 'couvade' women prolactin change: 41.11 ng/mL, SE = 10.98, n = 7; 'non couvade' women prolactin change: -1.34 ng/mL, SE = 7.14, n = 4).

3.3.6: Self-reported questionnaire rating

At the late postnatal stage, 21 sets of new parents rated how attractive they found baby smells to be since the birth of their own child, choices ranged from 'much more attractive', 'a little more attractive', or 'not really'. The magnitude of change of each hormone for fathers who found baby smells to be 'much more' and 'a little more' attractive combined (n = 10) were compared against those reporting 'not really' attractive (n = 11) using one-way ANOVAs. There were no differences for any hormone level changes, at any of the three stages, for either the fathers or their partners. This was further tested by comparing the change in hormone levels for fathers who found baby smells to be 'much more' attractive (n = 5) only with those reporting 'a little more' or 'not really' combined (n = 16). Again, there were no differences in the change of each hormone level for either the men or their partners. There were also no hormonal differences based upon the women's ratings of baby smells.

Men reporting their babies to be 'more difficult' than expected (n = 3) had no differences in any short-term hormone changes compared to those who found their babies to be 'average' or 'easier' (n = 18). Only one pregnant woman rated her own infant as more difficult than others at the late postnatal stage, so no parallel comparison could be made for the women.

Three fathers reported their infant to be more difficult than their partners did. The magnitude of change for their own hormone levels, as well as their partner's hormone levels, did not different from the couples that each provided the same rating for their infant's difficulty. When mothers rated their infant to be more difficult than their partner did (n = 4), there were also no differences in the change in hormone levels for mothers or fathers.

Only four fathers reported parenting to be more difficult than they expected, and there were no differences in their changes in hormone levels compared to men who did not report their infant to be difficult.

Men whose partners rated parenting to be more difficult than they expected had a different pattern of cortisol change during the late prenatal stage than men whose partners did not report difficulty with parenting ($F(1,9) = 7.734$, $p = 0.021$, mean cortisol change for partners of 'parenting more difficult' women: 3.55 μg/dL, SE = 0.1.61 μg/dL, n = 6; mean cortisol change for partners of 'parenting not difficult' women: -2.46 μg/dL, SE = 1.36 μg/dL, n = 5 There was also a difference in men's cortisol level change which approached statistical significance at the early postnatal stage ($f(1,14) = 4.474$, $p = 0.053$, mean cortisol change for partners of 'parenting more difficult' women: 14.44 μg/dL, SE

= 5.46 μg/dL, n = 7; mean cortisol change for partners of 'parenting not difficult' women: 2.92 μg/dL, SE = 0.97 μg/dL, n = 9).

When mothers or fathers rated difficulty with parenting to be higher than their mate's rating, the change in their hormone levels were compared to couples that each provided the same rating. The only difference between these groups for any of the short-term hormone changes was for women who rated parenting to be more difficult. These women had a different pattern of change in prolactin levels during the early postnatal stage ($F(1,10) = 5.311$, $p = 0.044$, change in prolactin levels for 'same rating' mothers: 37.53 ng/mL, SE = 29.41, n = 4; change in prolactin levels for 'rating higher than mate' mothers: -14.86 ng/mL, SE = 12.64, n = 8).

3.3.7: Response to recorded baby cries

Mothers and fathers checked off which responses they felt after listening to the taped baby cries, choosing from a list that included 'concerned, anxious, content, irritated, wanted to comfort baby, and other'. Respondents were divided into two groups based on whether they indicated a response, and one-way ANOVAs were carried out on the magnitude of change for the hormones measured (Table 3.5).

Fathers who reported concern in response to the baby cries had significant increases in prolactin levels during the early postnatal visit, compared to a decrease in men who did not report concern. They also showed a decrease in free testosterone levels during the late postnatal visit compared to the men who did not report concern. Fathers who reported excitement in response to the baby cries tape had significant short-term

increases in prolactin levels during the late postnatal stage when compared to fathers who did not report excitement.

Fathers who reported that they felt 'anxious', 'content', and 'wanted to comfort the baby' after hearing the recording of infant cries did not have any differences in magnitude of hormonal change from those fathers who did not report these responses.

No statistically significant differences in short-term hormonal changes were found between mothers who reported that they experienced any of the responses to the baby cries tape and those mothers who did not. There was a statistically non-significant trend towards partners of the women who reported 'wanting to comfort the baby' having a greater decrease in testosterone during the late postnatal stage than partners of those who did not ($F(1,12) = 4.707$, $p = 0.051$, mean testosterone change for partners of women who 'wanted to comfort': -0.035 pg/mL, SE = 0.45, n = 11; mean testosterone change for partners of women who did not 'want to comfort': 2.51 pg/mL, SE = 1.65, n = 3).

3.3.8: Heart rate responses

Heart rate monitors recorded the heart rate at each minute of the home visit for men. This information was recorded in order to ascertain whether certain events during the infant stimuli test (i.e. infant cries, having a finger stick sample taken) caused changes in heart rates. However, the rate per minute is quite a crude measure of changes over time, thus, the only information used from these recordings was the average heart rate per visit.

The average heart rate of men in the pregnant experimental group changed significantly over the three stages of pregnancy (repeated measures ANOVA: $F(2,22) = 5.069$, $p = 0.015$, $n = 12$, Figure 3.6). Post-hoc paired t-tests on the difference in heart rate from stage to stage showed a significant increase in heart rate from the late prenatal to the late postnatal stage (paired $t(12) = -2.917$, $p = 0.013$, late prenatal mean beats per minute: 71.00, SE = 2.26; late postnatal mean beats per minute: 77.92, SE = 2.89, $n = 13$) and a trend towards an increase in heart rate from the early to the late postnatal stage (paired $t(13) = -1.980$, $p = 0.069$, early postnatal mean beats per minute: 74.2, SE = 2.37; late postnatal mean beats per minute: 79.07, SE = 2.70, $n = 14$). There was no statistically significant difference between the late prenatal and the early postnatal stages (paired $t(11) = -1.468$, $p = 0.170$, late prenatal mean beats per minute: 70.75, SE = 2.45; early postnatal mean beats per minute: 73.58, SE = 2.74, $n = 12$).

