The University of Alberta

The Relationship between Ovarian Hormones and the Behavior of Japanese Macaque Females (*Macaca fuscata*) during the Mating Season

by

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ABSTRACT

The relationship between ovarian hormones and behavior of the Arashiyama West Japanese macaque females (*Macaca fuscata*) were examined using enzyme immunoassays (EIA) of fecal steroid metabolites. The hormone profiles of estrone-glucuronide (E1) and pregnanediol-glucuronide (PdG) delineated the three phases of ovarian cycles: follicular, periovulatory and luteal. The study females displayed increased attractivity and proceptivity, for both inter-sex and same-sex interactions, during the follicular and periovulatory phases of the ovarian cycle. Males and females also showed an increase in aggressive and affiliative patterns during the follicular and periovulatory phases, corresponding with increased inter-sex associations. Aggressive and affiliative behaviors among females also varied depending on the female's reproductive patterns. Post-conceptive mounting behavior showed a negative correlation with PdG levels, and females displayed a peak in sexual activity between the 6th and 10th weeks of gestation. This study demonstrates that ovarian hormones not only precipitate sexual behavior, but also elicit non-conceptive behavior in Japanese macaque females.

DEDICATION

This thesis is dedicated to
my daughter Sorcha

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Chapter One

GENERAL INTRODUCTION

Traditionally the study of non-human primate behavior has been divided into field studies and laboratory studies. This dichotomy has mainly been due to the difficulties associated with the collection of biological data from free-ranging primates, particularly for endocrine studies. Yet, without the examination of underlying physiological mechanisms, studies of primate behavior are incomplete. Field scientists have generally had to limit examinations of reproductive state and function to observations of sexual behavior and perineal swellings. However, gonadal hormones are a more direct index of ovarian activity, and hormone levels profile the actual endocrine function, giving a direct measure of physiological state. Serum hormone analysis has routinely been the most reliable indicator of reproductive state or ovarian function in most primate species, including humans (Speroff et al., 1989). Although this is frequently the method of choice for humans, it has been observed that venipuncture is not always a practical option for studying physiological function in nonhuman, free-ranging primates. However, measurable quantities of circulating steroids are excreted in both feces (Adlercreutz and Martin, 1976; 1978; Risler, et al., 1987) and urine (Eastman et al., 1984; Speroff et al., 1989). Circulating steroids are metabolized in the liver to less active and conjugated metabolites and excreted into bile. Conjugation reduces the lipid solubility of the steroids, preventing their reabsorption across the cell membranes. Intestinal ß-glucuronidase can hydrolyze conjugates to active and resorbable steroids (Adlercreutz et al., 1976). Once the steroids and steroid metabolites reach the bowel, they are excreted in feces.

An important aspect of bile excretion and fecal excretion of steroids and steroid metabolites, however, is that the process is species-specific. Therefore, identification of excreted steroids and steroid metabolites must be undertaken for each species examined. The proportions of conjugated and unconjugated steroids in the feces is also species specific. In humans, 98% of the fecal steroids and steroid metabolites excreted in the feces are unconjugated, in contrast to the high amounts (98%) of conjugated steroids and steroid metabolites in human urine (Adlercreutz and Martin, 1976). In fact some nonhuman primate species excrete even higher percentages of unconjugated urinary steroids and steroid metabolites (Ziegler et al., 1989).

Recent noninvasive methodologies have not only made it possible to study endocrine function in free-ranging primates, but also advanced the development of methods for extraction and preservation of estradiol and progesterone from fecal material. In Old and New World monkeys, fecal steroids are excreted in large quantities and are often unconjugated (Shideler et al., 1989). Recent developments in chromatography, radio and enzyme immunoassay techniques have made it feasible to identify and quantify even small quantities of these steroids. By correlating sexual behavior with physiological function, we are able to apply both a proximate and an ultimate approach to questions regarding the social and biological determinants of behavior. A fecal steroid extraction method can provide information for a socio-endocrinological approach to the study of free-ranging, nonhuman primates. By examining the interaction between hormones, physiologic regulation, and social environment we obtain a broader understanding of primate behavior.

Solubilization and enzyme immunoassay methods have proven successful on a number of New and Old World primates including *Macaca* (Shideler et al., 1990, 1993), *Pithecia pithecia* (Shideler et al., 1994), *Papio cynocephalus* (Wasser et al., 1991; Wasser, 1995), *Saimiri sciureus* (Moorman et al., 1994), *Brachyteles arachnoides* (Strier and Ziegler, 1997), and *Callithrix jacchus* (Sousa and Ziegler, 1998). These and other studies have reported highly successful results with the use of fecal steroid measurements on samples collected from free-ranging primates. However, to date there have been no studies on the measurement of fecal ovarian hormones in free-ranging Japanese macaques (*Macaca fuscata*).

According to Beach (1976), there are three observable characteristics of sexual behavior: "attractivity", which refers to a female's effectiveness in evoking sexual responses from the male; "proceptivity", which refers to a female's desire to initiate sexual interaction with a male; and "receptivity", which refers to a female's acceptance of the male's advances. The attractivity of a female is usually measured by the number of sexual solicitations received from males and, in primates, is considered to be regulated by estrogens. Although proceptivity in nonhuman primates has not been attributed to any one hormone (Johnson and Phoenix, 1976; Baum, 1983), Baum (1983) suggests that, unlike the case in non-primate females, progesterone production is dependent on the presence of estrogen in order to have an effect on proceptivity in primates. While female primates may only be receptive for a few days during their menstrual cycle, receptivity appears to be relatively free of hormonal control (Johnston and Phoenix, 1976).

Sexual behavior during the reproductive cycle has been well documented for Old World primates (e.g. Loy, 1987; Rowell, 1972), and estrogen has been shown to be responsible for attractivity in female rhesus macaques (Cochran, 1979; Herbert, 1970; Johnson and Phoenix, 1976; Keverne, 1979; Loy, 1970; Michael and Zumpe, 1970, 1993), Japanese macaques (Enomoto et al., 1979; Michael and Welagalla, 1968; Oshima et al., 1977; Wolfe, 1979), stumptail macaques (Murray et al., 1985), patas monkeys (Loy, 1981), Mangabeys (Gordon et al., 1991), Baboons (Saayman, 1970), and Talapoins (Herbert, 1970). Progesterone has been found to counteract the effects of estrogen, by decreasing the proceptivity and attractivity of the female (rhesus macaques: Baum et al., 1977; Japanese macaques: Enomoto et al., 1979; and stumptail macaques: Steklis and Fox, 1988).

Reproduction in the Female Primate

Three separate components are integrated in the reproductive system, the menstrual cycle, ovarian cycle, and sexual or estrus cycle. The menstrual cycle is a histological process that involves the monthly destruction and renewal of the endometrium (the inner lining of the uterus). The ovarian cycle is a physiological process designed to periodically produce and release mature eggs (ova). Estrus cycles, unlike menstrual and ovarian cycles, are defined behaviorally. Estrus in mammals refers to the period of behavioral and physiological change around the time of ovulation (Beach, 1976). The estrus period is characterized by a cyclic pattern of increased sexual behavior in females and their increased attractivity to males.

Chemical compounds known as hormones govern the reproductive life of nonhuman primate females, as well as human females. The hormonal process influencing reproduction for female primates is initiated from the hypothalamus. The hypothalamus releases *gonadotropin-releasing factor* (GnRH), which travels to the pituitary gland. The anterior of the pituitary gland is responsible for regulating the female cycle, and is a master switch in the hormonal circuitry of female reproduction. Initially the pituitary produces *follicle-stimulating hormone* (FSH) and releases it into the blood stream, where it travels to the target tissue, the ovary. The ovary responds by stimulating several ovum-containing follicles on its surface. One follicle will continue to grow under the stimulation of FSH, while the remainders discontinue their growth. The follicle wall thickens, forming a layer of connective tissue on the outer surface. The outer layer then begins to produce *estrogen*, a hormone important to both reproduction and behavior. During the female cycles, as the follicle grows larger, more estrogen is pumped into the blood stream. Estrogen flows up toward the pituitary day after day indicating the status of the follicle and its egg. When the estrogen levels reach certain levels, the pituitary responds by sending out a surge of *luteinizing hormone*

(LH). The LH surges toward the ovary where it bursts the swollen follicle, releasing the egg to travel down the fallopian tube.

The follicle continues to develop in the absence of the ovum, with some of the cells within the follicle dividing rapidly, filling it to form the *Corpus luteum* (yellow body) which continues the production of estrogen and also produces another hormone, progesterone. The function of the uterus and the cyclic changes in the endometrium are dependent on the presence of these two hormones. Initially estrogen is regulated by the follicle prompting the build up of the endometrium in preparation for conception. Estrogen also stimulates the midcycle surge in LH and FSH, which results in ovulation on or about day 14 of the cycle. Following ovulation, regulation of estrogen is transferred to the corpus luteum, assisting in the final preparation of the uterine wall. Progesterone is secreted during the post-ovulatory or luteal phase, late in the menstrual cycle. During the luteal phase, progesterone production is regulated by the corpus luteum and serves to complete the final maturation of the uterine wall after ovulation. If the egg is not fertilized, the corpus luteum regresses and ceases to excrete progesterone, leading to menstruation. The decline in circulating progesterone triggers the pituitary to secrete FSH and LH and a new cycle begins.

In the event of egg fertilization, the corpus luteum maintains pregnancy during early gestation since it is the primary source of progesterone and estrogen (Albrecht and Pepe, 1988). The length of time secretion continues varies among human and nonhuman primates. In rhesus macaques (*Macaca mulatta*), the ovary is essential until approximately days 14-21 (8% of gestation), after which, the placenta takes over production of progesterone (Albrecht and Pepe, 1988). The placenta assumes the production of estrogen slightly later than that of progesterone (after day 24 in rhesus macaques). In the rhesus monkey estrogen increases progressively with gestation, reaching peak concentrations by term (Hodgen et al., 1972). In rhesus macaques, progesterone values have been reported to fluctuate during the last two thirds of gestation, similar to those in the luteal phase of the ovarian cycle (Neill, et al. 1967). Rapid increases in progesterone immediately prior to parturition have been reported in the bonnet macaque (*Macaca fasicularis*: Stabenfelt and Hendrickx 1973).

The interaction between social, behavioral, environmental, and endocrine processes is responsible for driving reproduction in nonhuman primates. Although the hormonal element controls the physiology of reproduction, affecting behavior and physiology, conversely behavior, environment, and other physiological processes can drive endocrine functions and responses. Socioendocrinology is a conceptual framework, which examines the effects of social environment on the relationship between hormones and behavior (Bercovitch and Ziegler, 1990). This complex interaction between hormones and social environment demonstrates that primate reproductive behavior is not entirely dependent upon hormonal influences. Individual behavior and reproductive success are also products of environment and social context. Consequently, in order to fully understand primate behavior, it is necessary to have an understanding of the hormonal element that drives particular activities, and in turn is affected by those interactions.

The Study Species and Site

The Japanese macaque (*Macaca fuscata*) is indigenous to the islands of Japan, where they are exposed to snow and sub-zero temperatures. The genus *Macaca* exhibits the widest and most northern distribution of any nonhuman primate genus. Japanese macaques live in multi-male, multi-female social groups with female philopatry and male dispersal (Fedigan, 1992). The females typically remain within their natal group throughout their lives, maintaining close bonds with their female kin, resulting in the formation of distinct matrilines. Japanese macaques are considered semi-terrestrial, spending a substantial amount of their time on the ground. The Japanese macaque has been classified as omnivorous, with a diet consisting of fruit, insects, young leaves, crops, and, on occasion, small mammals. Japanese macaques display moderate sexual dimorphism, with males weighing around 15 kg. and females weighing around 12 kg.

The dominance rank among females is relatively stable, with daughters ranking below their mothers in reverse order of their ages (Fedigan, 1992). The dominance rank of males however, is unpredictable once they leave the natal group. Upon entering another troop the new male will typically have a low rank, regardless of their former rank. From there the male is then able to move up in the dominance hierarchy as other males leave or die.

In the early 1950's Japanese scientists began provisioning a troop of Japanese macaques in a mountainous region known as Arashiyama near the city of Kyoto on the island of Honshu. The troop consisted of approximately 45 individuals, which by 1966 had reached 163. At this time the troop fissioned into two smaller groups (Arashiyama A and B). By 1972 Arashiyama A had grown to a population of 159 and had spread its geographic range to include the suburbs of the city of Kyoto. The troop became such a nuisance to local farmers that it was decided the troop must be relocated. On February 23rd, 1972 the entire Arashiyama A troop was relocated to Texas, approximately 50 kilometers northwest of Laredo, and was officially renamed the Arashiyama West troop. The Arashiyama West troop was relocated twice more within Texas, once in 1980 approximately 20 kilometers southeast of the town of Dilley and then again in 1996 to their permanent home approximately 15 kilometers south of Dilley. The Arashiyama West troop resides in a semi free-ranging environment within a 65-acre enclosure, where they are provisioned on a daily basis and also enjoy much of the natural vegetation.

Research Questions

The primary goals of this study were to determine the reliability of fecal steroid analysis as an indication of reproductive status in Japanese macaques (*Macaca fuscata*), and to elucidate any relationship between the behavior of Japanese macaque females and their hormonal status during the mating season. I analyzed the relationship between behavioral and endocrine changes during the female's ovarian cycle in order to: (1) determine if specific male and female behavioral signals are good indicators of the female's reproductive status, (2) find out if there are any cyclic patterns in the social and aggressive behavior of the female Japanese macaque, and (3) determine the reliability of fecal analysis and enzyme immunoassay techniques for evaluating the hormonal status of Japanese macaque females. The following questions are of interest in the present study:

1. Does the frequency of specific sexual behaviors fluctuate over the course of the cycle in Japanese macaque females?
2. Do Japanese macaque females exhibit cycle-related variations in the frequency and duration of affiliative and aggressive behaviors?
3. How does non-conceptive mating behavior (i.e. homosexual mating and mating during pregnancy) relate to ovarian hormone levels in female Japanese macaques?

In chapter two, the influence of ovarian hormones on the sexual behavior of females during the breeding season are presented, through the examination of estrone and pregnanediol levels in the feces correlated with behavioral data. Chapter three discusses the affiliative and aggressive patterns during the mating season in relation to the reproductive status of the females. Chapter four looks at the influence of ovarian hormones on the non-conceptive mating behavior of the female, including post-conceptive and homosexual mating behavior.

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Chapter 2

The Relationship between Ovarian Cycle Phase and Sexual Behavior in Female Japanese Macaques (*Macaca fuscata*)

INTRODUCTION

The sexual behavior of non-primate mammals has been documented to be under relatively strict hormonal control (Baum et al., 1977; Beach, 1942, 1976). However, the extent to which primate sexual behavior is influenced by hormones is not yet fully known. Many laboratory studies have contributed to our knowledge of reproductive and physiological aspects of primate behavior, but field studies provide the essential foundation for investigating the behavioral repertoires and social organizations of primates in the environments for which they are adapted; they also provide the standard data against which results from all other studies can be assessed. However, few opportunities exist in the field for the interventions required to analyze underlying mechanisms. One exception is a set of recent techniques in fecal collection and extraction that allow us to monitor hormone levels of free-ranging animals through enzyme immunoassay (EIA) analysis of fecal steroid metabolites. This study employs such techniques in order to determine the influence of ovarian hormones on the sexual behavior of female Japanese macaques during the mating season.

Sexual behavior in Japanese macaques (*Macaca fuscata*) is concentrated in a discrete mating season that lasts 4 to 6 months beginning in early autumn (Lancaster and Lee, 1965). During the mating season, the monthly reproductive cycles of females take the form of estrous cycles, during which time females are periodically predisposed to mate. In seasonal breeders, the mating season is associated with increased aggression and increased sexual activity (Enomoto, 1981). Throughout the mating season, female Japanese macaques form close associations with males in consort pairs, which are inclined to isolate themselves from the rest of the group. The consort pair spends most of their time travelling, eating, grooming and copulating together. Consortships may last for as little as one hour to as long as two weeks or more. During estrous cycles the behavior of the female is modified, and she will actively seek out males, make sexual invitations and groom males more than at other times of the year (Enomoto, 1981). The male's behavior towards females also changes; he seeks females out, follows them closely, grooms them, and makes more sexual invitations.

During the mating season, female Japanese macaques experience periods of estrus which are associated with distinct changes in behavior. Following common practice, we define estrus as that period of time during which females are motivated and/or willing to mate (Fedigan, 1992). In Japanese macaques, estrus does not occur in a regular pattern; the female may show signs of being in estrus for one day, or for several weeks, and she may come into estrus only once during the mating season, or many times. The physical signs of estrus in Japanese macaque females include a red face and/or a red perineum. All mating activity is believed to take place when the female is in estrus, and it is therefore assumed that ovulation and conception occur during this time (Enomoto, 1981). However, there are no studies documenting any direct associations between the external signs of estrus and the internal endocrine conditions of the Japanese macaque females.

The ovarian cycle consists of three distinct phases: the follicular phase, governed by developing follicles; the periovulatory phase, during the days surrounding the time of ovulation; and the luteal phase, governed by the corpus luteum. The follicular phase is marked by minimal levels of circulating progesterone (often below 0.5 ng/ml), with increases in the progesterone concentrations occurring 1 or 2 days before ovulation. Progesterone secretion then increases markedly during the luteal phase of the cycle. The early follicular phase is also marked by moderate levels of estrogen, which increase by small degrees later in the follicular phase before reaching peak concentrations in the periovulatory phase. Estrogen concentrations then decrease to basal levels during the luteal phase (Figure 2-1).

Solicitations and copulation are known to vary with ovarian cycle phase in a number of primates (e.g. *Brachyteles arachnoides*, Strier and Ziegler 1997; *Cebus apella*, Linn et al. 1995; *Macaca mulatta*, Cochran 1979, Herbert 1970, Johnson and Phoenix 1976, Keverne 1979, Loy 1970, and Michael and Zumpe 1970; *Macaca fuscata*, Enomoto et al. 1979, Michael et al. 1968, Oshima et al. 1977, and Wolfe 1979; *Macaca arctoides*, Murray et al. 1985; *Erythrocebus patas*, Loy 1981; mangabeys, Gordon et al. 1991; baboons, Saayman 1970; and *Microcebus talapoin*, Herbert 1970). Changes in the intensity of sexual activity during the ovarian cycle are commonly observed in most non-human primates, with mating peaks typically occurring at midcycle, or in the periovulatory period, during the presumed time of ovulation (e.g., Beach, 1976; Bercovitch and Goy, 1990).

Beach (1976) described three patterns of the behavior of female mammals when in estrus; attractivity, proceptivity, and receptivity. Attractivity refers to the female's ability to evoke sexual responses from the male. Proceptivity refers to the female's actions toward the male, which correspond to her initiative to maintain or establish sexual interaction. Receptivity is defined as a female's willingness to accept the male's attempts

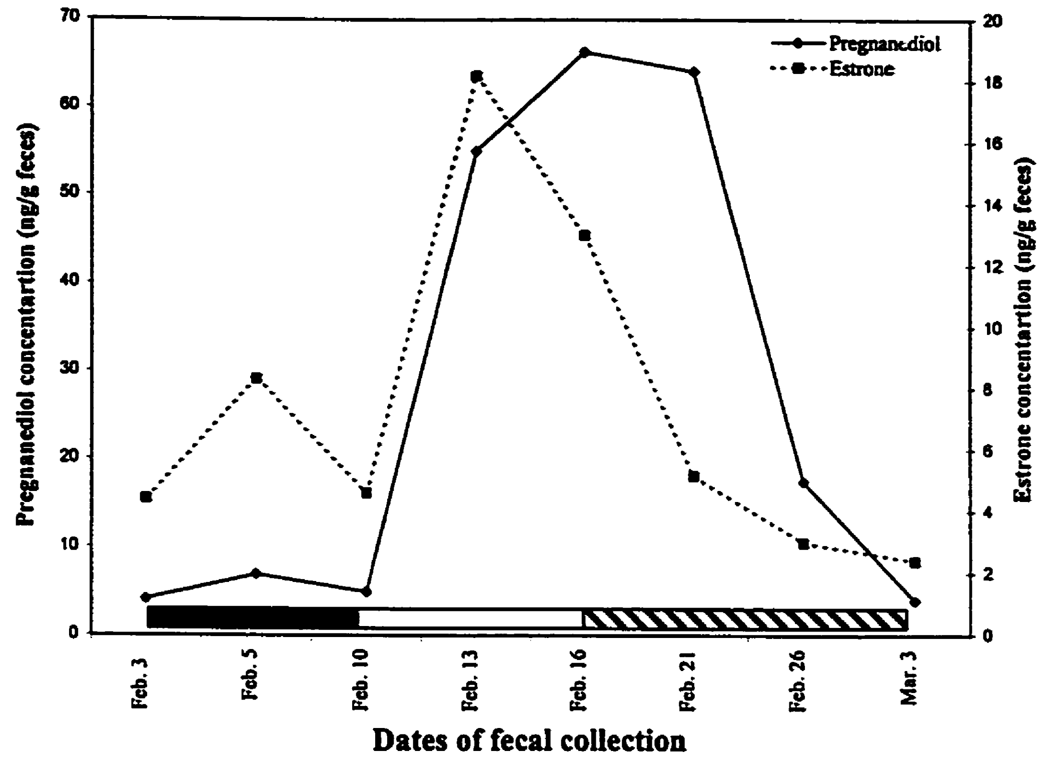

Figure 2-1. One representative ovarian cycle for a Japanese macaque female during the 1997/1998 mating season.

General Hormone levels during three phases of the ovarian cycle

Hormone	**Follicular** ■	**Periovulatory** □	**Luteal** ▨
Pregnanediol (PdG)	Low	Rising	Peak
Estrone (E1)	Rising	Peak	Low

to copulate. These three patterns have been linked to levels of circulating hormones in the following ways: Increased attractivity in females has been demonstrated during the periovulatory phase of the cycle in rhesus monkeys (Michael and Welegalla, 1968), at a time when estrogen levels reach their peak. In addition, the attractivity of ovariectomized monkeys increased significantly when injected with estrogen. It has also been demonstrated that estrogen-induced attractivity is reduced or eliminated by progesterone (Beach, 1976). Proceptivity is manifested through various behavioral interactions initiated by the female including contact, affiliative, and solicitational behaviors *directed* toward the male. Females tend to exhibit proceptive behaviors when they are stimulated by estrogen during the periovulatory phase of the cycle. Conversely, the increased levels of progesterone during the luteal phase function to decrease proceptive behavior in the female. Finally, it has been observed that sexual receptivity depends upon estrogenic stimulation, but the degree of dependence varies considerably in different species (Beach, 1976). However, it has also been suggested that testosterone, primarily of adrenal origin in the female, is the main libidinal hormone responsible for receptivity in primates (Trimble and Herbert, 1968). In some nonhuman primates, such as the chacma baboon, receptivity in the female is strictly limited to the periovulatory period, whereas rhesus females may permit copulation at any stage of the cycle (Saayman, 1970). Therefore, the functional significance of receptive behavior may vary greatly from species to species.

Many studies of ovarian steroids carried out on Japanese macaques and other macaque species have taken an invasive approach and have been accomplished in a captive environment. Although the use of blood serum levels is an effective means of measuring the hormones of interest, this requires that the animal undergo capture on a daily or near daily basis. This form of sample collection likely has an influence on the natural behavior and reproductive physiology of the subjects. In addition, captive populations of nonhuman primates are usually artificially formed and spatial relations among group members are severely altered by captivity. Some studies (e.g. Johnson and Phoenix, 1976; Wallen and Goy, 1977) have focused on the influence of steroids when administered to ovariectomized females. Although these studies have answered many questions about the physiological aspects of reproduction, the influences on the subject's natural behavior remain unresolved. Other studies have identified menstrual cycle characteristics for macaques using various methods such as vaginal smears or simply by examining animals daily for menstrual flow (e.g. Nigi, 1975; Oshima et al., 1977; Enomoto et al., 1979) Cytological changes seen in vaginal smears provide crude estimates of ovarian physiology but the subtlety of the transitions from one phase to the next prevents this technique from providing clear indices of ovarian activity. Although vaginal smears and observations of menstrual flow adequately describe the general pattern of the cycle, subtle daily changes, such as those occurring during the periovulatory phase, cannot be ascertained. Newer noninvasive techniques allow us to monitor the influence of endocrine levels on the reproductive activity of free-ranging populations, without disruption of normal behavior patterns.

