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Ovarian function and reproductive behaviors across the female orangutan life cycle

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OVARIAN FUNCTION AND REPRODUCTIVE BEHAVIORS ACROSS THE FEMALE ORANGUTAN LIFE CYCLE

by

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(Order No.)

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ABSTRACT

Due to their phylogenetic position as an outgroup to humans and the other African apes, empirical data from orangutans are an especially valuable comparative tool with which questions about the evolution of human life history and reproductive characteristics can be addressed. Yet few such data are available. In this dissertation, I use endocrinological and behavioral data from 7 female and 3 male orangutans housed at the Woodland Park Zoo in Washington and the Great Ape Trust in Iowa to characterize the ovarian function and reproductive behaviors of captive female orangutans at different points in the life cycle. Ovarian hormone measurements were achieved through the use of non-invasive urine sampling, and assays reveal both intra- and inter-individual variation in hormone production. Results indicate that (1) adolescent females in captivity do not experience a marked period of subfecund estrogen and progesterone levels in association with reproductive maturation, (2) individual females exhibit both "high quality" and "low quality" cycles, including instances of anovulation, in the absence of fluctuating dietary and environmental conditions, (3) mating behaviors vary between individuals and with cycle phase, but are not strongly influenced by absolute ovarian hormone concentrations, and (4) reproductive senescence does not significantly impact the ovarian function and mating behaviors of aging female orangutans. These results demonstrate that many aspects of human reproductive biology and behavior, such as an extended period of mating receptivity, are evolutionarily conserved. They suggest, however, that the mid-life decline in human ovarian function may be derived and of possible adaptive significance. The potential significance of differences between captive and wild ape populations, and the character, history, and familial relationships of the particular individuals discussed are considered in the interpretation of all data.

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Cr:	Creatinine
CRF:	Corticotropin-releasing factor
E_1C:	Estrone conjugates
EIA:	Enzyme immunoassay
EPC:	Extra-pair copulation
FSH:	Follicle-stimulating hormone
GnRH:	Gonadotropin-releasing hormone
HPA:	Hypothalamic-pituitary-adrenal
HPO:	Hypothalamic-pituitary-ovarian
IBI:	Interbirth interval
LH:	Luteinizing hormone
PdG:	Pregnanediol-3-glucuronide
POP:	Periovulatory period
WPZ:	Woodland Park Zoo

Chapter 1: Introduction and Background

Orangutans are highly sexually dimorphic, and have the longest average interbirth interval not only of any primate, but of any mammal (Galdikas and Wood 1990; van Noordwijk and van Schaik 2005; Knott and Kahlenberg 2011). Together, these facts have inspired most of the theoretical questions that drive research on orangutan mating behaviors, reproduction, and life history. The resulting data have increased our understanding of how habitat and dietary quality affect the fecundity of female orangutans (Knott 1998), how adult male orangutans differ in development, morphology, and the use of coercive mating behaviors (Maggioncalda et al. 1999; Utami et al. 2002; Emery Thompson et al. 2012), and how females' proceptive and receptive mating behaviors change across different phases of the ovulatory cycle (Maple et al.1979; Knott et al. 2010). Little is known, however, about changes in ovarian function across the life cycle, intra- and inter-individual variation in reproductive physiology, or the endocrinological factors underlying females' motivation to mate.

In this dissertation, I examine the reproductive hormones and behaviors of individual captive female orangutans to produce a more fine-grained picture of orangutan reproductive and behavioral endocrinology. Because orangutans serve as a phylogenetic outgroup to the African apes and humans, these data in turn allow me to consider how physiological and behavioral similarities and differences between orangutans and humans can shed light on the evolutionary history of several significant features of human life history and reproduction. I focus particularly on the adaptive importance of concealed ovulation and extended mating periods, characteristics that are not unique to humans (Hrdy and Whitten 1987; Andelman 1987; Pawlowski 1999; Fürtbauer et al. 2011) but are nonetheless frequently identified as selective pressures that favored pair-bonds and paternal investment in *H. sapiens* (Alexander and Noonan 1979; Strassman 1981), and on the presence or absence of a post-reproductive life history phase, which some hypothesize has been selected for in the human lineage because of its beneficial effects on inclusive fitness (Hawkes et al. 1997; Sear et al. 2000; Hawkes 2003; Lahdenperä et al. 2004).

This chapter reviews the present state of knowledge about orangutan life history parameters and reproductive physiology and behaviors, with particular attention paid to differences between free-ranging and captive animals. I also outline the current understanding of female reproductive hormone functionality throughout the life cycle, and review the theory and evidence pertaining to a direct link between ovarian hormone levels and mating behaviors in humans and non-human primates. To conclude, I argue that the dissertation results presented here not only provide new information about female orangutan physiology and behavior, but also broaden our understanding of how and why details of human life history and reproduction differ from those of the other apes.

Orangutan Socioecology and Reproduction

Genetic studies have revealed that there are two species of orangutan, *Pongo pygmaeus* and *Pongo abelii*, found exclusively on the islands of Borneo and Sumatra, respectively (Janczewski et al. 1990; Markham 1995; Zhi et al. 1996; Xu and Arnason 1996; Warren et al. 2001; Atmoko and van Schaik 2010). The least social of the great apes, orangutans are frequently described as “solitary” or “semi-solitary” (Nadler et al. 1986; van Noordwijk and van Schaik 2005; Atmoko and van Schaik 2010), although association patterns within and between islands vary to some degree (van Schaik 1999; van Schaik et al. 2009). In general, Bornean orangutans are less gregarious and are found at lower population densities than Sumatran orangutans, who are more likely to form traveling parties (e.g. several females and immature individuals) (van Schaik 1999; van Schaik et al. 2009; Atmoko and van Schaik 2010).

Differences in sociability likely relate to food availability on each island, and at different sites within each island. Orangutans are large-bodied and highly frugivorous (Wich et al. 2006), but live in a habitat in which fruit availability fluctuates substantially due to “mast fruiting” events, irregularly spaced periods of time during which almost 90% of tree species may simultaneously bear fruit after years of inactivity (Knott 1998). Although this phenomenon occurs on both islands, studies show that Sumatra is characterized by richer soils and higher plant productivity, and has a higher density of non-masting fruit trees (e.g. strangling figs) (Marshall et al. 2009; van Schaik et al. 2009; Atmoko and van

Schaik 2010). Sumatran orangutans therefore face relatively fewer months of low fruit availability and dependence on fallback foods and relatively fewer ecological constraints on sociability than their Bornean counterparts. On Borneo, social encounters may occur during times of fruit abundance (Sugardjito et al. 1987; Mitani et al. 1991) or if multiple animals are attracted to a single fruiting tree, but more frequently orangutans actively avoid conspecifics due to high levels of scramble and contest competition (Knott et al. 2008).

Outside of food-related aggregations, social interactions among orangutans primarily occur in the context of mating or intrasexual competition (Schürmann 1982; Atmoko and van Schaik 2010). Adult male orangutans exist in two different morphs: large males who display secondary sexual characteristics including cheek flanges and long calls, and unflanged males who are sexually mature but whose appearance does not differ appreciably from that of adult females (Utami et al. 2002; Knott and Kahlenberg 2011). Both are able to reproduce, but they differ in habitat use and mating strategy (Harrison and Chivers 2007; Atmoko and van Schaik 2010). Flanged adult males traverse large areas that encompass the ranges of several adult females. They are intolerant of other flanged males (Galdikas 1981; Atmoko and van Schaik 2010), spend the majority of their time alone, and most often mate by consorting with sexually mature females over a period of several days or weeks (Galdikas 1981; Nadler 1988; Atmoko and van Schaik 2010; but see Knott et al. 2010). Mature unflanged males, meanwhile, roam opportunistically throughout the ranges of their conspecifics (van Schaik 2004), and frequently achieve mating success through the use of forced copulations (Galdikas 1981; Atmoko et al. 2009; Knott and Kahlenberg 2011). Female mating strategies also play an important role in the occurrence and duration of encounters with both types of male (Fox 2002; Stumpf et al. 2008; Knott et al. 2010), as I will discuss further later in this chapter and throughout this dissertation.

In captivity, conditions necessitate more frequent contact between female orangutans, as well as between males and females. Though captive populations are usually small, spatial constraints limit avoidance behaviors and opportunities for solitude. Studies of orangutan sociality in captivity, however, suggest a capacity for behavioral plasticity that results in tolerant or prosocial dyadic/group relationships. Although captive orangutans may spend much of their time engaged in non-social activities (Classen 2011), they exhibit a high level of sociability relative to free-ranging individuals (Edwards 1982; Poole 2005; Classen 2011). No doubt this is due partially to the fact that captive orangutans are fully provisioned and experience little feeding competition. However, some of the variation underlying orangutan sociability may have a genetic basis, as suggested by recent data showing that captive orangutans of Bornean descent exhibit a stronger glucocorticoid (i.e. stress) response to increasing group size than those of Sumatran descent (Weingrill et al. 2011).

Female Orangutan Life History

Orangutans are characterized by an exceptionally slow life history pattern, with interbirth intervals (IBIs) that exceed any other extant ape (Galdikas and Wood 1990; Knott 2001; Knott et al. 2009). One consequence of this is that although they are long-lived, female orangutans produce few offspring during their lifespan, and long-term demographic data are difficult to collect. Conclusions about female life history parameters therefore rely on relatively few empirical data, and questions remain about the significance of differences between Bornean and Sumatran orangutans, and between females in the wild and in captivity (Anderson et al. 2008).