3.4: Discussion

Short-term prolactin levels increased for women who were exposed to the infant stimuli at the late prenatal stage, but there were no significant changes for the men at this stage. Fleming et *al.* (2002) also found that first-time fathers showed no prolactin increase in response to baby cries. However, Fleming et *al.* (2002) did find that experienced fathers who listened to recorded infant cries showed an increase in serum prolactin levels when compared to either first-time fathers or control men. The current results, in combination with those of Fleming et *al.* (2002), suggest that men may require more parental experience to respond hormonally to infant stimuli than do women.

The men did, however, exhibit significant short-term decreases in prolactin during the early postnatal visit. This stage is also where the men's prolactin baseline levels peaked (see Chapter 2). The new fathers' elevated prolactin baseline levels at this stage may represent a heightened responsiveness or reactivity to the newborn. The short-term declines in prolactin levels are likely a reflection of these baseline elevations. Previous work by Storey et *al.* (2000) showed serum prolactin decreases at each of the four stages that were tested, but the researchers hypothesized that a combination of the between-subjects design and anxiety-induced adrenal activation as a result of the venipuncture sampling procedure may have produced the prolactin decrease.

The observation that no changes in free testosterone occurred at any of the three stages measured was surprising, given that Fleming et *al.* (2002) reported increases in new father's salivary testosterone in response to baby cries, and Storey et *al.* (2000) reported that serum testosterone increased in men during the early postnatal stage. There were also no baseline testosterone changes for these men (see Chapter 2), though other studies have reported decreases in testosterone levels after the birth (Storey et *al.*, 2000; Berg & Wynne-Edwards, 2002).

Given that Storey et *al.* (2000) found overall short-term decreases in cortisol levels for men and women, with the largest decrease in men at the late prenatal stage, the absence of any short-term changes in cortisol levels was not predicted. There were technical difficulties involved in the measurement of blood spot cortisol (discussed in Chapter 2), which may have contributed to a decrease in power once the data was normalized. It is possible that there was an actual lack of short-term change in cortisol

levels, or alternatively, there may have been real differences that could not be detected as a result of the assay problems. The repeated measures design of the current study may also have contributed to cortisol levels remaining relatively stable. Couples had already been sampled once at the prenatal class, and were comfortable with the procedure by the late prenatal visit. Storey et *al.* (2000) used a between-subjects design, so the blood sampling was a novel event for those couples at each stage and cortisol may have been initially high as a result.

Cortisol has been shown to be positively associated with infant bonding in women (Fleming et *al.*, 1993; Fleming et *al.*, 1997), thus increases in short-term cortisol levels might be expected for 'couvade' men who were exposed to infant stimuli. However, men who were classified as 'couvade' based upon a higher number of self-reported 'pregnancy symptoms' had decreases in cortisol levels during the late prenatal visit, compared to short-term increases for 'non-couvade' men. It is not known why the pattern of cortisol change is opposite the predicted direction, though it could be a reflection of some factor besides bonding, such as feeling relaxed during the infant stimuli test. Alternatively, the self-reporting of physiological symptoms may not even be an indicator of a man's identification with his partner's pregnancy, or of his parenting readiness. Some men may simply be more willing to admit to physiological symptoms than others, which may be unrelated to hormones and parenting.

The 'non couvade' men had decreases in testosterone levels during the early postnatal visit, when they held their baby, and the 'couvade' men had relatively stable levels during this stage. Though lowered testosterone levels are often associated with

paternal care, short-term increases in fathers' testosterone levels in response to infant cries have also been previously been reported (Storey et *al.*, 2000; Fleming et *al.*, 2002).

Women who reported parenting to be more difficult than they expected had partners with increases in cortisol levels during the late prenatal stage compared to decreases for men whose mates did not report difficulty. The reason for these prenatal decreases in cortisol levels is unknown. If cortisol in men is associated with bonding, as has been indicated in women (Fleming et *al.*, 1993; Fleming et *al.*, 1997), those men with short-term declines in cortisol levels may not bond as well to their infant, requiring the mother to compensate and perhaps find parenting to be difficult as a result. However, this is purely speculation, and other evidence suggests less of a role for cortisol in fathers and infant bonding than for mothers (Fleming et *al.*, 2002).

Fathers who reported concern in response to the baby cries prenatally had a decrease in free testosterone levels during the late postnatal visit, and an increase in prolactin levels during the early postnatal visit compared to the men who did not report concern. This pattern of decreasing testosterone and increasing prolactin levels is consistent with other studies of fathers. Storey et *al.* (2000) reported that men who reported concern in response to the infant cries had greater short-term reductions in testosterone levels, as well as higher baseline prolactin levels. In the current study, the 'concerned' response occurs at the late prenatal test and the hormonal changes occur postnatally, which suggests that the 'concerned' men are reacting differently even before their babies are born. Whether this is a result of experience, personality, or other unknown factors is difficult to discern.

Similarly, fathers who reported excitement in response to infant cues had significant short-term increases in prolactin levels during the late postnatal stage when compared to fathers who did not report excitement. Again, men who respond in a positive way to infant stimuli at the prenatal stage may be predisposed to show prolactin increases after interacting with their newborns during the postnatal stages.

Other studies have found hormonal changes related to sympathy in mothers (Stallings et *al.*, 2002). The lack of significant changes in mothers is unexpected here, though a more sensitive scale to rate responses instead of a binomial 'yes/no' reply may have aided in detecting potential differences here.