Collection of simultaneous behavioral and hormonal data makes it possible to study the influence of ovarian hormones on the sexual and social behavior of female macaques during the mating season. This study examines the relationship between female sexual behavior and fluctuating hormonal levels during the mating season in a group of semi free-ranging Japanese macaques. The following questions with regard to hormonal influences on behavior are addressed: (1) does the attractivity of the female macaque vary depending on ovarian cycle phase? (2) does the proceptivity of the female macaque vary throughout the ovarian cycle? (3) does the female's spatial proximity in relation to male conspecifics vary according to cycle phase?

METHODS

Study Site and Animals

This study was carried out on a group of Japanese macaques (*Macaca fuscata*) kept under semi free-ranging conditions in a 65-acre enclosure at the Arashiyama West Texas Snow Monkey Sanctuary (TSMS). The group originated from the 1966 division of a troop in Kyoto, Japan, resulting in the establishment of the Arashiyama A and B troops. In 1972 the Arashiyama A troop, later referred to as Arashiyama West, was relocated to a ranch in South Texas. After a few more shifts they were settled into their permanent home approximately 15 km south of Dilley, Texas. The study group, composed of over 350 monkeys, is ideal for a behavioral study due to their semifree-ranging status, which makes them accessible to the researcher, while at the same time maintaining fairly normal behavior patterns in the animals. The Arashiyama West troop of Japanese macaques has been well studied, and the availability of complete genealogies and life histories on the monkeys plus the tattoo system of identification, provide valuable assistance to the field researchers.

The subjects for this study consisted of eight non-lactating Japanese macaque females. Each female was between 10 and 11 years of age during the study phase. All females were of similar rank (i.e. low ranking in the troop). Seven of the females were multiparous and one was primiparous. None of the eight females had offspring in the prior birth season (April-July, 1997).

Data and Fecal Sample Collection

Fecal samples were collected two to three times per week from the eight target females between the hours of 8:00 AM and 8:00 PM, during the 1997/1998 mating season (October to March). Fecal collection was carried out during focal animal follows, as well as opportunistically. Samples were collected within ten minutes of defecation and placed in 30-ml sterile plastic vials and frozen for preservation. Frozen samples were transported to the Wisconsin Regional Primate Research Center (WRPRC) in a plastic cooler containing dry ice, and stored in a freezer until analysis.

Behavioral data were collected using focal animal sampling (Altmann, 1974). In total, 352 hours of focal animal data were collected (ranging from 41 to 48 hours per subject) between October 1997 and April 1998. Only data collected prior to conception (239.5 hours) were used in this analysis. Normally, focal subjects were rotated throughout the week, although this depended somewhat on the schedule of fecal collection. The length of the focal samples was set at 30 minutes; however, some sessions were collected sequentially to allow for the collection of fecal samples. If the subject was lost prior to the end of the session, the session was terminated and the data discarded.

Sample Extraction and analysis

Extraction of ovarian steroids was performed according to the procedure described by Strier and Ziegler (1997). Aliquots of 0.1 g wet feces were extracted with 5 ml of distilled water and ethanol (50:50) and a portion of the supernatant, 500 µl was further extracted with 5ml diethyl ether. The samples were then vortexed, centrifuged, and the ether layer was aspirated off into clean vials, and resuspended in 500 µl ETOH.

High-pressure liquid chromatography (HPLC) was used to help identify which estrogens and progestins were excreted in higher amounts in the Japanese macaque feces. The technique used has been described in Strier and Ziegler (1997). For the Japanese macaque feces, estrone-glucuronide (E1) and pregnanediol-glucuronide (PdG), found in the highest quantities, were the most appropriate steroids to measure for this species by EIA.

The additions of tritiated steroids of known concentrations to a few representative fecal samples before extractions were used to determine procedural losses for extraction techniques. Tritiated 20 alpha hydroxy (for pregnanediol) and tritiated estrone (18,000 cpm) were added to 0.1 g samples of feces. Radioactivity was measured with a beta counter and procedural losses for extractions were calculated by taking the mean percentage of recoveries for each steroid.

Pooled fecal samples were used to validate the PdG and E1 assays. E1 and PdG concentrations were determined by EIA methods running assays with volumes from 5 to 100 µl from ether extracted pools. Adding small volumes of the fecal pool to the standard curve points assessed the accuracy with which the assay measures the steroids in each sample. Parallelism was determined through serial dilutions of the fecal pool along the standard curve. In addition to running a standard curve, fecal pool samples were run in duplicate on each assay in order to establish a mean intra- and interassay coefficient of variation (CV) for E1 and PdG. This procedure establishes the relative accuracy with which the steroid levels are being measured for all assays.

Estrone was measured in feces employing an E1 EIA previously reported (Ziegler et al., 1995). Pregnanediol-3-glucuronide was measured in feces by a PdG EIA also described previously (Carlson et al., 1996).

Due to the delayed excretion of E1 and the infrequency of fecal collection, the onset of PdG increase was considered to be the best estimate of the day of ovulation (Strier and Ziegler, 1997). Progesterone synthesis from the ovary actually begins to increase prior to ovulation, within hours of the gonadotropin surge (Espey and Lipner, 1994). Consequently, the sample preceding the first day of the PdG increase was considered to represent the most likely day of ovulation. The periovulatory phase was therefore conservatively defined as the estimated day of ovulation ± 3 days.

The earliest possible day of conception was considered to be the estimated day of ovulation if steroid levels failed to return to their lowest baseline levels. The length of gestation was calculated as the interval between estimated ovulation during the cycle in which conception occurred and the date of parturition. Parturition dates were defined as the first day in which a female was observed with a new infant (parturition dates for the troop were collected by Lou Griffin, Tracy Wyman, and Tanya Bell at the TSMS, and were accurate to within 2 days). Cycle lengths were calculated as the intervals between successive PdG surges. There were 1 to 5 cycle lengths calculated for each female (Figure 2-2).

Behavioral Analysis

Analyses were carried out on the behavioral variables listed in Table 2-1. Sexual behaviors were divided into those demonstrating attractivity to males and those indicating the proceptivity of the female. Those activities indicating the female's attractivity included sexual and non-sexual behaviors *received* by the target female from sexual partners. Proceptive activities included both sexual and non-sexual behaviors *directed* by the target female toward sexual partners. The sexual behavior of Japanese macaques is usually very subtle, particularly among the low ranking individuals in the group, making receptive behavior difficult to document. Consequently, only attractivity and proceptive behavior were considered for this analysis.

The results of the hormone analysis were used to create hormone profiles for each female (e.g. Figure 2-2). Hormone profiles were used to calculate ovulatory cycle and gestation lengths. The cycles were divided into the three phases: follicular, when PdG has dropped to base line levels; periovulatory, when PdG levels begin to slightly rise and E1 levels reach their peak; and luteal, when PdG levels stay well above base line for an

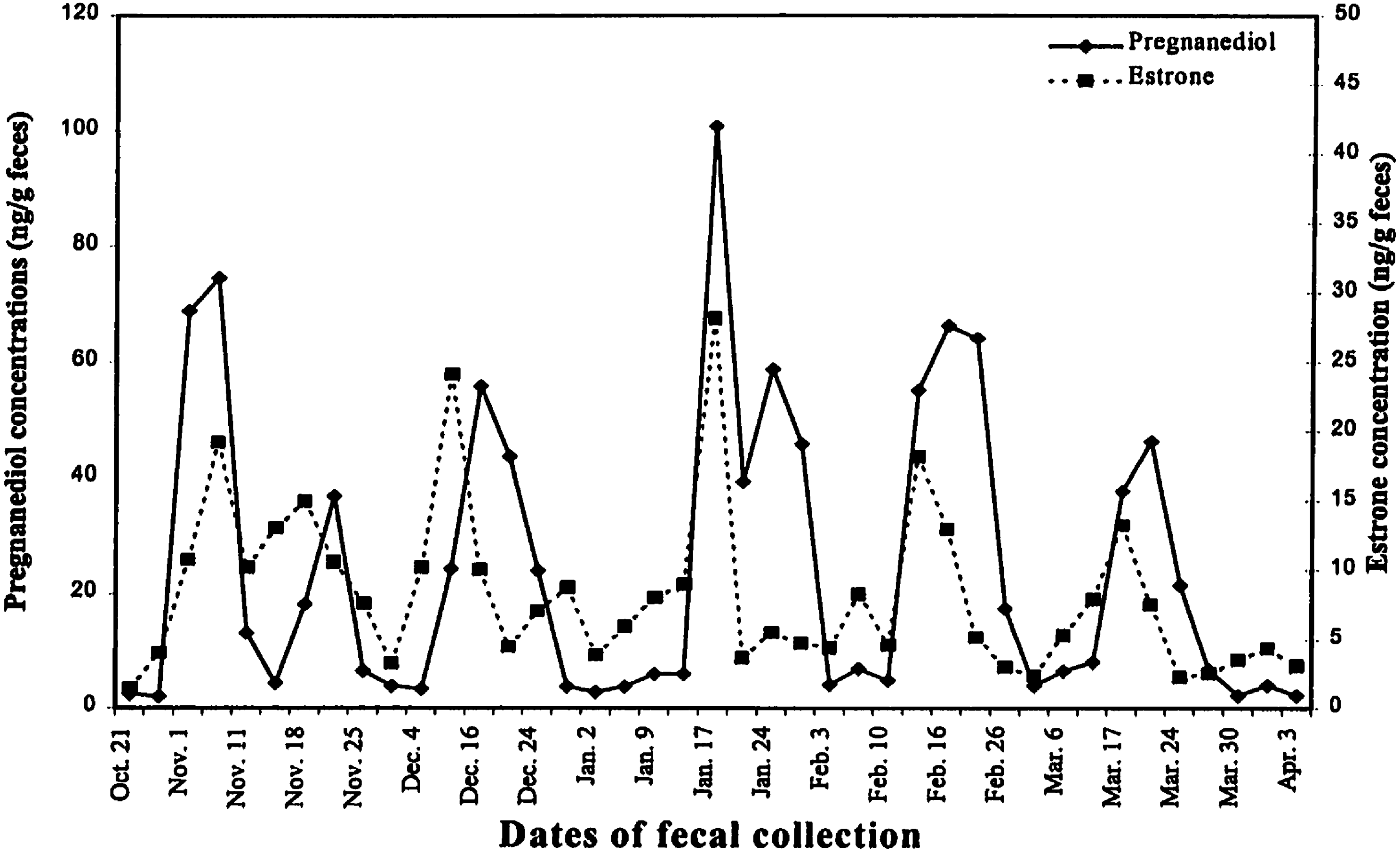

Figure 2-2. Representative hormone profile for one female Japanese macaque during the 1997/1998 mating season. Base levels of pregnanediol (PdG) indicate the follicular phase of the cycle, the day of the estrone surge (or the first day PdG levels begin to rise) ± Three days, represents the periovulatory phase, and a sustained increase in PdG levels represents the luteal phase.

Table 2-1. Sexual and non-sexual behavioral variables.

Behavior	Description
<u>**Attractivity**</u>	
Mount (receive)	Male* climbs either ventrodorsally or ventoventrally upon a standing female partner, with or without intravaginal penile insertion or pelvic thrusts
Hold (receive)	Male* sits in body contact behind the female with arms around female's waist. Usually observed in conjunction with a mount series.
Other (receive)	Other sexual behaviors including presenting, lip smacking, or hip touching received by the female from male* conspecifics
<u>**Proceptivity**</u>	
Mount (direct)	Female climbs ventrodorsally upon a standing partner, with or without pelvic thrusting
Hold (direct)	Female sits in body contact behind a partner with arms around the waist of the other animal. Usually observed in conjunction with a mount series.
Other (direct)	Other sexual behaviors including presenting, lip smacking, or hip touching directed by the female toward a partner
Estrus call	Loud staccato vocalization made by females in estrus.
<u>**Non-sexual Behaviors**</u>	
Groom†	One monkey inspects and cleans the fur of another. Sexual context is assumed if sexual behavior resumes or ensues.
Approach†	One monkey advances toward another to within one meter.
Spatial Association	Two monkeys maintain proximity to each other. Three criteria for proximity were used in the analysis: contact, less than one meter, and between one to four meters.

*May also be a female in homosexual consorts.

† These non-sexual behaviors are also distinguished as attractive (received by female) or proceptive (directed by female).

extended duration and E1 levels return to base line (Figure 2-1). In all, 26 complete cycles from eight females were identified and used in behavioral analyses for this study. Because females did not form consorts during every cycle, only those cycles in which the female was engaged in a consort were used in the statistical analysis for sexual behavior. In addition, one female was not observed in a consort during any focal sessions and was consequently removed from the analysis of sexual behavior.

Consorts and mounting behavior, however, are not restricted to male-female pairs in Japanese macaques. Female macaques have been observed to form consorts and mount with other females throughout the mating season (Fedigan and Gouzoules, 1978; Wolfe, 1976). Due to the occurrence of homosexual consorts among some of the females, mounts, holds, and "other" sexual behaviors were not distinguished by the sex of the partner. A mean frequency per hour score for each of the behavioral variables was calculated from each focal animal's interaction with male troop members (or females in the case of homosexual consorts).

Data were not normally distributed and consequently non-parametric statistics were used for this analysis. The mean scores obtained for each cycle phase were compared for cycle phase variation using Friedman analysis of variance by ranks (Siegal and Castellan, 1988). Behavioral variables showing statistical significance were further analyzed with Wilcoxon signed ranked tests (Siegal and Castellan, 1988) to determine where the variation existed. In addition to sexual behaviors, the frequency of approaches *directed* toward and *received* by the target female from male troop members were analyzed. Data were also analyzed to determine whether females were more likely to be found in proximity to males during certain phases of the ovarian cycle. This was accomplished by tallying the total time the target female was in proximity to a male during focal samples and calculating a mean percentage of time for each female for each phase of the cycle. Three criteria were used: contact, less than 1 meter, and from 1 to 4 meters. Friedman and Wilcoxon tests were also performed on spatial proximity data in order to detect variability between the phases. In addition, the grooming behavior of the target females in relation to male troop members was analyzed for cyclic patterns. This was achieved by calculating the mean percentage of time each female spent directing or receiving grooms from male troop members and separating the data into the three phases of the cycle. Friedman and Wilcoxon tests were then performed on cycle phases. Level of significance was set at $P<0.05$ and all tests were two-tailed. All statistical analyses were performed using an SPSS 8.0 statistical software package.

RESULTS

Validation and Hormone Profiles

The results of the validation procedures are summarized in Table 2-2. Mean steroid recoveries were found to be high (>90%). Accuracy assessment was also found to be within acceptable parameters (above 90 % accuracy). A mean percent accuracy was determined for PdG (102.69 ± 3.38%) and E1 (91.76 ± 1.44%) assays. The percent bound values obtained on serial dilutions of the extract in these assays closely paralleled the percent bound values of the standard curves (i.e. observed steroid measurements mirrored expected steroid levels). In addition, mean intra and interassay coefficient of variation (CV) values were found to be within acceptable limits (below 25% variation).

The profiles of immunoreactive E1 and PdG showed clear cyclic patterns in which the follicular and luteal components of the cycles could be clearly distinguished. One to five ovarian cycles were calculated for each of the target females for a total of 26 complete cycles (Table 2-3). The ovarian cycle lengths average 27.6 ± 4.2 (± SD) days (Table 2-4). Cycle lengths did not significantly differ among the eight females (χ^2=5.31, df=7, P=0.623). The lengths of the follicular and luteal phases average 8.4 ± 3.4, and 12.3 ± 3.8 (± SD) days, respectively (Table 2-4), and did not differ significantly among females (Follicular: χ^2=5.694, df=7, P=0.576; Luteal: χ^2=6.092, df=7, P=0.529). Four of the eight subjects conceived after one to three ovarian cycles. The mean gestation length for the females in this study was calculated as 175.25 ± 12.8 days (±SD; median=173 days).

Table 2-2. Summary of results of fecal assay validation procedures

	Pregnanediol	**Estrone**
Recoveries	90.00%	91.80%
Accuracy	102.69 ± 3.38%	91.76 ± 1.44%
Parallelism*	T=-0.50, df=28, $P<0.05$	T=-1.70, df=28, $P<0.05$
CV[†]: Intra	3.92% n=11	7.62% n=11
Inter	11.41% n=11	21.40% n=11

*Slopes do not differ.
†Coefficient of variation.

Table 2-3. Ovarian cycle and gestation lengths for Japanese macaque females.

Female	Cycle 1	Cycle 2	Cycle 3	Cycle 4	Cycle 5	Cycle 6	Gestation
Trisha	30	-	-	-	-	-	192
Saskia	35	22	25	-	-	-	170
Zoe	29	27	-	-	-	-	176
Tyler	33	-	-	-	-	-	163
Shy Mug	22	19	28	34	28	27	-
Tantalis	32	26	35	28	-	-	-
Lisa*	27	-	29	29	21	-	-
Bo Nose	23	24	27	31	28	28	-

* Sample collection for Lisa was very inconsistent for cycle 2 and was consequently removed from analysis.

Table 2-4. Hormone profile data for female Japanese macaques.

	N	#Cycles	X±SD (in days)	Range (in days)
Menstrual cycle	8	26	27.6 ± 4.2	19-35
Follicular	8	26	8.4 ± 3.4	3-15
Luteal	8	26	12.3 ± 3.8	7-22

Attractivity

Behavioral variables considered to indicate the attractivity of the female include mounts, holds, and other sexual behaviors *received* by the female from male troop members. Target females were observed to engage in sexual behavior during all three phases of the ovarian cycle (with the exception of one female who was not observed participating in sexual activity but did conceive during this mating season). The reception of mounts by females occurred frequently throughout the mating season and varied significantly throughout the ovarian cycle (Friedman's: χ^2=8.00, df=6, P=0.02; Figure 2-3a). Females were mounted significantly more during the follicular and periovulatory phases compared to the luteal phase of the cycle (Z=-2.20, P=0.03; Table 2-5), and although there was no significant difference in the rate of mounts *received* in the follicular phase compared to the periovulatory (P=0.53), the mean frequency showed a 56% increase in the periovulatory compared to the follicular phase.

Although males held females more frequently during the follicular and periovulatory phases compared to the luteal, the difference was not statistically significant (P=0.06; Table 2-5 and Figure 2-3b). The low frequency with which holding behavior occurred makes statistical analysis difficult. The reception by females of sexual behaviors listed in the category of "Other" (Table 2-1), also occurred rarely during focal observations. There were no significant difference among the three phases (χ^2=2.00, df=6, P=0.37). However, there was a trend for "other" sexual behaviors to be *received* more frequently during the periovulatory compared to the follicular and luteal phases (Figure 2-3c).

Proceptivity

The occurrence of mounts *directed* by females toward sexual partners varied considerably across the cycle phases (χ^2=6.91, df=6, P=0.03; Figure 2-4a). Females were

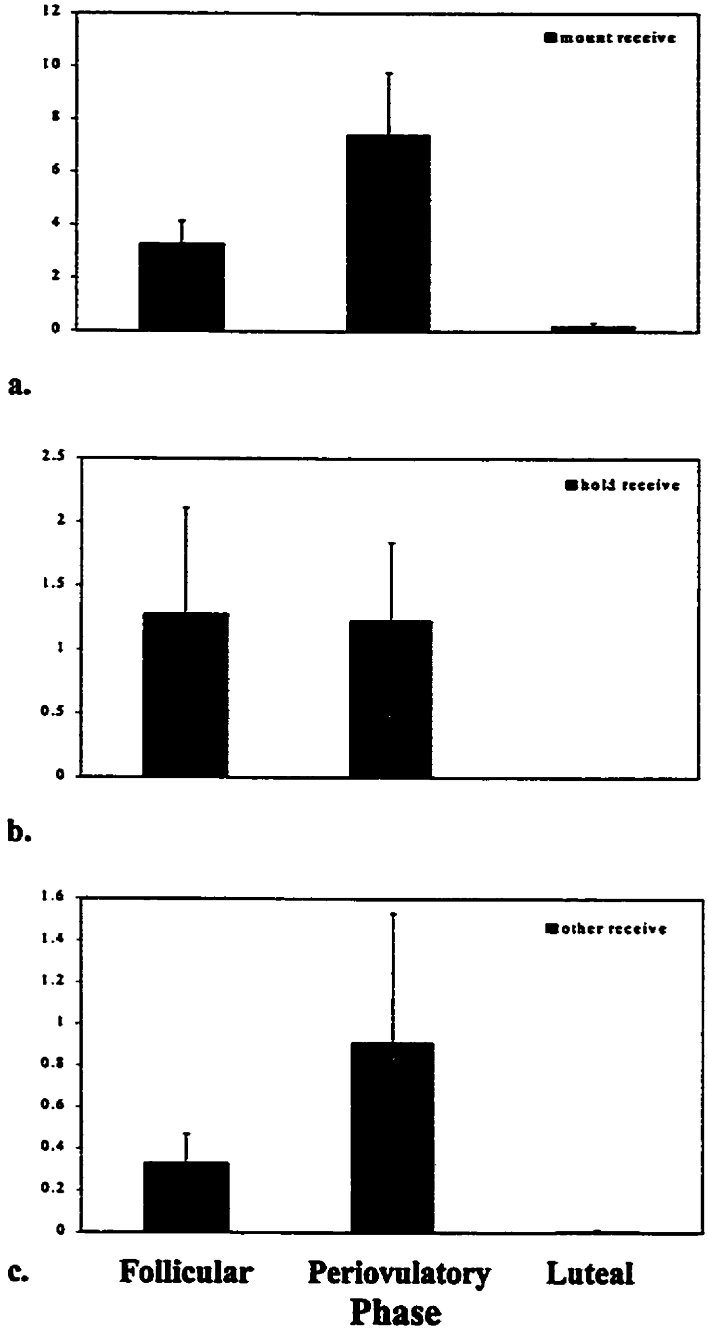

Figure 2-3. Attractivity of female Japanese macaques during three phases of the ovarian cycle (mean frequency ± SE).

a. Mounts received by focal females from their sexual partners.

b. Holds received by focal females from their sexual partners.

c. "Other sexual behaviors received by focal females from their sexual partners.

Friedman statistical analysis: a. χ^2=8.00, df=6, P=0.02; b. χ^2=5.77, df=6, P=0.06; c. χ^2=2.00, df=6, P=0.37.

*Scales for y-axis differ.

observed mounting males significantly more during the follicular and periovulatory phases of the cycle as compared to the luteal (Z=-2.00, P=0.05). However, there was no significant difference in the frequency of *directed* mounts between the follicular and periovulatory phases (P =0.91; Table 2-5). Nevertheless, the mean frequency of mounts *directed* by females increased by 20% during the periovulatory as compared to the follicular phase of the cycle (Figure 2-4a).

Although holds *directed* by target females toward sexual partners occurred infrequently, they were found to vary significantly among the three phases of the cycle (χ^2=9.00, df=6, P=0.01; Figure 2-4b). Females held sexual partners more frequently during the follicular and periovulatory compared to the luteal phase of the cycle (Z=-2.25, P=0.02). Sexual behaviors listed under the category of "Other" (Table 2-1) *directed* by target females toward sexual partners were found to vary significantly among the three phases (χ^2=6.33, df=6, P=0.04; Figure 2-4c). There was a notable, but non-significant increase in the frequency of *directed* occurrences of "other" sexual behaviors in the follicular and periovulatory compared to the luteal phase (P=0.06; Table 2-5).

Females showed a significant variability in the frequency of estrus calls exhibited throughout the cycle (χ^2=9.33, df=6, P=0.01; Figure 2-4d). The frequency of estrus calls did not differ significantly between the follicular and periovulatory phases of the cycle (P=0.41; Table 2-5), but did show a significant increase in frequency for the follicular and periovulatory phases compared to the luteal (Z=-2.27, P=0.02).

