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Body temperature and physical activity correlates of the menstrual cycle in female chacma baboons (Papio hamadryas ursinus)

Nyakudya, Trevor Tapiwa

2010-09-27
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CHAPTER 1

INTRODUCTION

1.1 Introduction

Mating and consortship behaviour in a number of baboon species vary greatly depending on the social structure of the troop (Manson, 1997). Chacma baboons, *Papio hamadryas ursinus* live in social groups composed of multiple males, females and their offspring (Saayman, 1970; Weingrill *et al*., 2000). Their troops are characterised by a dominance hierarchy (Duvall *et al*., 1976; Weingrill *et al*., 2004) and they have a promiscuous mating system which is based on the dominance relationship. Rank is a primary determinant of mating success (Saayman, 1970; Cowlishaw and Dunbar, 1991; Bulger, 1993) as a result, high-ranking chacma male baboons, referred to as the alpha males, maintain close proximity to females that are sexually receptive. They do this to manipulate and monopolise mating during consortships (Bercovitch, 1986; Weingrill *et al*., 2000; Bergman *et al*., 2005). Despite the presence of high-ranking males, low -ranking males may sometimes take the opportunity to mate when the alpha males are unaware. They also take their chance if there are more than two sexually receptive females in the troop (for critical review on primate consortship see Manson, 1997).

The presence of multiple reproductive males in a troop and the promiscuous mating behaviour of female baboons make the assignment of paternity and relatedness, even in captive baboons, difficult (Busse, 1985; Bruford and Altmann, 1993). In order to determine paternity of baboon offsprings, primatologists have to capture the baboon infants and their putative fathers and

use DNA analysis. Paternity determination using DNA analysis and fingerprinting has been done before in captive baboons (Bruford and Altmann, 1993; Smith *et al*., 1999). However capturing baboons and collecting samples for genetic analysis is difficult and expensive, particularly in free-ranging troops with a large number of possible sires (Smith *et al*., 1999). Capturing baboons is also labour-intensive and very stressful, especially to the young baboons, and this may result in social disruptions.

Primatologists determine paternity when addressing population diversity and differentiation, genetic variability, similarity indexes and to a lesser extent pedigree reconstruction in various captive and free-ranging non-human primate species (Pastorini *et al*., 2000). This data is used in the identification of parentage and assists in the classification of social relationship in baboon troops, especially in determining the male-to-infant relationship (Duvall *et al*., 1976).

The assignment of paternity without capturing baboons might be possible if the moment of ovulation could be identified remotely. Consequently there is a need to develop a technique that can be used by primatologists to estimate the day of ovulation in female baboons without disrupting the social lives of baboons. If the exact day of ovulation for a female baboon is known and the male baboon consorting with that particular female also is known, identifying the father of the offspring may be possible without having to capture the baboons and carry out genetic analysis (Bruford and Altmann, 2003; Weiss, 2005).

Apart from direct visual observation under microscope (laparotomy) (MacLennan and Wynn, 1971; Wildt *et al*., 1977), the standard method for determining the day of ovulation in ovulating primates is the measurement of plasma luteinising hormone (LH), progesterone and oestradiol concentrations. The collection of blood samples for hormonal measurements is stressful to the animals leading to the disruption of their social life and is not convenient when working with free-ranging and wild baboons. More convenient, less disruptive measures are faecal and urine ovarian steroid hormone concentrations as well as anogenital swelling changes that have been well validated against hormones in baboons (Wildt *et al*., 1977; Shaikh *et al*., 1982) and wild West African chimpanzees, *Pan troglodytes verus* (Deschner *et al*., 2003). The changes in abdominal temperature, physical activity and anogenital swellings are secondary effects of ovulation and apart from their intrinsic physiological importance, their measurement may be more convenient in estimating the time of ovulation, compared to faecal and urine hormonal measurements.

1.2 Menstrual cycle

1.2.1 Introduction

Here, I review knowledge of the baboon menstrual cycle and its regulation by the hormones. The subject of the estimation of the menstrual cycle phases and the time of ovulation, a central theme of my dissertation, is introduced and

discussed, for non-human primate species. I also point out shortcomings of previous methods used in estimating the time of ovulation as well as issues that still need to be addressed, some of which I hope to resolve.

The menstrual cycle is a recurring cycle of physiological, physical and psychological changes that occurs in females of most menstruating primates (Gillman and Gilbert, 1946; Graham 1981). Menstrual cycles begin at menarche and continue until menopause. Chacma baboons exhibit a menstrual cycle (Stevens, 1997) and they share many similarities in reproductive anatomy and physiology with humans (D'Hooghe *et al*., 2004; Dawood and Khan-Dawood, 2007). These similarities have been attributed to baboons being phylogenetically close to humans (Horie, 1983; D'Hooge *et al*., 2004) and they include the hormonal secretory patterns (Koyama *et al*., 1977; Goncharov *et al*., 1976; Stevens, 1997) and menstrual cycle events (Gillman and Gilbert, 1946; Goncharov *et al*., 1976). As such baboons are used in medical research as a surrogate for studying the human reproductive system (Horie, 1983; D'Hooghe *et al*., 2004).

The duration of the baboon menstrual cycle varies considerably within and between individuals of the same species (Stevens, 1997). This variability in the menstrual cycle length has been attributed to the variable length of the follicular phase. In captive yellow baboons, *Papio hamadryas cynocephalus*, the average menstrual cycle length is 33 days (Koyama *et al*., 1977), in captive olive

baboons, *Papio hamadryas anubis* the length is 35 days (Stevens, 1997), while in free-ranging chacma baboons, *Papio hamadryas ursinus*, the average length is 36 days (Skinner and Chimimba, 2005).

1.2.2 Events of the menstrual cycle

The reproductive cycle of menstruating primates can be divided into three major phases namely, the follicular, ovulation, and the luteal phases (Gillman, 1937; Gillman and Gilbert, 1946). The follicular phase is further divided into menstrual bleeding and non-menstrual bleeding periods. A normal baboon menstrual cycle begins with menstrual bleeding which marks the beginning of the follicular phase (Gillman and Gilbert, 1946). The follicular phase is defined as the interval between the first day of menstruation and the day before ovulation (Gear, 1926; Graham, 1981).

The follicular phase is also known as the proliferative phase because the lining of the uterus rapidly grows under the influence of oestradiol which is produced by the developing follicles. When the ovum is mature, under the influence of follicle stimulating hormone (FSH), oestradiol concentration reaches a threshold above which it stimulates the production of LH. The surge in LH causes the release of a mature oocyte from the follicle, that is, ovulation. The released ovum is captured by the fallopian tubes for fertilisation and if there is no fertilisation, the ovum disintegrates and dissolves in the uterus. The day of ovulation can be determined

by the surge in LH which occurs just before ovulation and around the mid-point of the menstrual cycle (Stevens, 1997; Hodges *et al*., 2005).

The luteal phase, also known as the secretory phase, is conventionally defined as the interval between the day after ovulation and the day before subsequent menstruation (Stevens, 1997). In vervet monkeys, *Chlorocebus aethiops*, FSH and LH produced after ovulation, cause the ruptured follicle to transform into a corpus luteum which secretes large amounts of progesterone and oestradiol for up to about two weeks (Molskness, 2007). During this two week period, progesterone makes the endometrium secretory and receptive to implantation and if implantation does not take place, the corpus luteum atrophies and degenerates leading to a decrease in concentration of progesterone (Graham, 1981). In baboons, the decrease in progesterone triggers the shedding of the endometrium leading to menstruation and the beginning of the next cycle (Gillman, 1940a, b; Graham 1981; Beehner *et al*., 2006).

1.3 Hormonal control of the menstrual cycle

1.3.1 Introduction

Hormonal profiles of the menstrual cycle of non-human primates have been reported in several species including the rhesus monkeys, *Macaca mulatta* (Hotchkiss *et al*., 1971), Tonkean macaques, *Macaca tonkeana* (Thierry *et al*.,

1996; Aujard *et al*., 1998) yellow baboons, *Papio hamadryas cynocephalus* (Kling and Westfahl, 1978; Stevens 1997) and the chimpanzees, *Pan troglodytes* (Graham *et al*., 1977). The hormonal regulation of the menstrual cycle of these non-human primates was found to be similar to the previously reported patterns in cycling women (Goncharov *et al*., 1976; D'Hooge *et al*., 2004; Dawood and Khan-Dawood, 2007).

1.3.2 Oestradiol

Oestradiol belongs to a group of oestrogens which include oestrone and oestriol. It is the most potent naturally-secreted oestrogen by the ovaries in non-pregnant baboons (Canez *et al*., 1992; Stevens 1997). In yellow baboons (Gillman, 1938; Stevens, 1970; Wasser *et al*., 1994) and howler monkeys, *Alouatta palliata* (Clarke *et al*., 1991), faecal concentration of oestradiol follow a cyclic pattern over the menstrual cycles. The concentration of oestradiol were reported to be low in the early part of the follicular phase followed by a rapid increase around the middle of the follicular phase, up to a mid-cycle peak which leads to a mid-cycle gonadotropin surge and ovulation (Stevens, 1997), Figure 1.1. The oestradiol concentration decrease after ovulation, but this decrease is followed by a second smaller rise which corresponds with the formation of the corpus luteum.

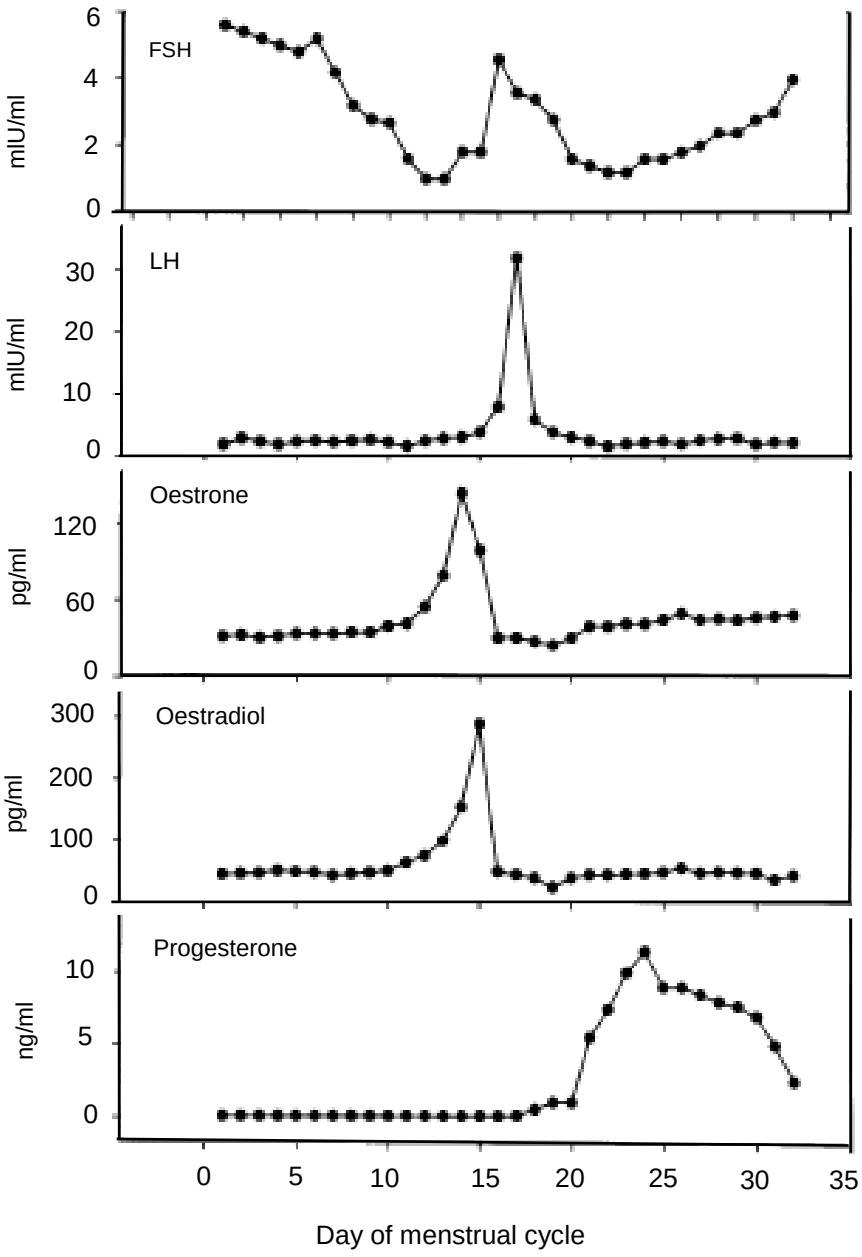


Figure 1.1 Serum hormone concentrations during the baboon, *Papio hamadryas cynocephalus*, menstrual cycle. Figure adapted and modified from Stevens, 1997.

1.3.3 Progesterone

Progesterone is one of the female sex-steroid hormones which are responsible for reproductive-related activities namely the cyclic changes in the uterine endometrium in preparation for implantation of the fertilised ovum, establishment and maintenance of pregnancy in baboons (Hodges *et al*., 2005). It is secreted from the corpus luteum and adrenal glands (in small amounts) in the luteal phase of a normal menstrual cycle and from the placenta during pregnancy in baboons (Goncharov *et al*., 1976). High progesterone in the luteal phase indicates the secretory capacity of the endometrium necessary for successful implantation (Gillman, 1940a; Dawood and Khan-Dawood, 2007).

During the menstrual cycles of baboons, plasma progesterone concentration remains consistently low and secretion is insignificant throughout the follicular phase (Stevens *et al*., 1970; Stevens, 1997), Figure 1.1. After ovulation, concentrations increase rapidly as more progesterone is released from the corpus luteum in preparation for potential implantation and pregnancy. Progesterone concentration reaches a peak in the middle of the luteal phase and if there is no implantation, the concentration decrease as the corpus luteum atrophies and degenerates (Stevens *et al*., 1970; Dawood and Khan-Dawood, 2007).

The decrease in progesterone concentration precedes the shedding of the uterus lining, a process known as menstruation (Gillman, 1935; Stevens, 1997). Studies have also shown that continuous administration of progesterone delayed, and in some cases, stopped the onset of menstrual bleeding in chacma baboons (Gillman, 1940a). It has also been shown that progesterone withdrawal, due to the absence of implantation, also induces menstruation in baboons (Gillman, 1940a; Gillman, 1940b).

1.3.4 Measurement of steroid hormones in non-human primates

Steroid hormone concentrations have been measured using various techniques. Current techniques used to assess reproductive hormones in non-human primates include blood sampling (Wildt *et al*., 1977) urine sampling (Dahl *et al*., 1987; Czekala *et al*., 1988) and faecal sampling (Ziegler *et al.* 1989; Heistermann *et al*., 1993; Wasser, 1996; Millspaugh and Washburn, 2004). These techniques employ either radioimmunoassay (RIA) or enzyme immunoassay (EIA) to determine steroid hormone concentrations. Blood samples have been used in baboons to ascertain hormone concentrations in reproductive cycles (Wildt *et al*., 1977). Although blood sampling is a reliable and sometimes practical method for assessing hormonal fluctuations in captive animals, it is unsuitable for use in free-ranging animals, due to its invasive nature and the stress associated with blood collection.

Urine samples have been used to quantify hormone concentrations reliably in captive gorillas, *Gorilla gorilla* (Czekala *et al.* 1988), baboons (French *et al*., 2004) and chimpanzee (Graham *et al*., 1977) over their menstrual cycle. It is relatively easy to collect urine from captive animals, for example by placing trays to catch the voided urine under cages, as previously done in cotton top tamarins, *Saguinus oedipus* (Ziegler *et al.* 1989). Urine is pooled in the bladder over several hours before urination and therefore represents the integrated secretion of hormones over several hours. In wild species, however, urine collection can be difficult because urine is absorbed in the ground before it can be collected (Kirkpatrick *et al.* 1988).

Faecal steroid analysis provides a non-invasive method for assessing endocrine and behavioural changes over the menstrual cycle in non-human primates (Ziegler *et al.* 1989; Heistermann *et al*., 1993; Wasser, 1996; Whitten and Russell, 1996; Beehner and Whitten, 2004). Faecal samples are easy to collect in captive and free-ranging animals and faecal steroids have been shown to provide accurate measures of reproductive status (Wasser *et al*., 1991; Clarke *et al*., 1991; Strier and Ziegler, 1994; Shideler *et al*., 1994; Heistermann *et al*., 1993) as well as information about pregnancy status before the appearance of perineal pregnancy signs (Stavisky *et al*., 1995). Like urine hormones, faecal hormones pool over time and therefore also represents the integrated secretion of faecal hormones over several hours. The collection of faecal samples from a

particular animal also requires the presence of an observer, which influences behaviour and probably stress hormones in unhabituated free-living primates.

Analyses of the relationships between concentrations of steroid hormones in urine or faeces reveal that there are different excretion rates for hormones in urine and faeces (Whitten *et al.* 1998). Urine steroids tend to be excreted within 4 to 8 hours with complete excretion by 24 hours (Ziegler *et al.* 1989; Wasser *et al.* 1994; Whitten *et al.* 1998) whereas faecal steroids can be excreted from 1 to 3 days after they have been secreted (Ziegler *et al.* 1989; Wasser *et al.*1994; Whitten *et al.* 1998).

1.4 Cortisol and the menstrual cycle

Cortisol and other glucocorticoids are secreted in response to environmental, psychological and physiological stress (Chernow *et al*., 1987; Millspaugh and Washburn, 2004). As a result, cortisol and other glucocorticoids is an indicator of a variety of ecological stressors in vertebrate species (Kreiger, 1975; Millspaugh and Washburn, 2004). Stavisky *et al*., (2001), have shown that in captive female cynomulgus monkeys, *Macaca fascicularis*, cortisol secretion differs in different phases of the menstrual cycle. They found that luteal phase cortisol was higher than at ovulation and during the follicular phase. In some callitrichid female primates, *Saguinus oedipus* and *Callithix jacchus*, variations of cortisol have been associated with reproductive status (Ziegler *et al*., 1996). These results

demonstrate that adrenal activity and hence cortisol secretion may be different in different phases of the menstrual cycle.

Physiological and psychological stress disrupts the reproductive physiology of non-human primates and may lead to the suppression of the ovarian cycle (Whitten *et al*., 1998; Bahr *et al.* 2000). In captive baboons that were subjected to physical stress by tethering and social isolation, the menstrual cycle was lengthened, especially the follicular phase (follicular phase defects) (Caperton *et al*., 2006). The follicular phase defects were induced by the influence of cortisol on the hypothalamic-pituitary-gonadal axis (Caperton *et al*., 2006). Inflammatory-like stress during the follicular phase in rhesus monkeys acutely activated the hypothalamic-pituitary-adrenal axis and affected the hypothalamic-pituitary-gonadal axis to influence the cyclicity of the menstrual cycle (Xiao *et al*., 1998).

The effects of cortisol on the menstrual cycle are believed to occur through the suppression of the gonadotropin-releasing hormone pulse generator by cortisol-releasing hormone and reduction in sensitivity of the pituitary to gonadotropin-releasing hormone (Loriaux and Nieman, 1990). Some studies in baboons, *Papio hamadyas ursinus* (Weingrill *et al*., 2004) and rhesus macaques (Smith and Norman, 1987) have shown that reproductive ovarian hormones, especially oestradiol, stimulate the hypothalamic-pituitary-adrenal axis leading to an increase in cortisol concentrations around the time of ovulation and pregnancy.

Determination of cortisol concentrations in faecal and urine samples may assist in assessing the adrenal activity over the menstrual cycle.

1.5 Anogenital swellings

During the menstrual cycle, conspicuous and prominent swellings of the anogenital area can be seen in female baboons (Gillman 1935; Domb and Pagel, 2001; Huchard *et al*., 2009). The anatomical and morphological changes occurring in the genital organs of baboons are considered to be hormone-dependent and play a role in determining sexual behaviour (Higham *et al*., 2008). The anogenital area in baboons that is involved in the cyclical changes during the menstrual cycle extends from the root of the tail to the lower part of the abdominal region (Gillman, 1935). Baboons exhibit the largest and most colourful sexual swellings of any primate species (Higham *et al*., 2008). In *Papio hamadryas cynocephalus*, swellings contribute about 14 % of the total body mass when at their maximum size (Domb and Pagel, 2001).

Anogenital swellings in baboons may have evolved as morphological signals of fertility (Saayman *et al*., 1973) which increase the female attractiveness and advertise the period during which males should consort with the females (Higham *et al*., 2008). Anogenital swellings are also believed to act as a visual cue and arousal stimulus for the male baboon (Girolami and Bielert, 1987) and they seem to be an indicator of the female's long-term reproductive value (Domb and Pagel,

2001; Huchard *et al*., 2009). In baboons and chimpanzees, anogenital swelling size indicate quality, with good quality females producing larger swellings and possessing higher reproductive success (Emery and Whitten, 2003; Domb and Pagel, 2001, Huchard *et al*., 2009).

An increase in female baboon anogenital swelling size and colour intensity has been related to changes in both male and female baboon sexual behaviour (Gesquiere *et al*., 2007). Male baboon mating-interest, consortship behaviour, masturbatory activity and male-male competition for females have been reported to increase around the time when the swellings are maximum (Bercovitch, 1987; Higham *et al*., 2008). The above information is evidence which suggests that anogenital swellings indicate the proximity and probability of ovulation and hence are used by female baboons to advertise their fertility period and thus increase their likelihood of getting pregnant (Domb and Pagel, 2001).