Female reproductive maturation begins with menarche, which researchers estimate occurs between the ages of 9 and 12 among wild orangutans (Markham 1995; Knott 2001; Cocks 2007; Shumaker et al. 2008). Age at first parturition is easier to document, and is estimated to occur at approximately 15-16 years among females on both Borneo and Sumatra (Tilson et al. 1993; Wich et al. 2004). Offspring typically nurse for 5-7 years, and associate closely with their mother for an additional 2-4 years before becoming wholly independent (Galdikas and Wood 1990; Markham 1995; van Noordwijk and van Schaik 2005; van Adrichem et al. 2006). This period of offspring dependence coincides with the timing of interbirth intervals, which range from a median of 7.7 years on Borneo (Galdikas and Wood 1990) to between 8.2 and 9.3 years on Sumatra (Knott 2001; Wich et al. 2004; van Noordwijk and van Schaik 2005). Orangutan longevity has proven difficult to assess, but male and female lifespans up to 60 years have been estimated (Markham 1995; Wich et al. 2004).

In captivity, reproductive maturation often happens at an accelerated pace, likely due to increased nutritional stability (Anderson et al. 2008). Like other mammals, the way in which orangutans allocate energy to biological processes differs throughout the life cycle: the substantial energetic investment in growth that occurs during the infant and juvenile periods ceases when physical maturation is achieved, and energetic investment is subsequently channeled into reproduction (Charnov and Berrigan 1993; Bentley 1999; Jones 2011). Because provisioning and good health in captivity enable young female orangutans to allocate abundant energy to growth, they reach adult size and undergo associated physiological transitions to reproductive maturity at a younger age than free-ranging animals (Knott 2001).

Unfortunately, as is the case with data from wild females, there are surprisingly few detailed accounts of puberty in individual captive female orangutans. The scant zoo data that have been published about menarche suggest that it occurs at approximately 7-8 years of age (Markham 1995; Knott 2001). Once again, records of first parturition are more thorough, but studies report different ages at first birth depending on the sample considered. While Shumaker *et al*. (2008) note that females can give birth as early as 7 years of age, Markham (1995) reports that data from the International Orangutan Studbook suggest a modal age at first birth of 9-10 years among captive females.

Anderson *et al*. (2008), however, also examined Studbook data, and separately analyzed records from genetically distinct Bornean and Sumatran individuals (individuals classified as genetic hybrids were excluded from their analyses). They calculated average ages at first birth of 15.5 and 16.4 years for Bornean and Sumatran females, respectively, results which mirror the parameters of free-ranging populations (see above). Despite this discrepancy, all studies on captive animals report interbirth intervals consistently shorter than 6 years (Markham 1995; Cocks 2007; Shumaker et al. 2008; Anderson et al. 2008), showing that reproduction occurs at a faster pace in captivity even if it does not start earlier. Reproduction by a captive female orangutan has not been documented past the age of 41 (Shumaker et al. 2008), but several captive orangutans have survived almost until the age of 60 (Leighton et al. 1995; Shumaker et al. 2008).

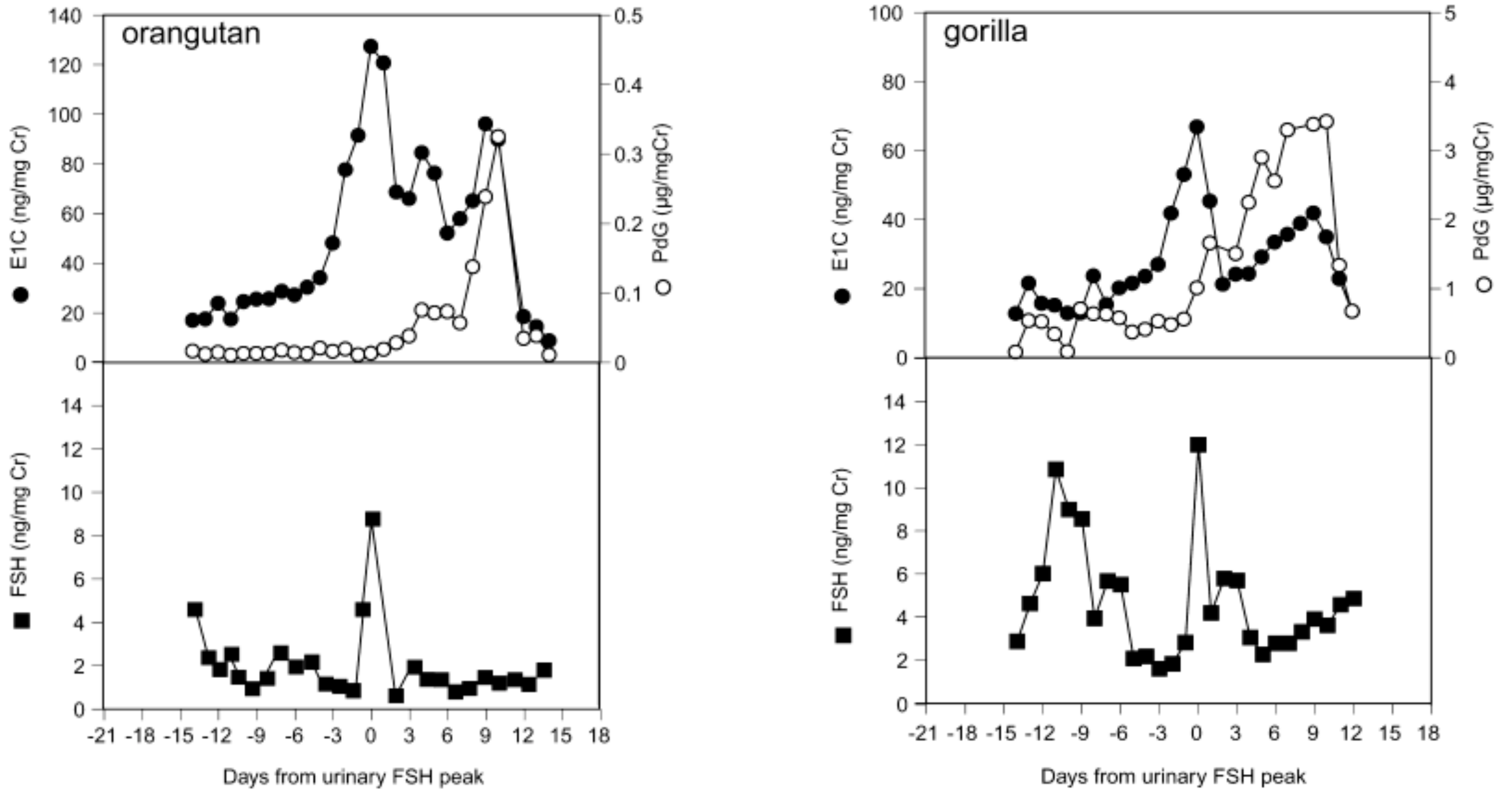
I will note the potential importance that differences in the timing of captive and wild female orangutans' major life history events have on the analysis of demographic or hormonal data (Shumaker et al. 2008) throughout this dissertation, particularly in the chapters that focus on puberty (Chapter 3) and reproductive senescence (Chapter 5).

Reproductive Physiology of Female Orangutans

The basic reproductive physiology of female orangutans is similar to that of gorilla, chimpanzee, and human females, suggesting that many reproductive traits have been conserved across the hominoid clade. A comparison of the ovulatory cycle of each species offers one example (Figure 1-1).