Non-Sexual Behavior

Non-sexual behaviors may also act as indicators of the attractivity, and the proceptivity of a female. Such non-sexual behaviors considered to indicate attractivity include those associated with consorts, such as grooms and approaches *received* from

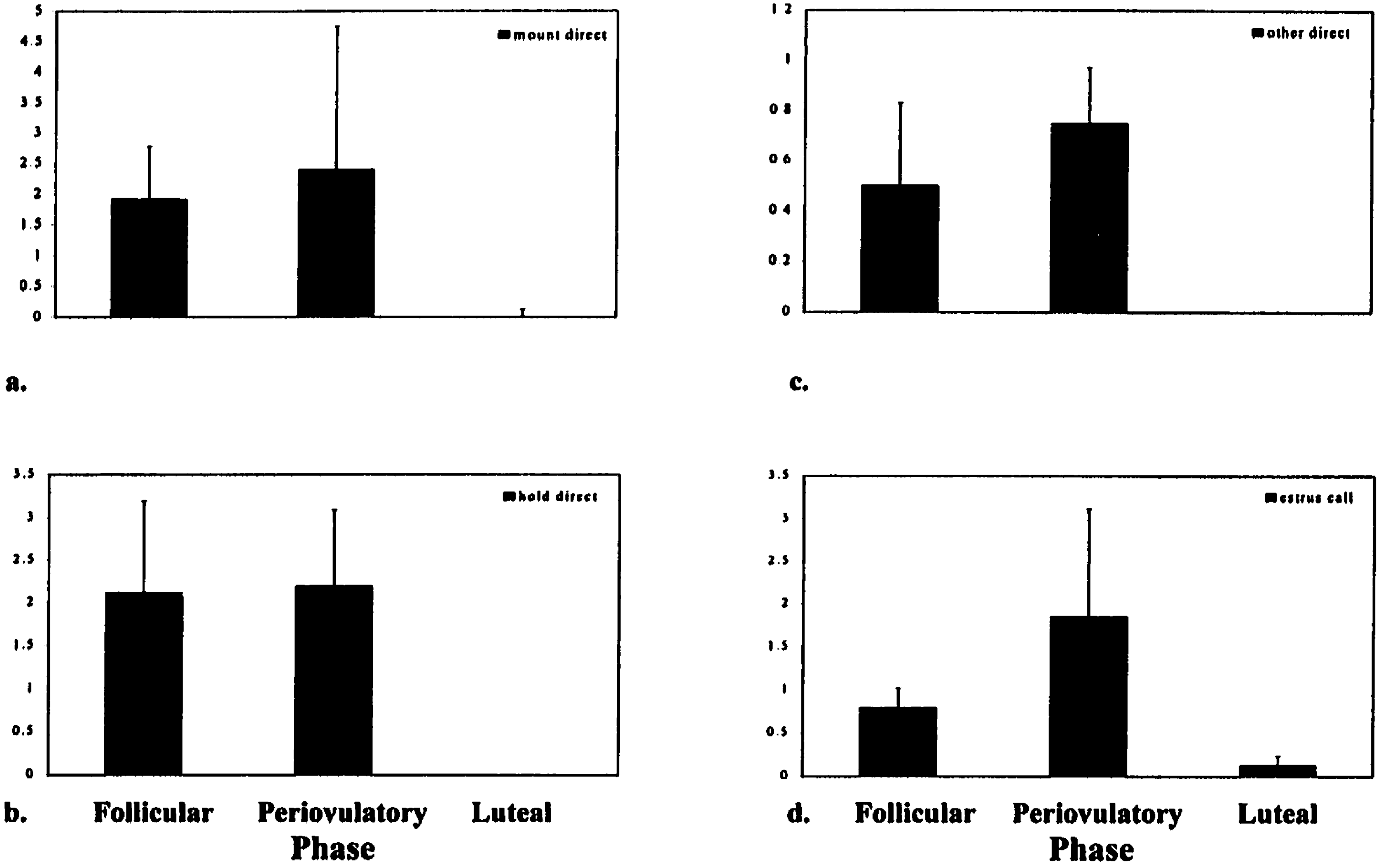

Figure 2-4. Proceptivity of female subjects during the three phases of the ovarian cycle (mean ± SE).

a. Mounts directed by focal females toward their sexual partners.

b. Holds directed by focal females toward their sexual partners.

c. "Other" sexual behaviors directed by focal females toward their sexual partners.

d. Estrus calls directed by females toward their sexual partners.

Friedman statistical analysis: **a.** χ^2=6.91, df=6, P=0.03; **b.** χ^2=9.00, df=6, P=0.01; **c.** χ^2=6.33, df=6, P=0.04; **d.** χ^2=9.33, df=6, P=0.01.
*Scales for y-axis differ.

Table 2-5. Wilcoxon signed rank analysis for sexual and non-sexual behaviors.

Sexual and Non-sexual Behaviors	follicular vs Periovulatory		Periovulatory vs luteal	
	Z	**P<**	**Z**	**P<**
Attractivity				
Mount receive	-0.63	0.53	-2.20	0.03 *
Hold receive	-0.96	0.33	-1.86	0.06
Other receive	-0.74	0.45	-1.23	0.22
Approach receive	-0.07	0.94	-1.13	0.26
Groom receive †	-1.93	0.05*	-2.41	0.02*
Proceptivity				
Mount direct	-0.12	0.91	-2.00	0.05*
Hold direct	0.00	1.00	-2.25	0.02*
Other direct	-0.83	0.41	-1.89	0.06
Estrus call	-0.82	0.41	-2.27	0.02*
Approach direct	0.00	1.00	-2.08	0.04*
Groom direct	-1.27	0.21	-2.43	0.02*
Spatial Proximity				
Contact	-0.30	0.76	-2.43	0.02*
<1m	-1.10	0.27	-2.25	0.02*
1-4m	-0.65	0.52	-2.20	0.03*

† Wilcoxon test for follicular vs luteal: Z=-2.12, P< 0.03.

* Statistically significant differences.

males. Behaviors indicating the proceptivity of the female include grooms and approaches *directed* toward male troop members by the female. The maintenance of proximity between two individuals requires the cooperation of both and spatial proximity may therefore be used as an indicator of the attractivity, as well as the proceptivity of the female.

Although the frequency of approaches *received* by target females from male troop members did not differ statistically among the three phases of the cycles (χ^2=1.23, df=7, P=0.54; Figure 2-5a), trends showed a 71% increase in the mean frequency of approaches *received* during the follicular compared to the luteal phase of the cycle, and a 69% increase in the periovulatory compared to the luteal phase (Figure 2-5a). The frequency of approaches *directed* by target females toward male troop members varied significantly across the three phases of the cycle (χ^2=6.07, df=7, P=0.05; Figure 2-5a), with the highest frequency occurring in the follicular and periovulatory compared to the luteal phase of the cycle (Z=-2.08, P=0.04).

The proportion of time females spent being groomed by male conspecifics varied throughout the cycle (χ^2=11.08, df=7, P<0.01; Figure 2-5b). Males groomed females significantly more during the periovulatory compared to the luteal phase of the cycle (Z=-2.41, P=0.02). There was also a significant increase in the periovulatory compared to the follicular phase of the cycle (Z=-1.93, P=0.05) and a significant increase in the frequency of grooms *received* in the follicular compared to the luteal phase (Z=-2.12, P=0.03). The proportion of time females spent grooming male conspecifics also varied significantly across the three phases of the cycle (χ^2=8.86, df=7, P=0.01; Figure 2-5b). Females groomed males significantly more in the follicular and periovulatory versus the luteal phase of the cycle (Z=-2.43, P=0.02; Figure 2-5b). Although there was no significant difference between the follicular and the periovulatory phases of the cycle (P=0.21), the mean proportion of time increased by 32% in the periovulatory phase over the follicular.

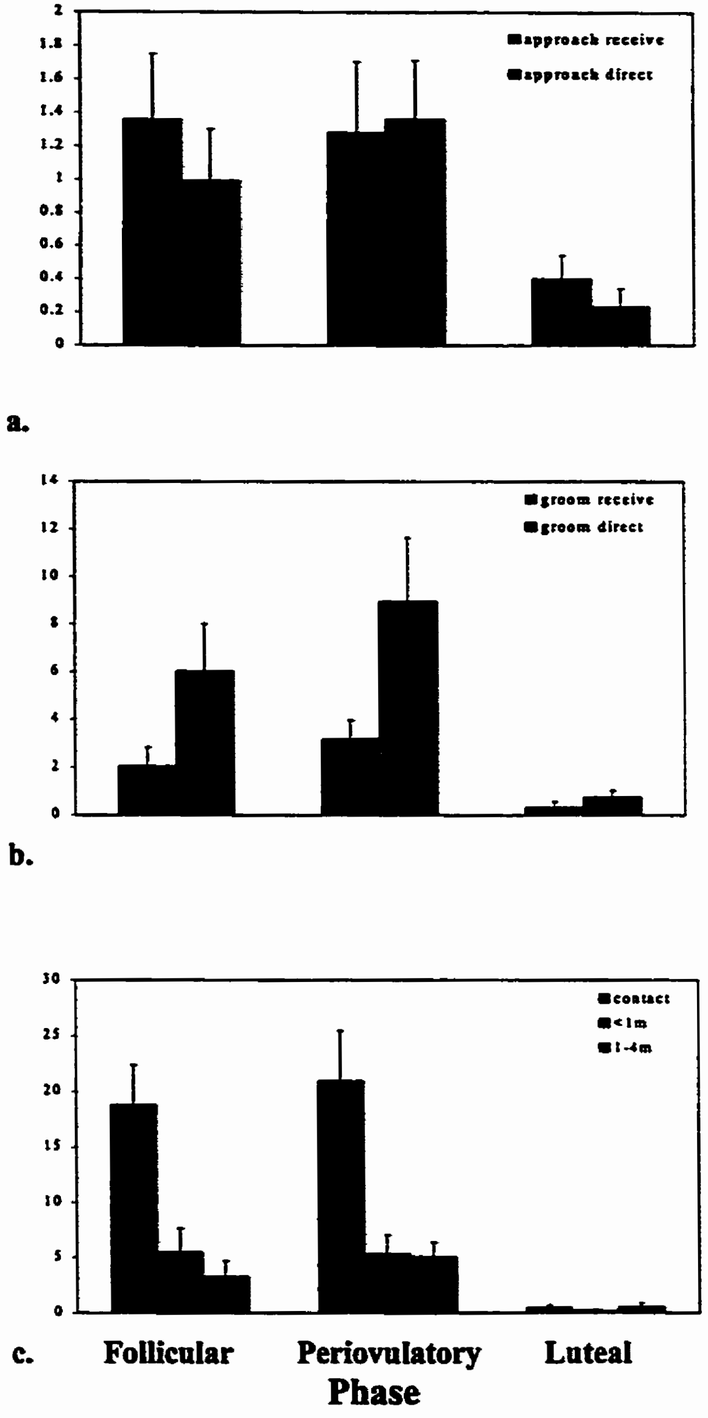

Figure 2-5. Non-sexual interactions between males and females associated with attractivity and proceptivity.

a. Approaches received and directed by focal females (mean frequency ± SE).

b. Grooms received and directed by focal females (mean proportion of time ± SE).

c. Proximity to male conspecifics (mean proportion of time ± SE).

Friedman statistical analysis: a. *received* χ^2=1.23, df=7, P=0.54, *directed* χ^2=6.07, df=7, P=0.05; b. *received* χ^2=11.08, df=7, P<0.01, *directed* χ^2=8.86, df=7, P=0.01; c. *contact* χ^2=10.90, df=7, P<<0.01; *<1m* χ^2=10.40, df=7, P=0.01; *1-4m* χ^2=6.65, df=7, P=0.04.

The proportion of time which females and males spent in close proximity varied considerably throughout the cycle for all three criteria (contact: χ^2=10.90, P=0.00; <1m: χ^2=10.40, P=0.01; 1-4m: χ^2=6.65, P=0.04; Figure 2-5c). Females were observed to spend significantly more time in proximity to males during the follicular and periovulatory compared to the luteal phase of the cycle (contact: Z=-2.43, P=0.02; <1m: Z=-2.25, P=0.02; 1-4m: Z=-2.20, P=0.03). However, there was no significant difference in the amount of time females spent in proximity to males during the follicular compared to the periovulatory phase of the cycle (contact: P=0.76; <1m: P=0.27; 1-4m: P=0.52).

DISCUSSION

Enzyme Immunoassays and Hormone Profiles

The employment of a simple extraction technique, coupled with established enzyme immunoassay methods, proved very successful for measuring fecal steroids in this species. This method yielded high total steroid recoveries (>90%). The sustained estrogen increase, which typically occurs in a 1 to 2 day period at the time of ovulation, was not always present in the sample collection. Therefore, given the irregular rate of fecal sample collection (i.e. not daily), PdG levels proved to be the most reliable means of determining cyclic patterns in the ovarian cycle. Average cycle length was calculated at 27.6 ± 4.2 days, which is comparable to earlier studies by Nigi (26.3 ± 5.4 days; 1975) that were based on vaginal swabs and sexual swellings. Although not statistically significant, ovarian cycle lengths were found to vary throughout the mating season for each individual female, as well as among the female subjects. The mean gestation length for the females in this study, as determined by hormonal profiles, was 175.25 ± 12.8 days (±SD; median=173 days), which was also comparable to earlier non-hormonal estimates of 173 ± 6.9 days (Nigi, 1976).

Attractivity

Previous studies have documented attractivity in primates as being regulated by circulating estrogen levels (Enomoto et al, 1979; Johnson and Phoenix, 1976; Michael and Zumpe, 1993). In this study, estrone levels were found to have a positive relationship with the frequency of sexual interactions in female Japanese macaques. Females *received* more mounts from male troop members during the follicular and periovulatory phases of the cycle, when E1 levels are increased. In addition, despite a lack of statistical significance, the copulatory rate increased further in the periovulatory phase, indicating an increase in attractivity toward midcycle; when E1 concentrations reach their peak and ovulation occurs. Although the variance in the numbers of copulations due to individual differences was large, the distribution of the copulations in relation to the phase of the ovarian cycle formed a meaningful pattern, indicating hormonal influence on the occurrence of mounting behavior. The increase in holds *received* by females during the follicular and periovulatory phases of the cycle, followed by an absence of this behavior in the luteal, also indicates a positive relationship between E1 and the attractivity of the female. Although the frequency of "other" sexual behaviors did not differ by phase, there was a trend toward increased frequency in the follicular and periovulatory phase of the cycle suggesting that E1 levels also affect this behavior.

The role of progesterone in the expression of sexual behavior is not entirely clear. This study found a significant negative relationship between pregnanediol (PdG) and the attractivity for all females, supporting earlier findings on rhesus macaques that demonstrated an inhibitory effect of progesterone on attractivity (Baum et al., 1977; Michael and Zumpe, 1993). Copulations were primarily restricted to the follicular and periovulatory phases, when PdG concentrations are low, followed by a significant decrease in the behavior during the luteal phase of the cycle when PdG levels reach their peak. Males generally held females during a mount series, and as a consequence the frequency of this behavior appeared to follow a similar pattern to that observed in "mounts received" by the female, with the highest frequency occurring in the follicular and periovulatory phases of the cycle, when PdG levels are at base line, followed by an absence of holding behavior in the luteal, when PdG levels peak. Other sexual behaviors, such as hip touches, lip smacking, and presents, were rarely observed in the target females. These behaviors did occur among higher-ranking females (personal observation), and may therefore be related to the rank of the female. Therefore, no significant patterns were detected in the frequency of these sexual behaviors *received* by the target females. Nevertheless, this category ("other" sexual behaviors) showed a trend toward increased frequency in the follicular, and particularly the periovulatory phases of the cycle.

A female's attractiveness to male troop members appears to be affected by the decrease in PdG levels at the onset of the follicular phase coupled with increasing E1 levels, and is further amplified by an E1 surge during the periovulatory phase of the cycle. The attractivity of the female also appears to be diminished by increased PdG levels in the luteal phase of the cycle. Results from this study suggest that both PdG and E1 levels influence attractivity in female Japanese macaques during the female's cycle.

Proceptivity

Proceptivity in primates has not been linked to any single hormone (Baum, 1983). However, it has been suggested that females are not only more attractive during the periovulatory phase, when estrogen levels are increased, but they also show increases in proceptive behavior during this phase(Beach, 1976; Wallen et al., 1986). In this study, the presence of E1 significantly predicted proceptivity in the females. There were increases in proceptive behaviors during the follicular and particularly the periovulatory phase, when E1 levels reach their peak. Females mounted male troop members more often in the periovulatory phase of the cycle, during the E1 peak. Females also held, and *directed* "other" sexual behaviors toward males during the follicular and periovulatory phases. And they showed a complete absence of these behaviors during the luteal phase, when E1 levels return to base line. Females also exhibit a higher frequency of estrus calls during the periovulatory phase, when E1 levels peak.

Progesterone has been observed to influence proceptivity in mammals, by inhibiting, or failing to stimulate, proceptivity (Baum, 1983). Some studies have suggested that, in primates, progesterone has no direct effect on proceptivity (Baum, 1983; Baum et al, 1977). However, other studies have found a negative relationship between proceptive behavior and progesterone levels (Wilson et al., 1982; Wallen et al., 1984). This study demonstrated a negative relationship between PdG and proceptive behavior in Japanese macaque females. Females displayed an increase in proceptive behavior during the follicular and periovulatory phases, when PdG levels are at their lowest, followed by a substantial decline in the behavior during the luteal phase, when PdG levels are elevated.

Only certain females engaged in holding behavior during copulatory activity. This behavior appeared to be highly variable in its expression among female Japanese macaques. Consequently the behavior was not observed frequently. There was, however, a significant cycle phase variability, with females holding males more during the follicular and periovulatory phases, when PdG levels are low. In addition, the luteal phase of the cycle was marked by an absence in the expression of this behavior, corresponding to elevated levels of PdG. Other sexual behaviors were not observed frequently, and were also highly variable among females, as well as among the individual cycles of a female. However, the display of these behaviors by target females also increased significantly during the follicular and periovulatory phases, dropping off sharply in the luteal phase of the cycle.

The expression of estrus calls appeared to be specific to certain females, and showed a trend toward increased frequency during the follicular and periovulatory phases, when PdG levels have decreased. Moreover, the display of estrus calls substantially declined during the luteal phase, when PdG levels reach their peak. Estrus calls generally occurred in conjunction with consortships, usually after the male had wandered off during the consort, or the consort ended abruptly with the male moving on to another female. This pattern of estrus calls supports previous observations that females use estrus calls to stimulate the continuation of series mounts (Fedigan and Gouzoules, 1978). As such, the occurrence of estrus calls was largely dependent on the stability of the consort and the individual temperament of the female.

It is difficult to ascertain whether the increase in proceptive behavior in the female macaque is due to the low PdG levels in the follicular and periovulatory phases, or the increased E1 levels during these phases. However, E1 levels were observed to fluctuate during the luteal phase of the cycle without any noticeable increase in proceptivity, suggesting that an increase in E1 alone does not trigger proceptive behavior. Low levels of PdG are also required to produce proceptivity in the female Japanese macaque. This is further evidenced by the presence of sexual activity during pregnancy, which occurs during periodic drops in PdG concentrations (see Chapter 4).

Non-Sexual Behavior

Although sexual activity is often the best indicator of the attractive and proceptive state of the female, other non-sexual behaviors also serve to identify attractivity and proceptivity in the female. The attractivity of the female can often be ascertained by the frequency with which male troop members approach her. In addition, grooming among consorts is frequently observed throughout the mating season and the amount of time that a male spends grooming a female may be an indicator of the female's attractiveness.

Although not significant, females were approached by males more during the follicular and periovulatory phases, when PdG levels are lowest and E1 levels peak, indicating an increased attractivity to males at this time. In addition, the rate at which males approached females decreased during the luteal phase, when PdG levels are at their peak and E1 levels reach base line. Grooming behavior between male and female Japanese monkeys during the mating season is closely linked to consortships. When a male and a female are engaged in a consort they spend most of their time together and groom each other frequently between mounts in a mount series. Grooming between male and female macaques is not always proportionate, and often the female directs significantly more grooming than she receives. This appears to be the case with the females in this study. Grooming patterns for some females were not consistent and not all females received grooming from male partners during consorts. Nevertheless, males groomed females significantly more during the follicular and particularly the periovulatory phase compared to the luteal. These results are consistent with other data displaying increased attractivity during midcycle, when E1 levels reach their peak.

Non-sexual behaviors may also function to indicate the proceptivity of the female Japanese macaque. Female Japanese macaques have been observed to approach and groom males more often, as well as spend more time in close proximity to males, during consorts (Enomoto, 1981). In this study, females approached male troop members increasingly more often in the follicular and periovulatory phases than in the luteal, indicating increased proceptivity toward midcycle, when E1 levels peak. In addition, females groomed males significantly more in the follicular and periovulatory phases of the cycle, with a notable, but not significant, increase in the periovulatory phase. Non-sexual behaviors indicate patterns of attractivity and proceptivity in female Japanese macaques, with the highest occurrences of these behaviors happening when E1 levels peak during the periovulatory phase of the cycle.

Because many approaches took place outside of the focal sessions, a further analysis was carried out in order to look at spatial proximity between males and females during the three phases of the cycle. Although, spatial proximity cannot be attributed to either individual, it does require the cooperation of both individuals to maintain proximity. Therefore, the proximity patterns may be attributed to both the attractivity of the female (i.e. male is actively maintaining proximity to the female), and the proceptivity of the female (i.e. female is actively maintaining proximity to the male). The results of this analysis clearly demonstrate that females are spending significantly more time in proximity (contact, <1m, and 1-4m) to male troop members during the follicular, and particularly the periovulatory phase, when E1 levels peak. Spatial proximity data supports the hypothesis that the proceptivity and attractivity of the female increases toward midcycle, suggesting that decreased PdG levels, along with the midcycle surge in E1, not only influence the proceptive behavior of the female, but also affect the attractivity of the female.

SUMMARY

The three phases of the ovarian cycle served as a physiological reference for the comparison of sexual behavior in *Macaca fuscata*. The results of this study indicate that most sexual behaviors occur at different rates during the follicular, periovulatory and luteal phases of the ovarian cycle in Japanese macaque females. Decreased PdG levels in the follicular and periovulatory phases, coupled with an increase in E1 appears to trigger attractivity and proceptivity in the female macaque, while increased PdG concentrations combined with base line E1 levels in the luteal phase inhibits the expression of proceptive behaviors, and reduces the attractivity of the female.

Many nonhuman primates engage in sexual activity far more often than is required for reproduction. This trend also seemed apparent among the females in this study. Females were observed engaging in sexual activity (to varied degrees) during all three phases of the cycle. Nevertheless, it is evident that circulating ovarian steroids play an important role in the reproductive patterns of Japanese macaque females.

CONCLUSIONS

1. The employment of simple extraction and enzyme immunoassay techniques proved very successful for measuring fecal steroids in Japanese macaque females.
2. The attractivity of female Japanese macaques increased significantly during the follicular, and particularly the periovulatory, phases of the ovarian cycle.
3. Females also displayed increased proceptive behavior during the follicular and periovulatory phases of the ovarian cycle.
4. The spatial proximity between males and females varied greatly depending on ovarian cycle phase, with females spending the largest percentage of time in proximity to male conspecifics during the periovulatory phase of the cycle.
5. Patterns of grooming behavior between male and female troop members were highly correlated with mating and consortships, and consequently showed similar patterns of significance with increases in grooming behavior between male and female troop members in the periovulatory phase of the cycle.