As with the baseline hormone levels in Chapter 2, there was no relationship between the magnitude of change in cortisol and affective ratings of baby smells for either parent. The one previous study that measured men's responses also did not find any such relationship (Fleming et *al.*, 2002). Another study found a relationship between women's baseline cortisol levels and their hedonic ratings of infant smells (Fleming et *al.*, 1997), but later work did not find any relationship (Stallings et *al.*, 2001). No studies have looked at cortisol change in relation to hedonic ratings for mothers. As discussed in Chapter 2, the current study may have asked parents to rate how attractive they found infant odours to be at an inappropriate time (i.e. too late with respect to the birth), as previous studies on this topic have been conducted shortly after birth (Fleming et *al.*, 1997; Stallings et *al.*, 2001; Fleming et *al.*, 2002).

Heart rate increased over time for the fathers, with the only post-hoc significant increase between the late prenatal and the late postnatal visits. According to Corter and

Fleming (1995), this increase may reflect the "salience" of the infant or "a preparatory response" to the infant. Alternatively, the increase may reflect increased physical movement by the fathers. These men hold their two-month old baby in the last visit, which presumably required some shifting and moving positions throughout the thirty-minute period. The increase, then, may not reflect a true physiological response to the infant, but rather an increase in physical exertion and, thus, subsequent increases in heart rate.

One problem with the current study was the lack of sensitivity in heart rate monitoring. Stallings et *al.* (2001) used sensitive heart rate monitors that recorded at 5-second intervals, and found that mothers who reported greater sympathy to recorded baby cries had higher heart rates. Heart rates were recorded here with the intention of finding out if certain events throughout the visit were related to either accelerations or decelerations in heart rates. However, one recording per minute was not sensitive enough to detect changes, and so an average value per visit was used instead. Future work on this topic will employ a more sensitive monitor to further explore what effect these stimuli have on parents', and especially fathers', autonomic response.

Another potential problem with the current study is that both hunger and pain cries were combined in the audio recording for the infant stimuli test. Previous work has shown fathers report a greater need to respond to pain cries than to hunger cries (Fleming et *al.*, 2002). Further, multiparous mothers responded more sympathetically than first-time mothers to pain cries relative to hunger cries (Stallings et *al.*, 2001). As the pain

cries invoke a more dramatic response in both mothers and fathers, it would have been better to only have the pain type cries on the test tape.

3.5: References

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Table 3.1 - Mean prolactin difference +/- standard error of the mean (n: number of participants) and percentage prolactin change from the first to the second finger stick blood sample.

	MEN		WOMEN	
	Pregnant experimental	*Non-pregnant control*	*Pregnant experimental*	*Non-pregnant control*
Late Prenatal	-0.29 +/- 0.58, n = 11 % change = 6.38 t(10) = -0.493 p = 0.633	-0.89 +/- 0.82, n = 7 % change = 6.58 t(6) = -1.087 p = 0.319	**25.88 +/- 9.69, n = 11 % change = 20.69 t(10) = 2.670 p = 0.024**	-0.52 +/- 2.31, n = 7 % change = 0.48 t(6) = -0.227 p = 0.828
Early Postnatal	**-1.35 +/- 0.49, n = 21 % change = 10.0 t(20) = -2.758 p = 0.012**	-1.07 +/- 0.66, n = 7 % change = 8.92 t(6) = -1.617 p = 0.157	4.41 +/- 7.93, n = 20 % change = 10.74 t(19) = 0.556 p = 0.585	-1.01 +/- 1.52, n = 7 % change = 8.52 t(6) = -0.664 p = 0.531
Late Postnatal	0.34 +/- 0.78, n = 16 % change = 1.05 t(15) = 0.441 p = 0.666		-5.03 +/- 5.29, n = 16 % change = 1.20 t(15) = -0.952 p = 0.356	

*** Significant results for paired t-tests ($p < 0.05$) are in bold text, units for prolactin are ng/mL.**

Table 3.2 - Mean free testosterone difference +/- standard error of the mean (n: number of participants) and percentage testosterone change from the first to the second finger stick blood sample.

	MEN	
	Pregnant experimental	*Non-pregnant control*
Late Prenatal	0.77 +/- 0.70, n = 11 % change = 9.82 t(10) = 1.091 p = 0.301	0.88 +/- 0.72, n = 7 % change = 4.51 t(6) = 1.215 p = 0.270
Early Postnatal	0.62 +/- 0.40, n = 21 % change = 8.62 t(20) = 1.524 p = 0.143	1.32 +/- 0.62, n = 7 % change = 16.62 t(6) = 2.117 p = 0.079
Late Postnatal	-0.25 +/- 0.70, n = 16 % change = 1.03 t(15) = -0.360 p = 0.724	

*** No significant t-tests ($p < 0.05$), free testosterone units are pg/mL**

Table 3.3 - Mean cortisol difference +/- standard error of the mean (n: number of participants) and percentage cortisol change from the first to the second finger stick blood sample.

	MEN		WOMEN	
	Pregnant experimental	*Non-pregnant control*	*Pregnant experimental*	*Non-pregnant control*
Late Prenatal	-0.78 +/- 1.19, n = 11 % change = 25 t(10) = -0.590 p = 0.568	1.39 +/- 1.21, n = 7 % change = 12.84 t(6) = 1.150 p = 0.294	-3.31 +/- 2.24, n = 11 % change = 5.78 t(10) = 1.409 p = 0.189	1.11 +/- 0.84, n = 7 % change = 6.13 t(6) = 1.323 p = 0.234
Early Postnatal	-0.62 +/- 0.65, n = 21 % change = 2.19 t(20) = 0.963 p = 0.347	2.95 +/- 1.86, n = 7 % change = 13.05 t(6) = 1.592 p = 0.162	-3.31 +/- 2.24, n = 20 % change = 4.49 t(19) = 0.449 p = 0.658	1.37 +/- 0.96, n = 7 % change = 11.18 t(6) = 1.427 p = 0.204
Late Postnatal	0.7220 +/- 2.45, n = 16 % change = 18.6 t(15) = -0.295 p = 0.772		-0.79 +/- 0.84, n = 16 % change = 3.01 t(15) = 0.946 p = 0.359	

*** No results are statistically significant ($p < 0.05$), units for cortisol are µg/dL.**

Table 3.4 – Correlations of the magnitude of change of each hormone for men and women at all stages (only statistically significant results at $p < 0.05$ are presented).