1.5.1 Effect of oestradiol and progesterone on the anogenital area

As would be expected of signals of ovulation, the size and colour of the anogenital swellings are determined by ovarian steroid hormones (Hendrickx, 1965; Wildt *et al*., 1977). Anogenital swellings have been defined as oestradiol-dependent oedema of the vulva and anal regions (Zuckerman and Parkes, 1939; Thompson, 2005). The anogenital skin tissue possesses oestradiol and

progesterone receptors which are believed to mediate the anogenital area response to oestradiol and progesterone in baboons (Gillman and Gilbert, 1946).

The oestradiol-sensitive tissue in the anogenital area of baboons swells and changes colour in response to elevated oestradiol during the follicular phase (Gillman, 1942; Gillman and Gilbert, 1946). Progesterone, the concentration of which increases during the luteal phase, reverses or antagonises the effects of oestradiol by causing localised oestradiol withdrawal and down-regulation of oestrodiol receptors in the anogenital area resulting in the decrease of the size of anogenital swellings, especially in the luteal phase (Gillman, 1940b; Graham *et al*., 1977; West *et al*., 1990; Gesquiere *et al*., 2007).

1.5.2 Estimation of ovulation using anogenital swelling changes

Anogenital swelling changes have been used to estimate the timing of ovulation in field studies in baboons, *Papio hamadryas ursinus* (Saayman, 1973; Weigrill *et al*., 2004). Previous studies have indicated that anogenital swellings are a reliable indicator of the fertile period within baboon menstrual cycles (Shaikh *et al*., 1982; Wildt, 1977). Gesquiere *et al*., (2007) showed that ovulation in yellow baboons, *Papio hamadryas cynocephalus* occurred when the anogenital swellings were at their maximum which also coincided with the highest faecal oestradiol concentration.

A study in chimpanzees (Emery and Whitten, 2003), in which progesterone and oestradiol profiles were combined with changes in anogenital swellings, has shown that ovulation occurred 1 to 2 days before the last day of maximum anogenital swelling (Figure 1.2). This evidence implies that anogenital swellings in baboons can be used as accurate and reliable indicators for estimating the time of ovulation. The main problem of using anogenital swellings to estimate the time of ovulation in unhabituated free-ranging baboons is that there is need for continuous close-up human observation, which may not be possible at all times and may also disturb the normal cyclicity of the menstrual cycle if the observer is perceived as a stressor.

1.6 Menstrual cycle related body temperature regulation

Mammals such as primates have evolved behavioural and autonomic motor outputs to regulate their body temperature within a narrow range, even when exposed to a wide range of ambient temperatures (Prosser and Heath, 1991). Temperature regulation is also affected by several intrinsic factors (Halbrock, 1945) and of particular interest to my study is the involvement of hormones in thermoregulation over the menstrual cycle.

Ovarian steroid hormones, progesterone and oestradiol, influence many behavioural and physiological variables in addition to their actions on reproductive organs (Yen *et al*., 1970; Czaja and Butera, 1986). Among the

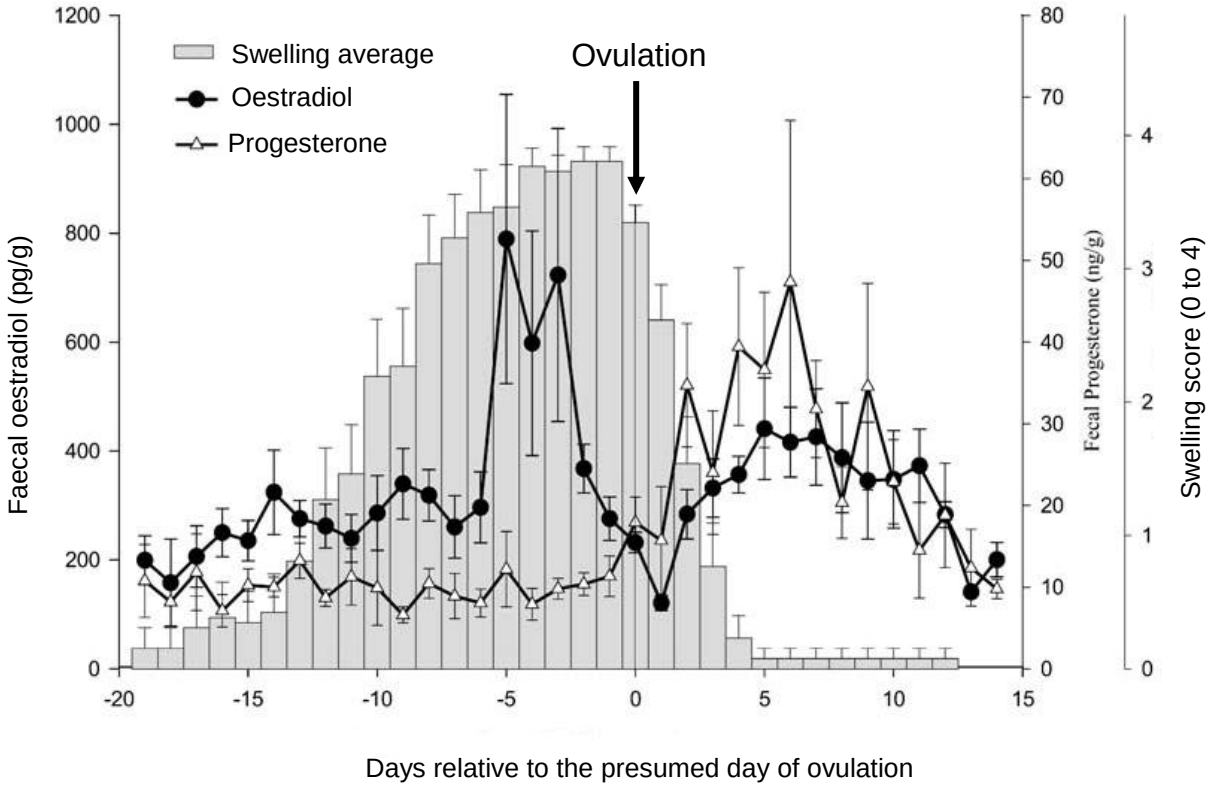


Figure 1.2 Hormonal and anogenital profiles in captive chimpanzee, *Pan troglodytes* over the menstrual cycle. Figure adapted and modified from Emery and Whitten, 2003.

variables affected by ovarian steroid hormones is body temperature, as shown in humans (Davies and Fugo, 1948; Israel and Schneller, 1950) and chimpanzees (Graham *et al*., 1977). Studies conducted in women have indicated that the nychthemeral 24-h profile of oral and rectal temperature is different in different phases of the menstrual cycle (Lee, 1988; Baker and Drive,r 2007). Normal ovulatory menstrual cycles in women show a cyclic pattern in mean 24-h morning rectal temperature, initially low in the follicular phase followed by a mid-cycle thermal shift to a sustained elevated temperature in the luteal phase before temperature falls again pre-menstrually (Marshall, 1963; Coyne *et al*., 2000), Figure 1.3.

The existence of a cyclic pattern in body temperature over the menstrual cycle was first documented by William Squire (1868). A possible association of this pattern in body temperature and the timing of ovulation was first proposed by Theodor va de Velde (Martinez *et al*., 1992), who suggested that the corpus luteum was responsible for the elevated temperature in the luteal phase. The cyclic variation in average daily rectal temperature over the menstrual cycle has been reported in chimpanzees, (Graham *et al*., 1977), Figure 1.4 and the rhesus monkeys (Balin and Wan, 1968).

A relationship between the ovarian cycle, progesterone and body temperature also has been seen in several mammalian species, such as Asian elephants, *Elephas maximus* (Kusuda *et al*., 2007), marine mammals like the captive

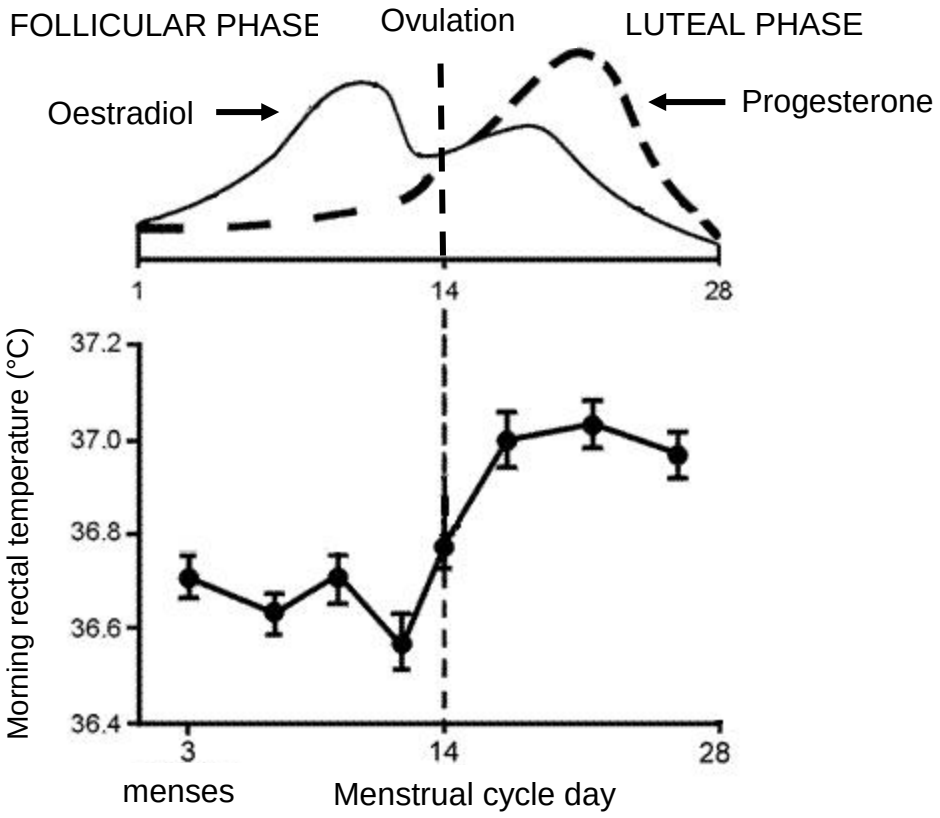


Figure 1.3 Variation of rectal temperature and ovarian steroid hormones over the menstrual cycle in women. Figure adapted and modified from Baker *et al*., 2007.

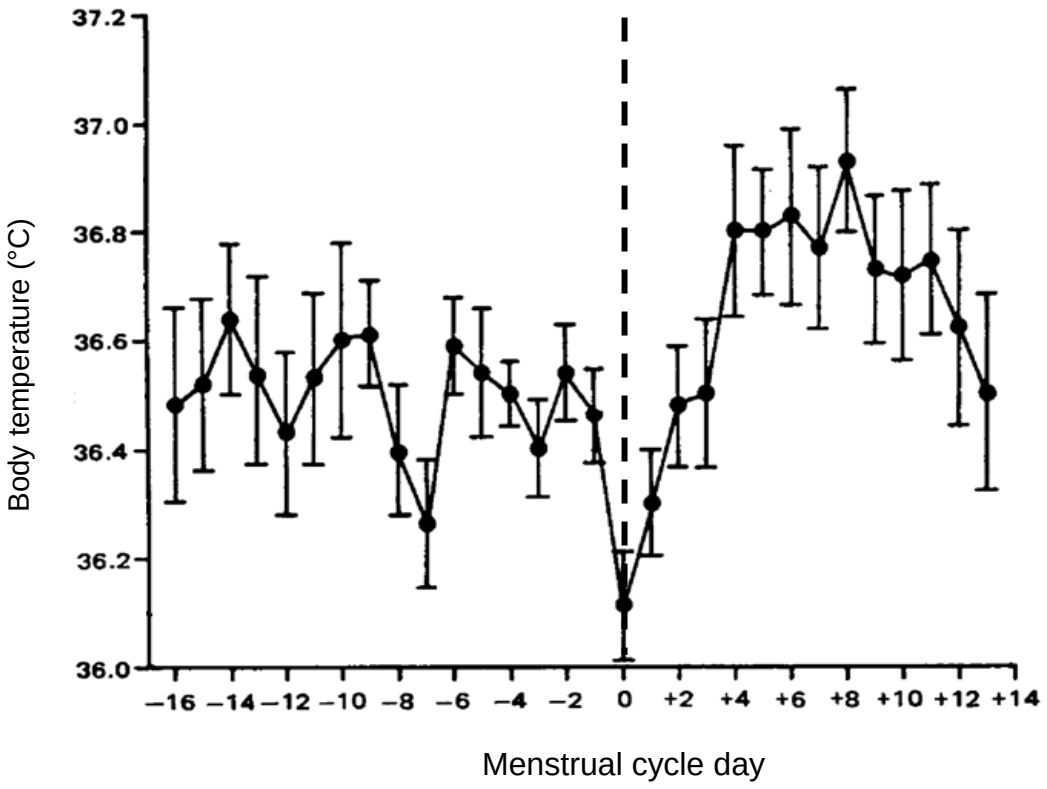


Figure 1.4 Nocturnal rectal temperature (mean ± SEM) in 12 chimpanzees, *Pan troglodytes*. Menstrual cycles were normalised to the mid-cycle temperature minimum (Day 0). Figure adapted and modified from Graham *et al*., 1977.

beluga, *Delphinapterus leucas* (Katsumata *et al*., 2006) and marsupials like Tasmanian bettong, *Bettongia gaimardi* (Rose and Jones, 1996). In all these species, the change in body temperature in their luteal phases has been linked to elevated concentrations of circulating progesterone and its thermogenic effect on the body (Barton and Wiesner, 1945; Davies and Fugo, 1948; Cagnacci *et al*., 1996, 1997; Coyne *et al*., 2000). The thermogenic mechanism of progesterone is not known but it is believed that progesterone possesses metabolic effects and induces an increase in body temperature (Hamp *et al*., 2006).

The presence of progesterone and oestradiol receptors in the pre-optic area of the anterior hypothalamus where temperature regulation takes place as well as the sensitivity of thermoreceptors to progesterone (Nakayama *et al*., 1975) may imply that progesterone and its metabolites act centrally to affect body thermoregulation (Israel and Schneller, 1950; Hamp *et al*., 2006). Progesterone is believed to rapidly increase the firing rate and pattern of hypothalamic pre-optic cold-sensitive neurons, resulting in a rise in body temperature (Israel and Schneller, 1950; Nakayama *et al*., 1975; Bentley-Condit and Smith, 1997). As a result of the association between body temperature and progesterone, the measurement of body temperature has been used as a retrospective diagnostic tool for assessing ovarian function and cyclicity (Czaja and Butera, 1986; Rose and Jones, 1996; Katsumata *et al*., 2006). In women rectal and oral temperature monitoring over the menstrual cycle has been used to detect impending ovulation (Kalant *et al*., 1956; Coyne *et al*., 2000). A general increase in mean body

temperature during the luteal compared to the other phases of the menstrual cycle, could be used as a possible non-invasive diagnostic indicator of ovulation (Lee, 1988; Cagnacci *et al*., 1996). Although studies have been done in chimpanzee and women, there is no information available on studies that were done in baboons, especially the use of body temperature measurements over the menstrual cycle in an effort to estimate the day of ovulation.

1.7 Physical activity patterns

Physical activity is an important variable which interacts with and affects several physiological systems in the body (Hunnell *et al*., 2007). Activity monitoring plays a fundamental physiological measure of the well-being and behaviour of animals (Mann *et al*., 2005). Studies have been done to monitor physical activity over the menstrual cycle in non-human primates using observational measures. In Tonkean macaques, *Macaca tonkeana* (Aujard *et al*., 1998) and baboons (Bielert and Busse, 1983) physical activity and restlessness was found to be associated with the menstrual cycle phase, with physical activity being highest in the follicular phase.

Field studies in which hormone-behaviour relationships in rhesus monkeys and chimpanzees were assessed have shown that the increase in physical activity in the follicular phase and around the time of ovulation is due to oestradiol (Rauth-Wildmann *et al*., 1996; Matsumoto-Oda and Oda, 1998). Some studies on

women in which subjective measures of physical activity were done have reported an increase in physical activity mid-cycle as well as in the luteal phase (Billings, 1934). Stenn and Klinge, (1972) however did not find any patterns in physical activity related to the menstrual cycle in women.

The use of activity data loggers is a standard objective locomotor analysis technique which quantifies gross motor movements in all directions. The activity data logger uses an omnidirectional accelerometer to monitor the occurrence and intensity of motion (Chantler *et al*., 2009). Activity data loggers were originally designed for the objective measurement of physical activity in humans but have been successfully used in non-human primates (Mann *et al*., 2005; Hunnell *et al*., 2007; Papailiou *et al*., 2008) and other mammalian species (Fick *et al*., 2006; Hetem *et al*., 2009). The main advantage of using activity data loggers is that a large amount of data can be collected within a short space of time (Sellers *et al*., 1998) and they are capable of recording physical activity without hindering movement. There is also minimum human contact once the data loggers have been implanted and therefore there is less disturbance of the social life of the animals.

Data loggers have also been used to detect and quantify the decrease in physical activity reported by women with a history of severe dysmenorrhoea (Chantler *et al*., 2009). It has been reported that women with primary dysmenorrhoea have decreased physical activity at menstruation compared to

women without a history of dysmenorrhoea. In rhesus monkeys, activity data loggers have been used to show that physical activity did not change across the menstrual cycle and was not affected by the concentration of circulating oestradiol (Hunnell *et al*., 2007). Physical activity was also monitored using activity data loggers in marmosets (Mann *et al*., 2005) to demonstrate the feasibility of using the data loggers to monitor activity in this species.

Although some studies in which observational methods for monitoring physical activity were used showed a menstrual cycle related pattern in physical activity (Erkert *et al*., 1986; Matsumoto-Oda and Oda, 1998), it is not clear whether such changes in physical activity occur in captive baboons and whether they can be quantified using data loggers. It would therefore be relevant to have a clear understanding of any regularly occurring changes in physical activity across the menstrual cycle of baboons when designing a method of estimating the time of ovulation.

1.8 Aims of the dissertation

The standard way of determining the time of ovulation in any ovulating mammal is to measure the hormone concentrations, which is difficult if not impossible in free-ranging baboons. I set out to measure abdominal temperature and physical activity, changes which may be secondary effects to ovulation. Measurements of abdominal temperature in captive and unrestrained baboons have been obtained before (Maloney *et al*., 2007; Mitchell *et al*., 2009), but abdominal temperature and physical activity correlates of the baboon menstrual cycle are unknown. In particular, no studies have correlated abdominal temperature, physical activity, anogenital swelling changes, faecal and urine ovarian steroid hormones.

The purpose of my study therefore was to investigate the temporal relationship between abdominal temperature, physical activity, anogenital swelling changes, faecal and urine steroid hormones over the menstrual cycle with the specific aim of exploring an alternative method of estimating the time of ovulation, without the need to measure ovarian steroid hormones in faeces and urine.

CHAPTER 2

MATERIALS AND METHODS

2.1. Experimental animals and housing

The experiments were performed on four captive, sexually mature and reproductive cycling female chacma baboons, *Papio hamadryas ursinus* (Figure 2.1). The baboons weighed between 12.9 and 19.9 kg at the beginning of the experiments. The animals were purchased from the Medical Research Council, Tygerberg, Cape Town (South Africa), where they had been captured from the wild and kept in captivity, and transported to the University of the Witwatersrand in Johannesburg, where they were quarantined in an indoor animal facility for six weeks. During this period, the animals were observed for overt signs of disease and were immobilised once, with an intramuscular injection of 10 $mg.kg^{-1}$ ketamine (Anaket-V®, Centaur Laboratories, Johannesburg, South Africa), for a general veterinary clinical health check. The animals were tested for tuberculosis using 0.1 ml of purified protein derivative tuberculin (Rinder-Tuberkulin®, PPD, Hoyerhagen, Germany) injected intradermally into the left upper eyelid, dewormed with an oral dose of 1 $ml.5\ kg^{-1}$ fenbendazole (Panacur®, Intervet, Edenvale, South Africa) and treated for ectoparasites with fipronil spray (Frontline Spray®, Merial, Halfway House, South Africa) by a veterinarian.

The baboons were housed individually in the same room. Each baboon had access to a set of three adjoining steel cages each, of which measured 1.2 x 0.9



Figure 2.1 An adult female chacma baboon, *Papio hamadryas ursinus* (Photo courtesy of Scotch Macaskill and used with permission)

x 0.9 m (Figure 2.2 A). The cages had an underfloor metal sluice which was modified by erecting a barrier (Figure 2.2 B) to allow for continuous collection and separation of faecal and urine samples. The cage layout allowed the baboons to have constant visual contact with each other and they were free to move around their home cages throughout the study period.

The ambient temperature in the indoor environment in which the animals were housed was regulated between 22-25°C and a 12 h light: 12 h dark cycle was maintained with the light cycle starting at 06:00. The animals were fed twice daily, in the morning, with a standardised diet of commercial pellets (H.J. Victor, Randfontein, South Africa), supplemented with fruits in the afternoon. The baboons had access to water *ad libitum*.

The experimental procedures were performed in accordance with the principles and procedures described in the University of the Witwatersrand Guide for the Care and Use of Laboratory Animals and approved by the Animal Ethics Screening Committee, of the University of the Witwatersrand (Animal Ethics Clearance number 2007/60/5).