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Chapter Three

The Relationship between Ovarian Cycle Phase and Aggressive and Affiliative Behavior in Female Japanese Macaques

INTRODUCTION

The behavior of female Japanese macaques (*Macaca fuscata*) changes as the mating season begins. Variations in the female's sexual behavior over the mating season have been reported (e.g. Beach, 1976; Fedigan and Gouzoules, 1978; Wolfe, 1976; Bercovitch and Goy, 1990), as well as in the occurrence of social behaviors and aggression (D'Amato et al, 1982; Enomoto, 1981; Zumpe and Michael, 1996). The mating season is known as a time when important changes in the social structure of Japanese macaque troops occurs, as well as increases in the occurrences of aggression (Eaton et al., 1981). Typical agonistic behaviors consist mostly of displays of dominance and submission, non-contact threats, chases and vocalizations. Seasonal variability in these non-contact forms of aggression has been reported for both male and female rhesus macaques (Mallow, 1981). In addition, increases in more serious forms of aggression during the mating season have been reported, as evidenced by the increased frequency of wounds (*Macaca mulatta*, Wilson and Boelkins, 1970). The precise relationship between the female's reproductive status and the affiliative and aggressive patterns of the female remain uncertain. Hormonal influences have frequently been suggested as the explanation for seasonal changes in the expression of aggressive behavior.

Fluctuations in hormone levels have also been linked to affiliative patterns in female macaques during the mating season (D'Amato et al, 1982). In many macaque species, "preferred" partners show lower levels of agonistic interactions toward one another, tend to spend more time in close proximity, groom each other more, and interact sexually more than other pairs. The social and sexual preferences of the partners for each other are usually temporary and under the influence of sexual attraction mediated by the hormonal changes of the female's ovarian cycle. However, definitive causal relationships between hormone levels and social and aggressive behaviors have yet to be demonstrated in Japanese macaques.

Agonistic behavior in primates can be observed in a diversity of contexts that stem from a variety of sources. A number of causal factors have been suggested, including proximate explanations (i.e., stimuli from the physical and social environment); ontogenetic or developmental factors; and ultimate explanations that emphasize factors influencing the evolution of aggression in primates. The increase of aggression during the mating season in some primates raises the question of whether male-male or female-female competition is an important factor influencing aggression in primates, and, if so, how hormones fit into this picture.

Although not all aggression toward females functions in a reproductive context, quantitative data from several species indicates that male aggression is more likely to be directed toward estrous females (macaques: Enomoto, 1981; Fedigan, 1992; Eaton, 1984. The widespread tendency for males to direct more aggression toward potentially fertile females is consistent with the hypothesis that male aggression often functions to increase access to mates (Smuts and Smuts, 1983). In effect, male aggression may represent a form of sexual harassment or coercion, functioning to increase the costs of non-cooperation, thereby increasing the probability that the female will yield to the aggressor and become a potential mate. The pattern of aggressive behavior displayed by males toward female macaques has been observed to increase during the female's midcycle (Michael and Zumpe, 1993).

In the past, females were often considered passive, cautious, and coy when it came to sexual interactions, but this assumption was challenged when sufficient evidence of the mating behavior of female primates was collected. Small (1993) indicates that female primates have been observed to mate frequently with multiple partners and often initiate copulations. It has also been sometimes assumed that female-female competition is either trivial or nonexistent. However, there is extensive evidence that female-female competition occurs throughout a wide variety of taxa, and that such competition can have important effects on a female's reproductive success (see Silk, 1992 for review). Female-female competition may take the form of female harassment of copulating pairs, and has been observed in a number of species including, Gray langurs (*Presbytis entelles*, Yoshiba, 1968), Patas monkeys (*Erythrocebus patas*, Loy and Loy, 1977), gelada baboons (*Theropithecus gelada*, Dunbar, 1980), savanna baboons (*Papio spp.*, Dunbar and Sharman, 1983), red howler monkeys (*Alouatta seniculus*, Crockett, 1984), rhesus macaques (*Macaca mulatta*, Zumpe and Michael, 1987), and bonnet macaques (*Macaca radiata*, Silk, 1989). Female macaques are also more inclined to be involved in agonistic encounters with other females during estrus. However, there are very few quantitative data on the subject.

There is some evidence that estrogen increases aggressiveness in female rhesus macaques (Michael and Zumpe, 1970), which supports colony studies suggesting increased aggression by females near midcycle. In addition, estradiol treatment of ovariectomized females has been reported to "protect" the female from direct aggression by the male, which is most likely a by-product of increasing his sexual interest in her. There is however, insufficient evidence from laboratory studies to determine whether progesterone affects the aggressiveness of ovariectomized females (Michael and Zumpe, 1993).

Grooming plays a very important role in the daily lives of many primate species and, as such, has been studied extensively in many primates. Grooming can be a form of tactile communication in a number of social contexts, and is very important in maintaining cohesion of the group. Grooming may be observed in a variety of contexts including mother-infant bonds, kinship bonds, as a means of mending ruptured bonds, or in mating bonds, as a prelude to mating. In some species male-female grooming may only occur in the context of mating and may decrease or disappear entirely after the courtship has ended. Spatial associations between group members also changes throughout the mating season as the female's priorities change with respect to mating opportunities and reproductive status. The study of allogrooming and proximity maintenance are effective tools for analyzing interindividual relationships and social dynamics within the group.

Grooming has been observed to increase among males and females during estrus (e.g. *Cebus apella*, Linn et al., 1995; *Macaca fuscata*, Enomoto et al., 1979), particularly during the periovulatory phase of the cycle. In Japanese macaques, allogrooming has been known to drastically change during the mating season, showing evidence of an increase, as well as a widening of an individual's range of interactions, and mature females become the main target of grooming from both male and female group members (D'Amato et al., 1982). In many Cercopithecine species, adult females have been observed to direct a high proportion of their allogrooming to kin and higher ranking nonkin. However, during midcycle, female rhesus macaques are known to interact less frequently with kin and other females and to direct significantly more behavior, including proceptive behavior, toward adult males (Wilson et al., 1981). A female may consort with one or several males during a 1-2 week period of the cycle, after which her sexual activity ceases and she again begins to associate more with the females than the males of the group (Michael and Zumpe, 1993).

The present study examines patterns of aggressive and affiliative behavior in female Japanese macaques across the ovarian cycle. I predict that (1) the frequency of aggression between males and females will vary throughout the ovarian cycle, with the highest rates occurring during the midcycle, or periovulatory phase. Given the likelihood of increased proximity and enhanced social interactions associated with mating, I predict that (2) increases in female aggression, both that directed to and that received from male conspecifics, will be a consequence of increased male-female interactions resulting from the female's attempt to copulate with a male and vis versa (that therefore, increased aggressive behaviors are only an indirect effect of hormone levels).

Elevated levels of female-female competition and female choice during the mating season may lead to increased aggression among female conspecifics. In addition, male-female consorts play an important role in the frequency in which a female directs and receives aggression, as well as the phase in which aggressive behavior occurs. Females appear to derive increased confidence from consorts, and females are also more likely to be involved in agonistic encounters during estrus. Therefore, I predict that (1) females will direct more aggression toward female conspecifics during the periovulatory phase, while engaged in a consort with a male; and (2) females will receive more aggression from female conspecifics during the luteal phase, when they are no longer involved in a consort.

The patterns of affiliative behavior toward different sex classes may vary according to cycle phase, dependent primarily upon the female's mating activity. I predict that (1) females will approach and be approached more frequently by female conspecifics during the luteal phase; (2) females will spend more time grooming and being groomed by female conspecifics during the luteal phase of the cycle, when the female's mating activity is decreased; and (3) proximity between female troop members will increase during the luteal phase (see Chapter 2 for analysis of male-female patterns of affiliation).

METHODS

The study was carried out on a group of Japanese macaques (*Macaca fuscata*) kept under semi free-ranging conditions in a 65-acre enclosure at the Arashiyama West Texas Snow Monkey Sanctuary (TSMS). At the time of the study, the group consisted of approximately 350 individuals of all sex and age classes. The subjects for this study consisted of eight non-lactating Japanese macaque females. All females were between 10 and 11 years of age during the study period, and were of similar rank. Seven females were multiparous, and one was primiparous. None of the eight females gave birth in the 1997 birth season (April-July).

Behavioral and fecal data collection

Behavioral data were collected during the 1997/1998 breeding season, from October to April. A total of 352 hours of focal data were collected on eight females (41 to 48 hours per subject). Only data collected prior to conception (239.5 hours) were used for analysis. Data were collected during 30-minute observations and were varied throughout the day for each female when possible. It was nevertheless, sometimes necessary to collect samples sequentially to allow for the collection of fecal samples. If the subject was lost prior to the end of the session, the session was terminated and the data discarded.

Fecal samples were collected two to three times per week from the eight target females between the hours of 8:00 AM and 6:00 PM. Fecal collection was carried out during focal animal follows, as well as opportunistically. Samples were collected within 10 minutes of defecation and placed in 30-ml sterile plastic vials and frozen for preservation. Frozen samples were transported to the Wisconsin Regional Primate Research Center (WRPRC) in a cooler containing dry ice, and stored in a freezer at WRPRC until analysis.

Sample Extraction and Analysis

Extraction of ovarian steroids was performed according to the procedure described by Strier and Ziegler (1997). See Chapter 2 for a detailed description of the procedure. Pooled fecal samples were used to validate the pregnanediol and estrone assays (see chapter 2 for validation procedures and results). Estrone concentrations were measured in feces employing an E1 EIA previously reported (Ziegler et al., 1995).

Pregnanediol-3-glucuronide was measured in feces by a PdG EIA also described previously (Carlson et al., 1996). EIA products were analyzed by ELISA for pregnanediol and estrone levels.

Behavioral Analysis

The results of the hormone analysis were used to create hormone profiles for each female (see Figure 2-1). Hormone profiles were used to calculate ovulatory cycle and gestation lengths. The cycles were divided into the three phases: follicular, when pregnanediol has dropped to base line levels; periovulatory, when pregnanediol levels begin to slightly rise and estrone levels reach their peak; and luteal, when pregnanediol levels stay well above base line for an extended duration and estrone levels return to base line (see Figure 2-2). In all, 26 complete cycles from eight females were identified and used in behavioral analyses for this study.

Analyses were carried out on the behavioral variables listed in Table 3-1. Behavioral variables used in the analyses included agonistic signals directed by target females toward male and female troop members, as well as agonistic signals received by target females from male and female troop members. Affiliative behaviors, such as approaches, spatial proximity, and grooming patterns, were also analyzed for phase variance (affiliative behaviors involving homosexual consortships were eliminated from the analysis). The frequency of approaches directed by target females toward male and female troop members, as well as received by target females from male and female troop members, were analyzed. Data were also analyzed to determine whether females were more likely to be found in proximity to male and female conspecifics during particular

Table 3-1. Agonistic and affiliative behavioral variables used for analyses.

Behavior*	Description
Agonistic Behaviors	
Chase	To pursue another monkey with accompanying agonistic signals.
Threat	Visual or vocal signal consisting of a stare, raised brows, or growl.
Supplant	One monkey moves toward another who immediately moves out of the former's way. The displacer frequently sits or stands in the exact spot vacated by the displaced monkey.
Other	Other agonistic signals, such as lunge, pinch, grab, or bite.
Affiliative Behaviors	
Groom	One monkey inspects and cleans the fur of another. Sexual context is assumed if sexual behavior resumes or ensues.
Approach	One monkey advances toward another to within one meter.
Spatial Association	Two monkeys maintain proximity to each other. Three criteria for proximity were used in the analysis: contact, less than one meter, and between one to four meters.

*All behaviors (with the exception of spatial associations) can either be directed or received by the target female.

phases of the ovarian cycle. This was accomplished by tallying the total time the target female was in proximity to other troop members during focal samples and calculating a mean percentage of time for each female for each phase of the cycle. Three criteria were used: contact, less than 1 meter, and from 1 to 4 meters. In addition, the grooming behavior of the target females in relation to other troop members were analyzed for cyclic patterns. This was achieved by calculating the mean percentage of time each female spent directing or receiving grooms from other troop members and separating the data into the three phases of the cycle. The mean scores obtained for each cycle phase were compared for cycle phase variation using Friedman analysis of variance by ranks (Siegel and Castellan, 1988). Behavioral variables showing statistical significance were further analyzed with Wilcoxon signed ranked tests (Siegel and Castellan, 1988) to determine where the variation existed. Level of significance was set at $P<0.05$. All statistical analyses were performed using an SPSS statistical software package.

RESULTS

Estrone and pregnanediol immunoassays showed clear cyclic patterns in which the follicular and luteal components of the cycles could be clearly distinguished. One to five ovarian cycles were calculated for each of the target females for a total of 26 complete cycles. See chapter 2 for detailed analysis of ovulatory cycles and validations for enzyme immunassays.

Male-Female Aggression

Since the target females for this study were all low ranking, occurrences of directed aggression toward male conspecifics were virtually non-existent. Females were not observed to supplant males during focal periods, and were rarely observed directing other agonistic signals toward males. Consequently, directed aggression by target females toward male conspecifics was not found to significantly differ across the three phases of the cycle (chases: $\chi^2=5.43$, df=7, P=0.07; threats: $\chi^2=2.30$, df=7, P=0.32; grab/bite: $\chi^2=5.20$, df=7, P=0.07; lunge: $\chi^2=2.36$, df=7, P=0.31). Nevertheless, there were some trends worth noting. The frequency of chases directed toward male troop members by target females showed a 50% increase in the mean value for the follicular over the periovulatory and an 83% increase over the luteal (Figure 3-1a). Mean values for threats directed toward male conspecifics indicate a 68% increase during the periovulatory compared to the luteal and a 57% increase in the follicular compared to the luteal phase of the cycle (Figure 3-2a). Females were observed to grab and/or bite male troop members more during the periovulatory phase of the cycle (Figure 3-2a). The frequency with which females lunged at male troop members showed a slight increase in mean values during the follicular phase (Figure 3-2a). Females were observed to avoid males in a relatively homogenous manner throughout the three phases of the cycle ($\chi^2=4.00$, df=7, P=0.14). Females also displayed fear grimaces toward males in a roughly consistent manner across the three phases ($\chi^2=4.75$, df=7, P=0.09), but did show a slight increase in the periovulatory.

The most predominant form of aggression between male and female Japanese macaques during the mating season involved males chasing females. Approximately 15% of chases included contact, whereby the male would catch the female and proceed to bite and pinch her. Males chased females frequently throughout the mating season and the occurrence of these chases varied significantly among the three phases of the cycle (χ^2 =9.75, df=7, P=0.01). Females were chased by male troop members significantly more often during the follicular and periovulatory compared to the luteal phase of the cycle (Z=-2.59, df=7, P=0.01; Figure 3-1b). The frequency of chases received during the follicular and periovulatory phases did not significantly differ (Z=-0.91, df=7, P=0.37), but mean values increased 35% from the follicular to the periovulatory phase. The occurrence of threats received by females from male troop members also did not vary significantly across the three phases of the cycle (χ^2 =1.20, df=7, P=0.55). Nevertheless, mean values demonstrate a 79% increase in frequency during the periovulatory compared to the follicular and a 75% in the luteal compared to the follicular (Figure 3-2b). The frequency with which females received grabs and/or bites from male conspecifics did not significantly differ among the three phases (χ^2 =1.08, df=7, P=0.58). Although not significant (χ^2 =5.20, df=7, P=0.07), females were observed to receive lunges by male conspecifics more during the periovulatory phase of the cycle (Figure 3-2b). Females were supplanted by male troop members during all three phases of the cycle, and the frequency of supplants did not differ significantly among the three phases (χ^2 =1.73, df=7, P=0.42).

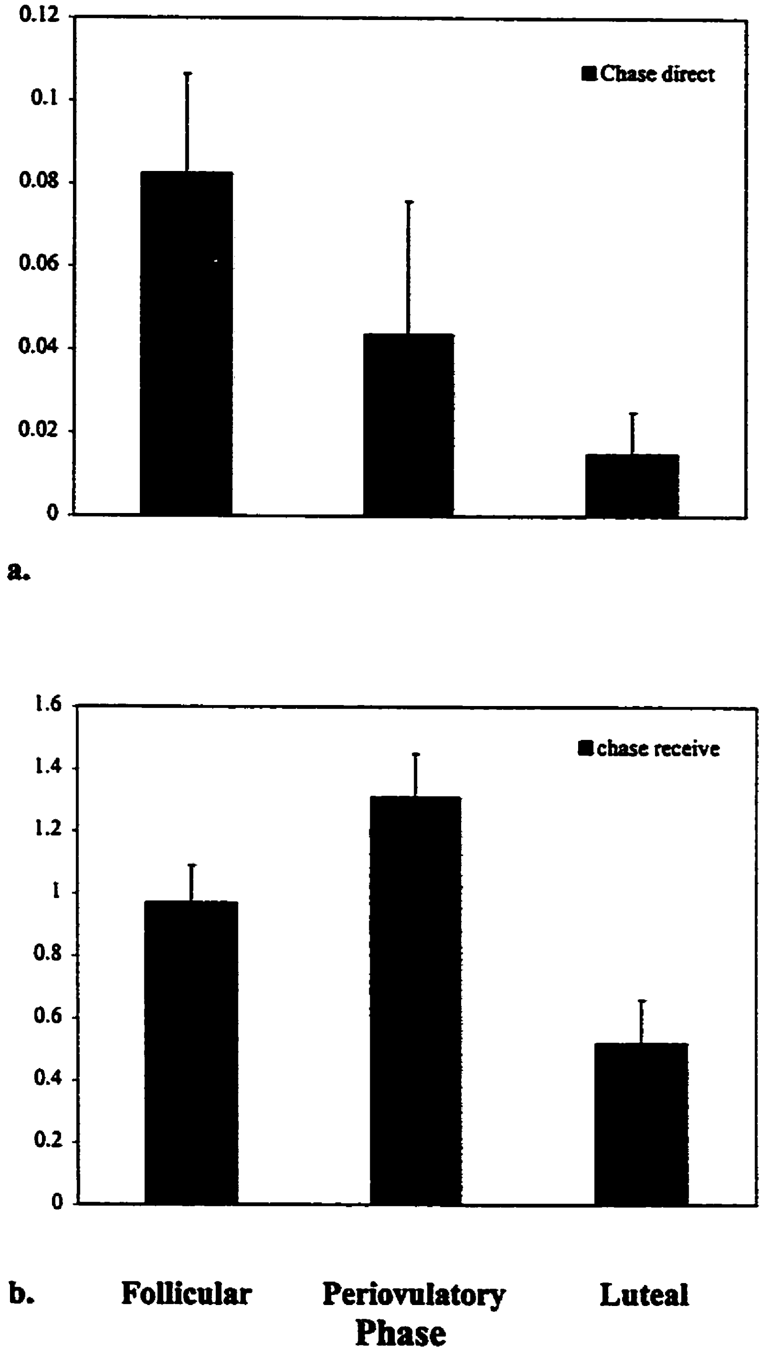

Figure 3-1. Chasing behavior displayed by target females, and received by target females from male conspecifics during three phases of the ovarian cycle.

a. Chases directed toward male conspecifics.
b. Chases received by male conspecifics.

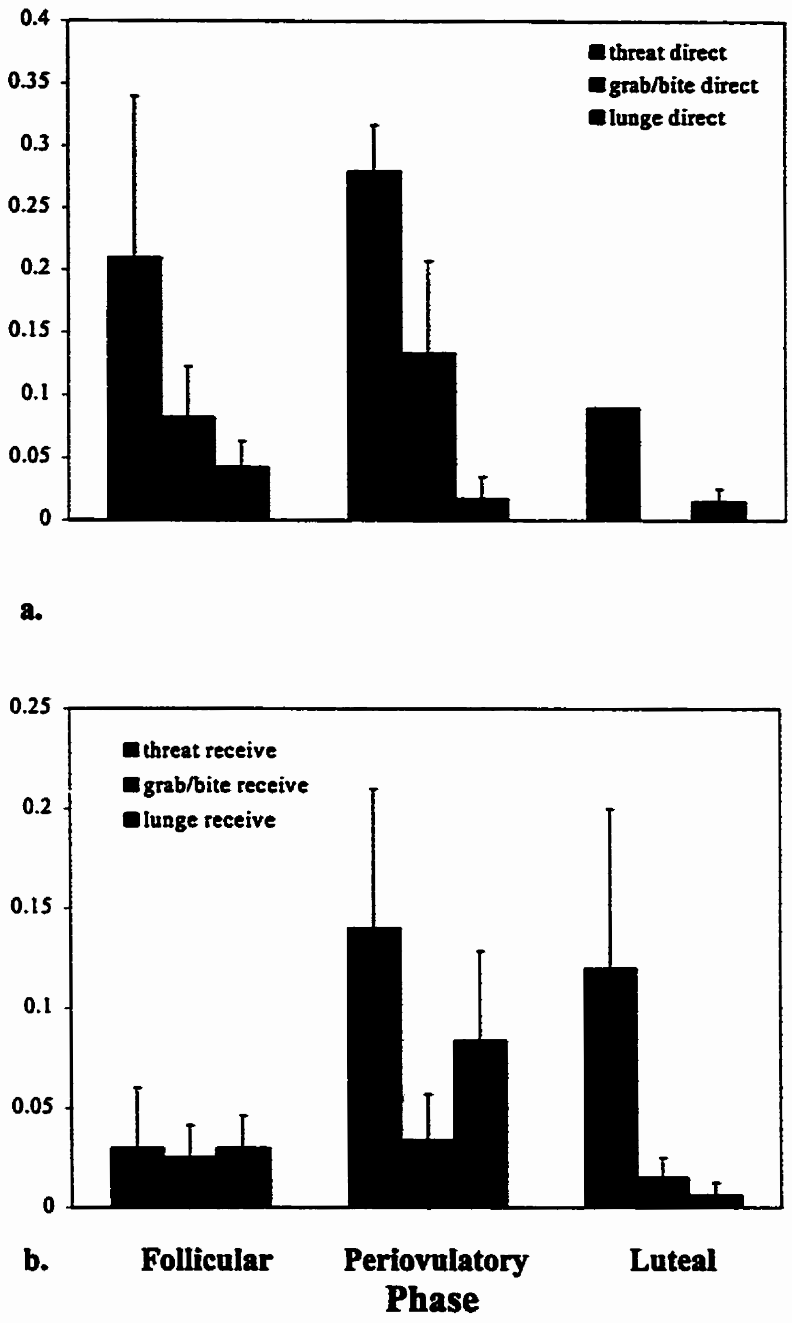

Figure 3-2. Aggressive behaviors displayed by Japanese macaque females, and received by females from male conspecifics during three phases of the ovarian cycle.
a. Aggressive behaviors directed toward male conspecifics.
b. Aggressive behaviors received by male conspecifics.

Female-Female Aggression

Aggression between female Japanese macaques often involves subtle facial threats, or supplants, but during the mating season aggression between females may escalate into chases, and occasional contact fights. The frequency of agonistic and submissive signals such as grabbing, biting, lunging, and fear grimaces occurred too infrequently between target females and other female conspecifics for statistical analysis. Therefore, statistical analyses were limited to chases, threats, supplants and avoids. Chases between female Japanese macaques occurred frequently throughout the mating season, and rarely resulted in physical contact. The frequency of chases directed by target females toward other females differed significantly among the three phases of the cycle (χ^2 =6.26, df=7, P=0.04). Target females were observed to chase other females more often during the periovulatory phase of the cycle (periovulatory Vs luteal: Z=-2.10, df=7, P=0.04; periovulatory Vs follicular: Z=-2.03, df=7, P=0.04; Figure 3-3a). The frequency of threats directed by target females toward other females differed significantly among the three phases of the cycle (χ^2 =5.85, df=7, P=0.05). The target females were observed to threaten (both facial and vocal) other females significantly more during the follicular phase of the cycle (Z=-2.46, df=7, P=0.01; Figure 3-3a). Due to the low rank of the target females, the occurrence of directed supplants toward other females were infrequent, and consequently did not significantly differ among the three phases (χ^2 =1.73, df=7, P=0.42). However, the mean frequency of directed supplants increased by 59% in the luteal, and 57% in the periovulatory compared to the follicular phase of the cycle, with little difference between the luteal and periovulatory phases (Figure 3-3a).