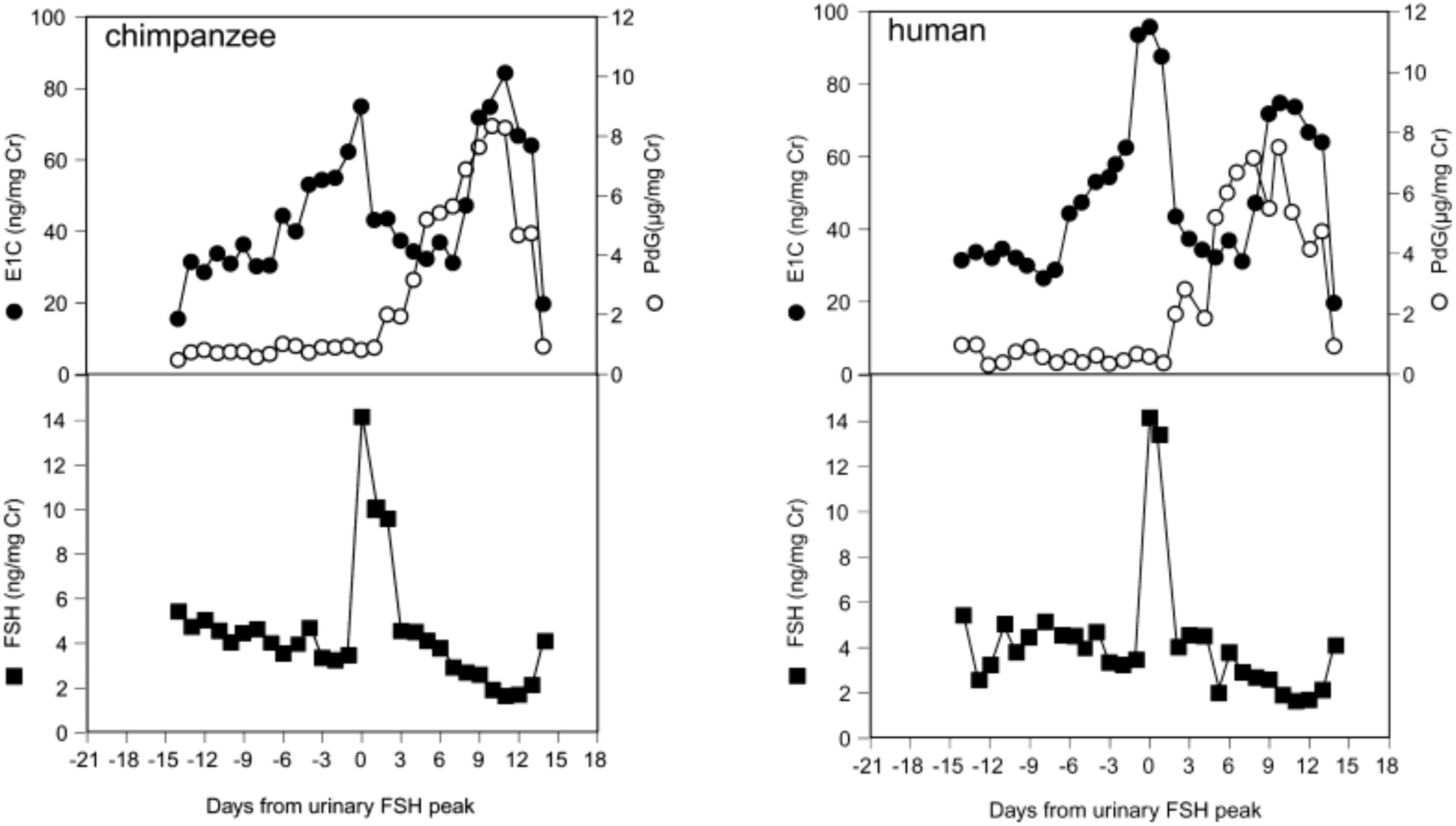
Each species exhibits an estradiol peak late in the follicular phase, which triggers the coincident surges in follicle-stimulating hormone (FSH) and luteinizing hormone (LH) that cause ovulation (Adashi 1994; Martin 2007). This is followed by sustained elevations in progesterone levels during the luteal phase. Interestingly, a second estrogenic peak is also evident in the cycle of each of the apes (Czekala et al. 1988; Shimizu et al. 2003), a characteristic that is found to a lesser extent, if at all, among Old World monkeys (Weick et al. 1973; Kling and Westfahl 1978; Monfort et al. 1987; Shideler et al. 1990; Aujard et al. 1999; Fujita et al. 2001). Luteal estrogen activity is important because it primes endometrial progesterone receptors, enabling the endometrium to thicken in response to post-ovulatory increases in circulating progesterone (Fritz et al. 1987; de Ziegler et al. 1998; Clancy 2009). However, the specific functional significance of the apes' pronounced secondary estrogen peak remains unclear, as do differences in the magnitude of the peak in each species (see Chapter 2).

More detailed examination of orangutan reproduction reveals some notable differences from humans. Maximum levels of progesterone production, for example, are up to ten times higher in the non-conceptive cycles of human females (Collins et al. 1975; Czekala et al. 1988; Shimizu et al. 2003; Venners et al. 2006). Additionally, as noted above, the average orangutan interbirth interval is significantly longer than that observed among human foraging populations



a. b.

Figure 1-1. Hormonal profiles of urinary ovarian steroid conjugates in individual menstrual cycles of an orangutan (a), gorilla (b), chimpanzee (c), and human (d). All data centered on mid-cycle follicle-stimulating-hormone (FSH) peak. E_1C = estrone conjugates; PdG = pregnanediol-3-glucuronide. Graphs reproduced from Shimizu *et al.* (2003).



c. d.

Figure 1-1. Hormonal profiles of urinary ovarian steroid conjugates in individual menstrual cycles of an orangutan (a), gorilla (b), chimpanzee (c), and human (d). All data centered on mid-cycle follicle-stimulating-hormone (FSH) peak. E_1C = estrone conjugates; PdG = pregnanediol-3-glucuronide. Graphs reproduced from Shimizu *et al.* (2003).

(7-9 years vs. 2-4 years) (Blurton-Jones 1986; Galdikas and Wood 1990; Knott 2001; Marlowe 2005). The shorter-than-expected human interbirth interval appears to be a unique adaptation that arose during the course of hominid evolution as the result of pooled energy resources and increased alloparental care of offspring (Knott 2001; Reiches et al. 2009; Kachel et al. 2011a).

Despite these differences, several aspects of orangutan reproductive biology resemble human characteristics more closely than do those of gorillas or chimpanzees. Most notably, neither orangutan nor human females exhibit any visible genital swelling associated with reproductive cycling (Graham 1981; Galdikas 1981; Graham 1988; Pagel 1994; Nadler 1995). Exaggerated mid-cycle swelling of the anogenital region is characteristic of many species of catarrhine (Graham 1981; Dixson 1983; Pagel 1994; Nadler 1995; Nunn 1999). Among the great apes, chimpanzees and bonobos display prominent swellings, and the period of maximal tumescence comprises approximately 20-35% of overall cycle length (Zuckerman and Fulton 1934; Graham 1982; Dahl et al. 1991; Heistermann et al. 1996; Deschner et al. 2004; Emery Thompson and Wrangham 2008). Gorillas exhibit a less exaggerated labial swelling that exists only for the few days surrounding ovulation (Nadler 1975; Graham 1981; Graham 1982; Czekala and Sicotte 2000). No genital swelling is observed near ovulation in orangutans, although, unlike humans, orangutan females develop a labial swelling early in pregnancy that is sustained through parturition (Schultz 1938; Graham 1981; Galdikas 1981; van der Werff ten Bosch 1982).

The degree of sexual swelling ancestral to the hominoid clade remains an open question, but theoretical reconstructions which indicate that the common ancestor of the apes possessed a slight swelling (Sillén-Tullberg and Moller 1993; Pawlowski 1999) suggest that both the evolution of exaggerated swellings in chimps and bonobos and the disappearance of visible signs of ovulation in orangutans and humans could require adaptive explanation.

A number of hypotheses have attempted to discern the adaptive function of sexual swellings in light of their taxonomic distribution and the relationship between a species' mating system and the size or absence of female swellings. In general, these hypotheses posit either that (a) sexual swellings evolved as a mechanism to concentrate paternity in a small number of dominant males (e.g. the "best male," "obvious ovulation," and "reliable quality indicator" hypotheses), or (b) swellings function to attract many mating partners and confuse paternity (e.g. the "many males" hypothesis) (Clutton-Brock and Harvey 1976; Hamilton 1984; Pagel 1994; Nunn 1999). Available data reveal support for both of these ideas. Swellings affect female attractivity and exert a measurable influence on male mating interest (Girolami and Bielert 1987; Aujard et al. 1999). However, swelling size has also been shown to serve as a reliable indicator of underlying reproductive hormone concentrations (Domb et al. 2001; Emery and Whitten 2003; Möhle et al. 2005; Gesquiere et al. 2007; Brauch et al. 2007), and dominant males show an ability to detect the peak conception period and adjust their mate guarding or consorting behaviors accordingly (Aujard et al. 1999;

Deschner et al. 2004; Brauch et al. 2007; Gesquiere et al. 2007; Emery Thompson and Wrangham 2008). As a result, Nunn (1999) suggests that swellings act as a “graded signal” that “[enables] females to manipulate male behavior by altering the costs and benefits of mate guarding, so that dominant males tend to guard only at peak swelling, but females can mate with multiple males outside peak swelling to confuse paternity.”

Hypotheses about the evolutionary origins of “concealed” ovulation have focused more extensively on humans than orangutans, and frequently emphasize monogamous pair bonds and paternal investment (Alexander and Noonan 1979; Strassman 1981). I will discuss concealed ovulation more extensively in the sections below, and in Chapter 4.

Other similarities in reproductive physiology between orangutans and humans include a close resemblance in estrogenic profiles during pregnancy, the result of a large fetal adrenal component relative to body size in each species (Czekala et al. 1983; Czekala et al. 1988), and similar post-partum profiles of gonadotropins and ovarian steroids (Nadler 1981). In addition, the average length of the menstrual cycle is approximately 29 days in orangutans (Table 1-1) and humans, shorter than the average cycle length of gorillas (31.1 days), bonobos (34 days), or chimpanzees (37.3 days) (Vervaecke et al. 1999; Martin 2007).

Table 1-1. Average Length of Ovarian Cycles Reported for Female Orangutans

Average Cycle Length (days)	Represented Individuals
27.8[1]	6 females, 35 cycles
28[2]	3 females, 6 cycles
29.6[3]	
30.5[4]	3 females, 8 cycles
29.7[5]	6 females, 92 cycles
1. (Graham 1988) 2. (Shumaker et al. 2008) 3. (Martin 2007) 4. (Nadler 1977) 5. Current study	

As the preceding paragraphs demonstrate, existing descriptions of the reproductive physiology and endocrine patterns of female orangutans provide a broad overview of the species, but offer little information about how ovarian function varies across the life cycle, between females, or across the cycles of an individual female. One of the primary goals of this dissertation is to characterize and interpret this variability.