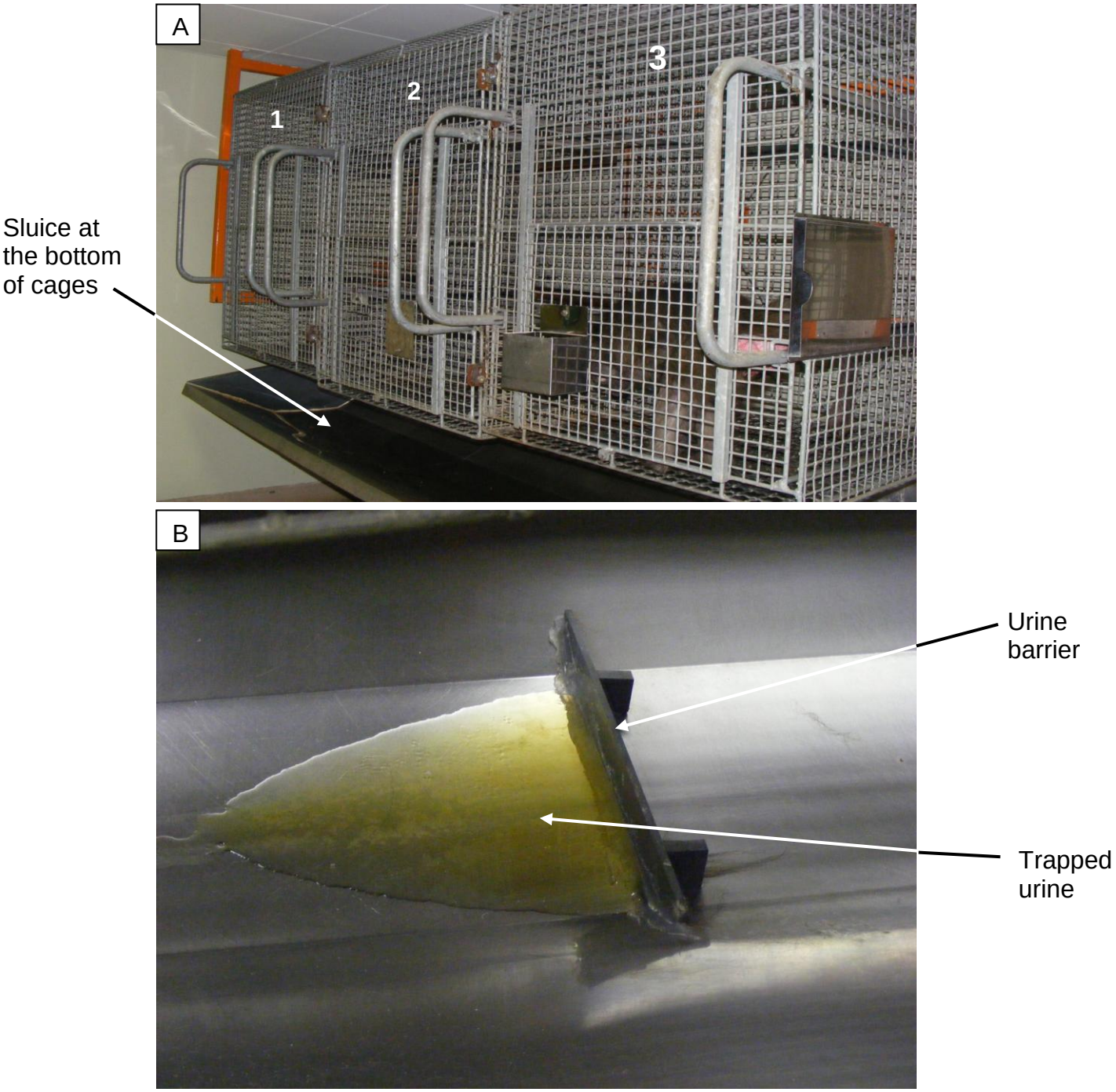


Figure 2.2 Photographs showing A) Three adjacent cages (1-3) and B) modified sluice under the cages to trap urine samples.

2.2. Surgery

2.2.1. Anaesthesia

The animals were anaesthetised with an intramuscular injection of 10 $mg.kg^{-1}$ ketamine (Anaket-V®, Centaur Laboratories, Johannesburg, South Africa) followed by an intravenous injection of 10 $mg.kg^{-1}$ thiopentone sodium (Intraval Sodium®, Merial, Johannesburg, South Africa). The animals were intubated endotracheally and anaesthesia was maintained during surgery with 1-3 % inhaled isofluorane (Isofluorane, Astra Zeneca Pharmaceuticals, Johannesburg, South Africa) in oxygen. Vital signs, namely respiratory rate, haemoglobin oxygen saturation, heart rate and blood pressure, were monitored and recorded throughout surgery using a vital signs monitor (Dinamap™ Portable Vital Signs Monitor 8100 Critikon, Florida, United States of America) and a veterinary pulse oximeter (Nonin 9847V, Nonin Medical, North Plymouth, United States of America). Rectal temperatures were measured during surgery with a thermocouple thermometer (BAT-12, Physitemp, Clifton, United States of America). Surgery lasted between 50 and 60 min for each animal.

2.2.2. Abdominal logger and telemeter implantation

The ventral abdominal area was shaved, cleaned, and sterilised with chlorhexidine gluconate (Hibitane®, Astra Zeneca, Johannesburg, South Africa)

before the animals were moved into a sterile surgical theatre. The surgical area was fully draped before the abdominal cavity was opened via a midline incision of about 20 cm. A thermometric data logger and a radiotelemeter (see section 2.4 below) were implanted into the abdominal cavity through the incision. The implanted radiotelemeters provided continuous real-time measurements of temperature allowing me to monitor the health of the animals. All the temperature-recording devices were dry-sterilised in a sealed drum containing paraformaldehyde tablets (Kyron Laboratories, Johannesburg, South Africa) for more than 24 h, before implantation into the baboons. Aseptic procedures were followed throughout surgery.

2.2.3. Activity logger implantation

Physical activity in each animal was measured by one activity logger (see section 2.5 below). Each activity logger was surgically implanted through the same incision made for the abdominal thermometric data loggers. The activity logger was positioned subcutaneously on the left side of the abdomen of each baboon and tethered to the abdominal wall using orthopaedic wire (Jayco International. Johannesburg, South Africa) and nylon sutures (Gabler Medical, Cape Town, South Africa), Figure 2.3. The activity logger was orientated horizontally for optimal sensitivity of the accelerometer (Fick *et al*., 2006).

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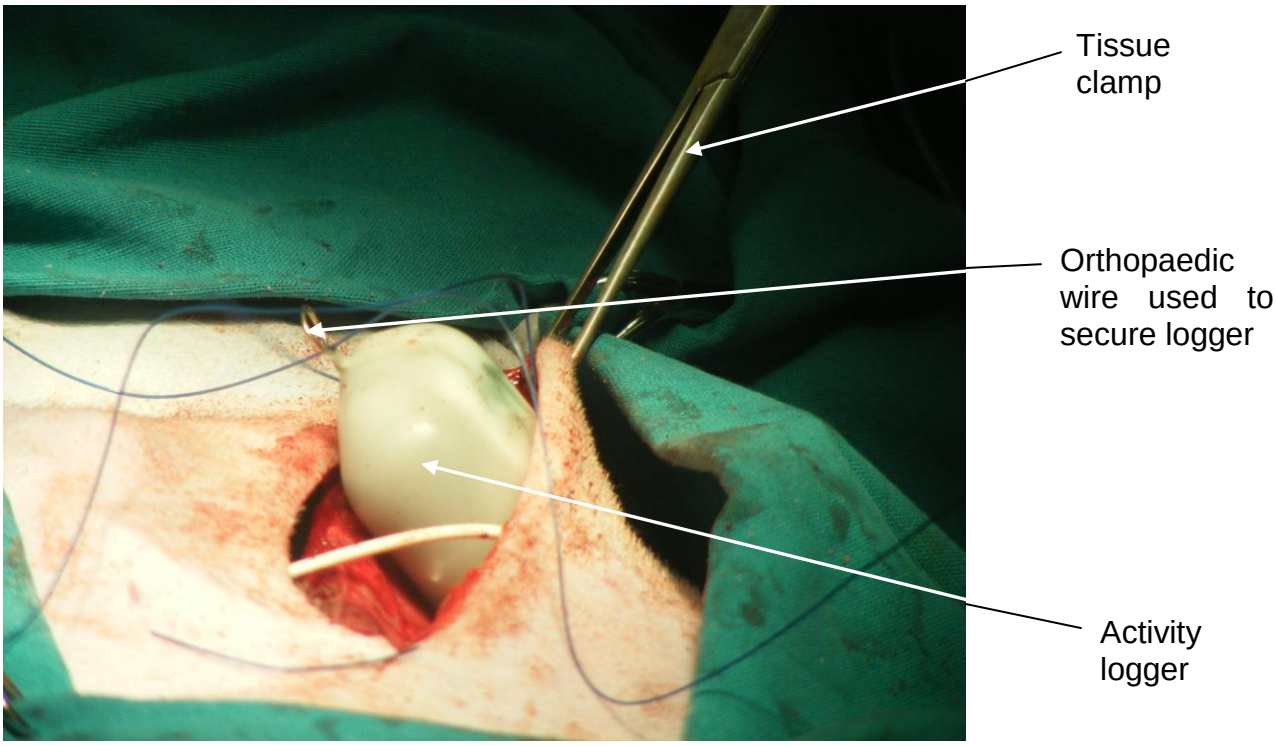


Figure 2.3 Implantation of an activity logger.

2.2.4. Post-surgical care

After surgery, the abdominal incision was closed back using visyn (Gabler Medical, Cape Town South Africa) for the inner muscle layer and nylon (Gabler Medical, Cape Town South Africa) for the outside skin. Each baboon was given an intramuscular injection of 0.3 mg opiate analgesic, buprenorphine hydrochloride (Temgesic®; Schering-Plough, Johannesburg, South Africa), 0.1ml.kg^{-1} intramuscular injection of a long-acting broad-spectrum antibiotic, procaine and benzathine benzyl penicillin, (Peni LA®, Phenix, Johannesburg, South Africa) and 2.2 mg.kg^{-1} of a non-steroidal anti-inflammatory drug, carprofen (Rimadyl®, Pfizer, Johannesburg, South Africa). Abdominal surgical wounds were sprayed with a topical antiseptic (oxytetracycline HCl and gentian violet, Necrospray®, Centaur Laboratories, Johannesburg, South Africa). The baboons were returned to their cages and were given a two-week post-surgery recovery period before the start of data collection.

2.3 Recovery of data loggers and radiotelemeters

The thermometric data loggers, radiotelemeters and activity loggers were recovered surgically after the six month study period. The animals were euthanased using a rapid 2 ml intravenous injection of 200 mg.ml^{-1} sodium pentobarbitone (Eutha-naze Solution®, Bayer Animal Health Division, South

Africa). The position of the activity loggers before implantation was compared to that after implantation and no logger migration had occurred.

2.4. Measurement of abdominal temperature

Abdominal temperature was measured with a thermometric data logger (Figure 2.4) with an internal temperature sensor (StowAway XTI, Onset Computer Corporation; Pocasset, United States of America) and a miniature temperature-sensitive radiotelemeter (Mini-Mitter, Sunriver, United States of America). The radiotelemeter was attached to the thermometric data logger using insulating, self-amalgamating, black tape (RS Components, Midrand, South Africa). The logger and radiotelemeter then were encased with wax (Sasolwax Exp 987, Sasol Wax, Johannesburg, South Africa) for waterproofing and to make them biologically inert. Together the devices had a final outside dimensions of 50 x 45 x 20 mm and a mass of about 55 g. The loggers were calibrated to an accuracy of 0.04°C against a high-accuracy thermometer (Quat 100, Heraeus, Hanan, Germany) in an insulated water bath.

The thermometric data loggers had a storage capacity of 32 kB and measured temperature within a range of 34 to 46°C every 10 min for the duration of the study. After the recovery of the loggers, the calibration procedure for temperature data was repeated to check whether any temperature drift had occurred during the study period by comparing the calibration equations obtained before and after

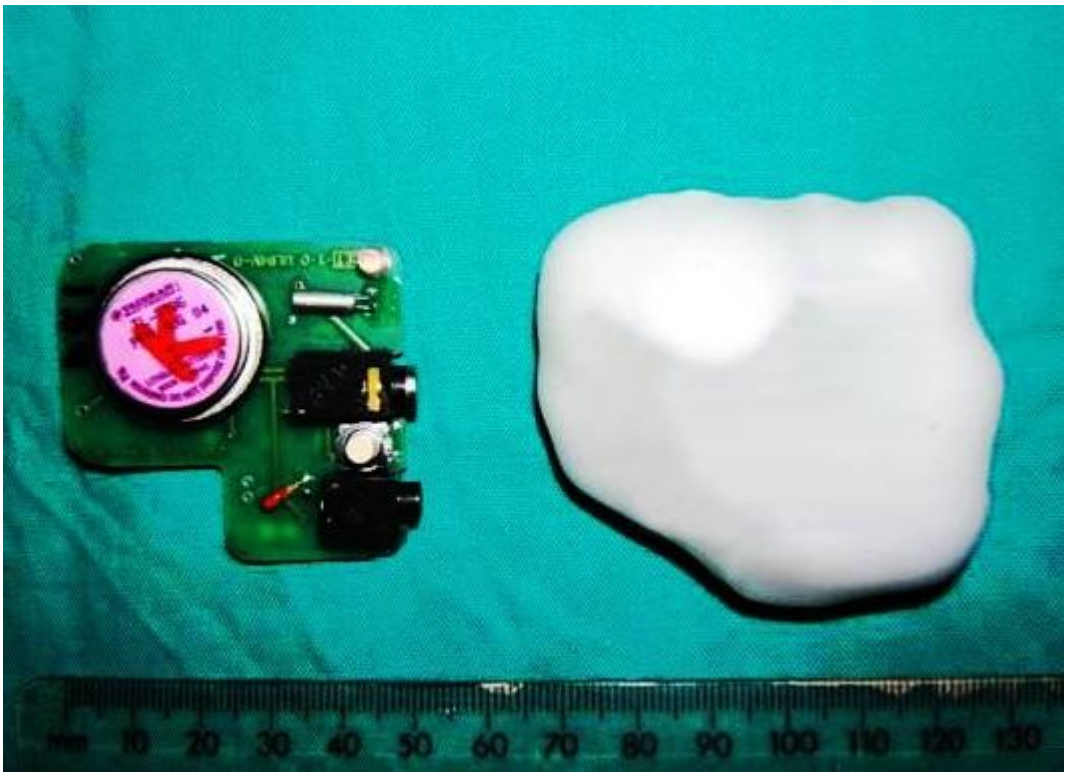


Figure 2.4 Thermometric data logger before waxing (left) and after waxing (right).

the study. After recovery of the data-loggers, I removed their wax coating and downloaded the data onto a personal computer, using custom-made software for temperature data (Boxcar 4.3®, Onset Computer Corporation, Pocasset, Massachusetts, United States of America). I exported the data files to a spreadsheet for further statistical analysis.

2.5. Measurement of physical activity

The level of physical activity was measured by an activity data logger (Actical™, Mini-Mitter Corporation, Oregon, United States of America). The activity data logger (Figure 2.5), an omnidirectional accelerometer, detected movement of the baboon and recorded it as the accumulated activity count over 10-min intervals for six months. The loggers had dimensions of 40 x 40 x 15 mm and weighed 28 g when covered in inert wax (Sasolwax Exp 987, Sasol Wax, Johannesburg, South Africa) for waterproofing and to protect the body tissue from the foreign implant.

After recovering the activity data-loggers, I removed their wax coating and downloaded the data onto a personal computer, using an ActiReader (Mini-Mitter, Oregon, United States of America) and custom-made software for physical activity data (Actical software®, version 2.0, Mini-Mitter, Oregon, United States of America). I exported the data files to a spreadsheet for further statistical analysis.

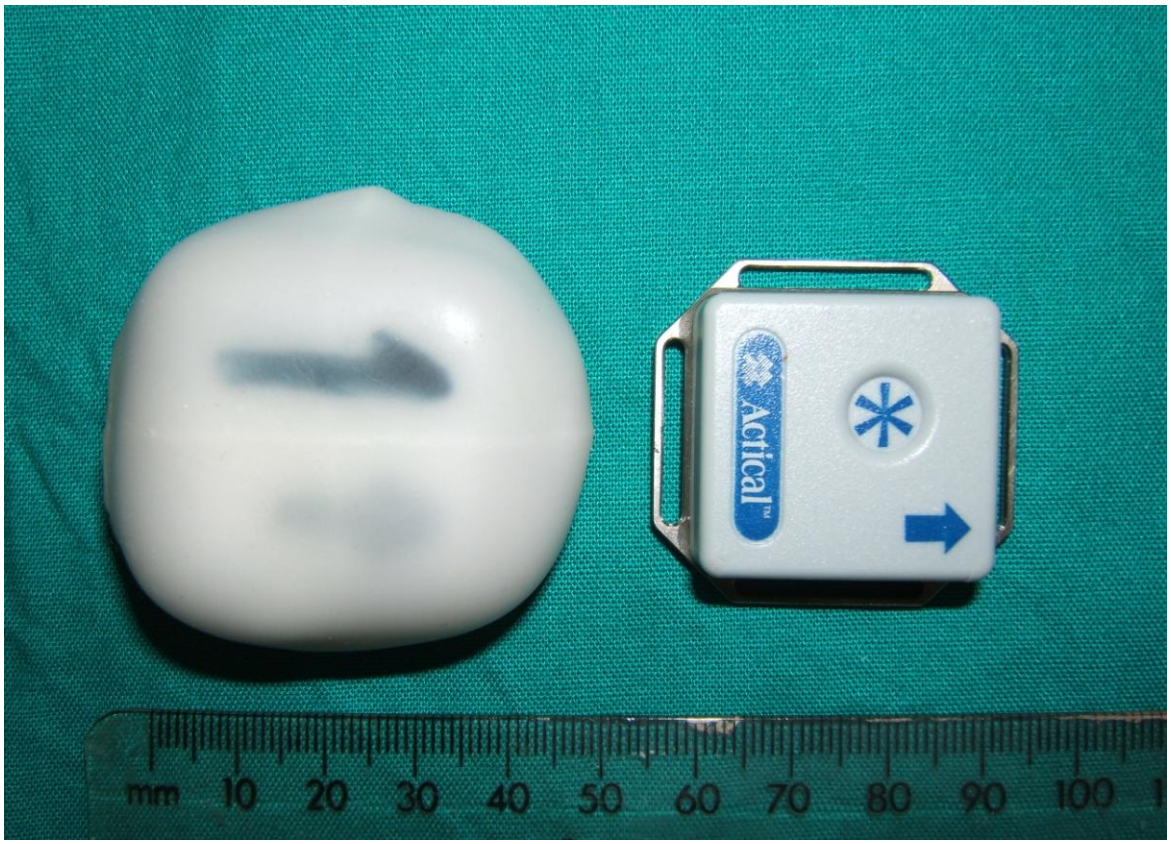


Figure 2.5 Activity data logger after waxing (left) and before waxing (right).

2.6 External reproductive changes

2.6.1. Menstrual bleeding

I recorded external reproductive changes each day between 08:00-09:00. Presence or absence of vaginal menstrual bleeding was determined by visual inspection of the anogenital area. Onset of menstrual bleeding indicated the beginning of a menstrual cycle. The cycle length was determined by counting the days between the onsets of two successive menstrual bleedings.

2.6.2. Anogenital swellings

The anogenital areas were observed daily between 08:00-09:00. The degree of wrinkling, colour and size of the vulva and anus (anogenital area) were used to evaluate changes in the anogenital swellings during the menstrual cycle (Aujard *et al*., 1998). These changes have been regarded as reliable indicators of the menstrual cycle status of baboons (Gillman, 1935; Hendrickx, 1965). Where possible and with attempts to reduce stress of the baboons, digital photographs of the anogenital swellings were taken (FinePix S5700, Fujifilm Corporation, Tokyo, Japan) as a record of external morphological changes.

I used the Emery and Whitten (2003) scoring system to subjectively quantify anogenital swellings. The scale had five levels which registered complete anogenital deturgescence as zero and maximum swelling as four (Figure 2.6). Intermediate stages were scored as 1, 2 and 3 corresponding to the ascending order of swelling. Observations and scoring were done only by me to avoid inter-observer variation.

2.7. Hormone analysis

2.7.1. Urine sample collection and processing

Urine samples were collected daily between 08:00-09:00, over the entire six-month study period. I stirred the trapped urine sample to homogenise it and collected 15 ml into a 15 ml polypropylene centrifuge tube (Greiger Bio-one GmbH; Frickenhausen, Germany). The samples were frozen immediately after collection and stored in a freezer at -20°C until hormonal and creatinine assays were performed. Urine samples contaminated with blood from menstrual flow were rejected. Before hormonal assays and creatinine measurements, I thawed the samples and centrifuged them for 10 min at 1 500 *x g* in a refrigerated centrifuge (Sorvall RT 6000B, DuPont Co., California, United States of America) to remove insoluble particulate organic matter. The urine samples then were aliquoted into 5 ml specimen tubes.