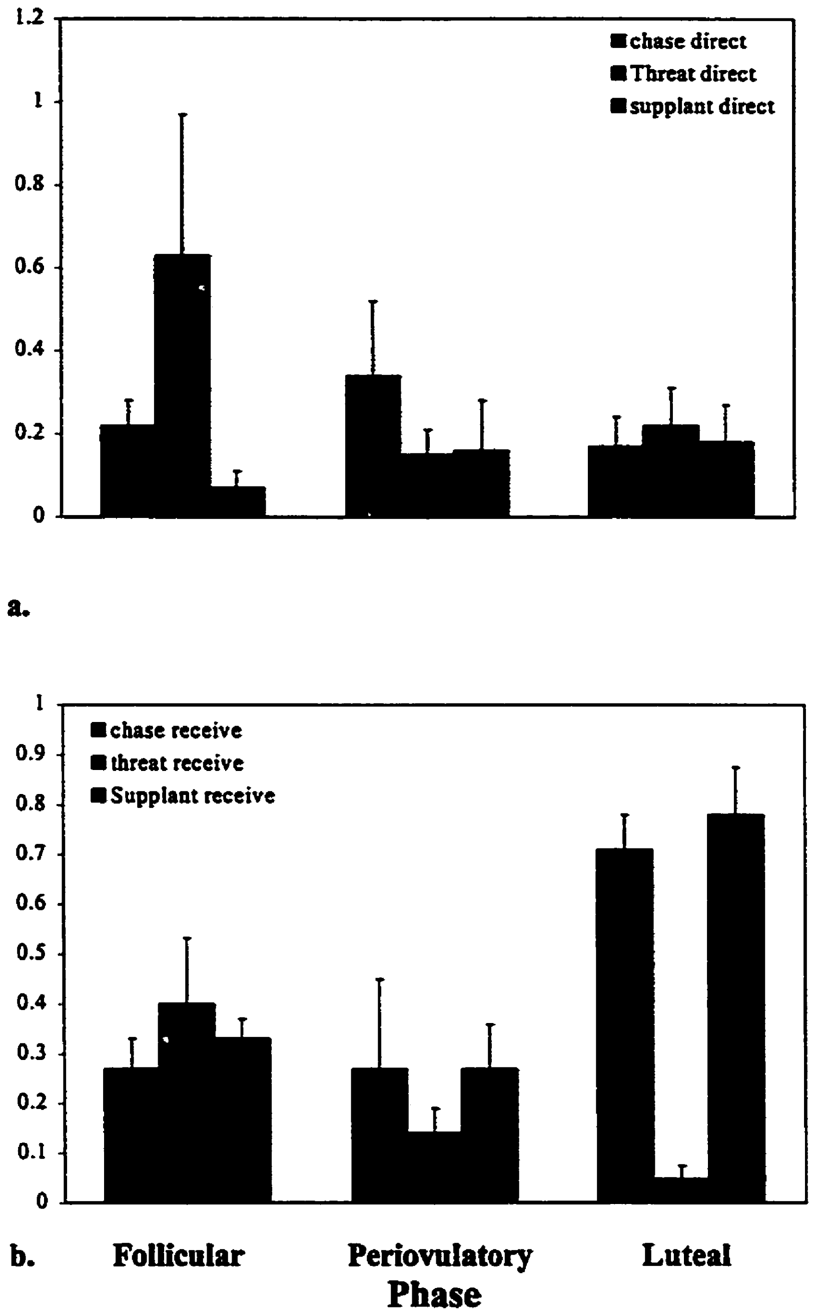

Figure 3-3. Aggressive behaviors displayed by Japanese macaque females toward female conspecifics.

a. Aggressive behaviors directed toward female conspecifics.

b. Aggressive behaviors received by female conspecifics.

The frequency of chases received by target females varied significantly across the three phases of the female's cycle (χ^2 =12.25, df=7, P<0.01). In contrast to directed chases, females were observed to be chased significantly more often by other females during the luteal phase of the cycle (luteal Vs periovulatory: Z=-2.25, df=7, P=0.01; luteal Vs follicular: Z=-2.25, df=7, P=0.01; Figure 3-3b). The occurrence of threats received by target females was also not found to vary significantly across the cycle (χ^2 =5.25, df=7, P=0.07). However, mean values indicate that females received 88% more threats from other females in the follicular, and 66% more in the periovulatory compared to the luteal phase of the cycle (Figure 3-3b). Although not statistically significant (χ^2 =1.93, df=7, P=0.38), females were also observed to be supplanted more often by other females in the luteal phase of the cycle (Figure 3-3b). Mean values denote a 65% increase in the frequency of supplants received by target females by other females in the luteal, compared to the follicular and a 58% increase in the luteal compared to the periovulatory phase of the cycle. As with avoids directed toward male troop members, the frequency of avoids directed at other females did not differ significantly among the three phases of the cycle (χ^2 =1.00, df=7, P=0.61).

Male-Female Affiliation

Affiliative patterns between male and female Japanese macaques are closely associated with copulatory activity during the mating season. During consorts, males and females are observed to spend more time in close proximity to each other and groom each other more (Enomoto, 1981). The affiliative patterns between male and female Japanese macaques is covered in more detail in chapter 2. The frequency of approaches directed toward male troop members by females differed significantly during the three phases of the cycle (see chapter 2 for statistical analysis). Target females approached males significantly more often in the follicular and periovulatory phases compared to the luteal (Chapter 2). Although the frequency of approaches received by females from male troop members did not vary significantly, there was a trend toward increased frequency in the follicular and periovulatory compared to the luteal (Chapter 2).

Females were observed to groom males significantly more during the follicular and periovulatory phases compared to the luteal (Chapter 2). In addition, females also received significantly more grooms from male troop members during the periovulatory phase of the cycle (Chapter 2). Spatial association patterns between male and female troop members also varied significantly among the three phases of the cycle, with the highest proportion of time spent in proximity to each other during the follicular and periovulatory phases of the cycle (Chapter 2).

Female-Female Affiliation

The frequency in which females approached other female troop members varied significantly throughout the three phases of the cycle ($\chi^2 = 10.75$, df=7, P=0.01). Females were observed to approach other females significantly more often during the luteal phase of the cycle (luteal Vs follicular: Z=-1.96, df=7, P=0.05; luteal Vs periovulatory: Z=-2.52, df=7, P=0.01; Figure 3-4a). The frequency in which females were approached by other females also varied significantly among the three phases of the cycle ($\chi^2 = 9.25$, df=7, P=0.01), with females being approached more often in the luteal compared to the follicular phase of the cycle (Z=-2.52, df=7, P=0.01; Figure 3-4a). Although there were no significant differences between the luteal and periovulatory phases (Z=-1.54, df=7, P=0.12), mean values demonstrate a 23% increase in the luteal compared to the periovulatory phase (Figure 3-4a).

The proportion of time females spent in close proximity to other female troop members varied significantly among the three phases of the cycle for all three criteria (contact: χ^2 =6.26, df=7, P=0.04; <1m: χ^2 =5.87, df=7, P=0.05; 1-4m: χ^2 =7.75, df=7, P=0.02). Target females were observed to spend the highest proportion of time in proximity to other females during the luteal compared to the follicular phase of the cycle (contact: Z=-2.38, df=7, P=0.02; <1m: Z=-2.38, df=7, P=0.02; 1-4m: Z=-2.20, df=7, P=0.03; Figure 3-4b). Although the proportion of time spent in proximity to other females did not differ significantly between the periovulatory and luteal phases (contact: Z=-1.26, df=7, P=0.21; <1m: Z=-1.40, df=7, P=0.16; 1-4m: Z=-1.52, df=7, P=0.13), mean values demonstrated a 31% increase in the amount of time females spent in contact with other females during the luteal phase compared to the periovulatory, as well as a 37% increase in the amount of time females spent within 1 meter of other females, and a 90% increase for time spent between 1 to 4 meters of other females (Figure 3-4b).

The proportion of time females spent grooming other females varied significantly among the three phases of the cycle (χ^2 =7.03, df=7, P=0.03). Target females were observed to groom other female troop members significantly more in the luteal phase of the cycle (luteal Vs follicular: Z=-2.24, df=7, P=0.03; luteal Vs periovulatory: Z=-1.96, df=7, P=0.05; Figure 3-4c). The proportion of time target females spent being groomed by other females also varied throughout the cycle (χ^2 =6.25, df=7, P=0.04), with the largest proportion of time spent during the luteal phase of the cycle (luteal Vs follicular: Z=-2.38, df=7, P=0.02; luteal Vs follicular: Z=-1.69, df=7, P=0.09; Figure 3-4c).

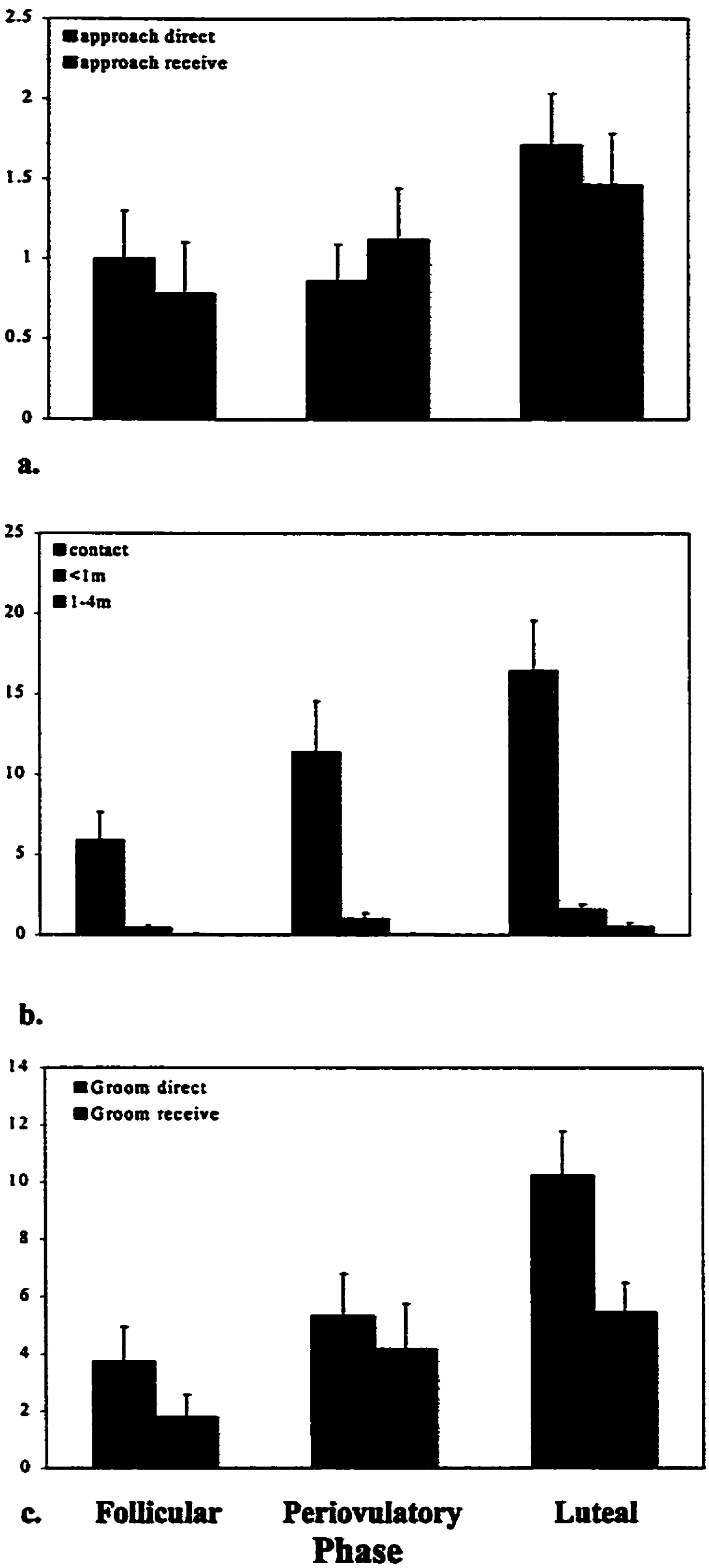

Figure 3-4. Affiliative behaviors during three phases of the ovarian cycle.
a. Approaches directed toward and received by female conspecifics.
b. Proximity to female conspecifics.
c. Grooms directed toward and received by female conspecifics.

DISCUSSION

This study examined the relationship between ovarian cycle phases and the aggressive and affiliative behaviors of female Japanese macaques during the mating season. The aggressive and affiliative patterns of Japanese macaques are complex and highly variable throughout the mating season. The direct and precise influence of progesterone and estrogen metabolites on aggressive and affiliative patterns remains obscure. Nevertheless, the aggressive and affiliative behaviors of Japanese macaque females did show cyclic changes in occurrence throughout the female's cycle and mating season.

Male-Female aggression

The target females for this study were all low ranking, and as such they rarely directed aggression toward male troop members. As a consequence, there were no statistically significant patterns. However, general trends in the data show an increase in aggression by females toward male conspecifics in the follicular and periovulatory phases of the cycle, coinciding with increased proximity to males at this time.

The most prevalent form of aggression received by the females from male troop members were chases. Chases occurred for various reasons throughout the mating season, but appeared for the most part to be linked to the female's copulatory period. Females became a target for male aggression during the follicular, and most notably the periovulatory phase of the cycle, coinciding with the period of female mating activity. The increased estrone levels, and decreased pregnanediol levels during these phases influence the attractivity of the female (Chapter 2), and consequently may increase the males inclination to mate with the female at this time. However, if the female is not receptive to the male's advances, he may use aggression to coerce the female into compliance. During the mating season, there were numerous wounds suffered by the female macaques. Four of the females in this study received serious wounds (i.e. had noticeable open wounds and bleeding) throughout the mating season. Of the six wounds documented during the study, four of them occurred during the late follicular phase, and the other two during the periovulatory phase. During the late follicular and periovulatory phases of the cycle females begin to increase their proximity to males, which in effect increases their vulnerability to attacks from male troop members.

Aggressive patterns between male and female Japanese macaques appear to be secondary to copulatory activity and increased proximity between males and females during the 1-2 week mating period (follicular and periovulatory). There is no evidence to support a direct influence of the female's hormonal levels and aggression. The data imply that agonism in a social context increases as the complexity of the social environment increases. Therefore, the increased proximity to males during the copulatory period increases the occurrence of aggression between male and female macaques.

Female-Female Aggression

Again, the low rank of the female contributes to an infrequent display of agonistic signals toward other female troop members. Target females rarely supplanted other females and were observed to be supplanted frequently throughout the cycle, with no significant pattern. Target females were observed to frequently avoid females, and this behavior also did not appear to be influenced by ovarian cycle phase. However, more direct forms of aggression, such as threats and chases, did appear to have a cyclic pattern throughout the mating season. Females threatened other female troop members more often during the follicular phase of the cycle. Also, females were observed to chase other females more often during the periovulatory phase of the cycle. The increased level of aggression during the periovulatory phase may indicate a lower tolerance of female conspecifics around the time of ovulation, and as suggested from studies with rhesus macaques (Michael and Zumpe, 1970), a possible influence by estrone in the aggressive tendencies of the female. Although, not analyzed statistically, females were observed to direct aggression toward other females more often when they were in a consortship with a male. With a male close by to back her up in a conflict, the increased aggression by the female during the periovulatory phase may be a function of increased confidence. It may also demonstrate a subtle form of competition between females. The close proximity of other females at this time may represent competition to the female in the consort, and the female in the consort may simply be protecting what is hers.

In contrast to directed chases, females were found to receive chases from other females more often in the luteal phase of the cycle. The increase in chases during the luteal phase may indicate greater vulnerability of the female at times when she is not engaged in a consort. The consort may also function to protect the female from agonistic encounters with other females. Further study on female-female aggression, during and after consortships is necessary to fully understand the functional significance of this behavior.

Male-Female Affiliation

Affiliative behaviors between male and female Japanese macaques appear to be primarily related to mating behavior. There are instances when the female grooms a male friend or relative, but most grooming tends to take place in the confines of a consortship. As indicated in chapter 2, females approach and groom males significantly more in the periovulatory phase of the cycle, when estrone levels peak, and pregnanediol levels are at base levels. In other words, when the female is most often engaged in a consort with a male. Males also approach and groom females significantly more during the periovulatory phase of the cycle (Chapter 2). In addition, male and female Japanese macaques are found to spend significantly more time in close proximity to each other during the periovulatory phase (Chapter 2). The affiliative patterns between male and female macaques appears to be under the influence of temporary sexual attraction mediated by the hormonal changes of the female's ovarian cycle.

Female-Female Affiliation

During the non-mating season female Japanese macaques form close matrilineal bonds, as well as other female-female friendships, and spend a great deal of time grooming, sleeping, eating, and traveling together. However, the behavior of the female macaque changes during the mating season. She spends more time with male troop members, and less time with female kin. Consequently, the female's social affiliations and partners vary across the ovarian cycle. The target females approached and groomed other female troop members more often in the luteal phase of the cycle, while male-female affiliations occurred more often in the periovulatory. Females were also observed to spend more time in close proximity to other females during the luteal phase of the cycle, and again, male-female proximity increased in the periovulatory phase. Females are spending more time engaged in affiliative interactions with other females during the luteal phase when they are not involved in consortships with male troop members. Therefore, the hormonal levels throughout the cycle only have an indirect affect on the affiliative behavior of the female. Affiliative patterns among female Japanese macaques appear to work themselves around the predominant copulatory activities of the female during the mating season, and as such, are not directly regulated by endocrine activity.

SUMMARY

The aggressive patterns of female Japanese macaques vary greatly depending on the phase of the cycle and the sex of the partner. Aggressive behavior between male and female monkeys is highly focused in the periovulatory phase of the cycle. However, aggressive behavior among female monkeys is more variable. Mild forms of aggression, such as threats (both directed and received), occur in higher frequencies during the follicular phase of the cycle. Yet, more intense forms of aggression, such as chases directed toward other females, occur at a higher frequency during the periovulatory, while chases received by other females have a higher frequency during the luteal phase of the cycle. As mentioned above, females may feel more confident during the periovulatory phase, when they are engaged in a consort with a male. Therefore the female may be more likely to display agonistic signals while they have a male close by to back them up in an ensuing conflict. As a result, females also receive fewer chases during the follicular and periovulatory, when they are more likely to be involved in a consort. Nevertheless, females may be more of a target for milder forms of aggression by other females when they are in the follicular and periovulatory phase of the cycle, nearing ovulation.

Affiliative behavior between male and female macaques varies across the ovarian cycle, with the highest frequencies occurring during the periovulatory phase. These patterns are consistent with consort behavior, with the female spending considerably more time interacting with male troop members. In contrast, affiliative behavior among female troop members occurs most frequently in the luteal phase when they are not involved in mating or consorts.

The influence that hormones have on the aggressive and affiliative patterns of Japanese macaque females is ambiguous. Increased aggression by males during the periovulatory phase, may simply be the result of the female exercising her choice in a male partner, or the mating strategy of the male. In other words, aggressive patterns during the mating season may be secondary to the females mating patterns, and not directly influenced by hormones. In addition, the increase in affiliative patterns between male and female macaques appears be the result of increased association between male and female troop members related to mating activity.

Patterns of aggression and affiliation among female Japanese macaques are also difficult to determine. There is some evidence that the female directs more aggression during the follicular and periovulatory phases, coinciding with increased competition among females for mating partners, as well as increased levels of estrone. However, higher levels of agonism are received in the luteal phase, when the female is not near ovulation, suggesting that the consort may protect the female somewhat from aggression. This is further evidenced by the increase in directed aggression, when the female is involved in a consort. Close qualitative observations of the females in this study suggested a shift in the female's rank when affiliated with higher-ranking males, initiating a more aggressive persona by the female. Affiliative behaviors among female macaques appears focused in the luteal phase, when the female is not mating, suggesting that mating activity influences interactions among females more than hormonal levels. The females are no longer pre-occupied with mating behavior during the luteal phase and therefore resume usual kin- or friendship-related activities.

CONCLUSIONS

1. Aggressive behavior between male and female Japanese macaques occurs at higher rates during the periovulatory phase of the cycle.
2. Female Japanese macaques direct milder forms of aggression toward female conspecifics at higher frequencies during the follicular phase, while directing more intense forms at higher rates during the periovulatory.
3. Females also received milder forms of aggression from other females at higher frequencies during the follicular phase, but other females received more intense forms of aggression during the luteal phase of the cycle.
4. Affiliative behavior among female Japanese macaques occurs at higher rates during the luteal phase of the cycle.

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Chapter Four

Hormonal Influences on Nonconceptive Mating In Japanese Macaque Females

INTRODUCTION

Sexual behavior in many primate species occurs at higher rates than required to ensure conception, and often occurs after the female has already conceived. Many macaque species exhibit nonconceptive forms of sexual behavior, such as post-conceptive mating and same-sex consortships. Although homosexual behavior is widespread across anthropoid primates, it is apparently completely absent among prosimians (Vasey, 1995). Of the 33 species in which homosexual behavior has been observed, only 12 commonly display this pattern under free-ranging conditions (Vasey, 1995). Homosexual behavior occurs in all the major primate social systems, but appears to be more common among multimale- multifemale groups (Smuts et al., 1986).

Same-sex consortships among primates is usually characterized by ventrodorsal mounting accompanied by thrusting, with occasional occurrences of ventroventral mounting (Vasey, 1995). Homosexual and heterosexual mounts are often indistinguishable and are associated with similar vocalizations and patterns of social behavior such as grooming and synchronized movements. In general, primates engaging in same-sex consorts appear to have highly flexible and varied sexual repertoires. Some studies have alluded to a positive correlation between estrus and homosexual activity in female Japanese macaques (e.g. Wolfe, 1979); however, no direct studies of the hormonal status of females during same-sex mating have been conducted.

The Arashiyama West (Texas) troop of Japanese macaques (*Macaca fuscata*) is known for its high rates of female homosexuality (Wolfe, 1979; Gouzoules and Goy, 1983). It should be noted however, that females who engage in such behavior are not exclusively homosexual; but also engage in heterosexual activity. The proportion of Arashiyama West females engaging in same-sex consorts appears to vary from year to year (61%: Fedigan and Gouzoules, 1978; 78%: Wolfe, 1979; 51%: Gouzoules and Goy, 1983). The Arashiyama B troop (Japan) displayed a somewhat lower, but also variable, proportion of females engaging in same-sex consorts (27%: Wolfe, 1986; 47%: Takahata, 1982). Wolfe (1984, 1986) proposed that the higher rates of homosexual activity expressed by the females in the Arashiyama West group represent a quest for sexual novelty and are related to a sex ratio skewed in favor of adult females (i.e. a "shortage" of adult males).

Another form of nonconceptive mating behavior observed in many primate species is post-conceptive mating activity. Although post-conceptive sexual activity has been described in the Japanese macaque (Eaton, 1978; Fedigan and Gouzoules, 1978; Hanby, 1972; Nigi, 1976; and Takahata, 1980; Wolfe, 1976, 1979), the relationship between this behavior and the ovarian status of the female has not been clarified. While female macaques spend a considerable amount of their adult lives pregnant, changes in social behavior in relation to pregnancy have been inadequately investigated. Pregnancy is associated with marked fluctuations in concentrations of ovarian hormones such as progesterone and estrogen, which are known to affect primate behavior (e.g. Chapter 2 and 3 of this thesis). Therefore, changes in the social behavior of females during pregnancy are to be expected.

Rhesus macaques (*Macaca mulatta*) have been observed to continue copulating during pregnancy, displaying a secondary peak in mating activity between the 6th and 10th week of gestation, when progesterone levels temporarily drop (Bielert et al., 1976). Continued copulation during early pregnancy has also been noted in field studies of the rhesus macaque (Loy, 1971; Lindburg, 1983). In contrast, female pig-tailed macaques do not show a decrease in progesterone levels until week 16, by which time the frequency of post-conceptive mating activity has all but ceased (Maestripieri, 1999). Nevertheless, pig-tailed macaques appear to show a similar peak in mounting behavior to that observed in the rhesus monkey between the 4th and 10th weeks of gestation (Maestripieri, 1999).

One hypothesis put forth to account for post-conceptive mating behavior in Hanuman langurs (*Presbytis entellus*) and some other primate species relates to the occurrence of infanticide. Hrdy (1974, 1977, 1979) views proceptivity and mating during pregnancy as a female tactic to confuse paternity. By mating with immigrant males after a group takeover, pregnant females reduce the risk of infanticide once their offspring are born. However, infanticide is absent in most multimale-multifemale primate societies, and is highest in polygynous, one-male groups (Dixson, 1998). Female Hanuman langurs show a peak in copulation between the 6th and 10th week of gestation (Sommer, 1993), coinciding with the time of peak post-conceptive mating also observed in rhesus macaques (Bielert et al., 1976), and the pig-tailed macaque (Maestripieri, 1999).