Reproductive Hormones across the Female Life Cycle

Reproductive Maturation

Neuroendocrine control of reproduction in female primates centers on the hypothalamic-pituitary-ovarian (HPO) axis, illustrated in Figure 1-2. The maturation of this axis, known as gonadarche, occurs at puberty and results in

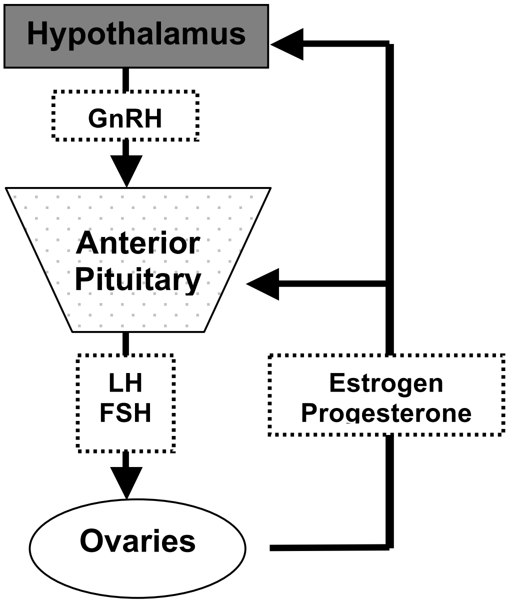


Figure 1-2. A Schematic Representation of the Hypothalamic-Pituitary-Ovarian (HPO) Axis. GnRH = gonadotropin-releasing hormone; LH = luteinizing hormone; FSH = follicle-stimulating hormone. Estrogen and progesterone have a negative feedback effect on gonadotropin production by the hypothalamus and pituitary.

the first significant production of LH, FSH, and ovarian steroids subsequent to a brief period of activity in infancy. Gonadarche is triggered by an increase in pulsatile GnRH secretion, which causes the release of LH and FSH, and consequently estrogen and progesterone (Bercovitch and Ziegler 2002; Plant 2002; Plant and Barker-Gibb 2004; Ebling 2005; Apter 2006; DiVall and Radovick 2008; Saltzman et al. 2010). However, evidence implies that the transition to full reproductive maturation requires some time, and that adolescent female primates frequently experience a period of subfecundity following menarche (Hartman

1931; Ashley-Montagu 1939a; Ashley-Montagu 1939b; Metcalf et al. 1983). Data from macaques, orangutans, gorillas, and chimpanzees indicate that the subfecund period in different species may last from one to five years (Galdikas 1981; Resko et al. 1982; Schurmann and van Hooff 1986; Pusey 1990; Markham 1990; Galdikas 1995; Wallis 1997; Knott 2001; Saltzman, et al. 2010), although these estimates almost always rely on observations of the amount of time that passes between the onset of sexual behavior and first parturition, rather than direct examination of hormonal patterns and concentrations (but see Resko et al. 1982). Hormone data from human adolescents are likewise rare, but similarly suggest a year or more of cycle irregularity and subfecundity (Metcalf et al. 1983; Saltzman et al. 2010).

Several researchers point to anovulatory cycles as the proximate cause of adolescent subfecundity, and suggest that anovulation is the result of a failure by the HPO axis to respond to ovarian estrogen production by releasing levels of LH and FSH sufficient to trigger ovulation (Hartman 1931; Ashley-Montagu 1939b; Resko et al. 1982; Apter 2006). In fact, the period of adolescent subfecundity is more likely characterized by a mix of ovulatory and anovulatory cycles (Vihko and Apter 1984; Knott 2001). Resko *et al*. (1982) found that among rhesus monkeys, 15% of the first 5 cycles following menarche were ovulatory; this number increased to 50% during the next 5 cycles. Subfecundity, however, may also occur in ovulatory cycles if luteal progesterone activity does not adequately prepare the uterus for implantation and pregnancy (Young and Yerkes 1943; Foster 1977; Apter 2006).

The various components of the female reproductive system, in other words, require synchronization, and it takes some time to effectively coordinate the glands and hormones of the HPO axis (Metcalf et al. 1983). Many therefore view adolescent subfecundity as a non-adaptive epiphenomenon resulting from the complexity of the system (Bercovitch and Ziegler 2002). Some, however, have suggested that adolescent subfecundity is adaptive for females because it enables them to be proceptive and to hone their mate selection judgment during a time in which they have a low risk of impregnation and its associated energetic costs (Galdikas 1995; Bercovitch and Ziegler 2002). This viewpoint is particularly interesting in light of the fact that an early primiparous age is frequently associated with negative maternal and fetal health outcomes (Ashley-Montagu 1939a; Ashley-Montagu 1939b; Fraser et al. 1995; Cocks 2007). In a study of captive orangutans, Cocks (2007) determined that 57% of females at or under the age of 10 at the time of first parturition survived less than 30 days, while no incidences of maternal death were recorded in primiparous females over the age of 10; he concluded that captive management practices should prevent females from breeding before the age of 12 (comparable to an early primiparous age among wild females).

As mentioned previously, reproductive parameters including age at menarche and age at first birth vary among free-ranging and captive primate populations, and between individuals. The duration of subfecund periods is also likely to vary as a function of mating system, local ecology, and individual genetics. Hormonal investigations of this phenomenon in wild and captive adolescents of different species will help determine the relative influence of these factors, will improve primatologists' understanding of how captivity affects reproductive physiology and maturation, and will provide important context that can be applied to observations of adolescent and adult reproductive strategies and behaviors. This project is the first to provide data regarding the existence and duration of a subfecund period in captive orangutans (Shumaker et al. 2008).

Reproductive Senescence

Changes in female reproductive physiology also occur later in life, driven by the depletion of the ovarian follicle pool (vom Saal et al. 1994; Kirkwood and Shanley 2010). In humans, this results in menopause, defined as the near or complete absence of oocytes and the complete cessation of menstrual cycling, around the age of 50 (O'Connor et al. 2001; Thomas et al. 2001; Wu et al. 2005; Walker and Herndon 2008). The presence or absence of a true menopausal transition in any non-human species is debated, as I will discuss further in Chapter 5. Evidence shows, however, that reproductive senescence is a gradual process, and alterations in hormone production and HPO axis functionality (i.e. perimenopause) begin many years before total follicular depletion (vom Saal et al. 1994; O'Connor et al. 2001; Kirkwood and Shanley 2010). We may therefore expect to observe some physiological effects of reproductive senescence in non-human species. Here, I offer an overview of the endocrinological changes associated with aging in human populations, from whom most of the data are derived. I then suggest that further examination of these factors in non-human primates is necessary to delineate the relationship between general and reproductive senescence, and to determine the evolutionary significance of a true post-reproductive life history phase.

Though definitions of the perimenopausal period are inconsistent in the literature, most researchers agree that an increase in cycle irregularity is one of the earliest signs of female reproductive senescence (Graham 1986; Santoro 2002; Ferrell et al. 2005; Hall 2007). Proximately, such irregularity can be attributed to an accelerated rate of follicular atresia after the age of 35 (Faddy et al. 1992; Richardson and Nelson 1990; vom Saal et al. 1994; O'Connor et al. 2001). As the follicle pool shrinks, ovarian estradiol production decreases, lessening negative feedback on the hypothalamus and pituitary and causing concentrations of FSH and LH to increase (Graham 1986; O'Connor et al 2001; Hall 2007). These glands also appear to become less sensitive to estradiol with age, such that estrogen levels sufficient to trigger an LH surge and ovulation in younger women do not do so in older women (Weiss et al. 2004). The result is a higher percentage of anovulatory cycles and an overall increase in average cycle length, although long cycles may be interspersed with shorter-than-usual cycles (vom Saal et al. 1994; O'Connor et al. 2001; Ferrell et al. 2005; Hall 2007).

Hormone levels continue to change as perimenopause progresses. Population level studies suggest that in aggregate, progesterone levels begin to decline when women reach their mid-thirties (Ferrell et al. 2005), although some women who continue to menstruate regularly past the age of 45 have luteal progesterone profiles comparable to those of younger women (Lee et al. 1988). Estradiol levels follow a more complex trajectory, and may temporarily increase in perimenopausal women before they begin to decline (Santoro et al. 1996; Ferrell et al. 2005; Hall 2007). Often estradiol concentrations do not reach their nadir until the year following the final menses (Hall 2007), especially in the case of women who retain some follicles and experience continued estradiol production after cycling ceases (Metcalf et al. 1982; Rannevik et al. 1986; Trevoux et al. 1986; Richardson and Nelson 1990). However, ovarian production of both estradiol and progesterone is undetectable within a few years after menstrual cycles stop (vom Saal et al. 1994). Concentrations of LH and FSH remain high in the years immediately following menopause, but may decline by up to 30% over the next several decades (Chakravarti et al. 1976; Hall 2007).