			MEN			WOMEN
			Late Prenatal		*Late Postnatal*	*Late Postnatal*
			ΔProlactin	ΔTest-osterone	ΔCortisol	ΔCortisol
MEN	*Late prenatal*	ΔCortisol			$R^2 = 0.636$, $p = 0.035$, $n = 11$	
	Early postnatal	ΔProlactin	$R^2 = -0.654$, $p = 0.029$, $n = 11$			
		ΔCortisol			$R^2 = 0.556$, $p = 0.025$, $n = 16$	
WOMEN	*Late prenatal*	ΔCortisol			$R^2 = -0.766$, $p = 0.006$, $n = 11$	
	Early postnatal	ΔProlactin				$R^2 = 0.562$, $p = 0.0029$, $n = 15$
	Late postnatal	ΔProlactin		$R^2 = -0.741$, $p = 0.009$, $n = 11$		

Table 3.5: Father's self-reported responses to recorded baby cries and their short-term hormonal change.

	Yes	No	t	P
EARLY POSTNATAL				
***'Concerned'* prolactin (ng/mL)**	0.98 +/- 1.04 N = 5	-1.58 +/- 0.52 N = 9	F(1,12) = 6.180	0.029
LATE POSTNATAL				
***'Concerned'* free testosterone (pg/mL)**	-2.64 +/- 0.84, N = 5	0.88 +/- 0.94, N = 9	F(1, 11.40) = 7.847	0.017
***'Excited'* prolactin (ng/mL)**	5.11 +/- 5.11, N = 2	-0.06 +/- 0.50, N = 12	F(1,12) = 6.431	0.026
OTHER RESPONSES (no statistically significant differences)				
Anxiety	N = 9	N = 5		
Content	N = 6	N = 8		
Wanted to comfort baby	N = 6	N = 8		

* Only the statistically significant results ($p < 0.05$) are reported.

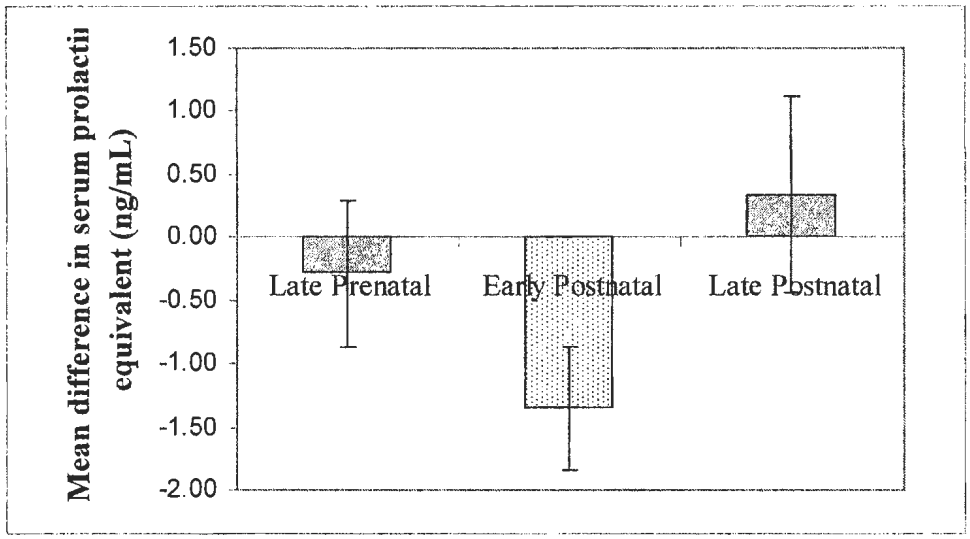

Figure 3.11 – Pregnant experimental group men's mean change in prolactin over a 30-minute test of infant stimuli. Prolactin significantly decreased in men at the early postnatal stage only (paired $t(20) = 2.758$, $p = 0.012$, $n = 21$). Error bars represent standard error.

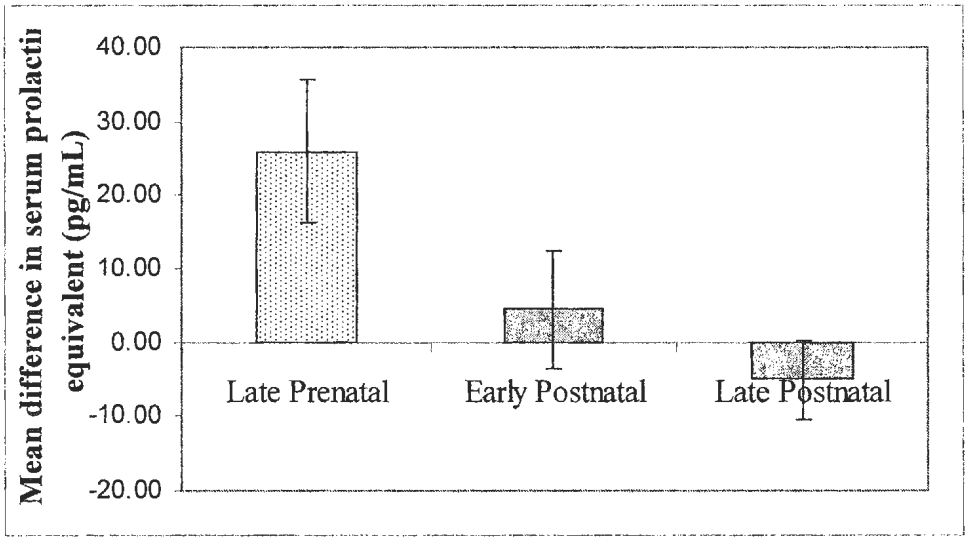

Figure 3.12 – Pregnant experimental group women's mean change in prolactin over a 30-minute test of infant stimuli. Prolactin significantly increased in women at the late prenatal stage only (paired t(10) = -2.670, p = 0.024, n =11). Error bars represent standard error.