The present study examines hormonal correlates of two aspects of nonconceptive mating in Japanese macaque females: (1) homosexual activity; and (2) post-conceptive mating. Homosexual activity is known to occur frequently among Japanese macaque females during the mating season, and studies have suggested that such behavior coincides with periods of estrus, near the time of suspected ovulation (Wolfe, 1979). Therefore, I predict that (1) same-sex mounts will occur more frequently during the follicular and periovulatory phases of the cycle; and (2) the frequency of same-sex mounting behavior will be comparable to the frequency of heterosexual mounts. Mating behavior in Japanese macaque females occurs more frequently during the follicular and periovulatory phases of the ovarian cycle, when pregnanediol levels drop to base line (Chapter 2). In addition, other *Cercopithecine* species display a peak in post-conceptive mating activity between weeks 6 and 10 of gestation (Bielert et al., 1976; Sommer, 1993). Therefore, I predict that (1) mating behavior during pregnancy will occur when pregnanediol levels are low; and (2) females will display a peak in copulatory activity between the 6th and 10th week of gestation.

METHODS

Subjects

The subjects were 8 adult female Japanese macaques (*Macaca fuscata*) enclosed in a 65 acre enclosure at the Arashiyama West Texas Snow Monkey Sanctuary (TSMS). At the time of the study, the group consisted of approximately 350 semi free-ranging individuals of all age and sex classes. All eight females were between 10 and 11 years of age during the study period, and were of similar rank in the group (low-ranking). Seven females were multiparous, and one was primiparous. None of the eight females had given birth during the prior 1997 birth season (April-July). During the study period, 4 of the 8 females conceived and maintained pregnancy to term. (One other female did conceive late in the mating season, but miscarried two weeks later; see below.)

Behavioral and fecal data collection

Behavioral data were collected during the 1997/1998 breeding season, from October to April. A total of 352 hours of focal data were collected on eight females (41 to 48 hours per subject). Data were collected during 30 minute focal animal observations and were varied throughout the day for each female when possible. It was nevertheless, sometimes necessary to collect samples sequentially to allow for the collection of fecal samples. If the subject was lost prior to the end of the session, the session was terminated and the data discarded.

Fecal samples were collected two to three times per week from the eight target females between the hours of 8:00 AM and 6:00 PM. Fecal collection was carried out during focal animal follows, as well as opportunistically. Samples were collected within 10 minutes of defecation and placed in 30 ml sterile plastic vials and frozen for preservation. Frozen samples were transported to the Wisconsin Regional Primate Research Center (WRPRC) in a cooler containing dry ice, and stored in a freezer at WRPRC until analysis.

Sample Extraction and Analysis

Extraction of ovarian steroids was performed according to the procedure described by Strier and Ziegler (1997). See Chapter 2 for a detailed description of the procedure. Pooled fecal samples were used to validate the pregnanediol and estrone assays (see chapter 2 for validation procedures and results). Estrone was measured in feces employing an E1 EIA previously reported (Ziegler et al., 1995). Pregnanediol-3-

glucuronide was measured in feces by a PdG EIA also described previously (Carlton et al., 1996). EIA products were analyzed by ELISA for pregnanediol and estrone levels.

Behavioral Analysis

The results of the hormone analysis were used to create hormone profiles for each female (Figure 4-1). Hormone profiles were used to calculate ovulatory cycle and gestation lengths. The cycles were divided into the three phases: follicular, when PdG has dropped to base line levels; periovulatory, when PdG levels begin to slightly rise and E1 levels reach their peak; and luteal, when PdG levels stay well above base line for an extended duration and E1 levels return to base line (see Figure 2-1). In all, 26 complete cycles from eight females were identified. Only the cycles where females engaged in sexual behavior were included in the analysis.

Copulatory activity during pregnancy was analyzed for the 4 females who conceived and carried to term during the 1997/1998 mating season. Profiles were also created for hormonal levels during gestation (Figure 4-1). Data were collected during the first 10 to 15 weeks of gestation for the four females. The earliest possible day of conception was considered to be the estimated day of ovulation if pregnanediol levels failed to return to their lowest baseline levels. The length of gestation was calculated as the interval between the estimated day of ovulation during the cycle in which conception occurred and the date of parturition. Parturition dates were defined as the first day in which the female was observed with a new infant (parturition dates were reported by Lou Griffin, Tracy Wyman, and Tanya Bell at the TSMS, and were accurate to within 2 days).

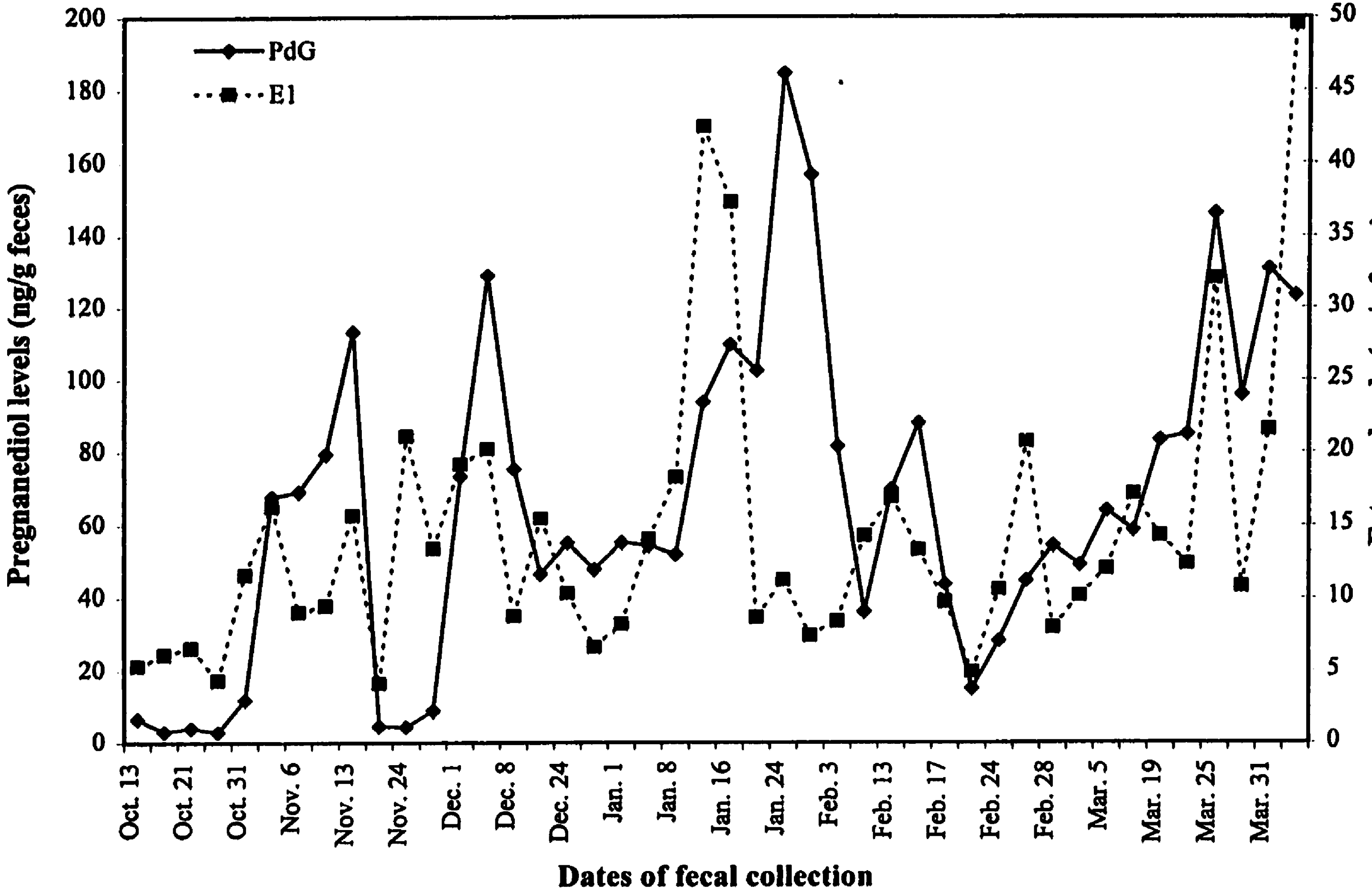

Figure 4-1. Representative hormone profile for one female Japanese macaque during the 1997/1998 mating season before and after conception.

A Spearman nonparametric correlation was used to analyze the relationship between hormone levels and sexual behavior during pregnancy (Siegal and Castellan 1988). Same-sex mounts were used in the analysis of homosexual behavior. Homosexual and heterosexual mounting frequencies were analyzed using Mann-Whitney U non-parametric tests (Siegal and Castellan 1988). All statistical analyses were performed using SPSS 8.0 for Windows.

RESULTS

Homosexual Activity

Five of the eight females engaged in homosexual consortships during the study period. However, of these five, only three of the females were involved in homosexual activity prior to conception. Table 4-1 summarizes the mounting patterns of the eight females during the study period. Analyses of homosexual and heterosexual activity were restricted to those individuals who engaged in same-sex consorts during regular cycling prior to conception.

Table 4-1. Number of homosexual and heterosexual consorts observed for each female during the 1997/1998 mating season.

	Pre-conceptive		**Post-conceptive**	
Female	**Heterosexual**	**Homosexual**	**Heterosexual**	**Homosexual**
Trisha*†	-	-	-	1
Saskia*	4	-	2	-
Zoe*	2	-	1	-
Tyler*	1	-	1	5
Tantalis**	11	-		
Shy Mug	8	1		
Lisa	4	1		
Bo Nose	6	3		

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* Females that conceived and carried to term.

** This female conceived late in the mating season, but miscarried after two weeks.

† This female was not observed mating prior to conception, but heterosexual activity is assumed due to conception.

The frequency of mounts directed toward female partners varied across cycle phase, although not significantly (χ^2=4.67, P=0.09), with the highest rates during the follicular and periovulatory (Figure 4-2a). The frequency of mounts received by female partners also varied across cycle phase, with the highest rate of mounts received during the follicular and periovulatory phases (χ^2=5.64, P=0.06; Figure 4-2b). The frequency with which females mounted female partners during the follicular phase of the cycle did not differ significantly from the frequency with which they mounted male partners (U=3.50, P=0.70; Figure 4-2a). Additionally, the frequency of directed heterosexual mounts versus homosexual mounts did not differ during the periovulatory phase of the cycle (U=2.00, P=0.40; Figure 4-2a). The frequency of mounts received by the target females by male partners did not differ significantly from the frequency of mounts received by female partners during the follicular phase of the cycle (P=0.70), nor did they differ during the periovulatory phase of the cycle (P=1.00; Figure 4-2b). None of the three females used in this analysis engaged in either heterosexual, or homosexual activity during the luteal phase of the cycle.

Hormone Levels During Early Pregnancy

The mean concentrations of estrone and pregnanediol in fecal samples, obtained two to three times weekly, fluctuated throughout the initial 110 days of gestation. Mean pregnanediol levels were 41.41 ng/g in the first week of gestation, increasing to 83.96 ng/g by the 4th week, followed by a drop to 45.61 ng/g by week 9, then steadily increasing to 105.20 ng/g by 16th week (Figure 4-3a). Concurrently, as shown in Figure 4-3b, mean estrone levels were 8.60 ng/g in the first week of gestation, but increased to a mean concentration of 19.64 ng/g by week 5, followed by a drop in mean concentration

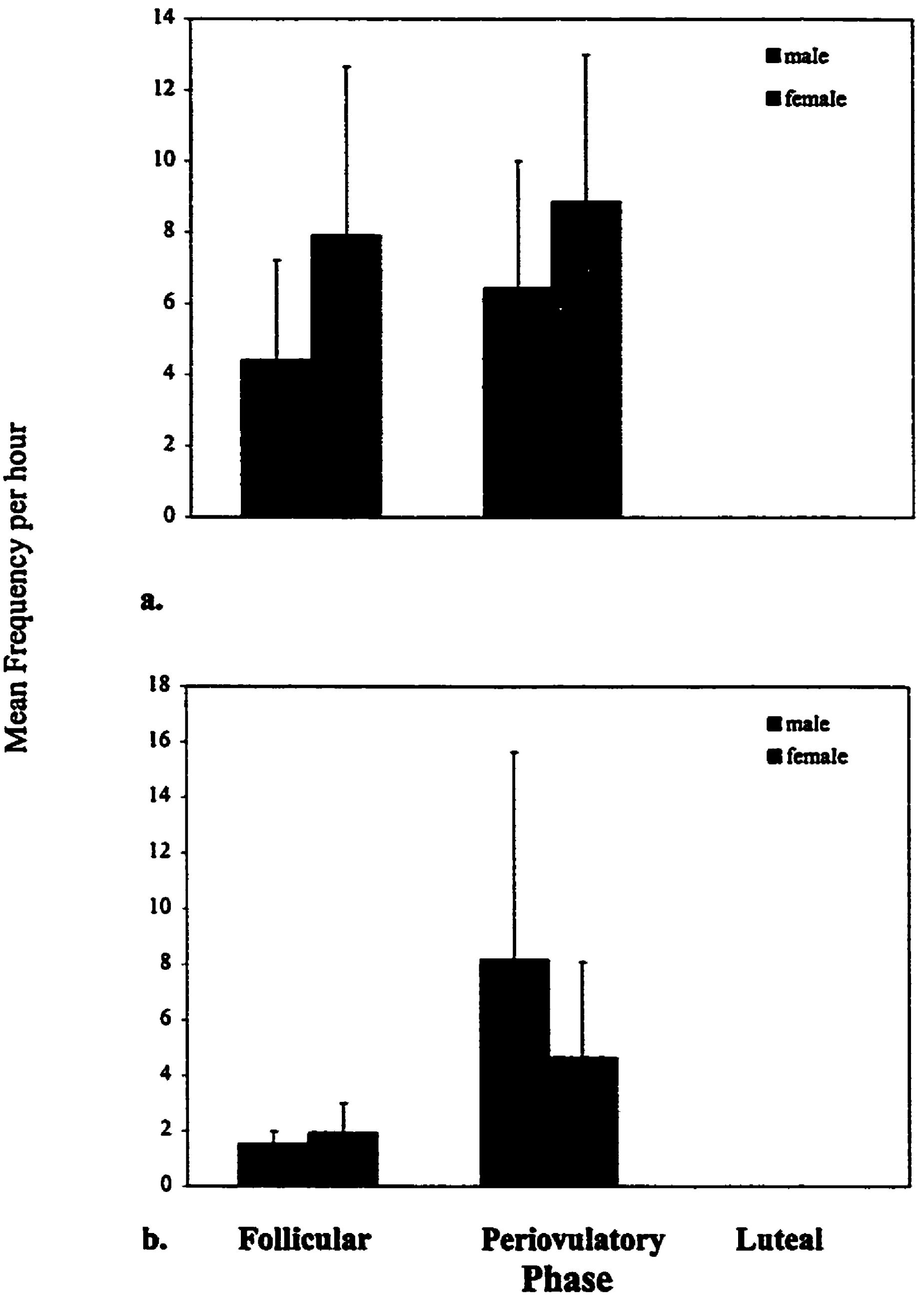


Figure 4-2. Heterosexual and homosexual mounting activity of female Japanese macaques during three phases of the cycle (X ± SE).

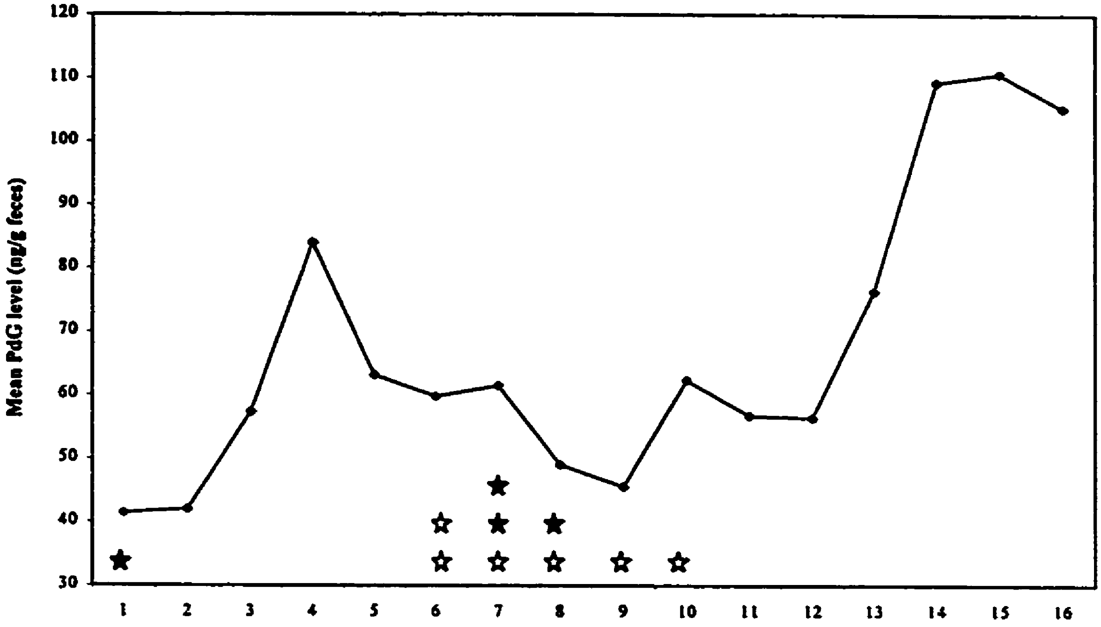
a. Mounts directed by target females toward male and female partners.

b. Mounts received by target females from male and female partners.

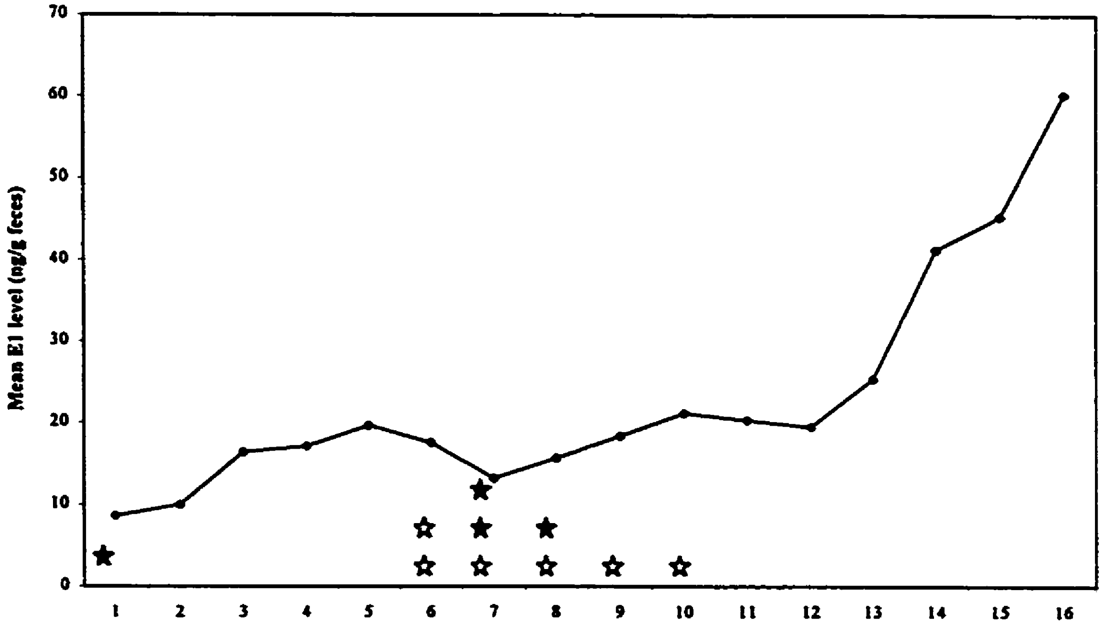
Mann-Whitney U statistical analysis:

a. Follicular U=4.00, df=2, P=1.00; periovulatory U=2.00, df=2, P=0.40.

b. Follicular U=3.50, df=2, P=0.70; periovulatory U=4.00, df=2, P=1.00.



a.



b.

Figure 4-3. Hormonal levels during the initial 16 weeks of gestation in four Japanese macaque females. ★ Indicates weeks in which heterosexual consorts took place.
☆ Indicates weeks in which homosexual consorts took place.

a. Mean weekly pregnanediol (PdG) levels (N=4)
b. B. Mean estrone (E1) levels (N=4)

to 13.23 ng/g in week 7, and then steadily rising to 60.21 ng/g by the 16th week. Hormone levels during pregnancy were found to be highly variable among individual females. Nevertheless, a positive correlation was found between PdG and E1 levels (Spearman: r_s= 0.37, P=0.006), showing a uniform rise in both hormones as the pregnancy nears term (Figure 4-1 and 4-3).

During the study period, four of the eight females conceived after 1 to 4 cycles. One other female conceived in the fifth cycle, but miscarried after only two weeks (Figure 4-4). This female displayed normal cyclic patterns in hormonal levels prior to conception followed by an increase in pregnanediol levels typical of the first few weeks of gestation. However, in the period from March 16 to April 4, 1998 the pregnanediol levels plummeted below baseline levels and remained low, indicating a termination of the pregnancy. This was further evidenced by the appearance of the female on March 15, 1998, when she was observed with blood spattered along her hind quarters (a larger quantity than would occur during menstrual flow). This female also miscarried in a prior year (1995) late in the mating season. The mean gestation length for the four females that carried to term was calculated to be 175 ± 12.8 days (±SD; median=173 days; range=163 to 192 days).

Post-conceptive mating

All pregnant females engaged in mating activity after conception. Of these four females, one was involved in both heterosexual and homosexual consorts, one was involved exclusively in homosexual consorts, and the other two exclusively in heterosexual consorts. The rate of mounts received by target females was similar for both

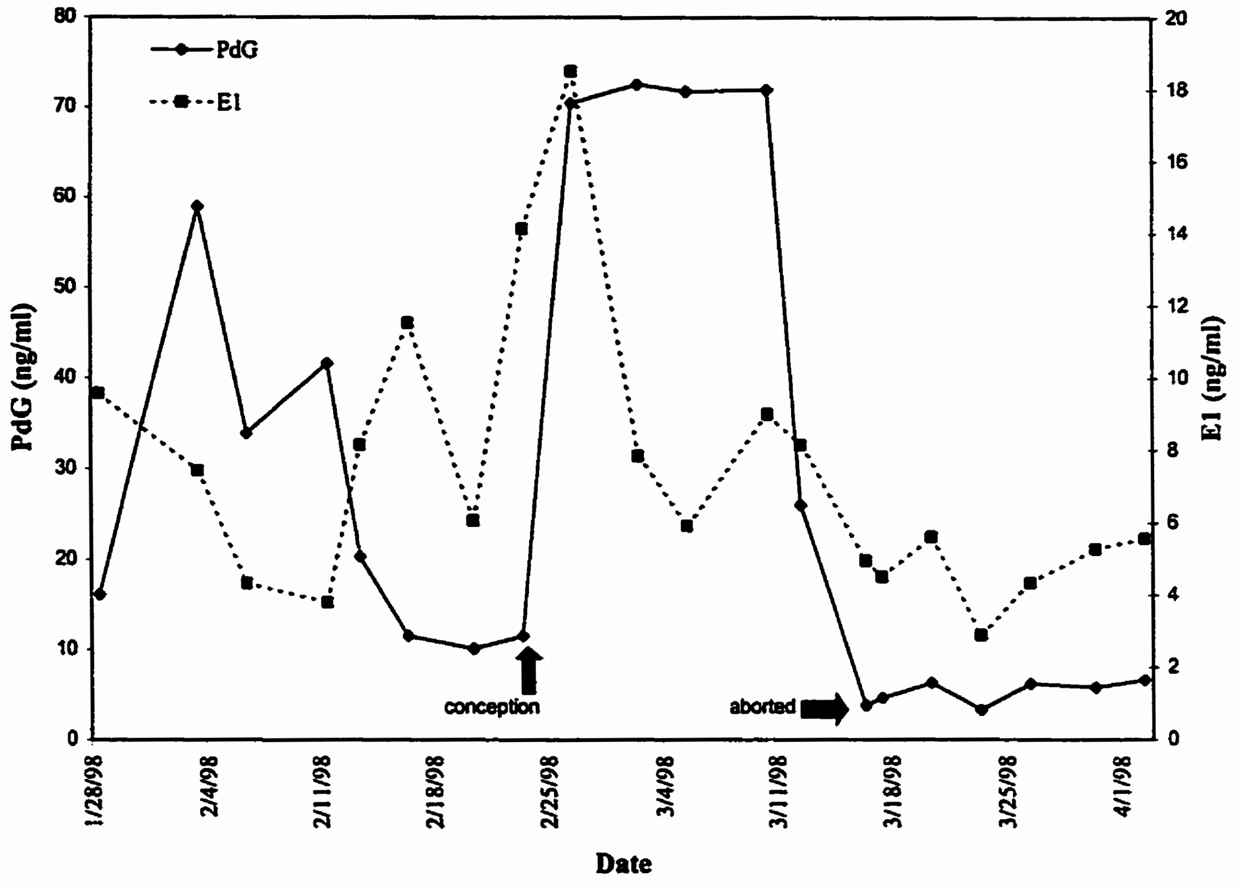


Figure 4-4. Profile of hormone levels for one female Japanese macaque who conceived and aborted during the 1997/1998 mating season. Depicted is the cycle immediately prior to conception, followed by the period of gestation, and the ensuing drop in pregnanediol concentrations following termination of the pregnancy.

heterosexual and homosexual consorts (13.17/hour for homosexual and 16.86/hour for heterosexual). The rate of mounts directed by target females were also comparable for both heterosexual and homosexual consorts (5.13/hour for homosexual and 5.67/hour for heterosexual). Therefore, heterosexual and homosexual mounting behavior were not considered independently in the analysis of post-conceptive mating activity.