Changes in ovarian steroid and gonadotropin levels thus offer an important window into the process of reproductive senescence in the years preceding and through menopause. In particular, while age at menopause appears to be highly conserved across human populations (Thomas et al. 2001), individual women can vary significantly in the timing, course, and duration of the perimenopausal stage. Neuroendocrine patterns of reproductive aging are likely to exhibit similar variability in non-human primate species, as well as in other long-lived mammals. Nonetheless, the analysis of age-related hormonal decline, particularly among the apes, offers a valuable, non-invasive alternative to follicular counts in efforts to study patterns of reproductive senescence across taxa, as well as the selective pressures that have shaped such patterns.

Ovarian Hormones and Mating Behaviors in Non-Humans

Throughout this dissertation, my consideration of the avenues by which ovarian hormones influence the mating behaviors of male and female orangutans is built upon the concepts of female attractivity, receptivity, and proceptivity delineated by Beach (1976). As he defines them for mammals,

- *Attractivity* "refers to the female's stimulus value in evoking sexual responses by the male,"
- *Receptivity* connotes "the female responses [that are] necessary and sufficient for the male's success in achieving intravaginal ejaculation," and
- *Proceptivity* includes "various reactions by the female toward the male which constitute her assumption of initiative in establishing or maintaining sexual interaction."

Male mating interest, in other words, varies with social and/or hormonal measures of female desirability, while female mating behaviors can be categorized as passive (i.e. cooperative), resistant, or actively solicitous. Due to the important role they play in maintaining and altering female fecundity, estrogen and progesterone are among the hormones that act as important mediators of these dimensions. In this section I briefly describe differences across species in the relative impact that ovarian hormones have on receptivity and attractivity, and pay particular attention to the theory and evidence pertaining to a link between hormones and proceptivity in non-human primates, especially apes.

Receptivity

In some mammalian species, ovarian hormones play a crucial permissive role in female receptivity by altering specific aspects of reproductive morphology or behavior. Among rodents, for example, estrogen levels regulate female lordosis behavior. Because female rodents adopt the lordosis posture, which allows for male intromission and ejaculation, only in response to estrogen activity during the ovulatory phase of the cycle, copulations are effectively restricted to this time (Beach 1976; Wallen 1990; Beyer et al. 2007). Similarly, copulations occur only in the breeding season and during the most fecund phase of the cycle in guinea pigs and many prosimian primates. In each of these cases the narrow window of receptivity is due to a vaginal membrane that opens to allow penile intromission only in response to elevated estrogen levels near ovulation (Doyle 1974; Wallen 1990; Ankel-Simons 2007; Dixson 2012).

Other female mammals may be physically capable of mating at any time, but nonetheless exhibit a defined period of estrus that coincides with periovulatory increases in estrogen and is characterized by increased receptivity. Table 1-2 provides several examples of mammals with limited periods of receptivity.

Table 1-2. Length of Estrus Period in Different Mammal Species

Species	**Average cycle length**	**Average length of estrus (receptive) period**[1]	**Mode of ovulation**
Rat	4-5 days	12-18 hours	Spontaneous
Sheep	17 days[2]	24-48 hours	Spontaneous
Pig	21 days	2-3 days	Spontaneous
Horse	21 days[2]	6 days	Spontaneous
Cat	14-21 days[2]	6-7 days	Induced
Rabbit	No regular cycles	Up to 1 month	Induced
1. All data from Merck Veterinary Manual, *http://www.merckvetmanual.com/mvm/htm/bc/trep03.htm* 2. Seasonally polyestrous			

Among primates, receptivity is often assessed by measuring copulation frequency across different phases of the ovulatory cycle. Copious evidence has established that mating occurs most frequently during the periovulatory stage of the cycle (e.g. Gordon 1981; Murray et al. 1985; Harris and Monfort 2006; Heistermann et al. 2007). Non-periovulatory receptivity, however, ranges broadly across females of different primate species (e.g. prosimians vs. bonobos; Dixson 2012). It is therefore likely that mid-cycle increases in ovarian hormone activity influence primate copulation frequency not by creating a limited window of receptivity, but by affecting female attractivity (i.e. male motivation to mate) and/or proceptivity (i.e. female motivation to mate) (Wallen 1990; Saltzman et al. 2010).

Attractivity

Many studies of mammalian reproduction have revealed that males exhibit more interest in mating with females during the periovulatory phase of the cycle, presumably responding to female attractivity as determined by visual, olfactory, or other signals of peak estrogen and progesterone production (review: Beach 1976). At first glance, such "choosiness" on the part of males may seem maladaptive. Because reproductive success in most mammals involves significantly lower energetic and parental investment from males than females (Trivers 1972), evolutionary theory predicts that males can maximize their fitness by taking advantage of every mating opportunity. Data show, however, that sperm availability may limit a male's ability to fertilize many females in a short time (Small et al. 1988; Stockley and Bro-Jørgensen 2010). In addition, any individual mating encounter has an opportunity cost for the male in that this time cannot be spent competing for, guarding, or mating with a potentially more fecund female. It is therefore adaptive for males to concentrate their mating efforts on the days of greatest female fecundability, particularly in species with short breeding seasons and a high degree of estrus synchronicity among females (Alfaro 2005; Stockley and Bro-Jørgensen 2010).

Among primates, the exaggerated female sexual swellings of many Old World species (see above) provide males with a visual cue of increased ovarian activity, and have been shown to be significantly associated with attractivity. Maximally swollen macaque, baboon, and chimpanzee females are subject to more frequent mating attempts and more aggressive mate-guarding by males than females with smaller or absent swellings (Beach 1976; Aujard et al. 1999; Domb et al. 2001; Deschner et al. 2004; Gesquiere et al. 2007; Brauch et al. 2007; Emery Thompson and Wrangham 2008; Garcia et al. 2009). The same pattern of increased male interest has also been documented in the absence of periovulatory swellings: male-initiated mating behaviors (e.g. approaching, displaying, investigating, mounting, consorting) are correlated with female hormonal status in tufted capuchins (Alfaro 2005), white-faced capuchins (Carnegie et al. 2005), squirrel monkeys (Mendoza et al. 1978), common marmosets (Converse et al. 1995; Smith and Abbott 1999), and Hanuman langurs (Ostner et al. 2006).

Few studies have directly investigated the relationship between ovarian hormone activity and attractivity in orangutans. In a series of laboratory pair-tests conducted by Nadler, the measure of copulations per day was highest mid-cycle, but male orangutans initiated copulations almost every day and responded forcefully to any female resistance (Nadler 1977, 1982, 1995). More recently, a collaborative analysis of multiple sites on Borneo and Sumatra indicated that flanged males at all sites copulate more frequently with cycling females than with pregnant or lactating females, but the data did not distinguish between females' cycle phases (Atmoko et al. 2009). To date, no researchers have investigated the possibility that the attractivity of individual females may change from one cycle to the next due to hormonal variation, or that male behavioral patterns may reveal consistent interindividual differences in attractivity.

Because female orangutans do not exhibit any visible genital swelling in association with the periovulatory period, changes in a female's ability to evoke a male sexual response must derive from alternate visual or olfactory stimuli, or from altered behavioral patterns exhibited by the female (Schürmann 1982; Nadler 1995). The latter is the focus of the next section.

Proceptivity

Recent years have seen a growing number of researchers adopt the perspective that mating outcomes represent a compromise between male and female mating strategies (Soltis et al. 2001; Nikitopoulos et al. 2005; Ostner et al. 2006; Knott et al. 2010; Stockley and Bro-Jørgensen 2010; Aloise King et al. 2011). In this context, females' proceptive behaviors can function either to concentrate or confuse paternity, and sexual selection theory predicts that proceptivity should vary accordingly across the phases of the ovulatory cycle: a female can improve her reproductive success by (a) soliciting copulations from the most fit males during times of peak fecundity, (b) avoiding copulations with less fit males during these times, and (c) soliciting copulations from non-preferred males outside of the fecund period. Female rodents, dogs, ungulates (review: Beach 1976), and elephants (Moss 1983; Poole 1989) all display patterns of proceptivity that conform to these predictions, suggesting that female-initiated mating behaviors in these species are influenced by the hormonal characteristics of the periovulatory period.

The complexity of primate social environments, coupled with the fact that many female primates mate throughout the ovulatory cycle, makes it difficult to determine how estrogen, progesterone, and androgens affect female sexual motivation in monkeys and apes (Wallen 1990; Takahashi 1991). Analyses of increased periovulatory proceptivity in tufted capuchins (Carosi et al. 1999), rhesus macaques (Wallen et al. 1984), Japanese macaques (O'Neill et al. 2004; Inoue and Takenaka 2008), mandrills (Setchell 2005), gorillas (Nadler et al. 1983), and chimpanzees (Nadler et al. 1994; Matsumoto-Oda 1999; Stumpf and Boesch 2005; Stumpf and Boesch 2006; Pieta 2008; Stumpf et al. 2008) suggest that higher levels of estradiol and testosterone are important proximate stimulators of female primates' motivation to mate, and that progesterone has an inhibitory effect (Baum et al. 1977; Nadler et al. 1983; Takahashi 1991; Nadler 1995; Zehr et al. 1998). However, several researchers have suggested that mating behavior may be influenced by several hormones acting in combination or sequentially (Baum et al. 1977; Gordon 1981). In addition, data from captive and free-ranging animals indicate that social and environmental circumstances (e.g. the number of males and females present, the rank of individuals) modulate endocrinological effects on primate behavior (Nadler et al. 1986; Wallen 1990, 1999, 2001).