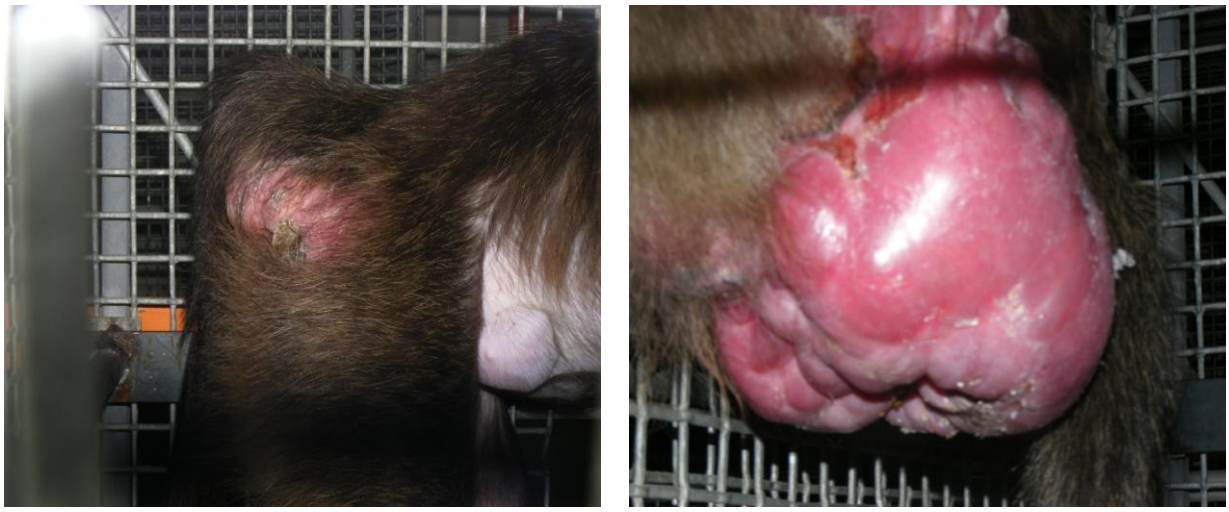


Figure 2.6 Different stages of the anogenital swellings during the menstrual cycle in baboons, complete anogenital deturgescence, score 0 (left) and maximum swelling, score 4 (right).

2.7.1.1 Extraction of urine steroid hormones

Individual urine samples were thawed and mixed with a vortex mixer (Model VM-300, Gemmy Industrial Corp, Taiwan) and 1 ml of the sample added to appropriately labelled 12 x 75 mm glass centrifuge tubes, to which 4 ml of methylene chloride (Sigma, Johannesburg, South Africa) was added. The tubes were capped and the mixture vortexed for 30 seconds, then centrifuged (Sorvall RT 6000B, DuPont Co., California, United States of America) at 1500 *x g* for 5 min. The upper aqueous layer was aspirated and the bottom non-aqueous organic layer evaporated to dryness overnight using a stream of air from a fan. The resultant extract was reconstituted in 200 µl of distilled water before I performed the radioimmunoassays. The extraction recovery for urinary steroid hormones was not done.

2.7.1.2 Measurement of creatinine

The 5 ml samples of the prepared urine in section 2.7.1 were sent to the National Health Laboratory Services, South Africa, for the determination of creatinine. The amount of creatinine in the urine samples was measured using the Creatinine_2 method (ADVIA® 1200 Chemistry Creatinine System, Siemens Medical Solutions Diagnostics (Pty) Ltd, Midrand, South Africa). This method is based on the reaction of picric acid with creatinine in an alkaline medium as described in the original method by Jaffe (Jaffe, 1886; Taussky, 1954; Kretochvila *et al*., 2007). In

the Jaffe's reaction, creatinine reacts with alkaline picrate to give an orange coloured creatinine-picrate complex. The intensity of the colour change and the rate of complex formation was measured at 505/571 nm and was proportional to the concentration of creatinine in the urine samples. I assumed that the rate of creatinine production was constant, though it may actually vary with muscle mass (Kretochvila *et al*., 2007). In this study I did not take into account the variation in muscle mass. The concentration of creatinine was reported in $mmol.l^{-1}$ and used to index the hormonal concentrations to control for variation in water intake. Samples that had levels of creatinine lower than 0.1 $mmol.l^{-1}$ were not used since they gave unreliable calculated values of hormone concentrations.

2.7.2. Faecal sample collection and processing

Fresh faeces, identified by observation of defaecation or by texture and colour, were collected daily between 08:00 and 09:00 over the six-month study period. Sampling time was kept constant to reduce variation due to circadian patterns of hormone excretion. The samples were collected in their entirety and placed in sealable plastic bags (Zip-loc®, SC Johnson, Johannesburg, South Africa). The faecal samples were frozen immediately after collection, to reduce the degradation of steroids hormones, and stored at -25°C until hormonal extractions were performed. Faecal samples contaminated with urine were not collected.

2.7.2.1. Drying

Before I performed hormonal extractions, I placed the frozen faecal samples into 50 ml plastic specimen bottles which I then placed in the freeze-drier (Plastomatic, Philips, South Africa) for freeze-drying. The faecal samples were freeze-dried for 12-14 h by which time they had reached constant weight. The dry faeces were ground using a coffee grinder (Sunbeam Designer Coffee Grinder®, Model SGC-2012; Nu-World Industries, Johannesburg, South Africa) to give a fine homogenised powder. The ground faecal samples were stored at -25 °C in 40 ml sterile specimen bottles until hormone extractions were performed.

2.7.2.2. Extraction of faecal steroid hormones

The extraction protocol for faecal steroids was modified from previously published methods (Ziegler *et al*., 1996). Briefly, 0.2 g of the dried, ground and powdered sample was placed into 15 ml polypropylene centrifuge tube (Greiger Bio-one GmbH; Frickenhausen, Germany). Five millilitres of aqueous 80% methanol solution (Merck Chemicals, Wadeville, South Africa) was added to the sample to extract the faecal steroid metabolites. The test tubes with the mixture of faecal sample and methanol were capped, mixed on a vortex mixer (Model VM-300, Gemmy Industrial Corp, Taiwan) for 30 seconds, and agitated on a shaker (Edmund Buhler, Germany) at 2000 revolutions.min^{-1} overnight. The mixture then was allowed to settle for 30 min and centrifuged (Sorvall RT 6000B,

DuPont Co., California, United States of America) at 1000 *x g* for 35 min. The extraction recovery for faecal steroid hormones was not done.

The supernatant from the samples was decanted and 2 ml freeze-dried in a freeze drier (Plastomatic, Philips, South Africa) in 15 ml polypropylene centrifuge tubes. The extract was reconstituted in 1 ml distilled water and stored at -25°C in 2 ml Epperndof tubes, which were stoppered and wrapped in plastic wrap (Parafilm M®; Pechinery Plastic Packaging, Illinois, United States of America) until the assays were conducted. Before I commenced with the assays, I diluted the steroid extract with distilled water to give a range of serial dilutions (10^{-1}, 10^{-2} and 10^{-3}) for the determination of the minimum and maximum detection limits.

2.7.3 Radioimmunoassays

I measured faecal and urine steroid hormonal concentrations using the 125-radioisotope of iodine (I^{125}) radioimmunoassay (RIA) that has been validated for baboons (Wasser, 1996; Hodges *et al*., 2005). The procedure follows the basic principle of solid-phase RIA where the (I^{125}) labelled analyte competes with the specific hormone for a fixed number of antibody binding sites (Yalow *et al*., 1971). Commercially-available progesterone, oestradiol, and cortisol RIA kits were used following the criteria described by Khan *et al*., (2002) and Weingrill *et al*., (2004). Faecal hormonal concentrations were expressed in $ng.g^{-1}$ of dry faecal matter and as $ng.mmol^{-1}$ of creatinine in urine samples.

2.7.3.1. Cortisol assays

I measured cortisol concentration in faecal and urine extracts using RIA cortisol kits (DSL- 2000, Diagnostic Systems Laboratory, Webster, Texas, United States of America) following the manufacturer's instructions. Reagents and samples were allowed to reach room temperature (25°C) and gently mixed by repeated inversion before use. Standards and controls were assayed in duplicate while samples were assayed singly. Standards, controls and samples were pipetted to the bottom of the 12 x 75 mm test tubes in 25 µl amounts. Immediately, 500 µl of the I^{125} cortisol reagent was added to all the test tubes except for the total count tubes. Total counts tubes represented the total amount of radioactivity added to the RIA tubes. All the tubes were vortexed and incubated in a water bath at 37 ± 2°C for 45 min. The tubes, excluding the total count tubes, then were centrifuged for 15 to 20 min at 1500 x *g* in a refrigerated centrifuge (CRU-5000, Damon/IEC Division, Needham, Massachusetts, United States of America). The supernatant from the tubes was decanted by simultaneous inversion, except for the total count tubes, and allowed to drain on absorbent paper for 20 seconds. The sediment in all the tubes was counted for radioactivity in a gamma counter (Cobra Auto-gamma B5002, Packard, Netherlands) for one minute.

According to the manufacturer, the antibody has a high affinity for cortisol and low cross-reactivity with other naturally-occurring steroids (100% cortisol; 33.33% prednisolone; 9.30% corticosterone; 3.80 % 11-deoxycortisol; 2.22 % cortisone;

1.42% prednisone; 1.00% 17α-hydroxyprogesterone; 0.61% 11-deoxycorticosterone; 0.38% dexamethasone; 0.14% testosterone; 0.12% progesterone; 0.04% epiandrosterone; 0.02%, dehydroepiandrosterone; 0.02% estradiol). According to the manufacturer the assay had a sensitivity of 0.11 $\mu g.dl^{-1}$, with the lowest standard used being 0.5 $\mu g.dl^{-1}$. The intra-assay coefficient of variation was 18.3% and the inter-assay coefficient of variation was 8%.

2.7.3.2 Progesterone assays

I measured urine and faecal progesterone using a commercially-available progesterone RIA human kit (DSL-3400, Diagnostic Systems Laboratory, Webster, Texas, United States of America). Briefly, reagents and samples were allowed to reach room temperature (25°C) and gently mixed by repeated inversion. Total counts, standards and controls were assayed in duplicate whereas samples were assayed singly. Progesterone standards, controls and samples were pipetted into test tubes in 25 µl amounts. To non-specific binding (NSB) tubes, 125 µl of the 0 $ng.ml^{-1}$ progesterone standard was added. Non-specific binding tubes are tubes that contain the labelled antigen but do not have any antibody. The counts from these tubes are subtracted from the counts of all the other tubes to obtain an accurate estimate of counts in the bound fraction. Immediately, 500 µl of the I^{125} progesterone reagent and 100 µl of progesterone anti-serum were added to each tube except total counts and non-specific binding

tubes. All the tubes were vortexed and incubated in a water bath at 37 ± 2°C for 60 to 70 min. The tubes, excluding the total count tubes, then were centrifuged, after I added a precipitating reagent provided by the manufacturer, for 15 to 20 min at 1500 *x g* in a refrigerated centrifuge (CRU-5000, Damon/IEC Division, Needham, Massachusetts, United States of America). The tubes were decanted by simultaneous inversion, except for the total count tubes, and allowed to drain on an absorbent paper for 15 to 20 sec. The sediment in all the tubes were counted for radioactivity in a gamma counter (Cobra Auto-gamma B5002, Packard, Netherlands) for one minute.

According to the manufacturer, the antibody cross reactivities were 100% progesterone; 5% 5α-pregnane-3, 20-dione; 0.35% 20α-dihydroprogesterone; 0.27% 11-deoxycortisol; 0.88% 11-deoxycorticosterone; 0.88% 5β-pregnane-3,20-dione; 0.88% 17α-hydroxyprogesterone; 0.35% corticosterone and 0.88% medroxyprogesterone. According to the manufacturer the assay has a sensitivity of 0.1 $ng.ml^{-1}$, with 0.3 $ng.ml^{-1}$ being the lowest standard used while the intra-assay coefficient of variation is 5.1% and the inter-assay coefficient of variation 2.5%.

2.7.3.3 Oestradiol assays

I measured urine and faecal oestrogen using a commercially-available Oestradiol RIA human kit (DSL-4400, Diagnostic Systems Laboratory, Webster, Texas,

United States of America). Briefly, reagents and samples were allowed to reach room temperature (25°C) and mixed thoroughly by inversion before use. Total counts, non-specific binding, standards and controls were assayed in duplicate whereas samples were assayed singly. Oestrogen standards, controls and samples were added to test tubes in 100 µl amounts. To non-specific binding tubes, 200 µl of the 0 $ng.ml^{-1}$ oestradiol standard and 100 µl of the I^{125} oestradiol reagent was added to each tube except and total counts and non-specific binding tubes. The tubes were vortexed and incubated in a water bath at 37 ± 2°C for 30 min and 1 ml of the precipitating reagent was added to all tubes except the total counts tubes. The tubes were vortexed again and allowed to stand at room temperature for 15-20 min. All the tubes excluding the total count tubes then were centrifuged for 15-20 min at 1500 *x g* in a refrigerated centrifuge (CRU-5000, Damon/IEC Division, Needham, Massachusetts, United States of America). The supernatant in all the tubes was decanted by simultaneous inversion, except for the total count tubes, and allowed to drain on absorbent paper for 15 to 20 seconds. The sediment in all the tubes was counted for radioactivity in a gamma counter (Cobra Auto-gamma B5002, Packard, Netherlands) for one minute.

According to the manufacturer, the antibody has a high affinity for oestradiol and low cross-reactivity with other naturally-occurring oestrogens; 100% 17-β oestradiol; 6.10% equelenin; 3.40% oilstone; 1.80% 17-β oestradiol-3-glucuronide; 0.84% equilin; 0.75% oestriol; 0.30% oestrone-β-D-glucuronide;

0.29% 16-keto-oestradiol; 0.26% 17α-oestradiol and 0.21% oestradiol-3-sulfate. According to the manufacturer, the assay had a sensitivity of 4.7 $pg.ml^{-1}$, with 20 $pg.ml^{-1}$ being the lowest standard used. The intra-assay coefficient of variation was 4.6 % and the inter-assay coefficient of variation of 8.5%.

2.8. Data analysis

All data are shown as mean ± standard deviation. Abdominal temperature (mean and amplitude) and physical activity were calculated from the original 144 data points recorded every 10-min within a 24-h period. Minima and maxima in abdominal temperature are the highest and lowest of individual 10 min values within each 24-h period. Comparisons of abdominal temperature, physical activity and steroid hormonal concentrations in different phases of the menstrual cycle were done using a repeated measures one way analysis of variance (ANOVA). Tukey multiple-comparison post-hoc test was used to compare individual means when significant differences were detected by ANOVA. The level of significance used was $p < 0.05$.

I did not retrieve physical activity data from the activity logger explanted from baboon A due to water entering the logger and causing failure, therefore I present physical activity data from three animals. To account for difference in sensitivity and variability between activity data loggers as well as for the inter-individual variability in the animals' physical activity, I expressed mean 24-h

physical activity counts as a percentage of the maximum count recorded by that logger; and the result of the calculation had dimensionless units and hence was reported as activity units (Chantler *et al*., 2009; Hetem *et al*., 2009).

I investigated the possibility of the utility of faecal hormones to predict urine hormonal patterns in baboons by comparing the hormonal patterns in faecal and urine samples. Pearson product-moment correlations were performed to find whether there was a significant correlation between the urine and faecal steroid hormonal concentrations.

I used data from the time when regular menstrual cycles were recorded as seen by regular menstrual bleeding and consistent anogenital swelling patterns. I divided the menstrual cycles into three phases; the follicular, ovulatory and luteal phases. The beginning of menstrual bleeding marked the start of the follicular phase while the onset of anogenital swelling deturgescence from 4 to 3 score marked the beginning of the luteal phase. The means for abdominal temperature, physical activity, and hormonal concentrations were calculated from five in the middle of each phase.

Estimation of the day of ovulation was done using data from three baboons that had both abdominal temperature and physical activity data and averaged across baboons. I constructed a 20-day composite menstrual cycle profile of abdominal temperature and physical activity anchored on the 4-3 transition in anogenital

swelling score. I used the 4-3 transition in anogenital swelling score to define the line fitting. Using abdominal temperature and physical activity data, I fitted straight lines by eye between 5-10 days before, and 5-10 days after the 4-3 transition in anogenital swelling score. Where these two fitted lines intersected was presumed to be the estimated day of ovulation because of the shift in abdominal temperature and physical activity from a mid-cycle minimum to elevated levels in the luteal phase. A paired Student's *t*-test was used to compare days of ovulation estimated from abdominal temperature changes and physical activity changes over the menstrual cycle.

CHAPTER 3

RESULTS

3.1 Menstrual cycle record

3.1.1 Menstrual bleeding

All the baboons menstruated at least twice within the six-month study period and each animal had at least one complete menstrual cycle. The four baboons had a total of nine menstrual cycles, with an average duration of 36 ± 2 days (Table 3.1), as determined from daily observations of menstrual bleeding and anogenital swellings. The duration of the menses ranged from 4-6 days.

3.2 Hormone analysis

3.2.1 Urine progesterone and oestradiol over the menstrual cycle

Urine oestradiol and progesterone concentration showed a cyclic pattern over the menstrual cycle (Figures 3.1 - 3.3). Urine oestradiol concentration increased after menstruation and was significantly higher during the follicular phase than in the ovulatory and the luteal phases, (ANOVA, $p = 0.01$; $F_{(2,4)} = 18.8$; Figure 3.4). Cycle 3 of baboon C had irregular peaks of oestradiol during the luteal phase. Progesterone concentration in urine samples increased after the ovulatory period and was higher during the luteal phase than in the follicular and ovulatory phases (ANOVA, $p = 0.01$; $F_{(2,4)} = 17.9$; Figure 3.4).

Table 3.1 Duration of the menstrual cycles (days) in baboons.

Baboon	Cycle 1	Cycle 2	Cycle 3
A	*51	-	-
B	*48	35	-
C	37	33	36
D	38	36	38
Mean ± SD	38 ± 1	35 ± 2	37 ± 1

*Menstrual cycles excluded as outliers as they were more than three standard deviations from the mean.

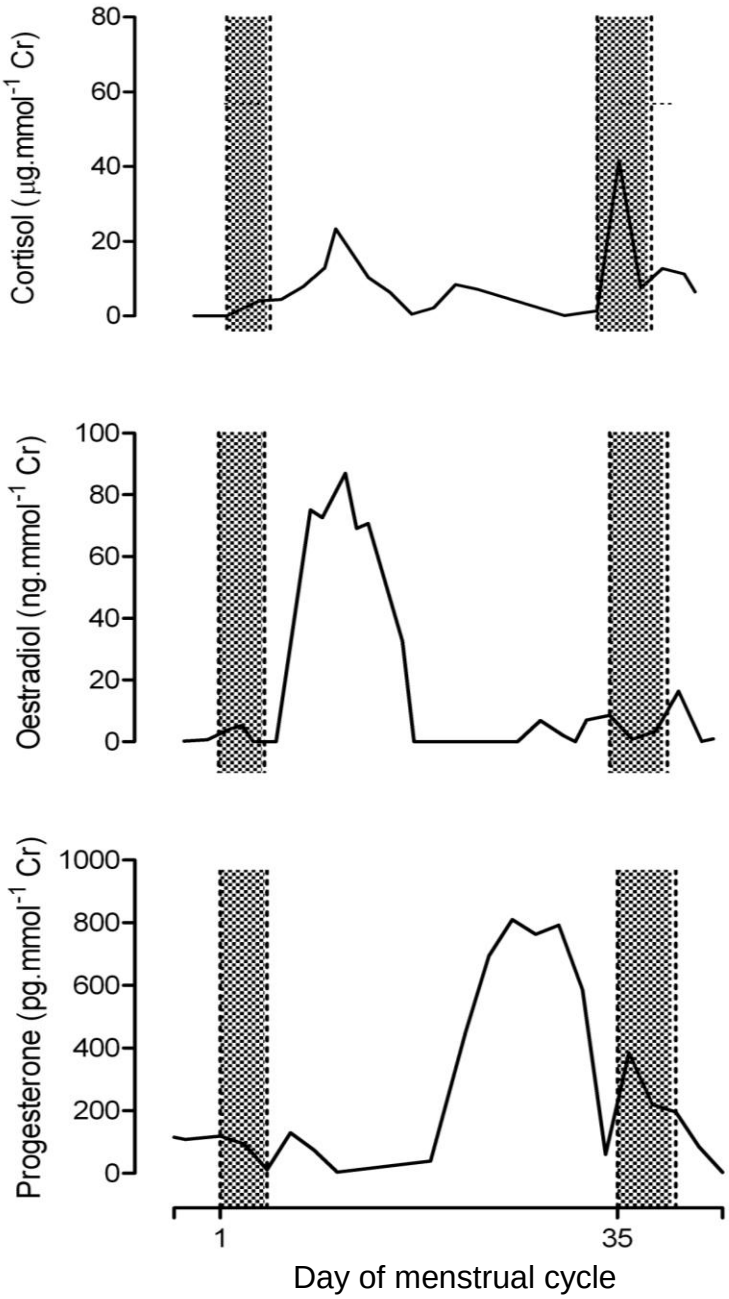


Figure 3.1 Urine steroid hormonal profiles, expressed per mmol of creatinine (Cr) in the same sample for baboon B, cycle 2. The vertical lines represent the beginning and end of menstrual bleeding while the shaded areas represent duration of menstrual bleeding.