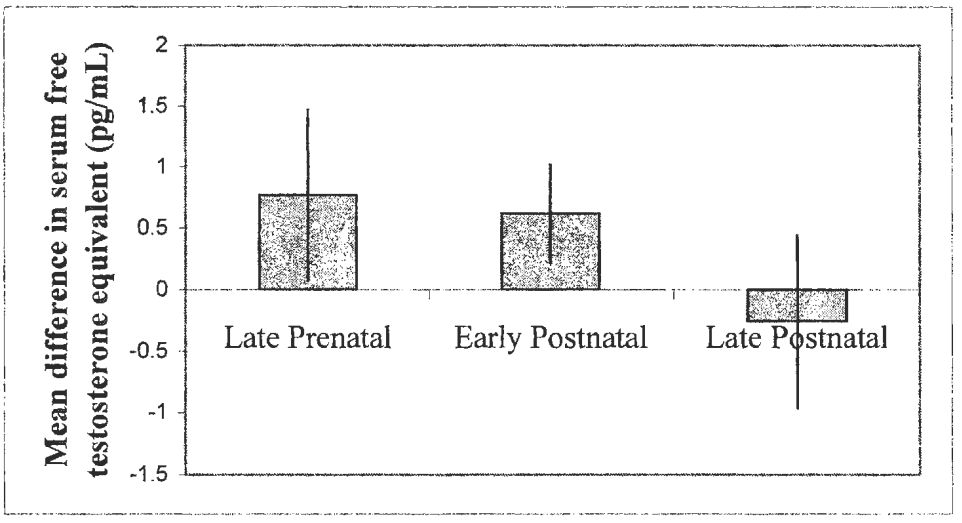

Figure 3.13 – Pregnant experimental group men's mean change in testosterone over a 30-minute test of infant stimuli. There were no significant short-term changes at any stages. Error bars represent standard error.

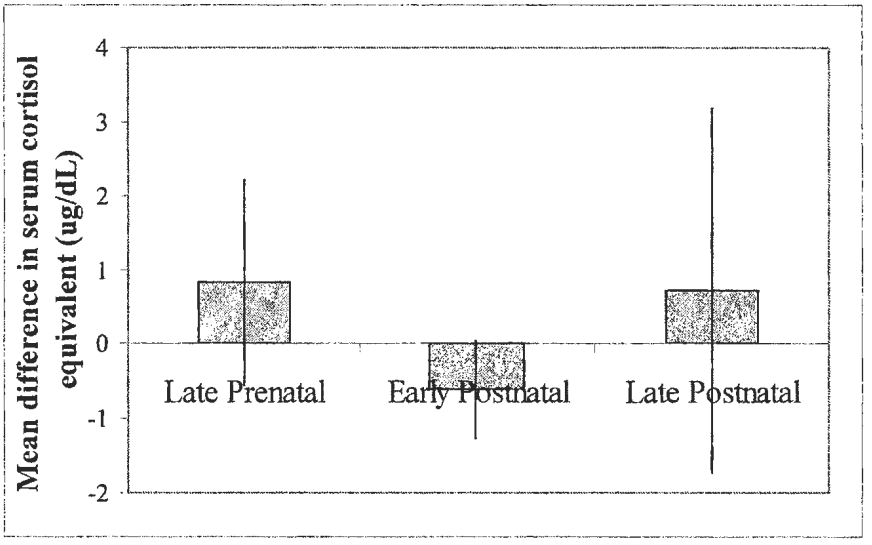

Figure 3.14 – Pregnant experimental group men's mean change in cortisol over a 30-minute test of infant stimuli. There were no significant changes at any stages. Error bars represent standard error.

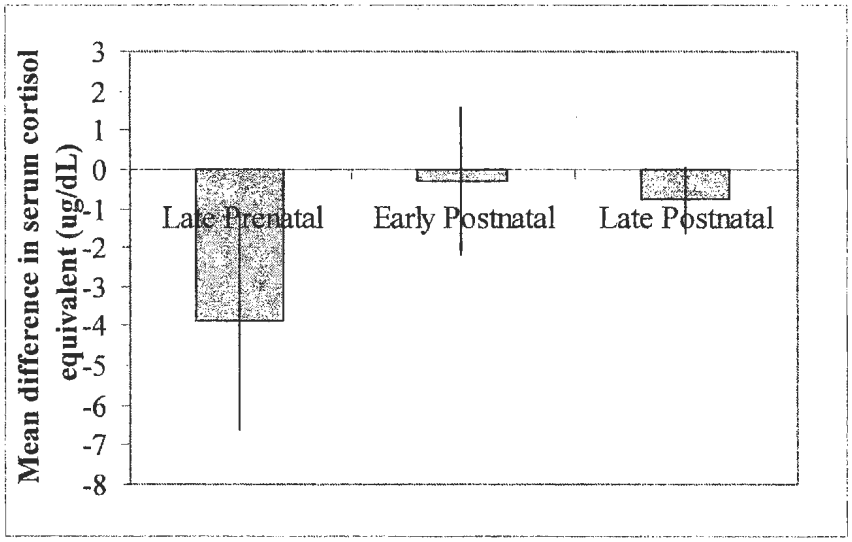

Figure 3.15 – Pregnant experimental group women's mean change in cortisol over a 30-minute test of infant stimuli. There were no significant changes at any stage. Error bars represent standard error.