Evidence of the relationship between reproductive hormones and behaviors in orangutans strengthens the argument that ovarian hormones primarily affect female sexual motivation, while the act of mating is freed from strict hormonal control (Wallen 1990, 2001). In captivity, traditional pair tests reveal that females reduce avoidance behaviors and increase grooming and solicitation of a male partner mid-cycle, while restricted-access pair tests show that copulations only occur during the periovulatory period when control over interactions rests entirely with the female (Nadler 1977; Maple et al. 1979; Nadler 1982, 1995). Free-ranging females similarly display changes in proceptivity in conjunction with cycle phase, soliciting only preferred males during the periovulatory period but mating cooperatively or proceptively with non-preferred males at other times (Knott et al. 2010).

Proceptive behaviors thus reveal that female “strategies” for reproductive success conform to the predictions of sexual selection theory. Females attempt to mate frequently and exclusively with the most fit males when conception is likely, but remain receptive throughout the ovulatory cycle. In fact, data suggest that several different selective pressures may have produced non-conceptive copulations as a widespread adaptation in the primate order. Constant receptivity may serve to reinforce and strengthen pair bonds, as in marmosets and tamarins (Ziegler 2007). It may serve as a mechanism of female competition, as appears to be the case among western lowland gorillas; a 2009 study by Doran-Sheehy *et al.* (2009) concluded that post-conception mating is a conditional strategy that females use to reinforce their own status and potentially delay conception in others. Lastly, mating outside of the conceptive period may help reduce the risk of infanticide by confusing paternity (Hrdy 1979; Hestermann et al. 2001; Borries et al. 2001; Engelhardt et al. 2007; Stumpf et al. 2008; Clarke et al. 2009; Knott et al. 2010).

Ovarian Hormones and Mating Behaviors in Humans

As mentioned previously, humans share numerous characteristics of reproductive biology and behavior with orangutans. Ovulation and peak fecundity are not physically advertised in either species, and women, like female orangutans, are receptive throughout the ovulatory cycle (Crews and Moore 1986; Pawlowski 1999). We may therefore expect to find similarities in the way that hormonal factors relate to male and female reproductive behaviors and motivation (i.e. a periovulatory increase in attractivity and proceptivity). Yet the evidence that human sexual behavior is not entirely emancipated from physiological control is divisive.

Disagreement on this subject is due partially to the fact that ‘concealed ovulation’ and an extended mating period have long been invoked as significant selective pressures in hypotheses about the evolution of human social and mating systems (Burley 1979; Strassman 1981). Though rare in mammals, monogamous pair-bonds and a high degree of paternal investment are widespread in humans (Quinlan 2008). In theory, sexual selection could have produced these features by favoring concealed ovulation and constant female receptivity. The evolution of these characteristics would limit males' ability to reliably determine the timing of ovulation, and consequently decrease mating effort and increase parenting effort among males (Alexander and Noonan 1979; Sillén-Tullberg and Moller 1993). This would have been especially beneficial in humans given the long-term energetic demands of our unique life history pattern, in which females have large-brained and highly altricial offspring but relatively short interbirth intervals (Knott 2001). Truly concealed ovulation, however, requires not only that women lack a periovulatory swelling, but also that they emit no olfactory or behavioral cues of underlying cycle phase.

Instead, data suggest that estrogen, progesterone, and androgen levels affect sexual motivation and behaviors, particularly in women (review: Gangestad and Thornhill 2008). In one of the earliest studies to examine male and female-initiated sexual behaviors separately, Adams *et al.* (1978) showed that while the frequency of male-initiated sexual behavior did not vary across cycle phases, female-initiated autosexual and heterosexual behaviors increased during the periovulatory period among married American women not using oral contraceptives (also see van Goozen et al. 1997). Additional research has since revealed that women near ovulation prefer the scent of more symmetrical men (Gangestad and Thornhill 1998), presumably because low fluctuating asymmetry serves as an indicator of underlying genetic quality (Cashdan 1996). Interest in extra-pair copulations (EPCs) is also highest at this time, particularly if a woman's current partner does not exhibit signs of 'good genes' (Gangestad et al. 2002; Haselton and Gangestad 2006).

Whether hormonally-induced changes in female sexual motivation result in higher or lower degrees of attractivity that might imply male awareness of ovulatory timing remains unclear (Marlowe 2004; Brewis and Meyer 2005). As mentioned above, married men initiate copulations throughout the cycle and thus do not appear to be aware of cycle phase (Adams et al. 1978). There is evidence, however, that males' mate-guarding efforts increase mid-cycle (Gangestad et al. 2002; Haselton and Gangestad 2006). This suggests that males register subtle changes in periovulatory women, and have evolved a mechanism of mate retention in response to increased female interest in EPCs at this time. A recent study which reported that observers who examined photographs of women at different points in the cycle most frequently identified women in the late follicular phase as "trying to look more attractive" offers one explanation of how women might consciously or subconsciously provide a signal of imminent ovulation (Haselton et al. 2007). Female body odor may also play a role (Singh and Bronstad 2001; Havlicek et al. 2006).

As many authors have noted, human sociocultural practices create a difficult framework within which the relationship between hormones and sexual behavior must be analyzed (Steklis and Whiteman 1989; Wallen 2001). Nonhormonal variables such as a woman's desire for or fear of conception and a society's 'norms' about the appropriate time and place for sex may create conditions in which hormonally-driven changes in female sexual motivation do not translate to actual changes in sexual behavior (Pawlowski 1999; Wallen 2001). These challenges are further compounded by the limitations of research design: most studies of the behavioral endocrinology of human sexuality rely on womens' self-reported data regarding cycle phase and sexual activity. That investigators have found evidence of estrus-like patterns in humans despite these difficulties suggests that ovarian hormones affect sexual motivation along a continuum that has been shaped by selection and conserved across hominoids.

Reproductive Endocrinology in Evolutionary Perspective

Reproductive hormones are intimately linked to the reproductive physiology, behaviors, and life history schedule of a species. Patterns of reproductive endocrinology are therefore an important reflection of the past actions of natural selection, and comparing these patterns across species can yield significant insight into the selective pressures that have shaped shared and derived characteristics of ovulatory cycles and reproductive behaviors. In this dissertation, I not only examine the variables involved in interactions between female orangutan ovarian function and reproductive behaviors across the life cycle (Figure 1-3); I also compare patterns of reproductive endocrinology in orangutans and humans to evaluate whether relevant similarities in the two species represent evolutionarily meaningful adaptations or are simply the result of convergent evolutionary processes. Of particular interest are those traits, such as the lack of a periovulatory swelling, which set orangutans and humans apart from the more derived human-chimpanzee clade.

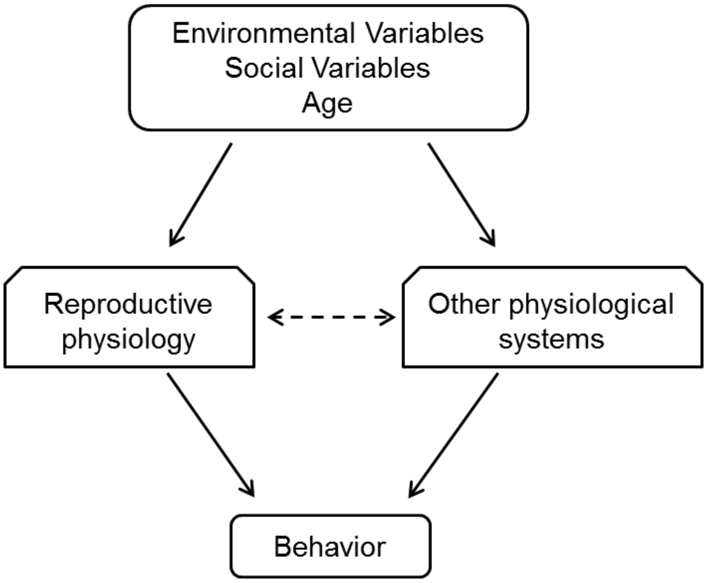


Figure 1-3. Factors Underlying Variability in Ovarian Function and Hormone-Behavior Relationships

Synopsis of Thesis Contents

Urine samples provide a valuable medium for non-invasively monitoring ovarian function in primates. Chapter 2 describes and validates the laboratory methodology used throughout this thesis, and provides an overview of the study sites and subjects involved in this project. Because this study has substantially increased the number of ovarian cycles that have been quantified for female orangutans, I also present a composite cycle profile of estrogen and progesterone that can be used in future comparative work.

Chapter 3 examines the ovarian function and reproductive behaviors of adolescent female orangutans in captivity. I place particular emphasis on differences in the developmental pacing of wild vs. captive individuals, and address the topic of adolescent subfecundity within this framework.

Chapter 4 assesses variation in cycle quality within this sample of nutritionally stable captive females, and considers possible non-energetic sources of intra- and inter-individual differences in ovarian function. I also examine the relationship between ovarian hormone activity and mating behaviors to (1) test whether orangutan ovulation can be classified as ‘concealed,’ and (2) measure the degree to which ovarian steroids influence female receptivity, attractivity, and proceptivity.

In Chapter 5, I investigate how the process of reproductive senescence affects the ovarian function and mating behaviors of older female orangutans. The results are discussed in the context of life history theory, the physiology of menopause, and the human post-reproductive period.