A negative correlation was found between pregnanediol (PdG) levels and the occurrence of mounts received and directed by the target females during pregnancy (Spearman: mounts received: r_s=-0.41, P=0.002; mounts directed: r_s=-0.41, P=0.002). Females engaged in mounting behavior (both directed and received) during periods when PdG levels were low (mean = 37.70 ng/ml; median = 35.13 ng/ml). No significant correlation was found between E1 levels and mounts received or directed by target females during pregnancy (Spearman: mounts received: r_s=-0.09, P=0.54; mounts directed: r_s=-0.08, P=0.54).

DISCUSSION

Homosexual Activity

Female homosexual behavior has been observed in most free-ranging and captive groups of Japanese macaques (Fedigan, and Gouzoules, 1978; Takahata, 1982; Vasey, 1996; Wolfe, 1984). Homosexual mounts between female Japanese macaques are considered sexual for a number of reasons (Wolfe, 1984). For example, reciprocal mounting interactions are confined to the mating season; the behavior occurs when the females are showing signs of estrus; same-sex mounting behavior is indistinguishable from heterosexual mounting; contact is maintained by mutual following and grooming, as in heterosexual consortships and; females avoid mounting interactions with close matrilateral female relatives, just as they avoid male relatives.

Homosexual mounting behavior occurred most frequently during the same phases of the cycle as heterosexual mounts (follicular and periovulatory), supporting earlier suggestions that homosexual activity is linked to periods of estrus, as well as to sexual proceptivity and receptivity (Fedigan and Gouzoules, 1978; Wolfe, 1984). Mounting events observed in this study included most of the behavioral elements typical of heterosexual interactions of Japanese macaques (multiple mountings, pelvic thrusts, consortships, etc.).

Three of the eight females engaged in homosexual consortships during regular cycling, and two other females were involved in homosexual consortships during pregnancy. All females who engaged in same-sex consorts during the mating season were also involved in a number of heterosexual consortships. It is worth noting that the three females who engaged in homosexual activity during regular cycling are also the only three females in my sample that did not conceive at any point during the mating season.

Homosexual activity in female Japanese macaques appears to follow a similar pattern as that seen in heterosexual consortships, suggesting a link between hormonal activity and sexual proceptivity and receptivity, regardless of partner preferences. Females frequently engaged in sexual activity with sub-adult males, and sub-adult males fathered the offspring of the four females that conceived. The occurrence of homosexual consortships during the same cycle phases as heterosexual consortships, along with evidence of increased mating activity with sub-adult males, supports the hypothesis that female homosexual activity may represent a quest for sexual novelty (Wolfe, 1984). This is further evidenced by the choice of partners. Those who engaged in same-sex consorts favored females that they normally did not interact with, and successive consortships were with different partners. Although adult males frequently interrupted consortships with sub-adult males, same-sex consortships were rarely interrupted. Therefore, females may have chosen same-sex partners for novelty, sexual gratification, defense from unwelcome male solicitors, or for all of these reasons. The occurrence of homosexual behavior among primates remains insufficiently documented and inadequately understood. More research is needed to better understand the hormonal influences on homosexuality, as well as the underlying factors influencing partner choice.

Hormones and Pregnancy

The mean gestation length of these Japanese macaque females was determined to be 175 ± 12.8 days (±SD; median=173 days; range=163 to 192 days), which is comparable to earlier findings based on non-hormonal studies. Nigi (1976) reported a mean gestation length of 173 ± 6.9 days, with a range from 161 to 188 days, Kawai (1966) reported a gestation period of 171-180 days, and Asakura (1960) found that the mean gestation period was 5.4 months (~162 days).

Pregnanediol and estrone levels showed patterned fluctuations in the initial 100 days of gestation. PdG concentrations rose steadily from the time of conception to approximately the 5th to 6th week of gestation, followed by a temporary drop in concentration between weeks 6 and 10, after which PdG levels continue to increase steadily toward term. Although E1 levels followed a similar pattern as that of PdG, the changes in concentration were not as marked during the temporary decline in the 6th to 10th week. This pattern of hormone fluctuations throughout pregnancy is comparable to results from other macaque species (*Macaca mulatta*: Hodgen et al., 1972; *Macaca fasicularis*: Stabenfelt and Hendrickx, 1973; *Macaca radiata*: Stabenfelt and Hendrickx, 1972).

Post-conceptive mating

There was a correlation between hormone levels and the expression of post-conceptive mating behavior in Japanese macaque females. Four of the eight females conceived and carried to term during the 1997/1998 mating season. All four of these females engaged in mounting interactions during pregnancy, either heterosexual, homosexual, or both. The rate of mounting behavior was very similar for both same-sex and heterosexual mounting interactions.

A significant negative correlation was found between pregnanediol levels during gestation and the occurrence of mounting interactions. However, there was no significant relationship between estrone concentrations and mating activity during pregnancy. Decreases in pregnanediol levels during gestation may allow for the expression of proceptive and receptive behavior in females, similar to the effects of decreased pregnanediol levels during the follicular and periovulatory phases of the cycle (see chapter 2).

All females showed a drop in pregnanediol levels between the 6th and 10th week of gestation, during which time the majority of mounting interactions occurred. These results are similar to earlier findings with rhesus monkeys (Bielert et al., 1976) and Hanuman langurs (Sommer, 1993), showing a peak in post-conceptive mating behavior between the 6th and 10th week of gestation.

The ovary is essential for the production of progestins and estrogens during the first few weeks of gestation, after which the placenta assumes the production of these hormones. The increase in PdG and E1 between the 3rd and 5th week of gestation may represent a temporary dual production of these hormones by both the ovary and the placenta. The subsequent drop in hormones between weeks 6 and 10 may represent a loss of the ovary's contribution, while the placenta attempts to take its place in the production of progestins and estrogens. Both PdG and E1 levels then begin to recover and steadily continue to rise toward term. Therefore, the temporary drop in pregnanediol levels during the 6th and 10th weeks of gestation may produce a false estrus or period of proceptivity in the female macaque. More quantitative data are required to determine whether hormone levels during pregnancy play a significant role in post-conceptive mating, and whether this peak in mating activity between weeks 6 and 10 is prevalent in other Old World monkey species.

SUMMARY

Five of the eight females were observed to engage in homosexual activity during the study period, but only three were involved in same-sex consorts prior to conception. During regular cycling, these three females showed similar patterns in homosexual mounting activity as that observed in heterosexual mounting, and as with heterosexual mounts, females displayed higher rates of same-sex mounts during the follicular and periovulatory phases of the cycle. The behavioral elements typical of heterosexual mounting (e.g. multiple mounting events, pelvic thrusts, formation of consort bonds, etc.)

were also present in homosexual mounting and were indistinguishable from heterosexual activity.

Four of the eight females conceived and carried to term during this study, and all four of these females engaged in either heterosexual or homosexual activity during pregnancy. The females displayed a peak in post-conceptive mating activity between the 6th and 10th week of gestation, coinciding with decreased pregnanediol levels. Although the particular factors responsible for the observed fluctuations in sexual activity after conception are not explicit, the present study indicates that post-conceptive mating activity is not independent of hormonal or reproductive condition.

CONCLUSIONS

1. Homosexual mounting behavior among female Japanese macaques occurred most frequently during the same phases of the cycle as heterosexual mounts (follicular and periovulatory).
2. The mean gestation length of the Japanese macaque females in this study was 175 ± 12.8 days.
3. A negative correlation was observed between pregnanediol levels during gestation and the occurrence of post-conceptive mounting activity.
4. Females displayed a peak in sexual activity between the 6th and 10th week of gestation, when pregnanediol levels were at a temporary low.

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Chapter Five

General Discussion and Conclusions

In this thesis, I have examined several aspects of Japanese macaque behavior as they relate to the hormonal status of the female during the mating season. In Chapter two I discussed the relationship between female sexual behavior and ovarian cycle phase. Questions relating to the influence of ovarian hormones on the female's proceptivity and attractivity across the ovarian cycle were addressed. In Chapter three I looked at the relationship between ovarian cycle phase and the female's aggressive and affiliative patterns. In Chapter four I considered the influence of ovarian hormone levels on nonconceptive copulatory behavior. Patterns of homosexual activity were analyzed for cyclic patterns during the ovarian cycle, as well as similarities to heterosexual mating behavior. Post-conceptive copulatory activity was analyzed for pregnanediol and estrone influences.

Chapter two presents evidence that ovarian cycle phase is a significant predictor of the presence of proceptivity and attractivity in the female. Females in this study engaged in higher rates of sexual activity during the follicular, and particularly the periovulatory phases of the cycle, indicating a higher level of attractivity to male troop members, as well as increased proceptivity. The proceptivity and attractivity of the female was positively correlated with decreased pregnanediol levels and increased estrone levels, hormonal patterns that are typical of the periovulatory phase of the cycle. In addition, females displayed an increase in other behaviors relating to proceptive behavior, such as approaches, consort grooming, and spatial proximity, during the follicular and periovulatory phases. Females were also approached and groomed more by sexual partners during these phases, indicating an increase in their attractivity.

The results of this study indicate that sexual behavior in Japanese macaque females occurs at variable rates during the follicular, periovulatory, and luteal phases of the ovarian cycle, and that sexual behavior is not independent of hormonal influences. The increase in estrone levels during the follicular and periovulatory phases, coupled with decreases in pregnanediol levels appears to stimulate proceptive behavior in the female, as well as to increase the female's attractiveness to males. With the increase in association between males and females during the follicular and periovulatory phases of the ovarian cycle, the affiliative behavior between male and female macaques also increases. Conversely, when pregnanediol levels increase and estrone levels decrease during the luteal phase of the cycle, copulatory activity virtually ceases. This study indicates that circulating ovarian steroids play a significant role in the expression of sexual behavior, as well as the reproductive patterns of female Japanese macaques.

In chapter three I examined the influence of hormonal status on the aggressive and affiliative behaviors of females during the mating season. Aggressive behavior between male and female troop members was more frequent during the periovulatory phase of the cycle, when most sexual interactions are occurring. Therefore, I conclude that aggression between male and female troop members appears to be the result of increased association related to mating activity, and not directly influenced by ovarian hormones. Females are involved in higher rates of agonistic encounters with males during the period when they are most fertile, suggesting a form of coercion, or mating strategy on the part of the male. Affiliative behavior between male and female troop members also appears to be secondary to mating activity. Affiliative behaviors are displayed at a higher rate during the fertile period, coinciding with consortships. As discussed in Chapter two, when engaged in a consortship, females spend considerably more time in the company of males, including traveling, foraging and grooming together. Consorts are formed more frequently during the follicular and periovulatory phases of the ovarian cycle, and therefore females were observed to engage in higher rates of affiliative behaviors with males at this time.

Aggressive patterns between female troop members is more varied. Females display higher rates of milder forms of aggression toward other females during the follicular phase, while more intense forms, such as chasing, occur more frequently during the periovulatory phase. In contrast, females receive more agonism from other females during the luteal phase of the cycle. Females also engage in more affiliative behaviors with other females during the luteal phase of the cycle. Again, it appears that the aggressive and affiliative behavior of the female toward other female troop members, is secondary to her mating patterns. Females direct more aggression toward other females when they are involved in a consortship, suggesting that they become more confident with a male to back them up, and they are also more threatened by female competition at this time. In contrast, females receive more aggression during the luteal, when they no longer have the male to back them up. The aggressive and affiliative patterns of Japanese macaque females appear to be independent of hormonal influence and more affected by the female's associations during the mating season.

In chapter four I examined evidence for hormonal influence on nonconceptive copulatory activity in females. Females engaged in homosexual activity more during the follicular and periovulatory phases of the cycle. Patterns of homosexual activity in female Japanese macaques were similar to heterosexual consortships, suggesting a link between hormonal activity and sexual proceptivity and receptivity, regardless of partner preferences. Post-conceptive copulatory activity occurred in all four of the females that carried to term. Females engaged in the majority of their post-conceptive sexual activity between the 6th and 10th week of gestation, when pregnanediol levels were temporarily low. This pattern of post-conceptive mating is similar to that found in other macaque species (*Macaca mulatta*: Bielert et al., 1976). The temporary drop in pregnanediol levels between the 6th and 10th week of gestation coincides with the transfer in the production of ovarian hormones from the ovaries to the placenta observed in early pregnancy. The fluctuations in the production of the ovarian steroids during this brief stage appear to result in a period of pseudo estrus, when females show an increase in attractivity and proceptivity, resulting in an increase in copulatory activity at this time.

Many nonhuman primates engage in sexual activity far more than required for the purposes of conception, and consequently a great deal of their sexuality is non-reproductive. Female Japanese macaques have a varied and complex behavioral repertoire. Nevertheless, it is evident that ovarian hormones play an important role in the expression of many of these behaviors, particularly sexual. Other activities, such as aggressive and affiliative behaviors, appear to be secondary to the females mating patterns. In addition, the presence of non-conceptive forms of sexual activity suggests that both reproductive and non-reproductive sexual behavior are an integral part of Japanese macaque society, and a necessary component of primate sociality.

This study has demonstrated the accuracy and predictive value of fecal steroid extraction for determining ovarian function in Japanese macaque females. The importance of this technique is not only an effective method for identifying and assessing hormone profiles in nonhuman primates, but is also an important tool for examining the relationship between hormonal patterns and social, behavioral and environmental factors.

The results of this study raise some interesting further questions such as: what influences do social factors, such as rank, age, or parity have on the reproductive behavior of females? What is the relationship between hormone levels and reproductive success of the female? What role, if any, does testosterone levels play in the sexual and aggressive patterns of the female? What is the hormonal status of the male during the mating season, and how does it influence the female's behavior? What role does the consortship play in the female's aggressive patterns? What is the relationship between homosexual behavior and the hormonal status of the partner? What is the extent of hormonal influence on post-conceptive copulatory behavior? Further studies are required to determine the full extent of hormonal influences on the social behavior patterns of Japanese macaques.

REFERENCES

Bielert, C.; Czaja, J.A.; Eisele, S.; Scheffler, G.; Robinson, J.A.; and Goy, R.W. 1976. Mating in the rhesus monkey (*Macaca mulatta*) after conception and its relationship to oestradiol and progesterone levels throughout pregnancy. *Journal of Reproduction and Fertility* 46: 179-187.

APPENDIX 1

Identification, matriline, age, and reproductive history for each female.

Subject	Matriline	Tattoo #	Year of Birth	Parity
Shy Mug	Wania	87	1987	Nulliparous
Tantalis	Matsu	163	1986	Primiparous
Lisa	Matsu	734	1987	Primiparous
Bo Nose	Nose	736	1987	Multiparous
Trisha	Wania	43	1987	Multiparous
Saskia	Petitemon	56	1987	Multiparous
Zoe	Matsu	800	1986	Multiparous
Tyler	Pelka	849	1986	Multiparous

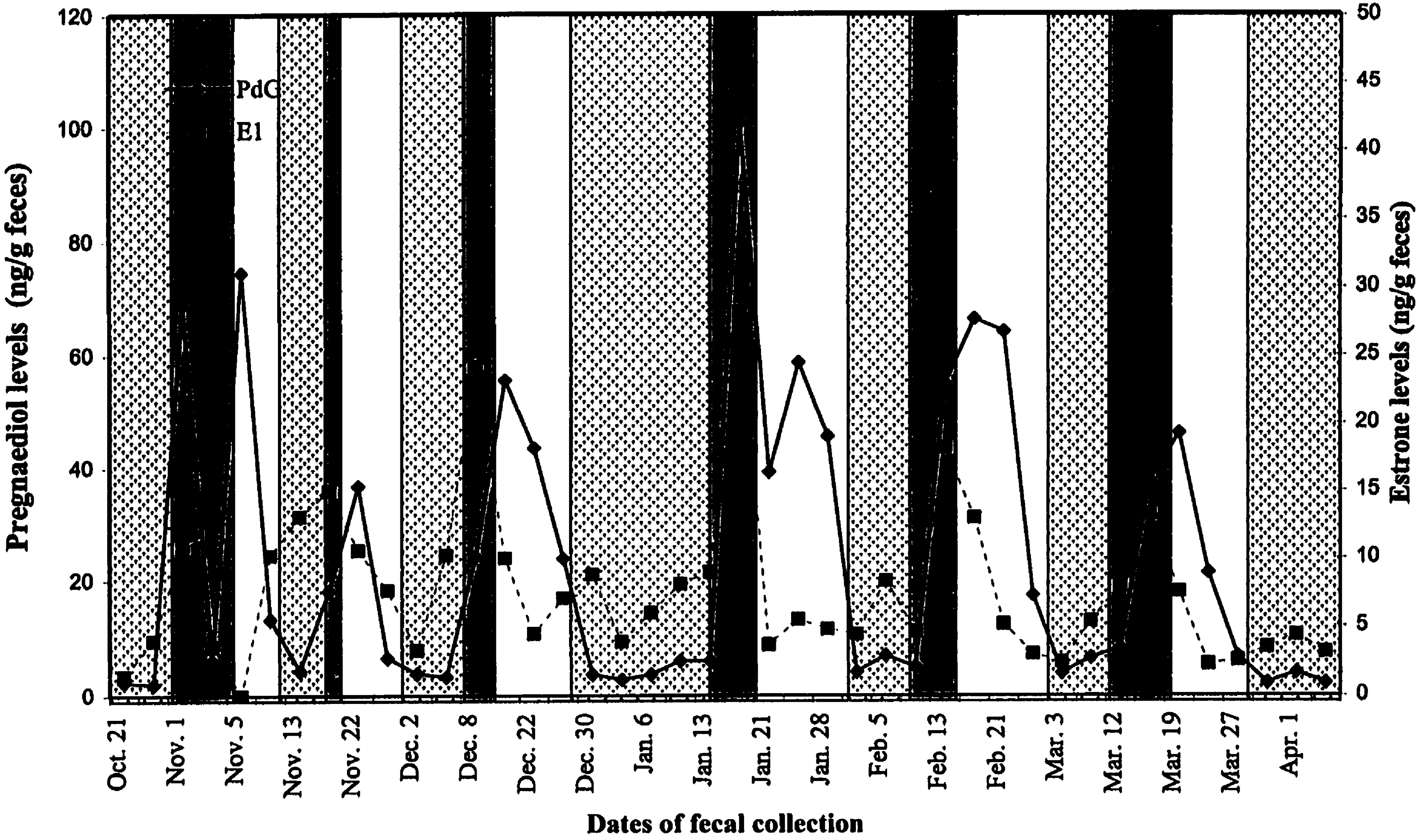
APPENDIX 2

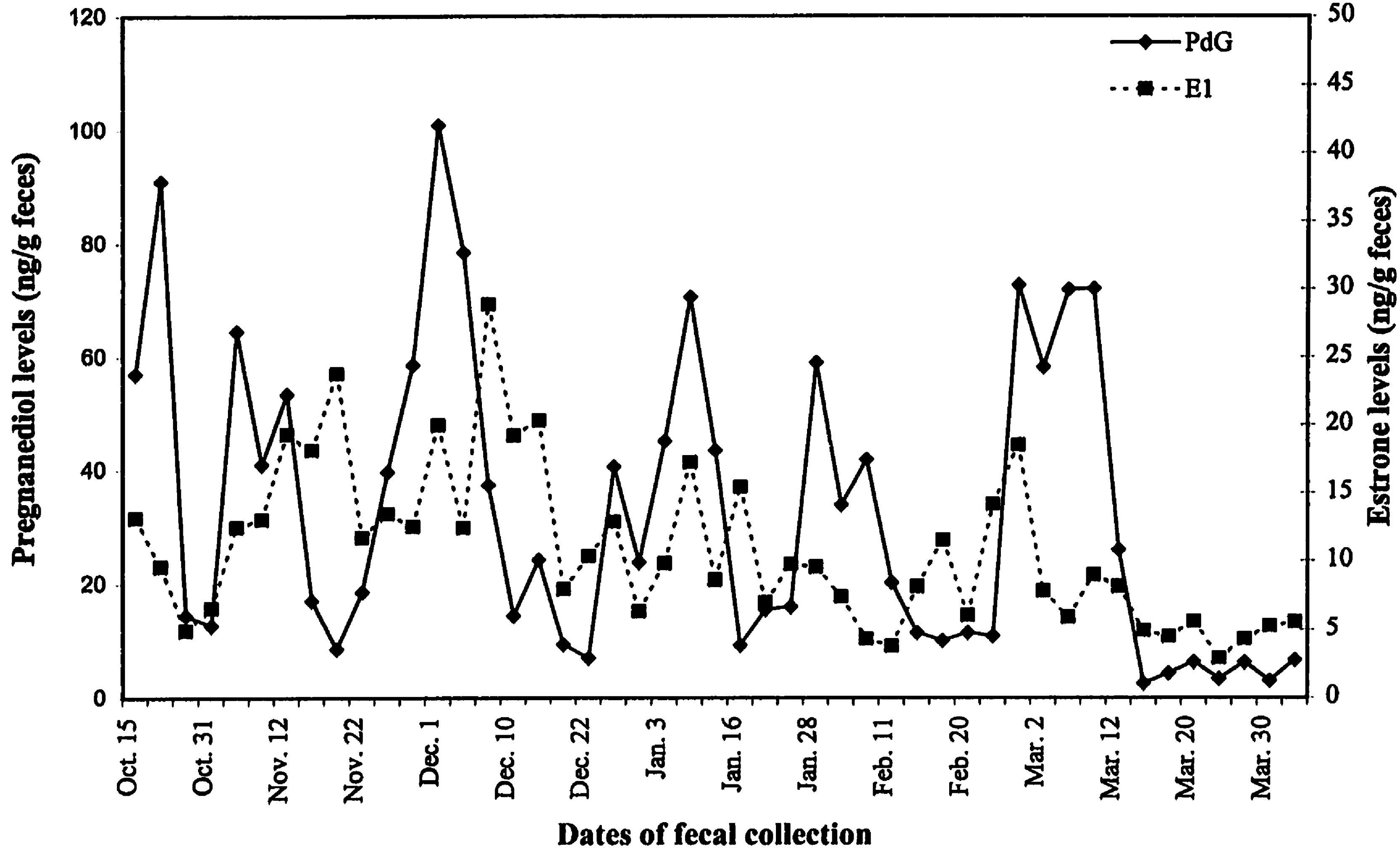
Individual hormone profiles for each subject.