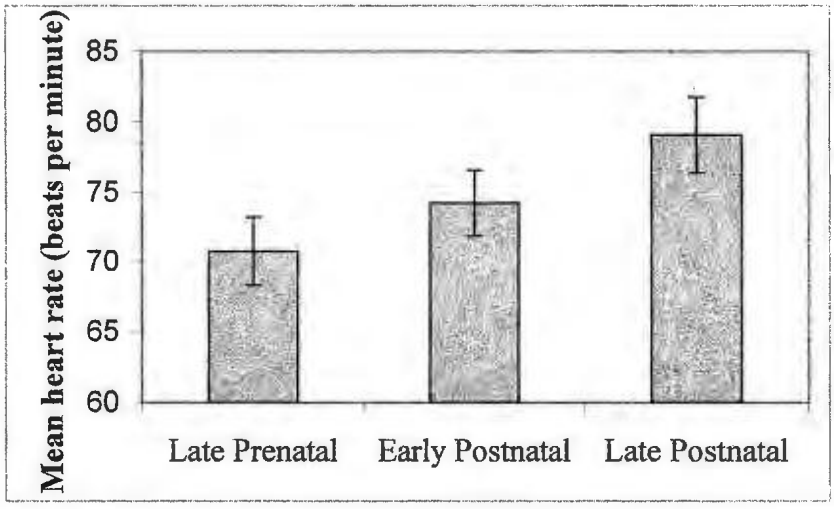

Figure 3.16 – Men's mean heart rate at each approximately 30-minute visit. Heart rate changed significantly over time ($F(2,22) = 5.069$, $p = 0.015$, $n = 12$). Post-hoc tests showed the significant change was between the late prenatal and the late postnatal stages. Error bars represent standard error.

Chapter 4 – Summary

Though men do not experience the dramatic hormonal changes that their partners do throughout pregnancy and the months following birth, it is clear that they do experience more subtle hormonal shifts throughout this period. These changes are linked to emotional responses to infant stimuli, and also to the match between their expectations about the difficulties of parenting and the subsequent realities.

Men showed significant baseline prolactin changes, with the highest levels in the two weeks after the birth. This peak occurred later than the women's prolactin peak, which was at the late prenatal stage. The increases in prolactin levels in women occur as a result of the pregnancy, and are well-established. Men may experience the highest levels of prolactin later than the women as a result of cues from her, from his newborn baby, or most probably from a combination of the two. Regardless of the stimuli, men do experience significant physiological changes around the time of birth of their first child. In contrast, men in the non-pregnant control group showed no changes in prolactin levels.

Men's prolactin levels decreased after they held their babies for 30 minutes during the early postnatal visit. Since increases in prolactin are usually associated with parental activities in most species, the decrease for men at this stage was unexpected. The baseline prolactin levels, however, were highest for men at this stage, and the short-term decrease may actually reflect these heightened baseline levels.

In the baseline cortisol levels, there is evidence that the men may be taking some type of cue, either social/emotional or physiological, from their partners. The baseline cortisol levels were correlated for partners at all four stages in the pregnant group, but not

for non-pregnant control couples. Though difficulties were encountered in the measurement of cortisol, the data for both the pregnant and the control groups was normalized in the same manner, and thus, the significant correlation for one group and not the other indicates a true difference.

The most meaningful results from the ratings that couples provided on the questionnaire were the ratings of parenting difficulty. Women who reported parenting to be more difficult than they expected had higher baseline cortisol levels at all stages except the late prenatal stage, and their partners had higher baseline cortisol levels at the early prenatal and early postnatal stages. The question remains as to whether these elevated cortisol levels result from stress about parenting difficulties post-birth and/or expected difficulties in the prenatal stages. Alternatively, these elevated cortisol levels may indicate a high level of anxiety that eventually results in the parenting difficulties.

Changes in short-term hormone levels differed for individuals with different emotional responses to the baby cries tape. The men who reported nurturing responses to the baby cries tape exhibited a pattern of hormonal change that is consistent with predictions for biparental species. Men who reported 'concern' showed a short-term increase in prolactin levels at the early postnatal stage, and a decrease in testosterone levels at the late postnatal stage.

This is the first study to investigate the longitudinal changes in prolactin levels for men and women around the time of birth of their first child. Further work to replicate the results is necessary. The current study has begun to investigate which factors are

important in the hormonal and physiological responses of men, and future work can build upon this knowledge.

Newer blood collection products are now available that allow for serum to be obtained from taking finger sticks (BD Microtainer™ tubes). This will allow future research to continue to utilize the minimally-invasive method of finger sticks, but to obtain serum instead of whole blood dried on filter paper. This method avoids the pitfalls of saliva sampling, and also avoids the pitfalls of the modified cortisol measurement method used here.

Though 21 pregnant couples were involved in the study, obtaining repeated data throughout all the sampling stages was a problem. Future work should focus on recruiting couples earlier in their pregnancy, as the number of subjects at the early prenatal stage was small. Additionally, when women give birth later than their expected due date, a second late prenatal blood sample should be collected to ensure that one baseline sample falls within the last two weeks before birth.

The infant stimuli test should be modified based on information in other studies that have been published since this work began. First, Fleming et *al.* (2002) have shown a difference in hormonal reactivity to infant hunger and pain-type cries, with pain cries eliciting a more dramatic response. These two types of cries are mixed together in our tape, and perhaps testing couples using only one type of cry would show clearer differences in hormonal reactivity. Second, Berg & Wynne-Edwards (2002) have reported that men had a drop in salivary testosterone levels in the week before birth to the week after. The current study did not take blood samples that close to the time of birth,

but the drop in men's free testosterone from the late prenatal to the early postnatal stage was almost significant. Had more men been included in the repeated-measures test, or perhaps if free testosterone had been measured a little closer to the time of birth, the difference may have been significant. Finally, if future studies plan to measure heart rates in men, heart rate monitors that are capable of sensitive recording and data storage should be used.

In summary, men experience clear hormonal changes around the time of birth of their first child. This research is just the beginning of investigating these patterns of hormonal change that men experience during this period. With more sensitive collection methods, and building upon the current findings, a clearer picture can emerge in future research.