Chapter 2: General Methods & Laboratory Validation

Project History

I am able to present the results of this dissertation in part due to the long-term collaborative efforts of my adviser, Dr. Cheryl Knott, with several captive-animal institutions in the United States. Her relationships with the Woodland Park Zoo in Seattle and the Great Ape Trust in Des Moines enabled me to amass a substantial collection of orangutan urine samples in our laboratory, and helped me foster the partnerships necessary to coordinate the sharing of behavioral and veterinary records.

Urine collection at the Woodland Park Zoo initially took place from February 1992 through March 1994, and was resumed in September 2008. It continues today in collaboration with Libby Lawson and other zoo staff and administration. Urine collection at the Great Ape Trust began in September 2004 in partnership with Dr. Rob Shumaker and Dr. Serge Wich. It ended at this facility in March 2009, but several of the orangutans involved now reside at the Indianapolis Zoo and I anticipate collecting additional samples and behavioral data from them in the future.

In this chapter I will provide background information on the institutions and individual animals involved in this research. I will also detail the methodology applied through the remainder of this thesis and validate the procedures used for data collection and analysis.

Study Sites and Subjects

Woodland Park Zoo, Seattle

The Woodland Park Zoo has housed orangutans since the mid twentieth century (c. 1960; L. Lawson, personal communication) and is currently home to five orangutans, all of whom contributed hormonal and/or behavioral data to this project. The three females at the zoo are Chinta (44 years old[1]), Melati (41 years old), and Belawan (31 years old; offspring of Melati and Towan). The two males are Towan (44 years old; Chinta's twin), and Heran (23 years old; offspring of Melati and Towan). Further biographical information for each of these individuals is provided in Table 2-1.

Chinta, Towan, Belawan, and Heran have all resided at the Woodland Park Zoo since birth. Melati was born at the National Zoo in Washington, DC but was transferred to Woodland Park at the age of two and a half. Until 1998 the orangutans were all housed as one social group, but at that time Belawan began acting aggressively toward Chinta and as a result was removed from the group and placed into a separate, adjoining enclosure. Following this separation, either Towan or Heran would spend most of the day with Belawan before moving back into the other enclosure in the afternoon. However, in 2001 Towan became resistant to returning to the group and the zoo staff decided to allow Towan to stay with Belawan full-time. The orangutans are still divided into two social groups at present, occupying separate but adjoining enclosures. Diagrams of the

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[1] All ages as of 1/1/13

indoor and outdoor orangutan enclosures at Woodland Park Zoo are provided in Appendix A.

The orangutans' diet consists of a daily biscuit ration for each individual (calculated based on weight) along with a combination of fruits, vegetables, and leafy greens. These foods are served throughout the day and complemented by occasional enrichment and training reinforcement items such as yogurt, sunflower seeds, and frozen blueberries (L. Lawson, personal communication). None of the individuals at WPZ is overweight (L. Lawson, personal communication), a problem that can result in health complications among captive orangutans (Jones 1982; Knott 1999) and that is known to negatively affect ovarian function in humans (Hartz et al 1979; Rachón and Teede 2008). A complete overview of the orangutan diet at the WPZ is provided in Appendix A.

Urine samples from an additional female orangutan named Kelly, who lived at Woodland Park Zoo during the renovation of the orangutan exhibit at Houston Zoo, were collected from February 1992 through December 1992. Kelly, who was 11 years old at the time of her arrival, had visual access to the resident Woodland Park orangutans, but no physical access. Further biographical information for Kelly is provided in Table 2-1.

No form of birth control was given to any of the female orangutans at the Woodland Park Zoo during either period of urine collection (L. Lawson, personal communication).

Great Ape Trust, Des Moines

The Great Ape Trust is a non-invasive primate research facility, founded in 2002 with the goal of “understanding the origins and future of culture, language, tools and intelligence, and [the] preservation of endangered great apes in their natural habitats” (Great Ape Trust). Over the course of the data collection period, it housed five orangutans: Azy (male, 35 years old), Knobi (female, 33 years old), Allie (female, 18 years old), Katy (female, 24 years old), and Katy’s son Rocky (8 years old). A female named Popi (41 years old) also resided at the Great Ape Trust for a portion of the time in question, but she has not been included in any of the data analyses for this dissertation due to inadequate sampling.

Due to the recent origins of the facility, each of these orangutans was transferred to the Great Ape Trust from another captive location: Azy moved from the National Zoo in September 2004; Knobi transferred from the Henry Doorly Zoo in Omaha in February 2005 and was introduced to Azy in March; Allie arrived from the Denver Zoo in October 2005 and joined Azy and Knobi in November; and Katy and Rocky came to the Trust in July 2008 after retiring from the entertainment industry. Further biographical information for each of the individuals involved in this project is provided in Table 2-1.

All of the orangutans at the Great Ape Trust were socialized with one another and could move and interact freely at all times. Their enclosure, comprised of indoor and outdoor areas each measuring approximately 30 feet wide, 50 feet long, and 25 feet high, is illustrated in Appendix A. The orangutans had unrestricted access to all of this enclosure space, with the exception of a small kitchen, restroom, and entry area. Unlike the Woodland Park Zoo, the Great Ape Trust is not open to public visitation, so keepers and researchers were the only humans present on a daily basis.

The diet of the Great Ape Trust orangutans was similar to that of the Woodland Park Zoo apes, consisting of a daily selection of fresh produce and a small portion of biscuits (R. Shumaker, personal communication).

Because Azy was vasectomized years prior to his arrival at the Great Ape Trust, no form of birth control was given to any of the female orangutans at the Trust during the period of urine collection (R. Shumaker, personal communication).

Knobi

Knobi merits special mention because I consider her hormonal and behavioral data separately from the other female orangutans throughout this dissertation. I chose to do this for two reasons. First, Knobi has a history of urinary tract infections. This increases the likelihood of finding blood in her urine samples, which makes it difficult to delineate menstrual periods. Hormone levels also cannot be accurately assayed in urine that has been contaminated with blood. Second, in August 2007, after more than 8 months of registering blood in her urine almost daily, veterinarians performed an ovariectomy on Knobi (R.

Table 2-1. Biographical Information for All Study Subjects

Name	Sex	Date of Birth	Place of Birth	Genetic Background	Age at first parturition	Offspring	Current Location[1]
Chinta	F	2/19/1968	WPZ, Seattle	Hybrid	11	Birute (F, 1979)	WPZ, Seattle
Towan	M	2/19/1968	WPZ, Seattle	Hybrid		Birute (F, 1979) Rusti (M, 1980) Belawan (F, 1981) Heran (M, 1989)	WPZ, Seattle
Melati	F	12/27/1971	National Zoo, Washington	Hybrid	8	Rusti (M, 1980) Belawan (F, 1981) Heran (M, 1989)	WPZ, Seattle
Azy	M	12/14/1977	National Zoo, Washington	Hybrid		None	Indianapolis Zoo
Knobi	F	9/30/1979	Henry Doorly Zoo, Omaha	Hybrid		None	Indianapolis Zoo
Kelly	F	9/22/1980	San Diego Zoo	Bornean	16	Luna Bela (F, 1997) Solaris (M, 2003) Aurora (F, 2011)	Houston Zoo
Belawan	F	5/17/1981	WPZ, Seattle	Hybrid		None	WPZ, Seattle
Katy	F	12/18/1988		Hybrid	16	Rocky (M, 2004)	Indianapolis Zoo
Heran	M	2/19/1989	WPZ, Seattle	Hybrid		None	WPZ, Seattle
Allie	F	12/30/1994	Yerkes Primate Center, Atlanta	Sumatran		None	Center for Great Apes Wauchula, FL

Data taken from the International Orangutan Stud Book, provided by Lori Perkins of Zoo Atlanta (Orangutan SSP coordinator).

1. As of September 2012

Figure 2-1. Select Orangutan Subjects, from left to right (photo source in parentheses): Chinta (Woodland Park Zoo), Melati (The Telegraph), Belawan (Woodland Park Zoo), Towan (The Seattle Times), Knobi (Indianapolis Zoo), Katy (Indianapolis Zoo), Allie (Center for Great Apes), Azy (CBC/Radio-Canada)

Shumaker, personal communication). Thus, although I only examined behavioral and hormonal data from Knobi from 2005-2006, I have excluded her from group comparisons due to the possibility of species-atypical hormone levels and reproductive functioning.

Endocrine Methods

Urine Collection and Preservation

Urine samples for this research were collected by keeper staff at the Woodland Park Zoo and the Great Ape Trust. Urine sampling is an excellent technique for ovarian hormone analyses for several reasons. It is non-invasive, only small volumes of urine are needed to measure steroid conjugates, and both the steroid conjugates and creatinine in urine appear to remain stable over time (Kesner et al. 1995; van Noordwijk and van Schaik 2005; Valeggia 2007; see below). Urine sampling is also advantageous because it yields results that reflect hormone concentrations integrated over several hours of production. This means that although steroids are excreted in urine within hours of production (Crockett et al. 1993; Ziegler et al. 1989; Heistermann 2010), urine sampling is not as sensitive to acute changes in steroid concentrations as serum sampling (Whitten et al. 1998; O’Connor et al. 2003). Lastly, the materials required to conduct enzyme immunoassay analyses of urine samples are fairly inexpensive (Wheaton et al. 2011).