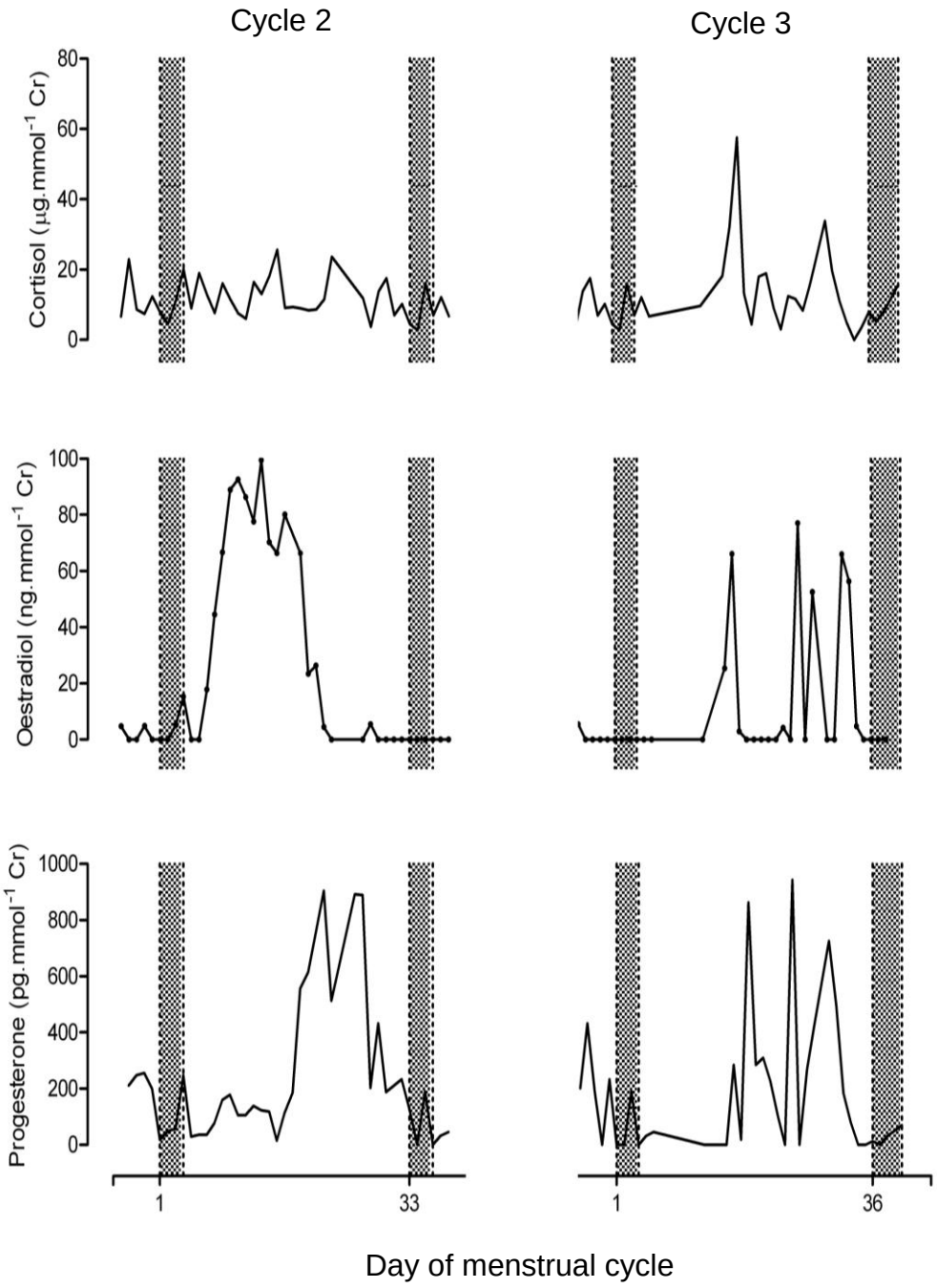


Figure 3.2 Urine steroid hormonal profiles, expressed per mmol of creatinine (Cr) in the same sample for two consecutive cycles in baboon C. The vertical lines represent the beginning and end of menstrual bleeding while the shaded areas represent duration of menstrual bleeding.

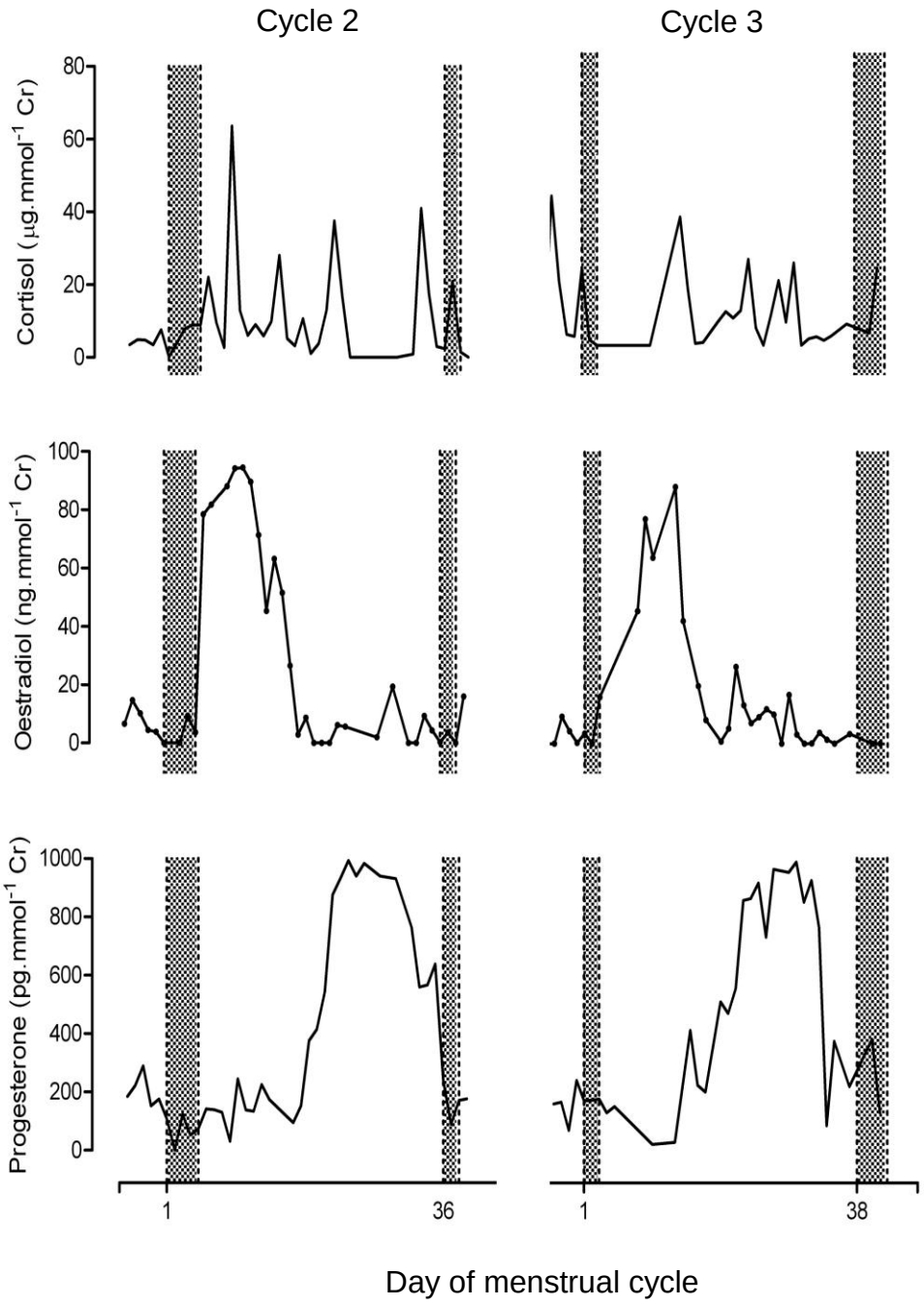


Figure 3.3 Urine steroid hormonal profiles, expressed per mmol of creatinine (Cr) in the same sample for two consecutive cycles in baboon D. The vertical lines represent the beginning and end of menstrual bleeding while the shaded areas represent duration of menstrual bleeding.

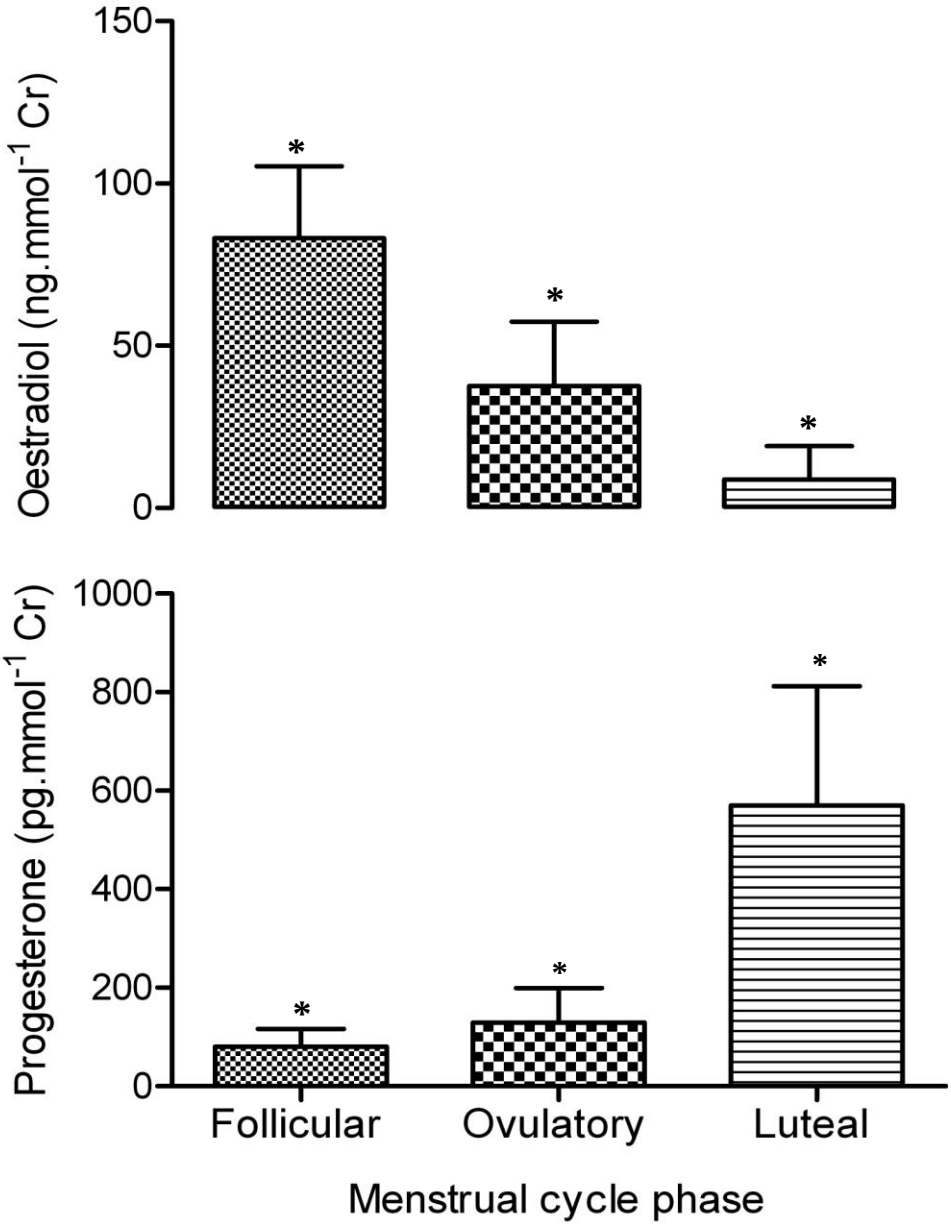


Figure 3.4 Oestradiol (mean ± SD) and progesterone (mean ± SD) concentrations, expressed per mmol of creatinine in the same sample in different phases of the menstrual cycle. ANOVA, ***** p = 0.01, n = 3.

Progesterone concentrations reached a peak in the second half of the menstrual cycle and decreased before menstruation (Figure 3.1 - 3.3).

Cortisol concentration was low throughout the menstrual cycle with some peaks that occurred at irregular intervals. Cortisol concentration in urine samples did not appear to be associated with the phase of the menstrual cycle.

3.2.2 Comparison of urine and faecal steroid hormones

The concentrations and patterns of ovarian steroid hormones in faecal compared to urine samples are shown in Figure 3.5. Faecal oestradiol and progesterone concentrations expressed per gram of dry faecal matter, showed similar cyclic patterns to those in urine samples expressed per mmol of creatinine. In both urine and faecal samples, oestradiol was higher in the follicular phase than in the luteal and ovulatory phases while progesterone was higher in the luteal phase than in the follicular and ovulatory phases.

I found a significant relationship between urine and faecal oestradiol concentrations (Baboon B, $r^2 = 0.46$; Baboon D, $r^2 = 0.21$) as well as urine and faecal progesterone concentrations (Baboon B, $r^2 = 0.42$; Baboon D, $r^2 = 0.54$), Figure 3.6. There were random peaks in the concentrations of cortisol in faecal samples over the menstrual cycle. The changes in faecal cortisol concentration did not show any menstrual cycle-related patterns. As in urine samples, section

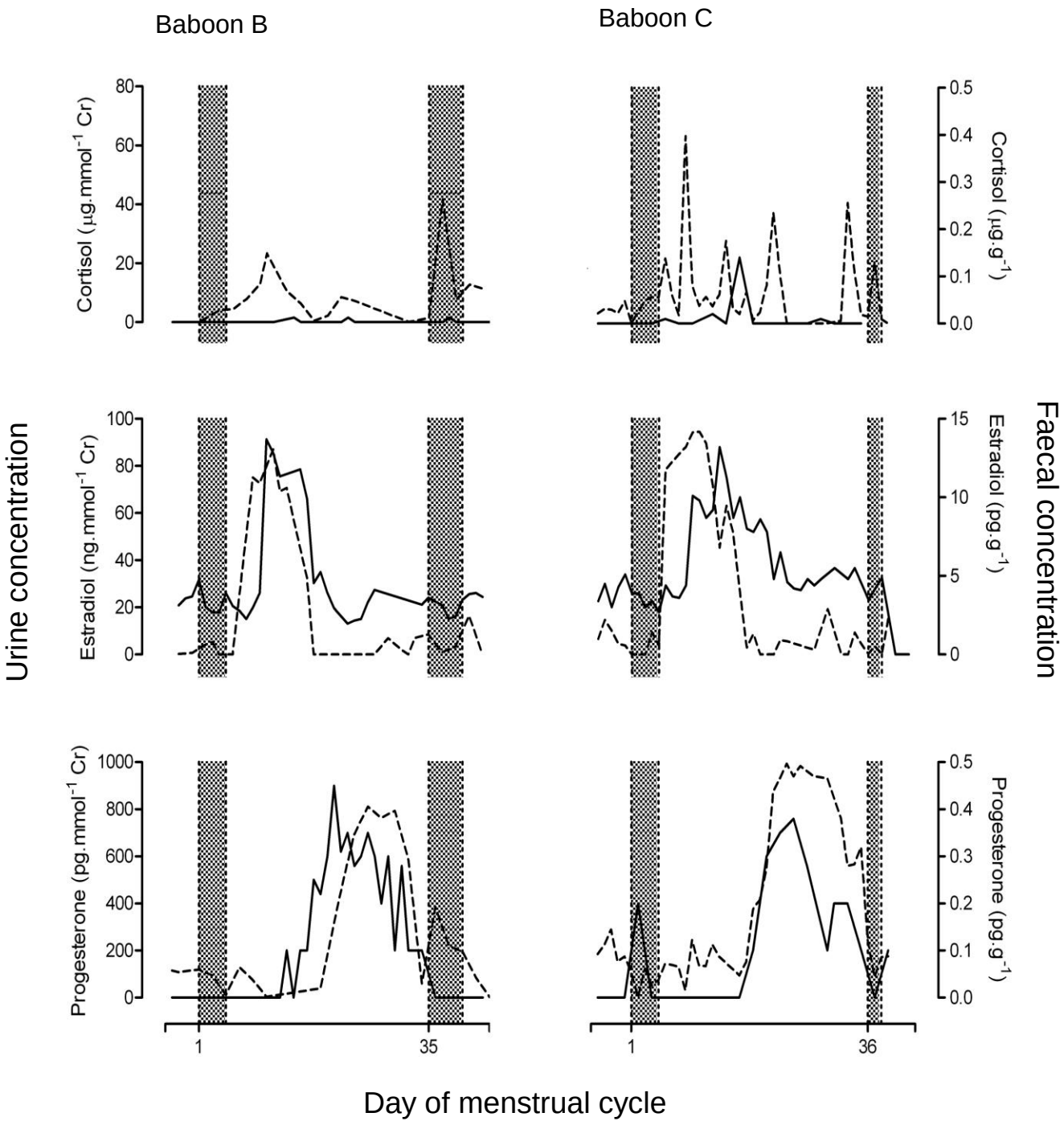


Figure 3.5 Urine (broken line) and faecal (solid line) steroid hormonal profiles during the menstrual cycles of two baboons.

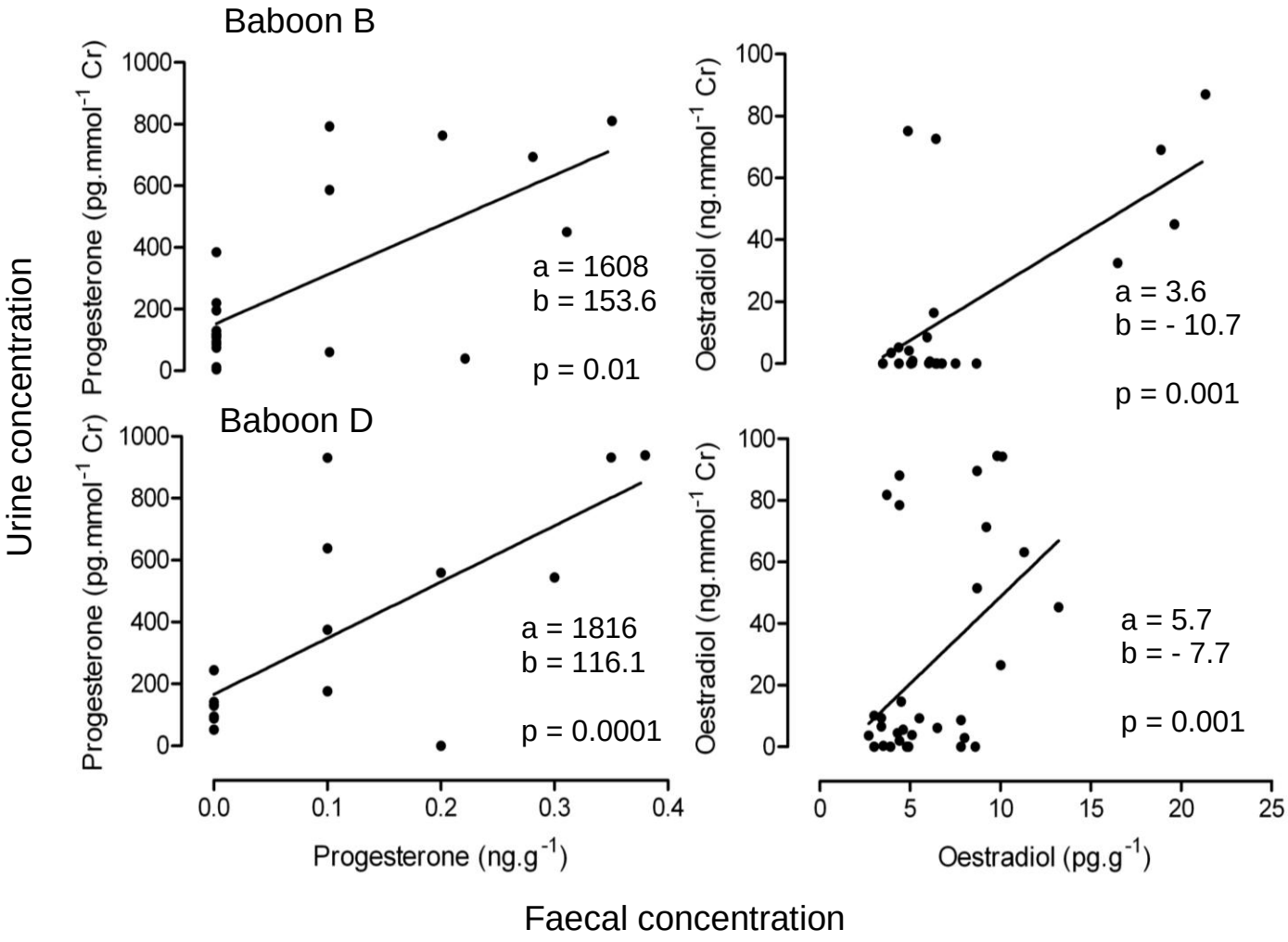


Figure 3.6 Correlation of progesterone and oestradiol concentrations in urine and faecal samples for two baboons. There was a significant relationship between progesterone and oestradiol concentrations in urine and faecal samples. This relationship is given as: y = ax + b, where;

y = progesterone or oestradiol concentration in urine samples.

x = progesterone or oestradiol concentration in faecal samples

a = slope

b = progesterone or oestradiol concentration in urine when progesterone or oestradiol in faeces is zero.

3.2.1., there were cortisol peaks at irregular intervals over the menstrual cycle.

3.3 Anogenital swelling changes over the menstrual cycle

I recorded five different stages of anogenital swelling changes over the nine menstrual cycles of the study animals (Figure 3.7). All the animals exhibited at least one complete cycle of anogenital swellings and deturgescence during the six-month study-period. The change in anogenital swelling from the 4-3 score occurred within one day. The change in size and duration of each stage of anogenital swelling varied among individual baboons (Table 3.2).

Figure 3.8 shows the pattern of anogenital swellings over one menstrual cycle, while Figure 3.9 shows the change of anogenital swellings over consecutive menstrual cycles in three of the animals as a function of time.

3.4 Nychthemeral rhythms of abdominal temperature and physical activity

Baboons displayed a nychthemeral rhythm of abdominal temperature and physical activity (Figure 3.10). Abdominal temperature started increasing before the lights came on reaching an average maximum of ~ 38.6°C at midday. Physical activity followed the same pattern and also reached a maximum at about 12:00. Abdominal temperature and physical activity started dropping before the lights went off and they both reached a minimum at night.