4.1 References

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Appendix 1 - Late prenatal home visit questionnaire – both mother and father

Date_____ Sex_____ Couple Code _____

(1) Check all of the following changes, if any, that *you* have experienced since the pregnancy began:

Anxiety _____
Happiness _____
Nausea _____
Weight loss _____
Decrease in appetite _____
Indigestion _____
Irritability _____
Depression _____
Increase in appetite _
Fatigue _____
Weight gain _____
Muscle aches _____

(2) Check all of the following changes, if any, that you think *your partner* has experienced since the pregnancy began:

Anxiety _____
Happiness _____
Nausea _____
Weight loss _____
Decrease in appetite _____
Indigestion _____
Irritability _____
Depression _____
Increase in appetite _
Fatigue _____
Weight gain _____
Muscle aches _____

(3) How much time do you expect to spend caring for your baby (e.g., feeding, changing diapers, bathing, etc.)

1	2	3	4	5
much less than my partner	somewhat less than my partner	same amount as my partner	somewhat more than my partner	much more than my partner

(4) How much time do you expect to spend playing with your baby?

1	2	3	4	5
much less than my partner	somewhat less than my partner	same amount as my partner	somewhat more than my partner	much more than my partner

(5) Compared to when you first found out about the pregnancy, do you now think more about being a good parent?

1	2	3	4
no change	a little more	moderately more	much more

(6) Do you now feel closer to, or more attached to, your unborn child compared to when you first found out about the pregnancy?

1	2	3	4
no change	a little more	moderately more	much more

(7) Since the last time you completed our questionnaire, have you and your partner spent any extended period of time away from each other (more than a three day period)?

1	2
yes	no

How long was this period? _____

Approximately which dates?_____

(8) From now until two months postnatal, do you and your partner anticipate having any extended period of time apart (more than a three day period)?

1	2
yes	no

Approximately which dates will this occur?

_____

For how long? _____

(9) Compared to before the pregnancy, do you now feel closer to your partner?

1	2	3	4	5
less close	no change	somewhat closer	moderately closer	much closer

(10) How did you feel when you were listening to the tape of the baby cries? Check as many as you feel are appropriate:

Initially, I felt:

Concerned _____ Content _____ Irritated _____ Excited _____ Anxious _____ Wanted to comfort baby _____ Other (please specify)_____

Was there any change by the end of the tape?

_____
_____
_____
_____
_____

(11) How would you rate your anxiety about the fingerstick procedure?

1	2	3	4	5
none	very low	low	high	very high

Appendix 2 Late postnatal home visit questionnaire – father

Postnatal Questionnaire - DAD

Date _____ Couple Code _____

(1.) Were you present for your baby's birth? _____

(2.) Approximately how long after the birth did you first hold your baby?

_____

(3.) If your baby is (sometimes or always) bottle-fed, how recently did you feed your baby?

1	2	3	4
in the past 2 hours	2-4 hours ago	4-6 hours ago	more than 6 hours ago

(4.) Approximately how much time have you spent holding your baby in the past four hours?

1	2	3	4
less than 15 minutes	about 30 minutes	about an hour	more than an hour

(5.) Roughly how many hours in total have you spent in contact with your baby *today*?

_____

(6.) Approximately how much of the **daytime feeding** are you currently doing?

1	2	3	4	5
very little or none	somewhat less than my partner	about the same as my partner	somewhat more than my partner	most or all of the feeding

(7.) Approximately how much of the **nighttime feeding** are you currently doing?

1	2	3	4	5
very little or none	somewhat less than my partner	about the same as my partner	somewhat more than my partner	most or all of the feeding

(8.) Approximately how much of the **bathing and diaper changing** are you currently doing?

1	2	3	4	5
very little or none	somewhat less than my partner	about the same as my partner	somewhat more than my partner	most or all

(9.) Approximately how much **playing with or talking to** your baby are you currently doing?

1	2	3	4	5
very little or none	somewhat less than my partner	about the same as my partner	somewhat more than my partner	most or all

(10) Approximately how much **household chores** (cleaning and tidying the house, doing laundry, etc.) are you currently doing?

1	2	3	4	5
very little or none	somewhat less than my partner	about the same as my partner	somewhat more than my partner	most or all of the chores

(11) How has the time you spend doing household chores changed from before the baby was born?

1	2	3	4
I am doing less	No change at all	I'm doing a bit more	I'm doing a lot more

(12) Approximately how much **cooking/ meal preparation** are you currently doing?

1	2	3	4	5
very little or none	somewhat less than my partner	about the same as my partner	somewhat more than my partner	most or all of the cooking

(13) How has the time you spend cooking or preparing meals changed from before the baby was born?

1	2	3	4
I am doing less	No change at all	I'm doing a bit more	I'm doing a lot more

(14) How has the time you spend involved in social or community organizations and hobbies changed from before the baby was born?

1	2	3	4
I am doing less	No change at all	I'm doing a bit more	I'm doing a lot more

(15) How much help are you getting from people other than you and your partner?

1	2	3
none	some	lots

(16) Are the other helpers mostly relatives? Yes_____ No_____

(17) Compared to before the pregnancy and birth of your baby, how has your feeling of closeness towards your partner changed?

1	2	3	4	5
I don't feel as close at all	I feel a little less close	There has been no change	feel a little more close	I feel much more close

(18) In the first weeks after you brought your baby home, did you feel that you were well prepared for parenthood?

1	2	3	4
I felt really unprepared	I felt a bit unprepared	There were no major surprises	I felt really well prepared

(19) How do you feel when you hear your baby cry?

1	2	3
Slightly concerned, want to comfort	Moderately concerned, want to comfort	Really concerned, need to immediately respond

(20) Since the birth of your baby:

Are you more responsive to **other** babies?	Much more ___	A little more ___	Not really __
Do you **talk about** babies more?	Much more __	A little more __	Not really __
Do you **think about** babies more?	Much more __	A little more ___	Not really __
Do you find baby smells to be more attractive?	Much more __	A little more ___	Not really __

(21) Would you describe your baby as being easier, about average, or more difficult to care for than other babies you've known?