Phases for each cycle were determined from hormone profiles as follows: The day preceding the first day of pregnanediol increase was considered to represent the most likely day of ovulation (see strier and Ziegler, 1997, and Espey and Lipner, 1994 listed in Chapter two references). Because samples were not collected on a daily basis, the periovulatory phase was therefore conservatively defined as the estimated day of ovulation ± 3 days. The follicular phase was considered to be from the first day when pregnanediol levels dropped to base line to the day preceding the start of the periovulatory phase. The luteal phase was determined to be between the last day of the periovulatory phase to the day before pregnanediol levels drop to base line.

When cycle phases were ambiguous, Dr. Toni Ziegler of the Wisconsin Regional Primate Research Center assisted in the interpretation of the phases from hormone profiles. The division of the phases was not always explicit, and as a consequent some data were not used in the analysis. Data used were distinctly identified within a specific phase of the cycle. The first profile demonstrates the demarcation of the phases within each cycle (▒ Follicular phase ■ Periovulatory phase □ Luteal Phase).

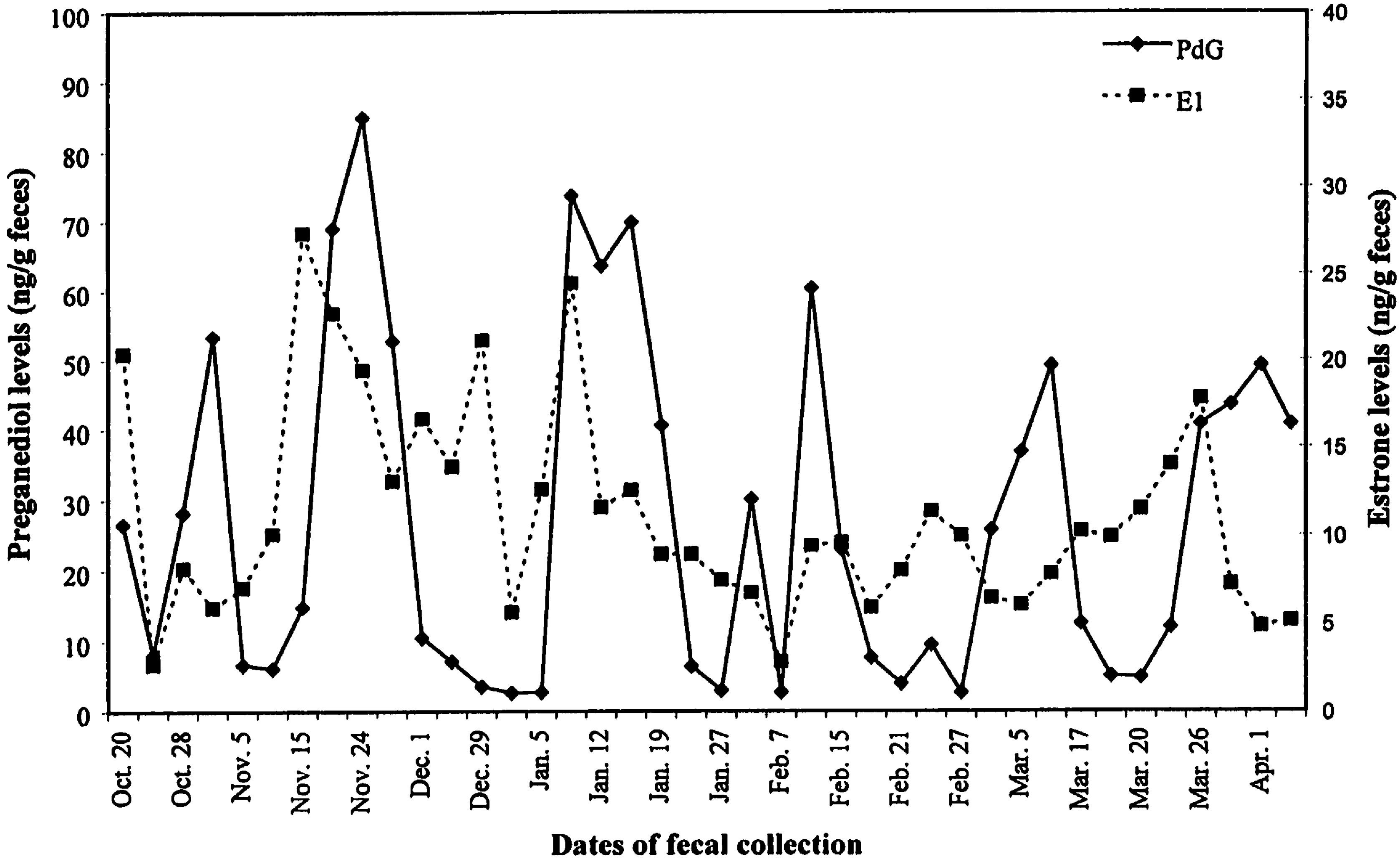
Hormone Profile for Shy Mug



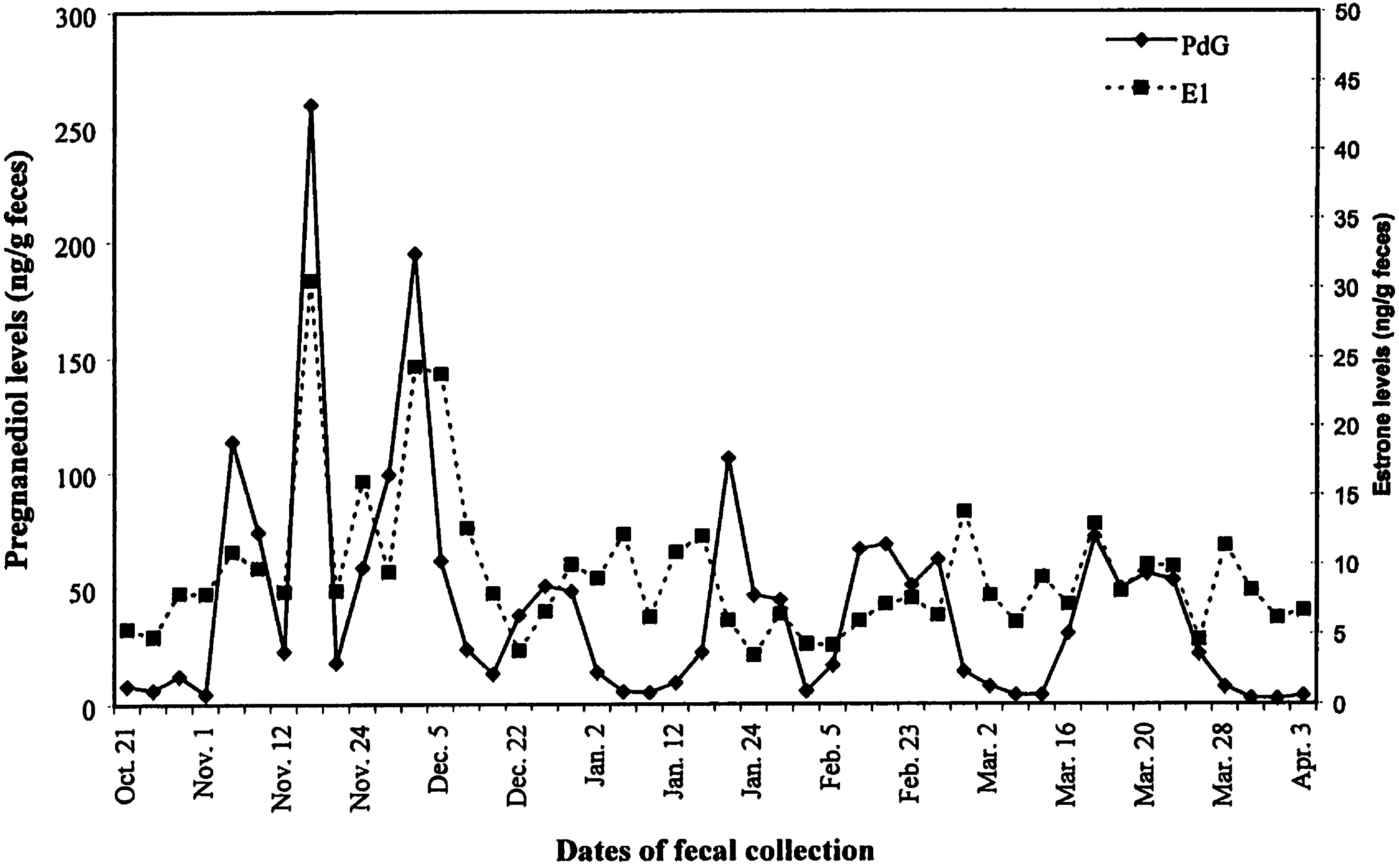


Hormone Profile for Tantalis

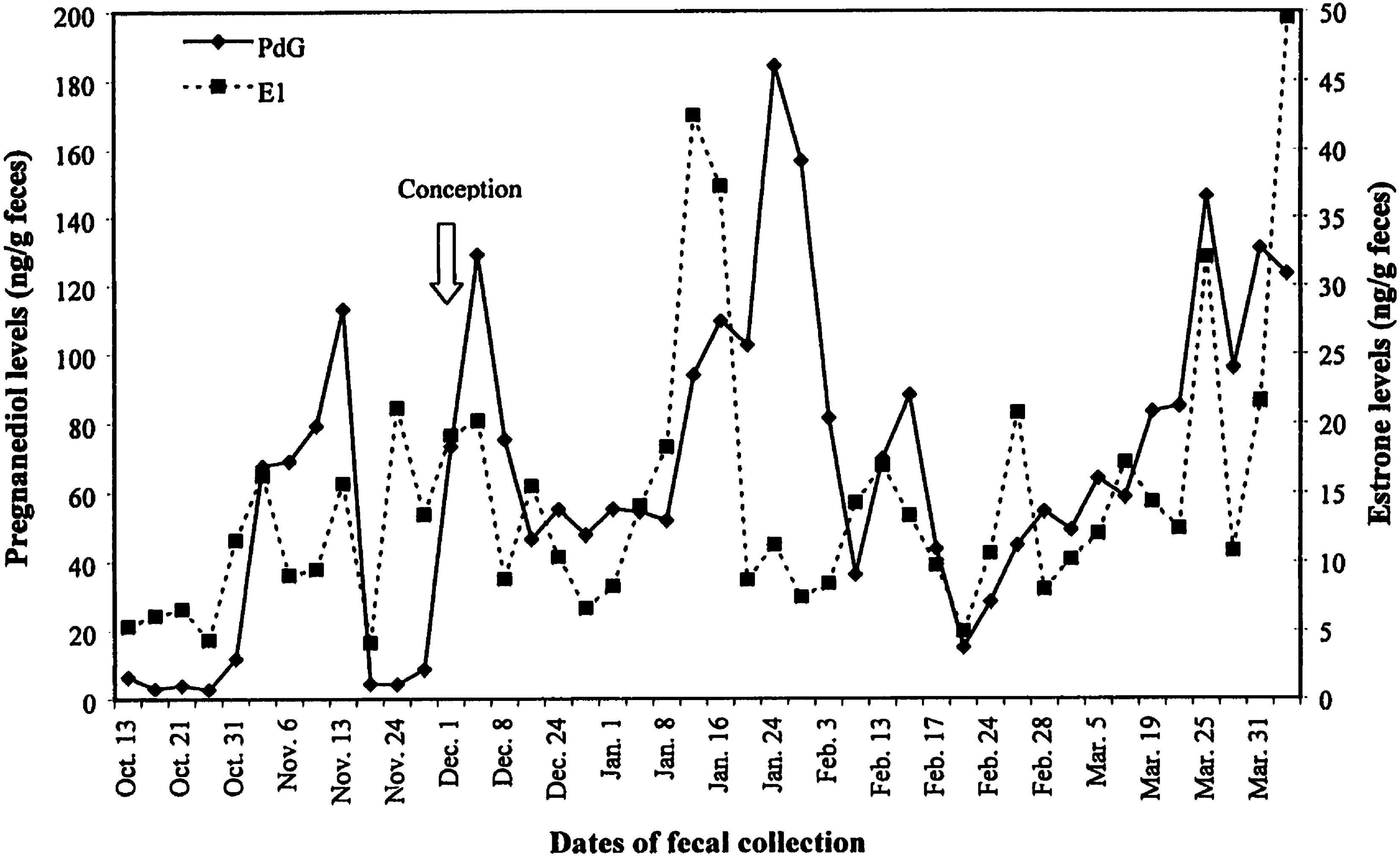
Hormone Profile for Lisa



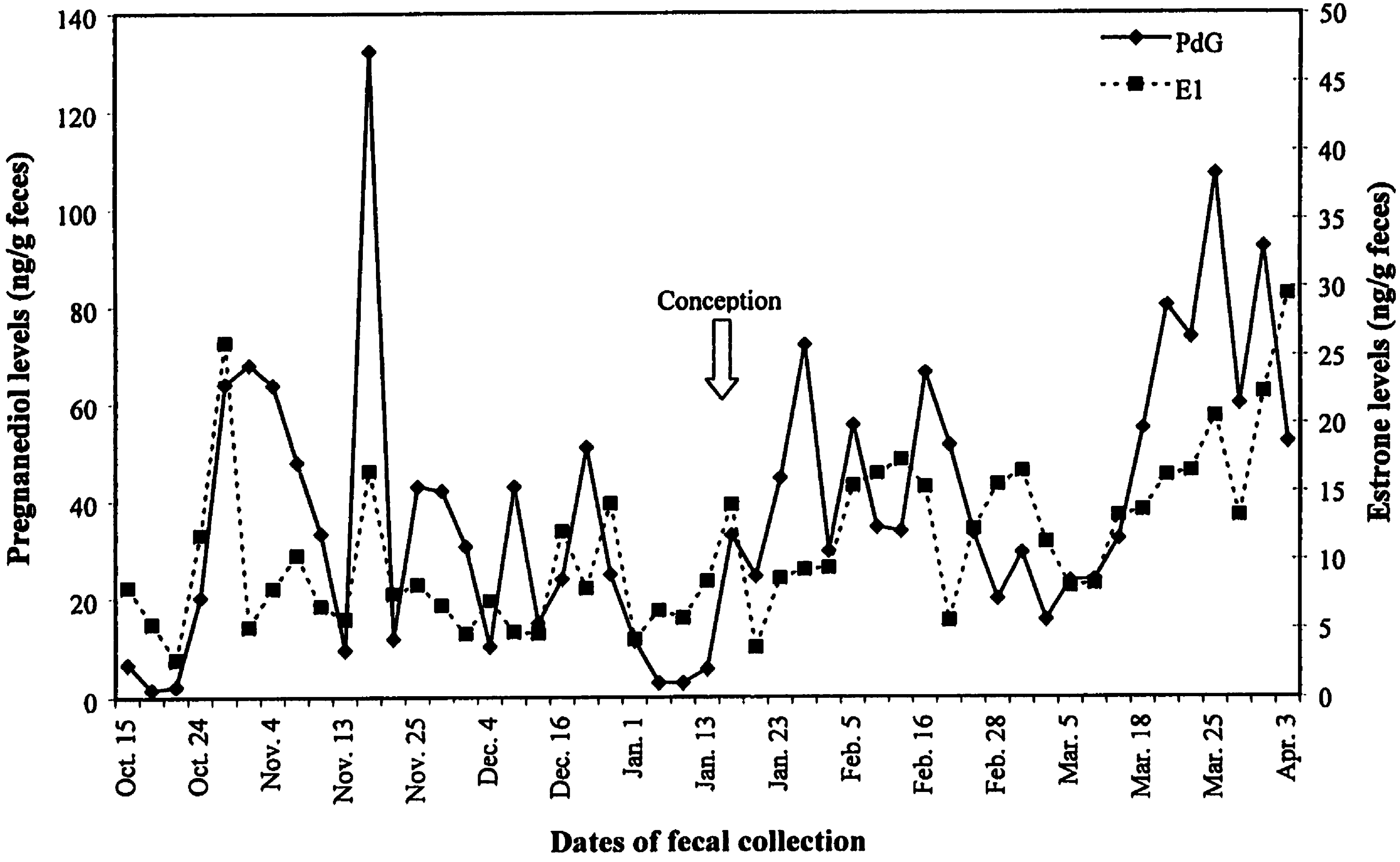
Hormone Profile for Bo Nose



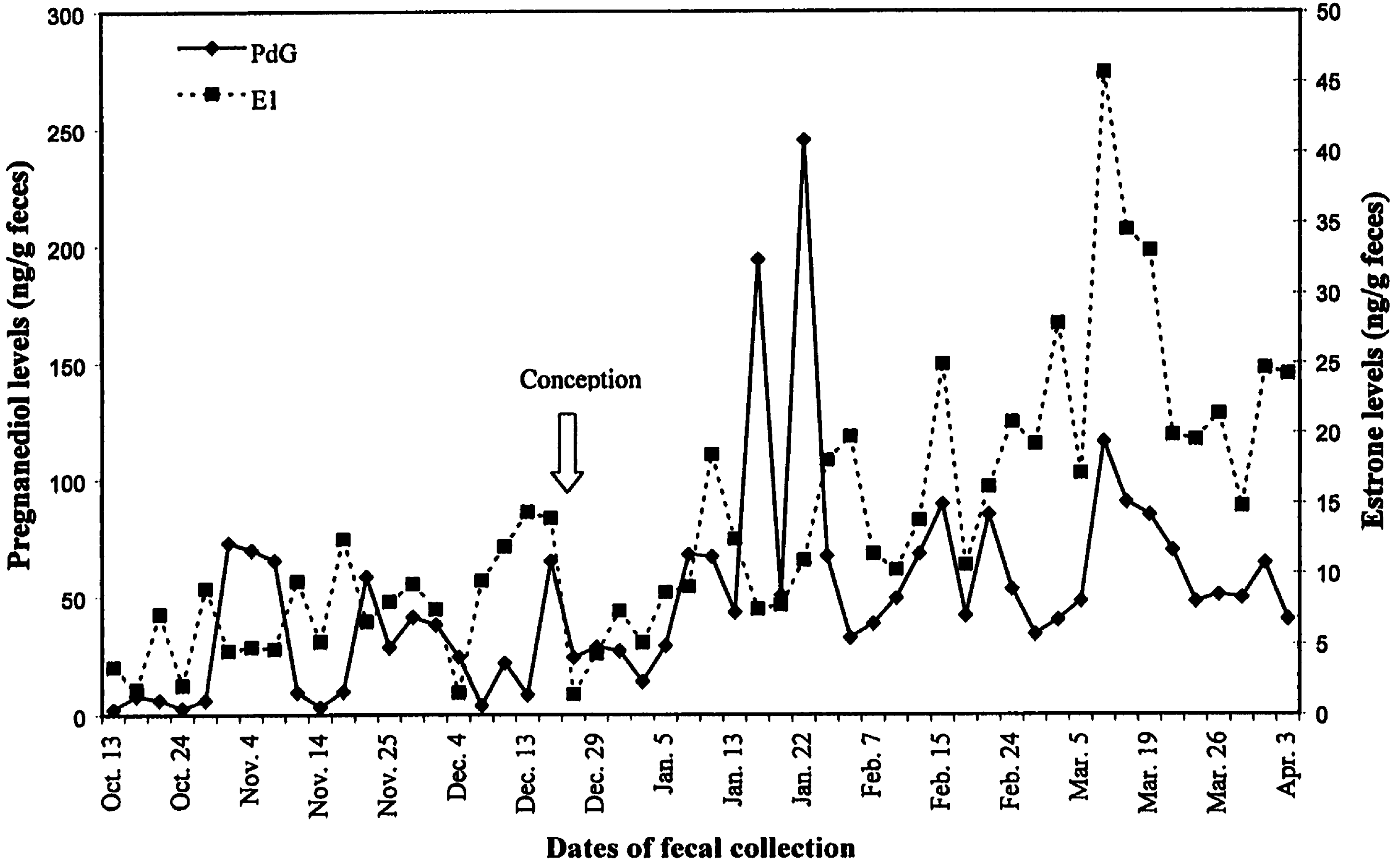
Hormone Profile for Trisha

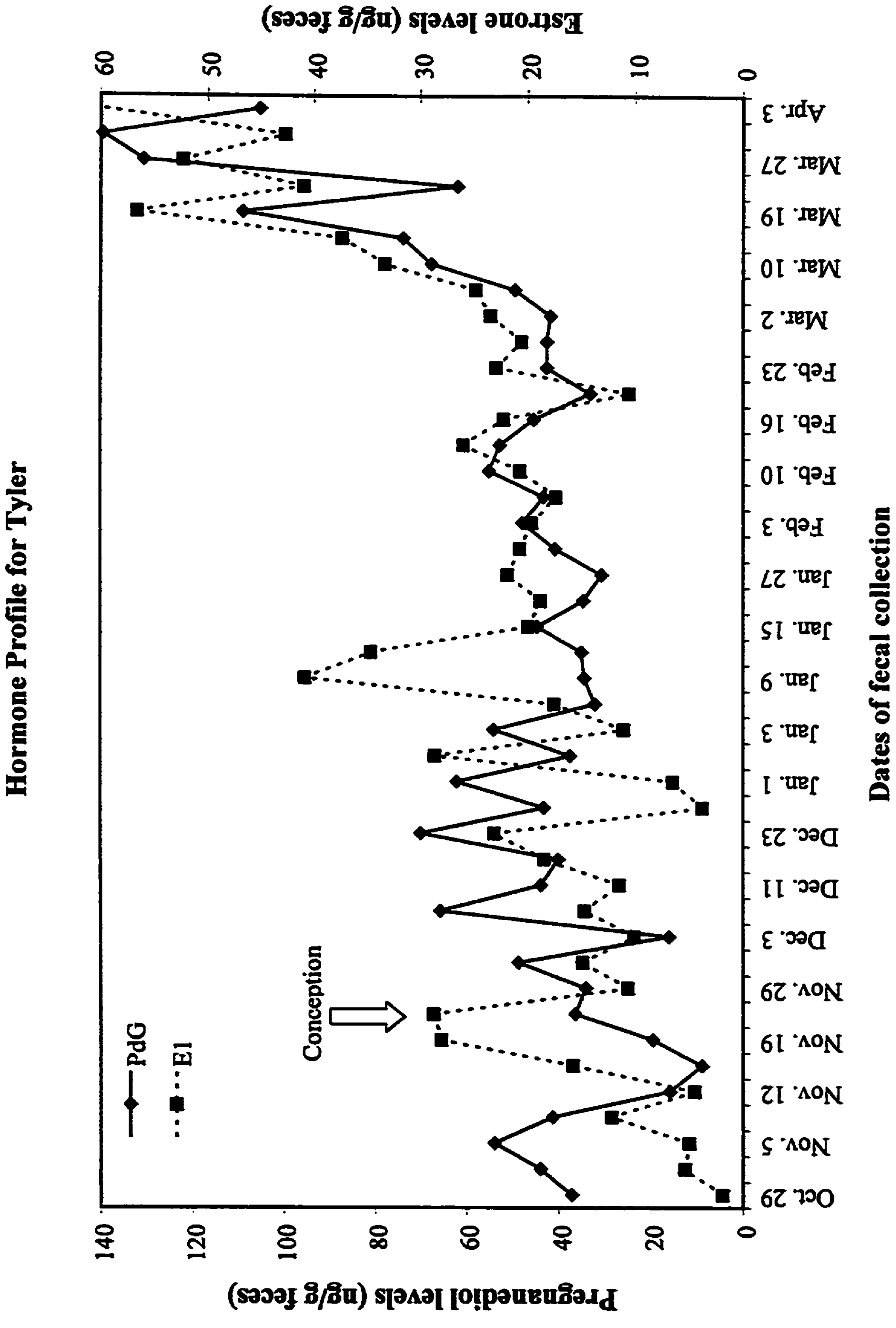


Hormone Profile for Saskia



Hormone Profile for Zoe





Hormone Profile for Tyler