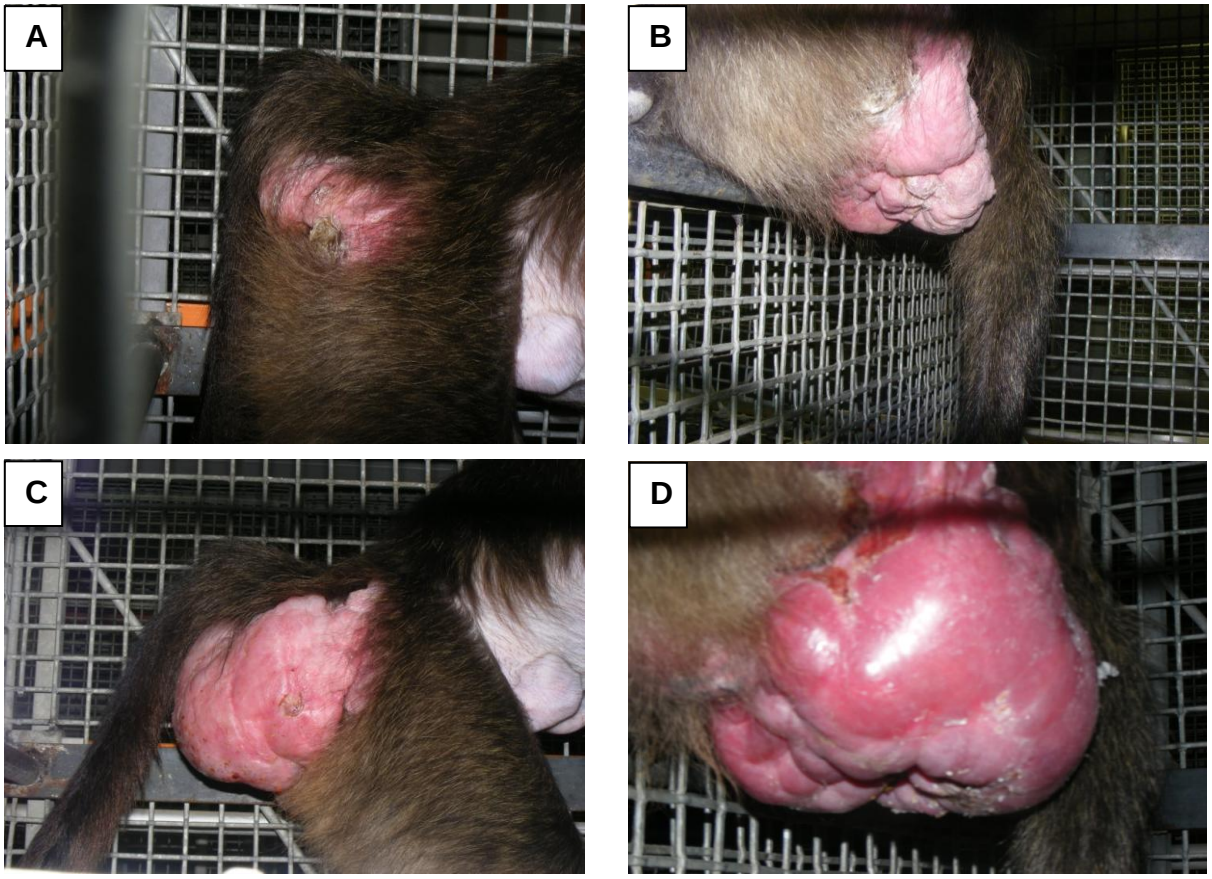


Figure 3.7 Digital photographs showing different stages of the anogenital swellings during the menstrual cycle. A = score 0, B = score 1-2, C = score 3, D = score 4. The anogenital swellings and colour changes over the menstrual cycle followed a similar pattern in all the study animals.

Key

A = complete deturgescence (score 0)

B = partial swelling (score 1 or 2)

C = near maximum swelling (score 3)

D = maximum swelling (score 4)

Table 3.2 Duration of different stages of anogenital swellings.

Baboon	Duration of different stages of anogenital swellings (days)		
	Quiescent	Swelling	Maximum swelling
A*	-	-	
B	8	6	10
C	9	12	10
D	10	5	12
Mean ± SD	9 ± 1	8 ± 4	11 ± 1

*The cycle from this baboon was an outlier (see Table 3.1) and was not used in the calculation.

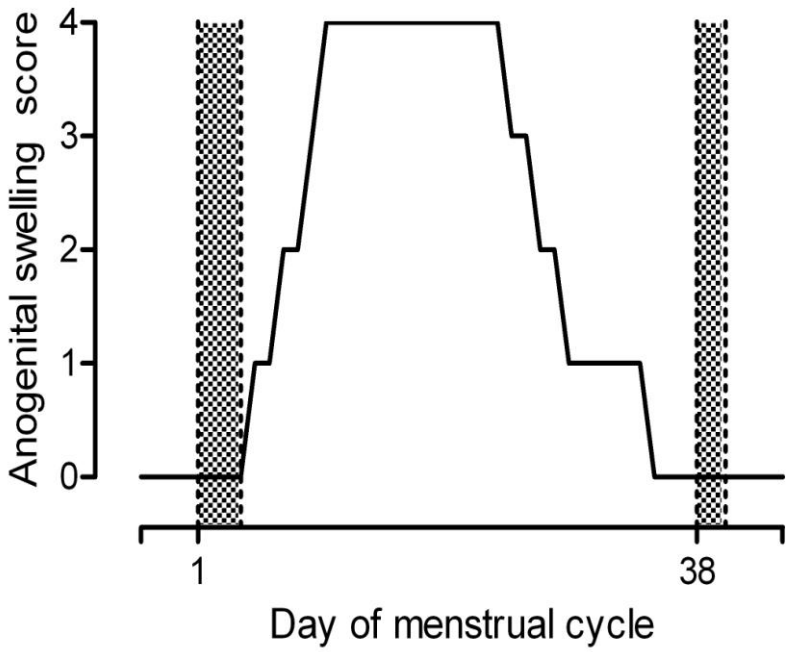


Figure 3.8 Anogenital swelling changes over one menstrual cycle in one of the study animals. The vertical lines represent the beginning and end of menstrual bleeding while the shaded areas represent duration of menstrual bleeding. Days 1 and 38 are the first days of menstrual bleeding in two successive menstrual cycles.

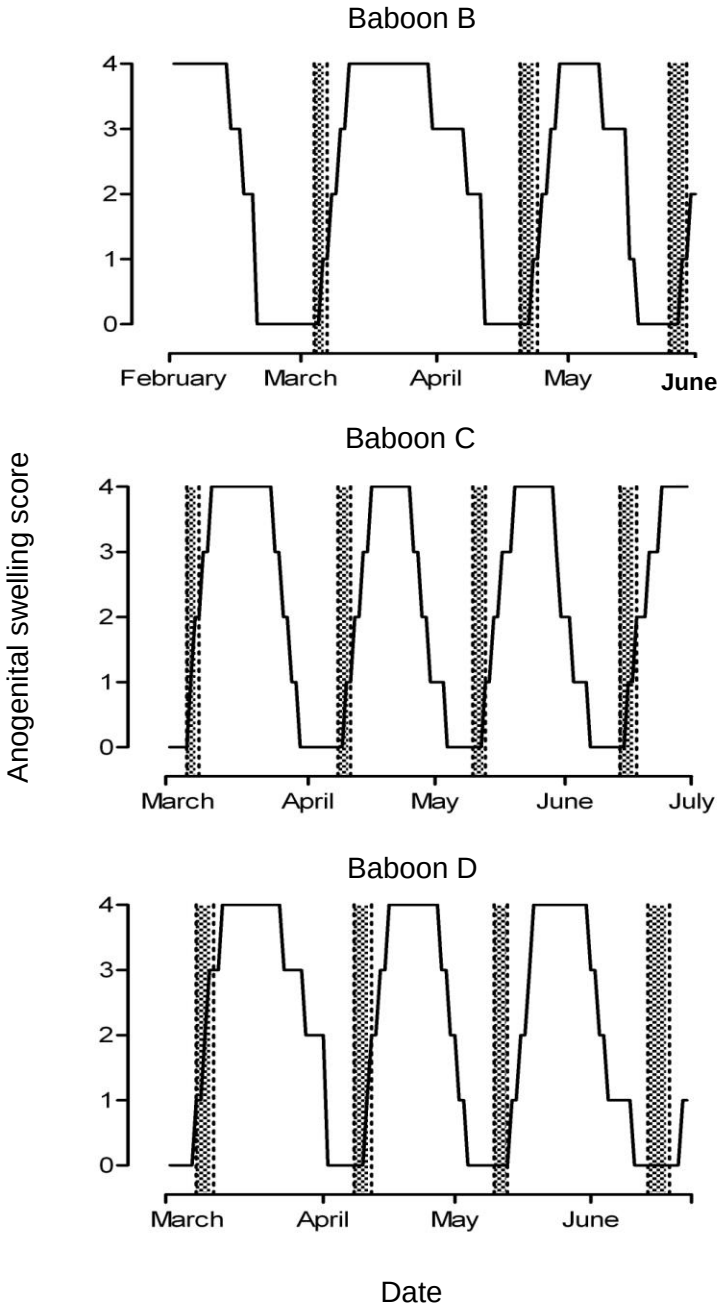


Figure 3.9 Cyclic anogenital swelling changes over menstrual cycles in three of the study animals as a function of time. The vertical lines marks the beginning and end of menses while shaded area represents the duration of menstrual bleeding.

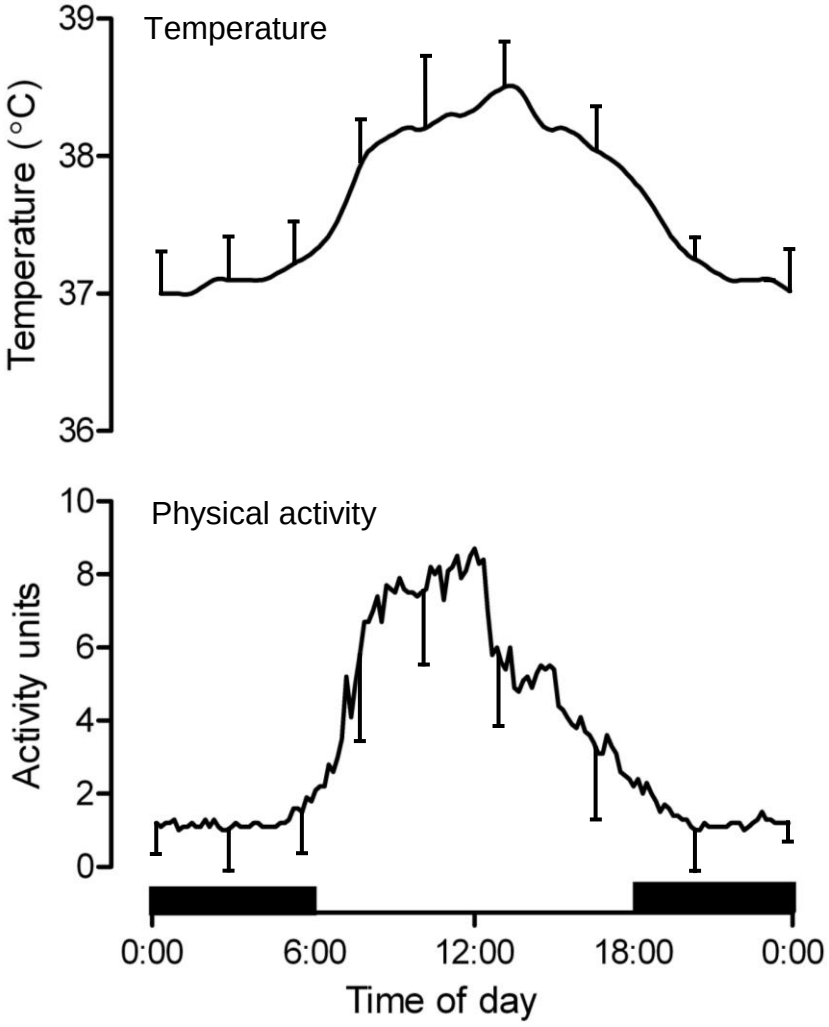


Figure 3.10 Nychthemeral rhythms of 10-min recordings of abdominal temperature (mean ± SD) and physical activity (mean ± SD), as a function of time from one baboon. The black bars on the time axis are showing the periods when the lights were switched off.

3.5 Abdominal temperature over the menstrual cycle

Mean 24-h abdominal temperature gradually decreased from that prevailing during menstruation to a minimum near the middle of the cycle (Figure 3.11). From the mid-cycle minimum, mean abdominal temperature increased and was maintained up to the end of the menstrual cycle. This pattern was common in all of the menstrual cycles, except for baboon A whose abdominal temperature data was too few to discern any pattern over the menstrual cycle as seen in Figure 3.12. Table 3.3 summarises the abdominal temperature data obtained from the 10-min recordings in all the animals. Mean 24-h abdominal temperature from the 10-min recordings during the luteal phase was significantly higher than during the ovulatory phase (ANOVA, $p = 0.04$; $F_{(2, 9)} = 4.7$; Figure 3.13). There were no significant differences however, between the mean 24-h abdominal temperature during the follicular and during the luteal phase.

Maxima and minima were calculated from the 24-h values. Maximum daily abdominal temperature followed the same pattern as mean daily abdominal temperature, with the luteal phase maximum abdominal temperature being significantly higher than that of the ovulatory phase (ANOVA, $p = 0.005$; $F_{(2, 9)} = 10.1$; Figure 3.13). I found no significant differences in mean 24-h minimum abdominal temperature in different phases of the menstrual cycles (ANOVA, $p = 0.2$; $F_{(2, 9)} = 1.7$), Figure 3.13. Mean 24-h amplitude in abdominal temperature did not vary with the phase of the menstrual cycle (ANOVA, $p = 0.2$; $F_{(2, 9)} = 1.7$).

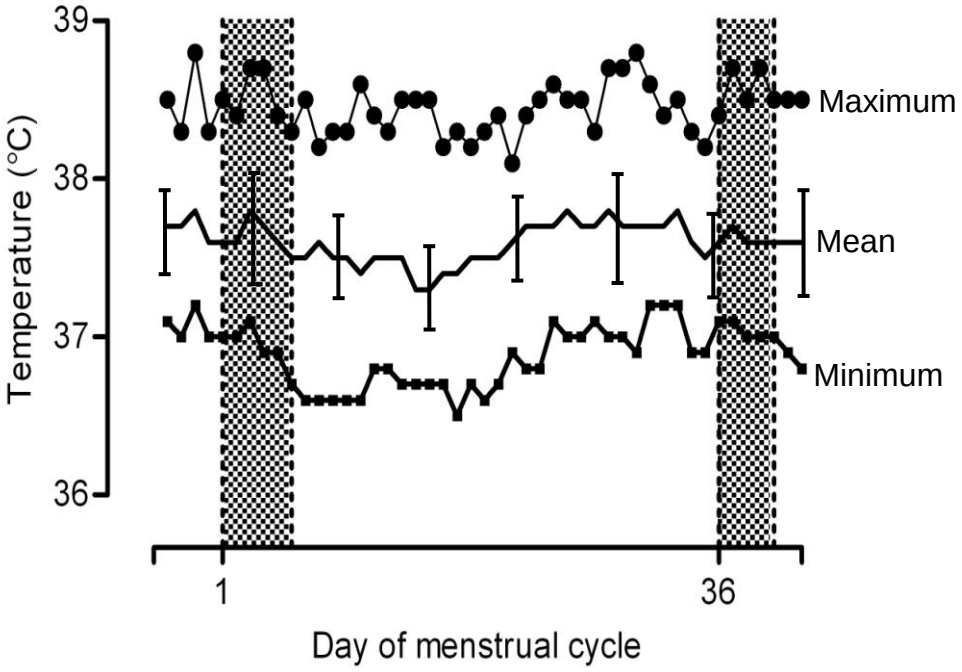


Figure 3.11 A typical example of mean ± SD, minimum and maximum 24-h abdominal temperature changes over one menstrual cycle from one of the animals. The mean ± SD was calculated from 144 10-min values within a 24-h period. Maxima and minima are the highest and lowest of individual 10-min values within each 24-h period. Days 1 and 36 are the first days of menstrual bleeding in two successive menstrual cycles.

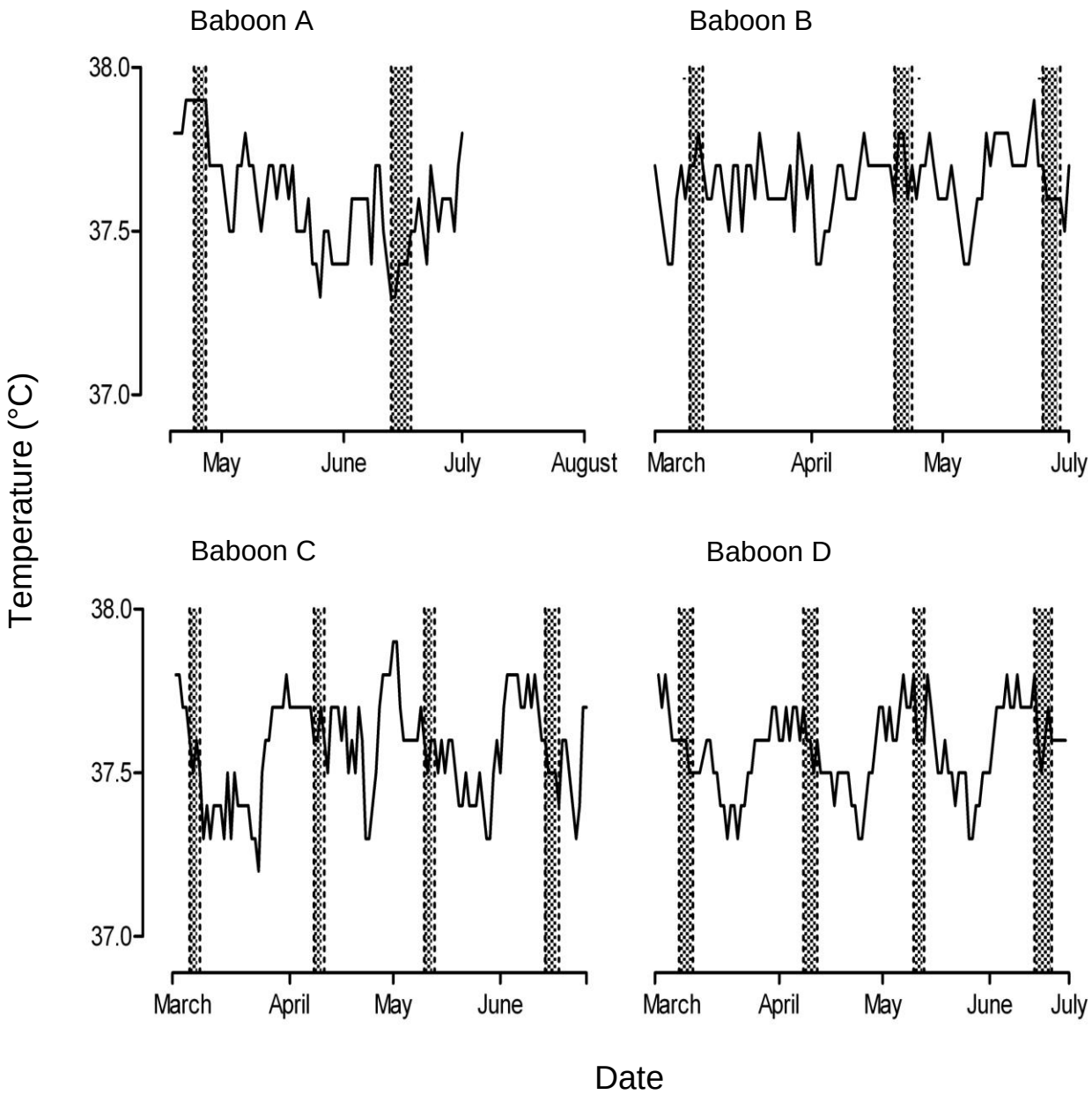


Figure 3.12 Mean 24-h abdominal temperatures calculated from the 10-min recordings over menstrual cycles in four baboons (standard deviations were omitted for clarity). The shaded areas between the vertical lines indicate duration of the menstrual bleeding period.

Table 3.3 Abdominal temperature data for all the animals.