1	2	3
Easier	About average	More difficult

(22) Is being a parent as difficult as you expected it would be?

1	2	3	4	5
Much less difficult	Somewhat less difficult	About what I expected	A little more difficult	Much more difficult

(23) Prescription medications containing some steroids (cortisol, hydrocortisone, prednisone, and prednisolone) interfere with the way we measure the cortisol blood hormone in your blood sample. These include inhalers containing steroids, as well as some prescription cremes, and eye, ear, and nasal suspensions. Since the first time we took a finger-prick blood sample from you, have you been taking either of these medications? (If you are not certain if a drug you were taking contained steroids, consult one of the investigators for a full list)

Yes, I took a medication containing steroids _____ No, I did not _____

If you answered yes to the above, approximately what dates were (or are) you taking the medication ?

_____

(24) How would you rate your anxiety about the fingerstick procedure?

1	2	3	4	5
none	low	moderate	high	very high

Postnatal Questionnaire - MOM

Date _____ Couple Code

_____

(1) What date was your baby born? _____

(2) What was your due date? _____

(3) Was your baby a boy or a girl? _____

(4) Was your labour induced? _____

(5) Did you have a Cesarean section? _____

(6) Did you initially try breast feeding your baby? _____

(7) Have you continued to breast feed your baby? _____

(8) If you are still breast feeding, how is it going?

1	2	3	4
no problems	initially difficult, but fine now	still minor problems	still major problems

(9) If you are breast feeding, when was the last time you nursed?

_____ When was the last time you expressed or pumped milk?_____

(10) Is your baby regularly given any food or formula? _____

(11.) If your baby is (sometimes or always) bottle-fed, how recently did you feed your baby?

1	2	3	4
in the past 2 hours	2-4 hours ago	4-6 hours ago	more than 6 hours ago

(12.) Approximately how much time have you spent holding your baby in the past four hours?

1	2	3	4
less than 15 minutes	about 30 minutes	about an hour	more than an hour

(13.) Roughly how many hours in total have you spent in contact with your baby *today*?

_____

(14.) Approximately how much of the **daytime feeding** are you currently doing?

1	2	3	4	5
very little or none	somewhat less than my partner	about the same as my partner	somewhat more than my partner	most or all of the feeding

(15.) Approximately how much of the **nighttime feeding** are you currently doing?

1	2	3	4	5
very little or none	somewhat less than my partner	about the same as my partner	somewhat more than my partner	most or all of the feeding

(16.) Approximately how much of the **bathing and diaper changing** are you currently doing?

1	2	3	4	5
very little or none	somewhat less than my partner	about the same as my partner	somewhat more than my partner	most or all

(17.) Approximately how much **playing with or talking to** your baby are you currently doing?

1	2	3	4	5
very little or none	somewhat less than my partner	about the same as my partner	somewhat more than my partner	most or all

(18) Approximately how much **household chores** (cleaning and tidying the house, doing laundry, etc.) are you currently doing?

1	2	3	4	5
very little or none	somewhat less than my partner	about the same as my partner	somewhat more than my partner	most or all of the chores

(19) How has the time you spend doing household chores changed from before the baby was born?

1	2	3	4
I am doing less	No change at all	I'm doing a bit more	I'm doing a lot more

(20) Approximately how much **cooking/ meal preparation** are you currently doing?

1	2	3	4	5
very little or none	somewhat less than my partner	about the same as my partner	somewhat more than my partner	most or all of the cooking

(21) How has the time you spend cooking or preparing meals changed from before the baby was born?

1	2	3	4
I am doing less	No change at all	I'm doing a bit more	I'm doing a lot more

(22) How has the time you spend involved in social or community organizations and hobbies changed from before the baby was born?

1	2	3	4
I am doing less	No change at all	I'm doing a bit more	I'm doing a lot more

(23) How much help are you getting from people other than you and your partner?

1	2	3
none	some	lots

(24) Are the other helpers mostly relatives? Yes_____ No_____

(25) Compared to before the pregnancy and birth of your baby, how has your feeling of closeness towards your partner changed?

1	2	3	4	5
I don't feel as close at all	I feel a little less close	There has been no change	feel a little more close	I feel much more close

(26) In the first weeks after you brought your baby home, did you feel that you were well prepared for parenthood?

1	2	3	4
I felt really unprepared	I felt a bit unprepared	There were no major surprises	I felt really well prepared

(27) How do you feel when you hear your baby cry?

1	2	3
Slightly concerned, want to comfort	Moderately concerned, want to comfort	Really concerned, need to immediately respond

(28) Since the birth of your baby:

Are you more responsive to **other** babies?	Much more ___	A little more ___	Not really __
Do you **talk about** babies more?	Much more __	A little more __	Not really __
Do you **think about** babies more?	Much more __	A little more ___	Not really __
Do you find baby smells to be more attractive?	Much more __	A little more ___	Not really __

(29) Would you describe your baby as being easier, about average, or more difficult to care for than other babies you've known?

1	2	3
Easier	About average	More difficult

(30) Is being a parent as difficult as you expected it would be?

1	2	3	4	5
Much less difficult	Somewhat less difficult	About what I expected	A little more difficult	Much more difficult

(31) Prescription medications containing some steroids (cortisol, hydrocortisone, prednisone, and prednisolone) interfere with the way we measure the cortisol blood hormone in your blood sample. These include inhalers containing steroids, as well as some prescription cremes, and eye, ear, and nasal suspensions. Since the first time we took a finger-prick blood sample from you, have you been taking either of these medications? (If you are not certain if a drug you were taking contained steroids, consult one of the investigators for a full list)

Yes, I took a medication containing steroids _____ No, I did not _____

If you answered yes to the above, approximately what dates were (or are) you taking the medication ? _____

(32) How would you rate your anxiety about the fingerstick procedure?

1	2	3	4	5
none	low	moderate	high	very high