Baboon	Body temperature (°C)			
	Maximum	Minimum	Mean	Amplitude
A	38.6	36.8	37.7	1.9
B	38.6	36.7	37.7	1.9
C	38.5	36.8	37.6	1.8
D	38.4	36.8	37.5	1.6
Mean ± SD	38.5 ± 0.1	36.8 ± 0.1	37.6 ± 0.1	1.8 ± 0.1

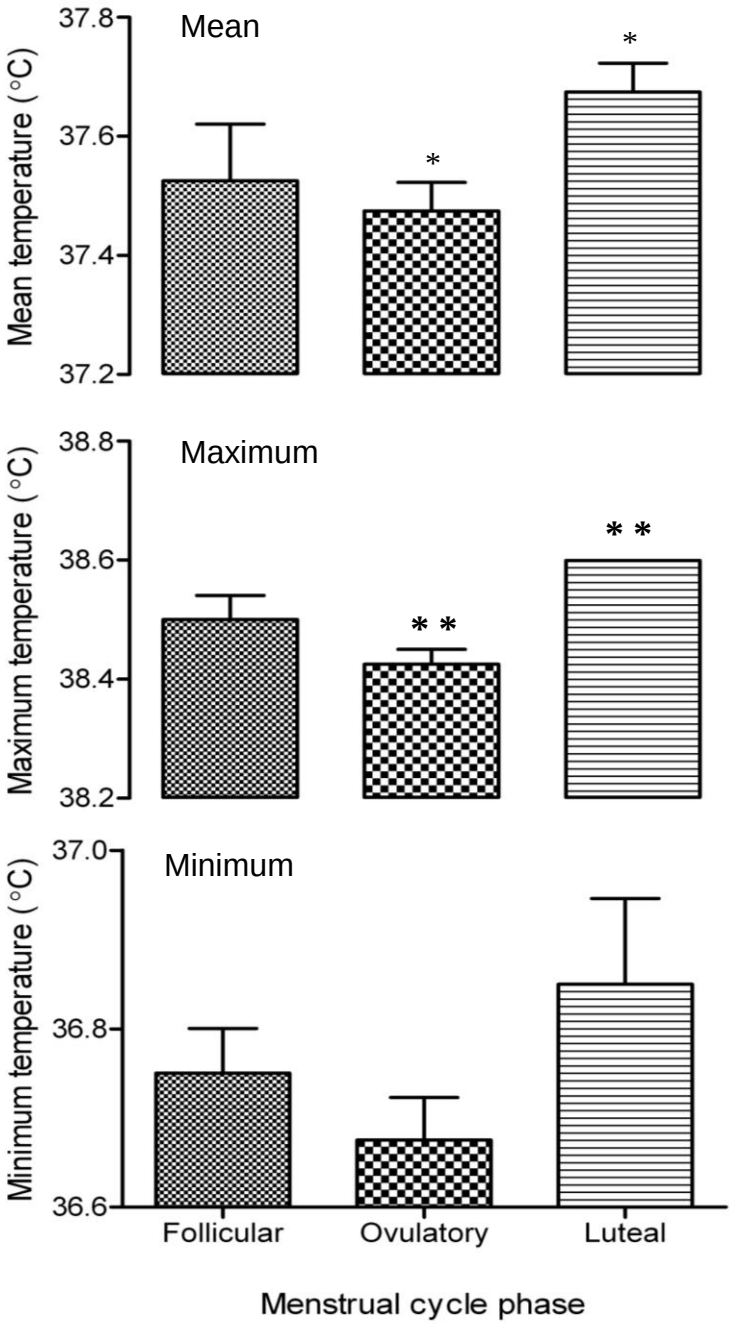


Figure 3.13 Mean ± SD, maximum ± SD and minimum ± SD 24-h abdominal temperature in different phases of the menstrual cycle. ANOVA, * p = 0.04, **p = 0.01, n = 3.

3.6 Physical activity over the menstrual cycle

Physical activity was high during the follicular phase and decreased rapidly as soon as menstruation ended to an approximately constant level maintained until after the middle of the cycle, before it increased at the end of the menstrual cycle (luteal phase) with some variability (Figure 3.14 and 3.15).

Mean 24-h physical activity was higher in the luteal ($p = 0.02$; $F_{(2, 12)} = 5.8$), compared to the ovulatory period (Figure 3.16). There were no significant differences between the mean 24-h physical activity during the follicular and the luteal phases ($p = 0.02$; $F_{(2, 12)} = 5.8$).

3.7 Correlation of abdominal temperature and physical activity with day of ovulation

I used the method which I described in section 2.8, (Figure 3.17) and found that the day of ovulation, estimated from mean 24-h abdominal temperature and physical activity changes, occurred between two to three days before the day of the 4-3 transition in anogenital swelling score. Table 3.4 summarises the days of ovulation obtained from abdominal temperature and physical activity data.

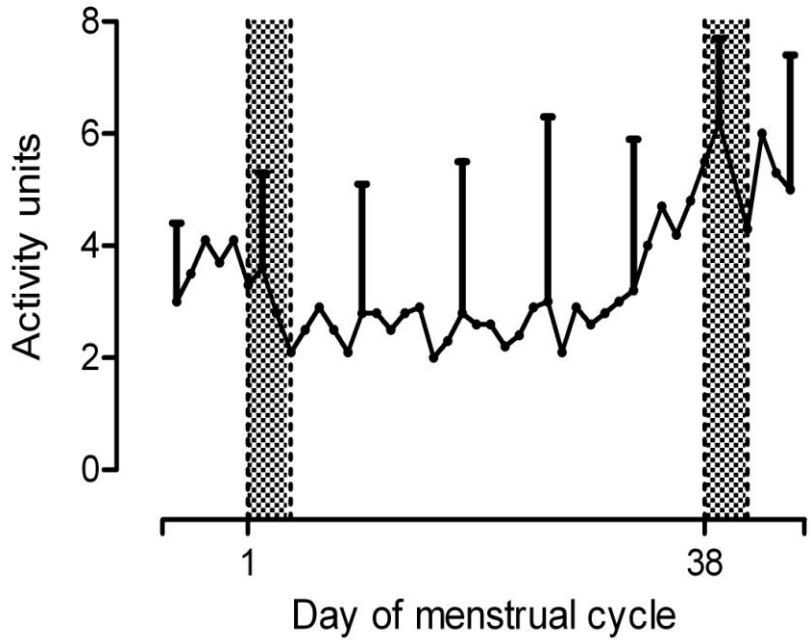


Figure 3.14 Typical mean ± SD 24-h physical activity changes over one menstrual cycle from one of the animal. The mean ± SD was calculated from 144 10-min values within a 24-h period. Days 1 and 38 are the first days of menstrual bleeding in two successive menstrual cycles.

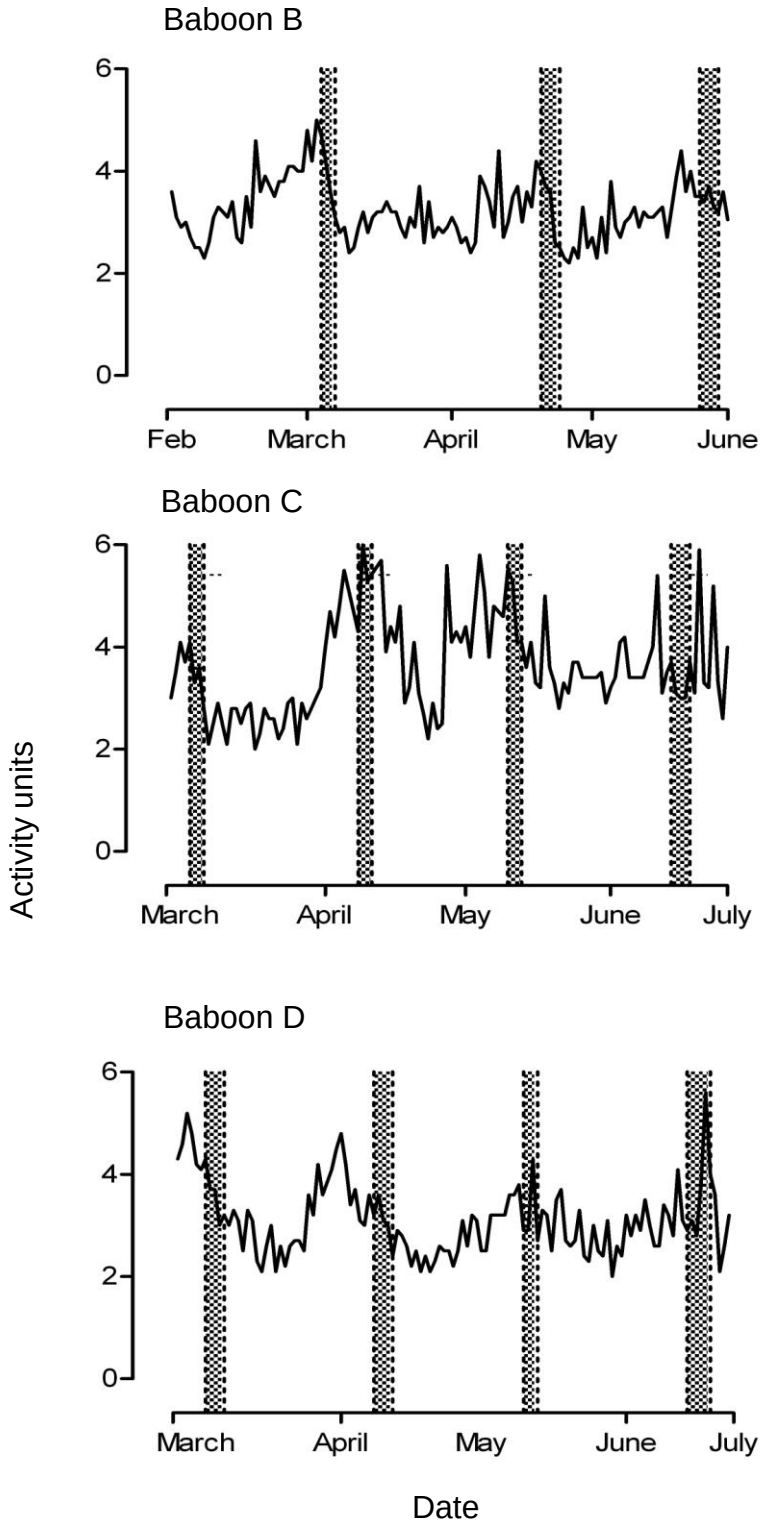


Figure 3.15 Mean 24-h physical activity over menstrual cycles in three baboons over a period of four months. Each data point is the mean of 144 10-min values (standard deviations omitted for clarity).

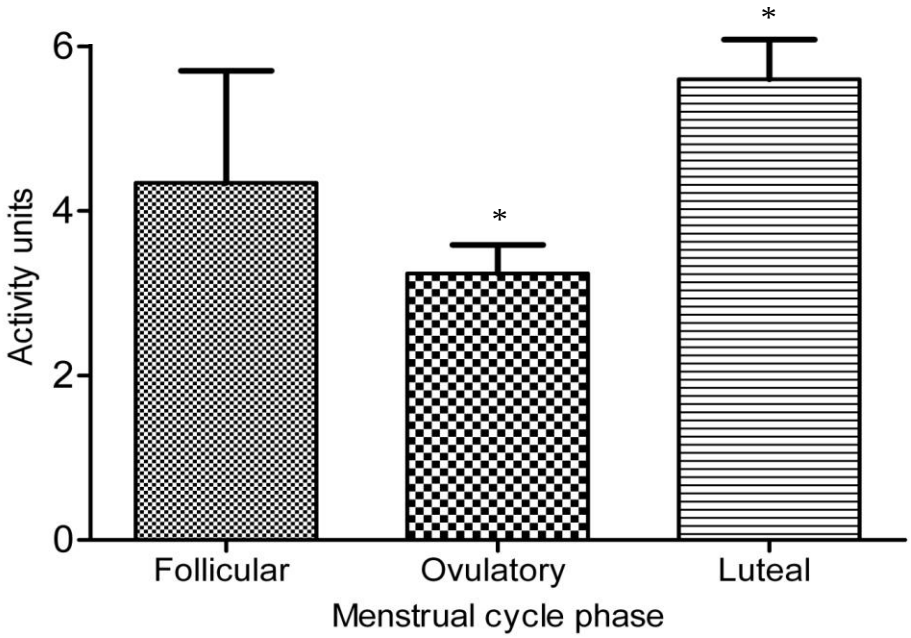


Figure 3.16 Mean ± SD 24-h physical activity in different phases of the menstrual cycle. There were significant differences between physical activity in the luteal and ovulatory phase and no significant difference between follicular phase and the luteal phase or the follicular phase and the ovulatory phase. ANOVA, * $p = 0.02$, $n = 3$.

Mean 24-h abdominal temperature and physical activity over the 20-day period, increased from a mid-cycle minimum before the ovulatory period, and increased in the luteal phase (Figure 3.17). There were no significant differences between days of ovulation that were estimated from abdominal temperature and those from physical activity data (Student's t-test, $p = 1$).

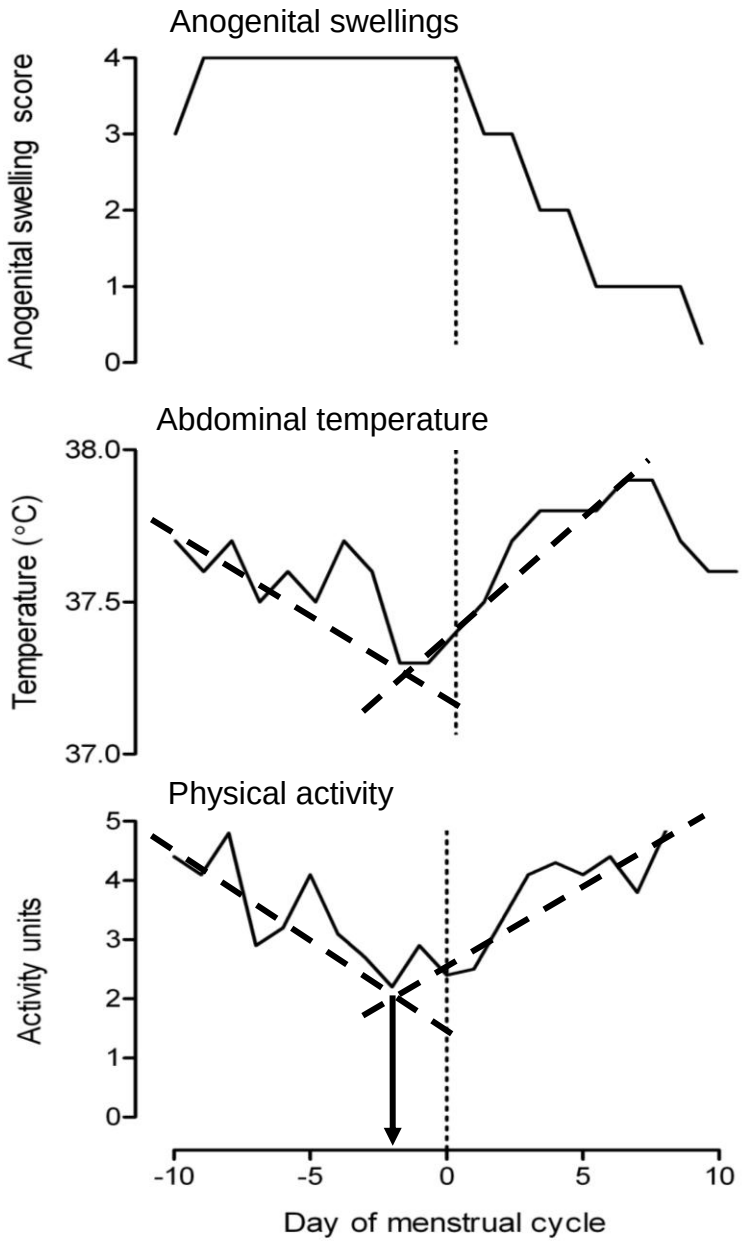


Figure 3.17 Estimation of the day of ovulation for one cycle in baboon C. Anogenital swelling changes, mean 24-h abdominal temperature and physical activity profiles over a 20-day period. The straight lines were fitted by eye between 5-10 days before, and 5-10 days after the day of the 4-3 transition in anogenital swelling score (day 0).

Table 3.4 Estimation of the day of ovulation from abdominal temperature and physical activity data as number of days prior to anogenital deturgescence.

Baboon	Abdominal temperature	Physical activity
A[*] [#]	-	-
B	3	3
C	3	2
D	2	2

*Menstrual cycle considered an outlier.

[#] Physical activity data not available due to activity data logger failure.

CHAPTER 4

DISCUSSION

I investigated and characterised the temporal relationship between the correlates of the menstrual cycle namely abdominal temperature, physical activity, anogenital swelling changes and ovarian steroid hormones. As far as I am aware, I have shown for the first time in unrestrained captive baboons, a menstrual cycle related pattern in physical activity, using activity data loggers. I have also confirmed that there are measurable events in abdominal temperature and physical activity patterns that occur 2 to 3 days before deturgescence of anogenital swellings which I believe are related to ovulation. Abdominal temperature, physical activity and ovarian steroid hormonal concentrations showed a cyclic change over the menstrual cycle.

I combined digital photography and observational data on external reproductive changes like menstrual bleeding and anogenital swelling changes, to confirm that baboons exhibit variable menstrual cycles between 33 to 51 days in length. There was variability in the length of the menstrual cycle within and between individual animals. Two of the menstrual cycles in two baboons after surgery were longer than the subsequent cycles and were regarded as outliers.

Baboons also exhibited variable cyclic patterns of anogenital swelling changes over the menstrual cycle. The patterns of anogenital swellings appeared to coincide with the patterns of urine and faecal progesterone and oestradiol concentrations. Maximum anogenital swellings were recorded in the follicular phase, while deturgescence of anogenital swellings was observed in the luteal

phase and corresponded with rising concentrations of progesterone. I used the decrease in anogenital swellings from a 4 to 3 score, which corresponded with declining levels of oestradiol, in the development of my proposed procedure for estimating the time of ovulation. In parallel with the changes in anogenital swellings, my baboons displayed a cyclic rhythm in mean 24-h abdominal temperature which was associated with the phase of the menstrual cycle. Mean 24-h abdominal temperature increased from a minimum during the ovulatory period and remained elevated in the luteal phase.

I used activity data loggers to detect and quantify the level of physical activity and have shown that physical activity follows a menstrual cycle related rhythm in captive baboons. Mean 24-h physical activity gradually increased from a minimum during the ovulatory period up to a maximum in the luteal phase. The cyclic pattern in physical activity over the menstrual cycle was similar to that of abdominal temperature. No one has ever measured physical activity using activity data loggers in unrestrained captive baboons, and as far as I can establish, I have obtained the first continuous measurements of 10-min values using an objective technique over the menstrual cycle.

I have also confirmed that captive female baboons exhibit a characteristic nychthemeral rhythm of abdominal temperature and shown for the first time, using non-observational means, a nychthemeral rhythm in 24-h physical activity as expected for a diurnal or nocturnal mammal. Abdominal temperature and

physical activity were higher during the light period compared to the dark period. The nychthemeral rhythm in abdominal temperature and physical activity varied between baboons and did not seem to be affected by the phase of the menstrual cycle.

In addition to the anogenital swelling changes, abdominal temperature and physical activity changes, I have confirmed that urine and faecal progesterone and oestradiol concentrations also show a cyclic change over the menstrual cycle. Oestradiol concentrations were higher in the follicular than in the ovulatory and luteal phases, while progesterone concentrations were higher in the luteal than in the follicular and ovulatory phases. The patterns of oestradiol and progesterone concentrations which I recorded in urine and faeces seem to confirm that ovulation occurs between 2 to 3 days before deturgescence of anogenital swellings. Urine and faecal cortisol concentrations did not show any menstrual cycle related changes.

The sample size of four animals used in this study may have decreased the statistical power of my analysis, a problem which could have been addressed by using more animals. The baboons were housed in an indoor environment where ambient environmental conditions like temperature, humidity and light intensity were kept constant. As a result, the contribution of the ambient environmental conditions to the behaviour of the baboons was not considered. There is supportive evidence from previous studies which suggests that social and

reproductive behaviour in captive animals differs from that of free-living animals under natural conditions (Knott, 2001).

Another important constraining factor in my study was the absence of male baboons in the vicinity of the study animals. The characteristics of the menstrual cycle of captive baboons might be different in the presence of a male, even without mating. In future it would be ideal to determine the correlates of the menstrual cycle in free-living animals under natural conditions in the presence of male baboons.

I experienced failure of one of the activity data loggers and could not retrieve physical activity data from the logger. The main disadvantage of data logging technology is that it is impossible to tell, without surgically explanting the loggers and downloading the temperature and physical activity data, whether the equipment is working properly.

The duration of the menstrual cycle in my study corroborates findings from previous studies in captive baboons. These studies showed that menstrual cycle length was 35 ± 3 days for the *Papio hamadryas anubis* (Koyama *et al*., 1977) and 33 ± 4 days for the *Papio hamadryas cynocephalus* species (Stevens, 1997) compared to 36 ± 2 days which I reported from my baboons. The length of the menstrual cycles of two of my baboons post-surgery were abnormally long presumably as a consequence of the interaction of glucocorticoids, produced in

response to the surgical procedure, with the hypothalamic-pituitary-gonadal axis resulting in irregular ovarian cyclicity (Loriaux and Nieman, 1990; Caperton *et al*., 2006).

As with studies done in baboons (Stevens, 1997), chimpanzees (Graham, 1981; Emery and Whitten, 2003) and macaques (Thierry *et al*., 1996), I have confirmed that the cyclic change of the anogenital swellings evident in free-living primates is preserved in captive baboons. The individual differences and variations in anogenital swelling patterns observed in my study are consistent with findings reported by Domb and Pagel (2001). This variability in anogenital swelling changes is probably due to the difference in response to oestradiol and progesterone by the tissue of the anogenital area in different animals (Whitten and Russell, 1996).

The nychthemeral rhythm in abdominal temperature which I reported in this study is similar to that which was previously reported in free-living (Brain and Mitchell, 1999) and captive baboons (Maloney *et al*., 2007; Mitchell *et al*., 2009) as well as in women (Baker and Driver, 2007) Figure 4.1. The menstrual cycle phase did not seem to have an effect on the amplitude of the nychthemeral rhythm of abdominal temperature and physical activity. Due to the high-amplitude in the nychthemeral rhythms, I recommend that in future, anyone using the temperature or physical activity data to study the menstrual cycle rhythms must gather 24-h

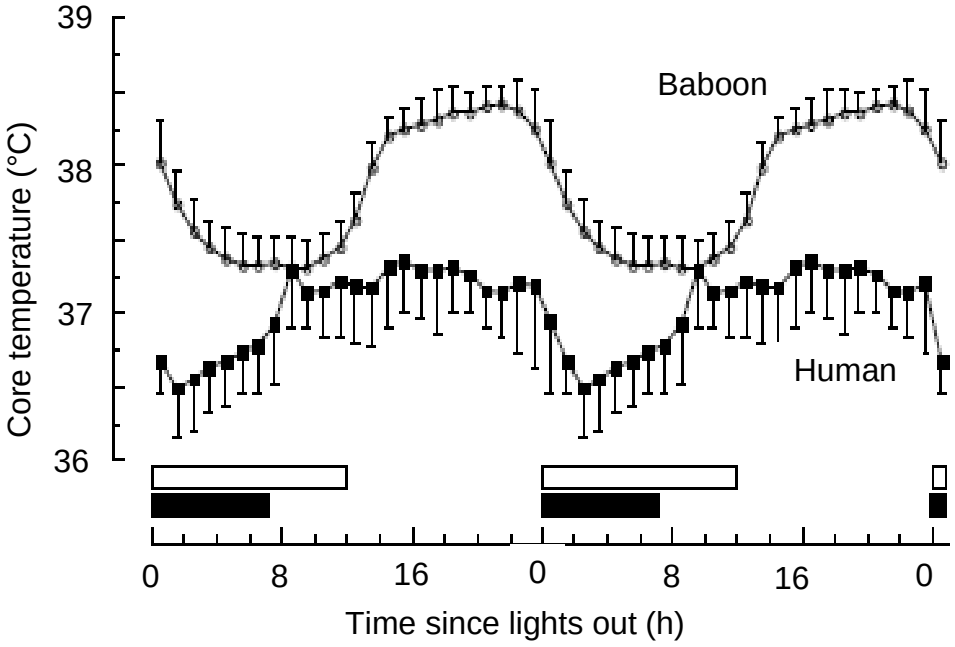


Figure 4.1 Double plots of the nychthemeral rhythm of core body temperature of baboons and a woman. Abdominal temperature (‘‘Baboon,’’ mean ± SD) of unrestrained baboons, averaged over each hour of the day, on the thirteen days when the baboons were exposed to a constant 22 °C environment. Open bars on the time axis indicate times of complete darkness in the climatic chamber. The temperatures of the adult female (‘‘Human,’’ mean ± SD) are rectal temperatures that were measured at 22°C at night. The Figure was adapted and modified from Mitchell *et al*., 2009.

data or do all the measurements of either body temperature or physical activity at the same time of the day.

The cyclic change in mean 24-h abdominal temperature over the menstrual cycle reported in this study is comparable and similar to that which was reported in a colony of captive baboons (Gillman, 1938), nocturnal rectal temperature in chimpanzees (Graham *et al*., 1977) Figure 1.4, and morning oral temperature in women, Figure 1.3 (Coyne *et al*., 2000; Baker *et al*., 2007). The abdominal temperature measurements from my baboons confirmed that there is a significant drop in the abdominal temperature around the ovulatory period which is followed by an increase in the luteal phase (Figure 4.2). The drop in oestradiol before ovulation and the increase in progesterone cause a sudden change in the ratio of progesterone and oestradiol resulting in the drop in body temperature before ovulation (Stephenson and Kolka, 1999).

Using the continuous measurements of abdominal temperature, I was successful in confirming that the day of ovulation is between 2 to 3 days before deturgescence of anogenital swellings. The change in body temperature during a normal menstrual cycle is well-established in humans and is frequently used diagnostically and therapeutically (Buxton and Atkinson, 1948). Time of ovulation in fertility studies of women also has been estimated from morning oral temperature changes (Buxton and Engle, 1950; Coyne *et al*., 2000). It has been shown that ovulation in women is preceded by a decrease in rectal temperature

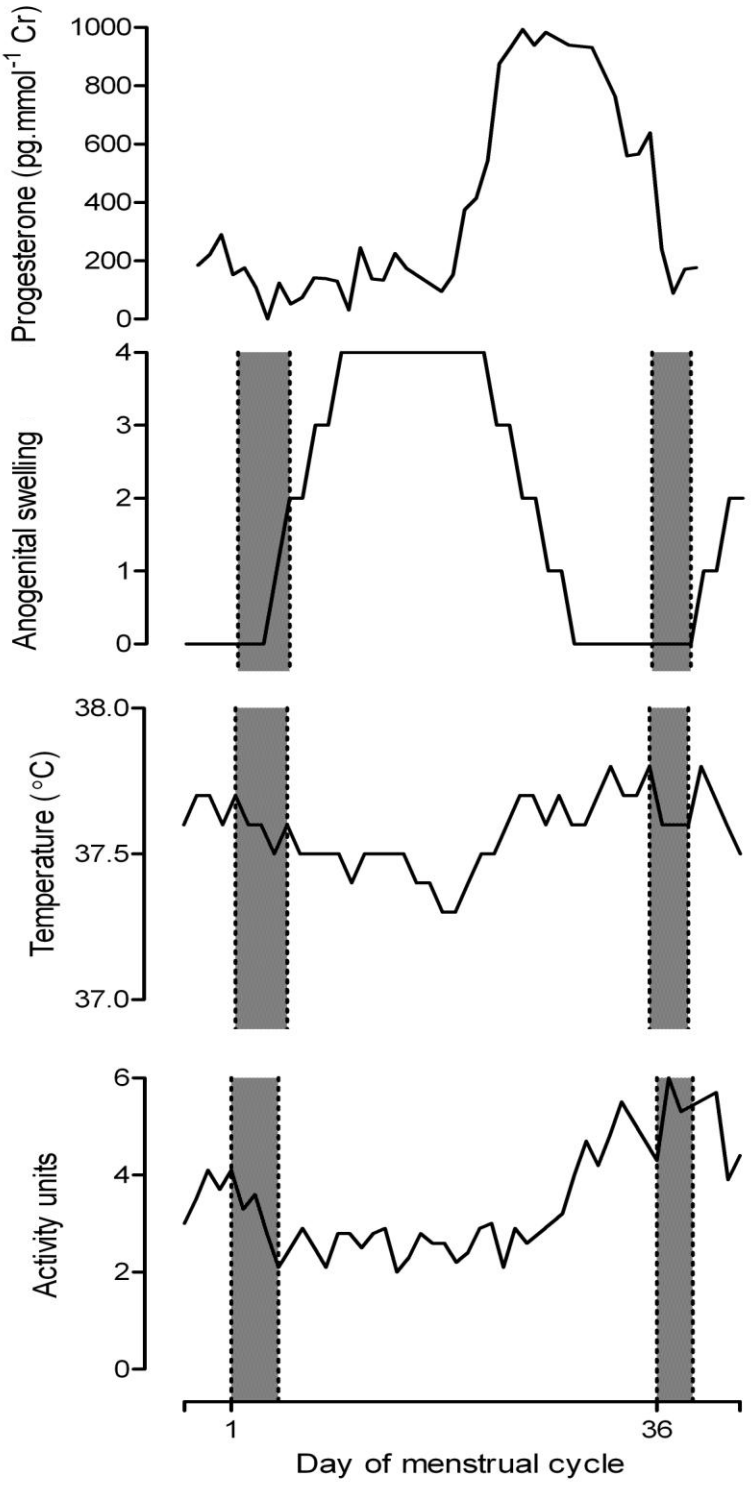


Figure 4.2 Progesterone, anogenital swellings, abdominal temperature and physical activity changes over one menstrual cycle in one of the study animals.

which increases within a day of ovulation and remains elevated in the luteal phase until the concentration of progesterone has decreased (Simpson and Halberg, 1974). Confirmation of menstrual cycle related abdominal temperature changes in my baboons will assist in future studies of the baboon menstrual cycle especially in the determination of the time of ovulation without having to measure hormonal concentrations in faecal and urine samples.

The increase in abdominal temperature in my animals, especially in the luteal phase can be ascribed to the thermogenic effect of progesterone and its metabolites, which are produced by the corpus luteum after ovulation (Barton and Wiesner, 1945; Israel and Schneller, 1950). It is believed that progesterone affects heat production by regulating the activity of numerous enzymes of oxidative metabolism and membrane protein carriers (Kalkhoff, 1982; Hamp *et al*., 2006). Because progesterone is produced after ovulation, a shift in body temperature can be used as a retrospective indicator of ovulation (Graham *et al*., 1977).

To confirm the occurrence of ovulation, I also measured the concentrations of progesterone and oestradiol over the menstrual cycle in urine and faecal samples. The recorded patterns of urine and faecal progesterone and oestradiol concentrations during the follicular and luteal phases in this study concur with those found in other baboon (Saayman, 1973; Nulsen and Peluso, 1992) and gorilla studies (Czekala *et al*., 1988). Except for the irregular peaks of oestradiol

in the luteal phase of cycle 3 for baboon 3 which may suggest that the corpus luteum did not become fully functional, the hormonal patterns that I recorded are also similar to the patterns that were reported in captive baboons (Stevens, 1997), Figure 1.1 and in chimpanzee (Emery and Whitten, 2003), Figure 1.2. Despite similar hormonal patterns, the concentrations of oestradiol, progesterone and cortisol recorded in my study were however lower than those from other studies. This is possibly due to the fact that I used radioimmunoassays and a different sub-species of baboons, while enzyme-immunoassays and different sub-species of baboons were used in other studies. In mammalian species, the standard and most accurate method of monitoring hormonal concentrations over the menstrual cycle would be to measure plasma LH, oestradiol and progesterone. But because of the problems associated with measuring plasma hormones, which include confining the animals to collect blood samples, urine samples are a good alternative. In the field it is much easier to collect faecal samples compared to urine samples and in some studies of non-human primates, steroid hormones measured in faecal samples have been used to monitor ovarian cyclicity (Wasser, 1996; Weingrill *et al*., 2004). The patterns of ovarian steroid hormones in urine and faecal samples from my baboons were similar, which confirms that the use of faecal samples in the wild will be as reliable as using urine samples in captive animals.

In addition to the measurement of ovarian steroids, it was also important to determine the effect of the menstrual cycle phases on the secretion of cortisol.

Determination of cortisol levels plays fundamental role in assessing the functional disturbances of the hypothalamic-pituitary-adrenal axis and stress levels in animals (Stewart, 1988; Caperton *et al*., 2006). Previous studies on rhesus and cynomolgus monkeys have shown that there is a possible interaction between hypothalamic-pituitary-adrenal axis oestradiol which would elicit the production of corticotropin-releasing hormone from the hypothalamus and cortisol from the adrenal gland (Xiao *et al*., 1998; Stavisky *et al*., 2001). My study however did not show any menstrual cycle changes in urine and faecal cortisol concentrations over the menstrual cycle. The absence of menstrual cycle related patterns in cortisol concentrations may be of relevance to researchers who use cortisol as an index of stress in baboons because they may not need to be concerned about the menstrual cycle effects on cortisol secretion.

The cyclic changes in physical activity over the menstrual cycle were similar to the abdominal temperature patterns. I used the changes of physical activity before and after the ovulatory period and also confirmed, as with abdominal temperature data, that ovulation occurs between 2 to 3 days before anogenital deturgescence from 4 to 3 score. The behaviour and physical activity of rhesus monkeys (Rauth-Wildmann *et al*., 1996) and chimpanzees (Matsumoto-Oda and Oda, 1998) is believed to be modified by oestradiol. It has been proposed that the level of physical activity increases in the follicular phase when oestradiol concentration is high (Matsumoto-Oda and Oda, 1998).

Physical activity in animals has always been measured using observational methods (Aujard *et al*., 1998). Recently omnidirectional activity data loggers, which were originally designed for human studies (Chantler *et al*., 2009), have been used in non-human primates (Hunnell *et al*., 2007; Papailiou *et al*., 2008) to measure physical activity over the menstrual cycle. In rhesus monkeys, activity data loggers have been used to show that no menstrual related patterns occur (Hunnell *et al*., 2007). Chantler *et al*., (2009) used activity data loggers to show that there were no significant differences in physical activity between two groups of women when they were not menstruating. However during menstruation, physical activity was significantly reduced in the women with a history of dysmenorrhoea, compared to the women without a history of dysmenorrhoea (Figure 4.2). Women without dysmenorrhoea had no patterns in physical activity over the menstrual cycle.

I would have hypothesised that physical activity in female baboons over the menstrual cycle would not show any menstrual cycle related pattern as in women without a history of dysmenorrhoea and rhesus monkeys (Hunnell *et al*., 2007). My findings however contradict the previous findings from rhesus monkeys, obtained using activity data loggers, which did not reveal any pattern in physical activity related to the menstrual cycle (Hunnell *et al*., 2007), and seem to support the idea that physical activity is influenced by oestradiol.

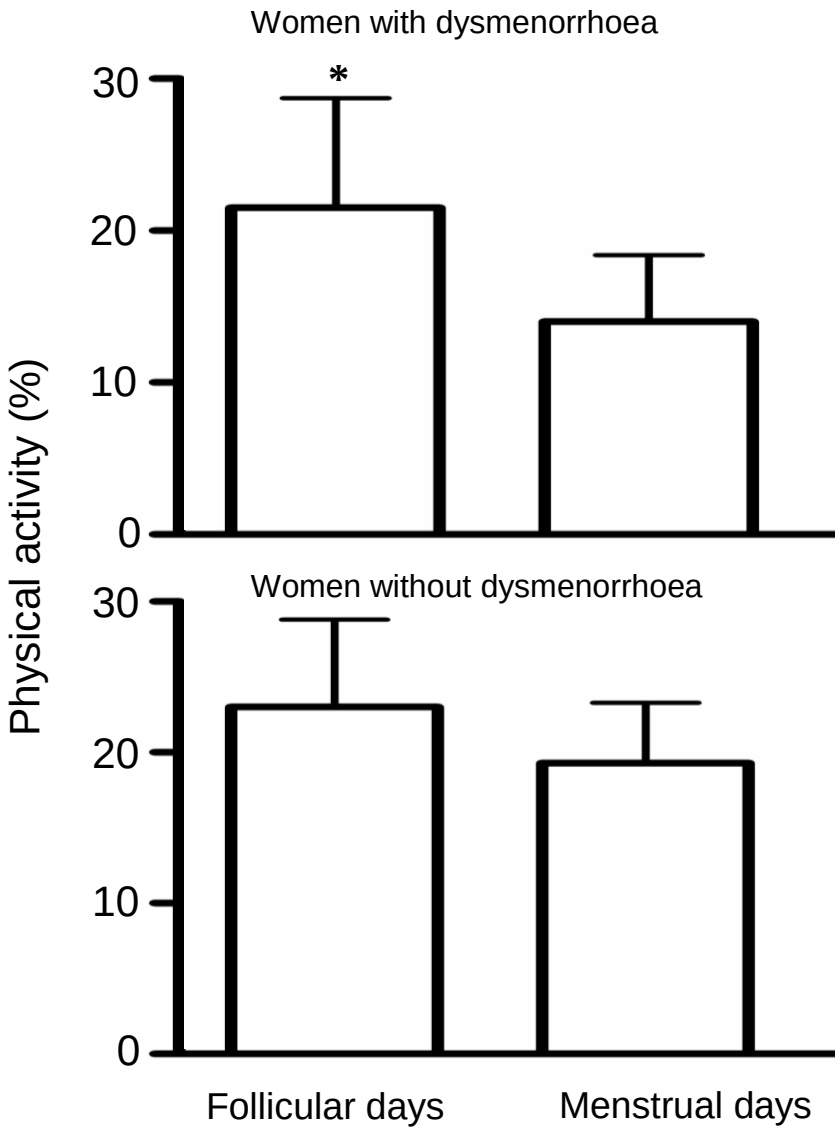


Figure 4.3 The physical activity (percentage of maximum recorded activity for each woman, mean ± SD) of the women with a history of dysmenorrhoea and women without a history of dysmenorrhoea when not menstruating and during menstruation. * $p < 0.001$ compared to menstrual days. Figure adapted and modified from Chantler *et al*., 2009.

CHAPTER 5

CONCLUSIONS

Primatologists need to determine paternity of primate offspring in order to assess social relationships and analyse pedigree reconstruction in captive and wild baboon troops. The presence of many reproductively-active males, especially in a wild troop of baboons, and the promiscuous behaviour of female baboons makes it difficult for primatologists to assign paternity to baboon offspring. The current method of determining paternity in baboons involves capturing the baboon offspring and their putative fathers for DNA analysis. If the day of ovulation for a particular female is known and behavioural observations can confirm the male baboon consorting with that female at the time of ovulation, then identifying the father of the offspring may be possible without capturing the baboons for genetic analysis. It would therefore be valuable for primatologists to remotely determine ovulation by assessing variables which are relatively easy to measure and correlate with reproductive hormones. Abdominal temperature and physical activity provide such variables.

In my study, I have demonstrated the feasibility of using implantable thermometric and activity data loggers (bio-logging) to measure abdominal temperature and physical activity over the menstrual cycle of unrestrained captive chacma baboons. Using measurements of abdominal temperature and physical activity, I was successful in confirming that ovulation occurs between 2 to 3 days before the 4 to 3 anogenital deturgescence, and that there were detectable changes at that time in ovarian steroid hormonal concentrations. As far as I can gather from the literature, no one has ever shown a menstrual cycle

related cyclic rhythm in physical activity using activity data loggers in any non-human primate species. Based on the association between abdominal temperature, physical activity and ovarian steroid hormonal profiles from my study, I recommend that it is possible to use the 4 to 3 deturgescence in anogenital swellings, whenever it is possible to observe the anogenital swellings without stressing the animals, to predict the ovulatory period in captive and wild troops of baboons.

The use of observations of anogenital swelling changes over the menstrual cycle to estimate the time of ovulation for baboons in the field is possible, but requires the close-up presence of human observers. The presence of human observers is not a problem for habituated troops of baboons which are accustomed to the presence of humans. However in unhabituated troops, close-up observation of anogenital swelling changes remains particularly challenging as the baboons are not accustomed to human presence, therefore may not behave naturally and may run away from the observers. Human presence in wild unhabituated troops may also cause stress to the animals, which may affect the cyclicity and duration of the menstrual cycle (Caperton *et al*., 2006).

If the observation of changes in anogenital swellings is not possible, the patterns of abdominal temperature and physical activity are also useful in detecting ovulation. Although the measurement of physical activity and abdominal temperature requires surgical implantation of data loggers, it subsequently would

provide a method to estimate ovulation in free-living undisturbed baboons. The main advantage of using bio-logging to measure abdominal temperature and physical activity is the reduced human presence which is known to have an effect on the behaviour of the unhabituated animals. I believe that I have shown that retrospective analysis of measurements of physical activity and abdominal temperature, made possible by bio-logging technology, can be used to estimate the time of ovulation in wild baboon troops, without requiring the measurement of ovarian steroid hormones in faeces or urine and the presence of human observers to monitor anogenital swelling changes. In order to determine the male baboon consorting with a particular female baboon at the time of ovulation without close-up observations, the technology already exists, which makes use of implantable tags that sends signals to remotely detect the proximity of animals to each other. This method of estimating the time of ovulation and determining paternity may not be fail safe since there is possible sperm competition, especially in a troop with promiscuous females and several potential sires.

The thermal consequences of ovulation are well-known for ovulating mammals and I have demonstrated that building baboon abdominal temperature and physical activity profiles and understanding how they relate to the menstrual cycle can be a very important research tool for developing a “thermal fingerprint” associated with ovulation, which may also be used in other non-human primate species. Bio-logging of physical activity and abdominal temperature also provides a platform for developing wireless radio-link techniques and novel computerised

devices, which can be used to download this type of data remotely in baboons and other non-human primate species. Remote temperature data logging using temperature data loggers that are worn under the arm in women in fertility studies (O'Carroll and Smith, 2009) provides a good example of how ovulation is already being detected using bio-logging. Bio-logging also provides avenues for developing advanced techniques for monitoring other physiological variables like heart rate and blood pressure, associated with the menstrual cycle.

Although measurement of physical activity and abdominal temperature are useful in my study, relationships may be different in wild primates because behavioural mechanisms of thermoregulation are limited in animals that are housed in cages, even if they are unrestrained (Mitchell *et al*., 2009). The ambient environment in laboratories also is constant compared to natural environments where the animals are exposed to fluctuating climatic conditions. In future, studies should focus on determining correlates of the menstrual cycle using bio-logging in free-ranging, wild troops of baboons in their natural environment where fluctuating climatic conditions may have influences on the menstrual cycle.

When I determined the correlates of the menstrual cycle there were no male baboons near the study animals. The presence of male baboons may affect the characteristics of the menstrual cycle (Seyfarth, 1978; Girolami and Bielert, 1987) and sexual behaviour of female baboons. It therefore also would be important for

future studies to be done with male baboons within the vicinity of the study animals.

I believe that my study on the correlates of the menstrual cycle in captive baboons, made possible by the availability of bio-logging technology, has contributed towards the current knowledge of reproductive and thermal physiology of one of Africa's most successful diurnal primates.

My finding that captive baboons exhibit cyclic patterns in abdominal temperature and physical activity over the menstrual cycle and that such changes correlate with ovulation, is important for primatologists, as the day of ovulation no longer needs to be estimated using the measurement of ovarian steroid hormones. Remote estimation of the time of ovulation using bio-logging of temperature and physical activity can also be an invaluable tool for primatologists in estimating the day of ovulation and determining paternity of baboon offspring without having to capture them and their potential fathers for DNA analysis and fingerprinting.

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