Urine sampling is an especially effective method in captivity because conditions allow for the collection of samples from the same individuals at the same time each day (Heistermann 2010). This provides a robust picture of individuals' hormone profiles that is difficult to obtain for free-ranging animals due to the logistical challenge of collecting daily samples from select individuals. Consistency in the timing of sample collection also controls for any circadian effects, although studies of humans and callitrichids have found minimal diurnal fluctuations in ovarian hormone secretion (Bös et al. 1993; Norjavaara et al. 1996).

Woodland Park Zoo Procedures

The urine samples used for this project were collected from female orangutans at the Woodland Park Zoo from February 1992 to May 1993 and from September 2008 to March 2011. Staff members usually collect urine from the first morning evacuation by catching it as the orangutans travel through an overhead tunnel from their sleeping areas to the rest of the enclosure. In recent years, staff members have also trained the orangutans to provide samples upon request; the orangutans urinate directly into a clean paper cup and are rewarded with a desired food item or cup of juice (L Lawson, personal communication).

Once collected, a staff member pipettes the sample into a 2ml microtube and labels it with the orangutan's name as well as the date (1992-1993 samples) or date and time (2008-2011 samples). Samples are then frozen. When enough samples have been collected to fill several microtube boxes, these are packaged with dry ice and sent to Boston University using overnight shipping. The boxes of samples are then transferred to one of the freezers in the biological anthropology laboratory, which are kept at -20°C.

Great Ape Trust Procedures

The urine samples used for this project were collected from female orangutans at the Great Ape Trust from August 2005 to March 2009. All of these females had been trained previously to provide samples upon request by urinating into a clean paper cup. Once collected, a staff member transferred the sample into a 15ml tube and labeled it with the female's name along with the date and time. Samples were then frozen, and the same procedure described above for the Woodland Park Zoo was used to move boxes of samples to Boston University.

Great Ape Trust practices often resulted in multiple samples collected from the same individual on a given day. In these cases, I assayed the sample collected earliest in the day. In total, 84% of all of the samples assayed from the Great Ape Trust were collected before 11:00 AM.

Urinary Enzyme-immunoassay and Creatinine Standardization

I analyzed all urine samples in the biological anthropology laboratory at Boston University using enzyme immunoassay (EIA) procedures, which are commonly employed in primate research (Czekala and Sicotte 2000; Shimizu et al. 2003; Emery Thompson 2005b). Specifically, I performed assays to measure urinary estrone conjugates (E_1C) and pregnanediol-3-glucuronide (PdG), the primary ovarian steroid metabolites that are excreted in urine (Lasley and Kirkpatrick 1991; Munro et al. 1991). Although the presence of these conjugated steroid forms in urine can also result from the breakdown and conversion of estrogens and androgens of non-ovarian origin, E_1C and PdG have been shown to accurately reflect circulating concentrations of estradiol and progesterone (O'Connor et al. 2003). The reagents for these assays were provided by University of California, Davis, Department of Population Health and Reproduction (C. Munro).

The EIA procedure is described in detail in Appendix B. I assayed all samples in duplicate, starting with dilutions that I calculated with Dr. Melissa Emery Thompson at the University of New Mexico Hominoid Reproductive Ecology Laboratory. I reassayed samples if (a) duplicate results were drastically different from one another, (b) the standard curves from the plates did not conform to a set precedent, (c) the internal controls on a plate fell outside of the expected ranges, or (d) the dilution used produced an optical density higher or lower than the standard range. In general, I began by conducting assays chronologically for one individual at a time; however, many of the later assays I performed contained samples from two or more individuals.

All urinary conjugate results are expressed as mass per mg creatinine (Cr). Creatinine is a byproduct of muscle metabolism that is excreted at a constant rate in individuals with normal kidney function (Brown et al. 2004). It therefore serves as a reliable indicator of the amount of time over which steroids have been metabolized into the urine, regardless of sample volume, and is a convenient way to control for water content and to standardize E_1C and PdG results (Heistermann 2010). I measured creatinine using an assay that exploits the Jaffe reaction, in which creatinine reacts with picric acid in an alkaline solution to produce a color change (Taussky et al. 1954) and resulting optical densities are calibrated to three standards (Sigma-Aldrich, St. Louis, MO). In my analyses, I discounted any urine samples with Cr values below 0.1 mg/ml, as these produced significantly inflated steroid conjugate results.

Validation Methods

Many animal researchers collect and analyze urine as a means of studying variation in reproductive hormones levels and associated outcomes (Whitten et al. 1998; Valeggia 2007; Heistermann 2010). Primatologists are no exception, and urine has been used to successfully monitor ovarian function in a variety of monkeys (Heistermann et al. 1995; Aujard et al. 1999; Carosi et al. 1999; He et al. 2001; Fujita et al. 2001; Harris and Monfort 2006) as well as among chimpanzees (Dahl et al. 1991; Deschner et al. 2003; Emery Thompson 2005b), gorillas (Czekala et al. 1986; Bellem et al. 1995; Czekala and Sicotte

2000), and orangutans (Collins et al. 1975). However, specific methods of urine preservation and steroid quantification can vary greatly. I therefore conducted a variety of validation procedures to examine the efficacy of the assays performed during this study. Table 2-2 presents the results of my assay evaluations, using standard criteria that are defined as follows (Porstmann and Kiessig 1992; Brown et al. 2004; Ederveen 2010):

- Assay *specificity* is the ability to quantify a specific analyte while excluding other substances in the sample matrix. This information was provided by the assay manufacturer, who measured the antibody's discrimination of the target antigen versus other potential cross-reactants.
- An assay's *sensitivity* is defined as the minimum quantity of analyte detectable by the assay.
- Assay *precision* is defined as the repeatability of measurements, or the agreement among individual test results. I calculated assay precision by determining coefficients of variation (CV) for (a) samples repeated within an assay and (b) internal controls repeated in each assay in the study. Internal controls were derived from various dilutions of a human urine sample, and CV was calculated across assays (1-5 plates per assay).
- *Accuracy* is defined as the proximity of the results of an assay to the true concentration. I evaluated accuracy by measuring the average recovery of steroid standards added in duplicate to a sample.

- *Parallelism* examinations exclude interference of the sample medium with the assay reaction. I examined parallelism by calculating the regression of percent binding to log-transformed steroid doses; assay validation was confirmed because slopes for the standard curves did not differ significantly from those generated by serial dilution of a sample.

Long-term Storage and Sample Viability

One of the challenges of working with older samples is that little information is available about the effects that long-term storage has on the viability of urine samples used for hormonal assay. Some studies have reported loss of gonadotropin immunoreactivity in continuously frozen samples (Livesey et al. 1983), suggesting that protein hormones may not remain stable over time. Investigations of ovarian steroids, however, have reported that multiple freeze-thaw cycles have no impact on the ability to measure estrogen and progesterone (O’Connor et al. 2003; Wheaton et al. 2011). A literature search revealed no studies investigating the likelihood of E_1C or PdG degradation in urine samples frozen for 10+ years, as were those used in this dissertation.

Table 2-2. Validation of Assays

Analyte	**Sensitivity**	**Accuracy (% Recoveries)**	**Parallelism**		**Intra-assay CV (%)**	**Inter-assay CV (%)**	
			Standards	**Sample**		**Low Sample**	**High Sample**
uE$_1$C	175 pg/ml	93.6 ± 19.7	$y=-0.49x+1.35$ $R^2=0.895$ $p=0.005$	$y=-0.42x+1.37$ $R^2=0.914$ $p=0.0004$	5.4	14.6	12.7
uPdG	5 ng/ml	97.9 ± 21.5	$y=-0.30x+0.46$ $R^2=0.922$ $p=0.0001$	$y=-0.37x+0.29$ $R^2=.952$ $p=0.004$	7.1	13.3	13.7

Accuracy was tested by recovery of standards added in duplicate to samples and is expressed as mean ± standard deviation. Parallelism was examined by comparing the regression of percent binding on log-transformed dose for the standard curve and serial dilution of a sample. All tests for parallelism were successful in that they produced samples curves not significantly different from the standard curve.

It is currently recommended that urine be frozen as soon as possible following collection, and stored at or below -20°C (Brown et al. 2004; Valeggia 2007; Heistermann 2010). These protocols were followed for all samples used in this project. Nonetheless, I investigated the possibility that the older samples used in my analyses might yield inaccurate hormone measurements by calculating average E_1C and PdG concentrations for the 1992-93 and 2008-11 samples of Belawan, Melati, and Chinta, and comparing the values (Figure 2-2). The results demonstrate that while the 1992-93 samples of Chinta and Melati contain lower hormone concentrations than the more recent samples (as might be expected if analytes degrade over time), Belawan's samples show the opposite pattern. I interpret this as evidence that there is no uniform steroid deterioration as a result of long-term freezer storage. Additional support for the viability of the older samples comes from a visual analysis of two representations of Chinta's March-April 1993 cycle, one derived from my own assays and the other from analyses done by Dr. Cheryl Knott in 1993. Although different assay techniques (radioimmunoassay vs. enzyme immunoassay) make a direct comparison of absolute hormone values difficult, the relative pattern of E_1C measurements remains consistent across the two assays (Figure 2-3).

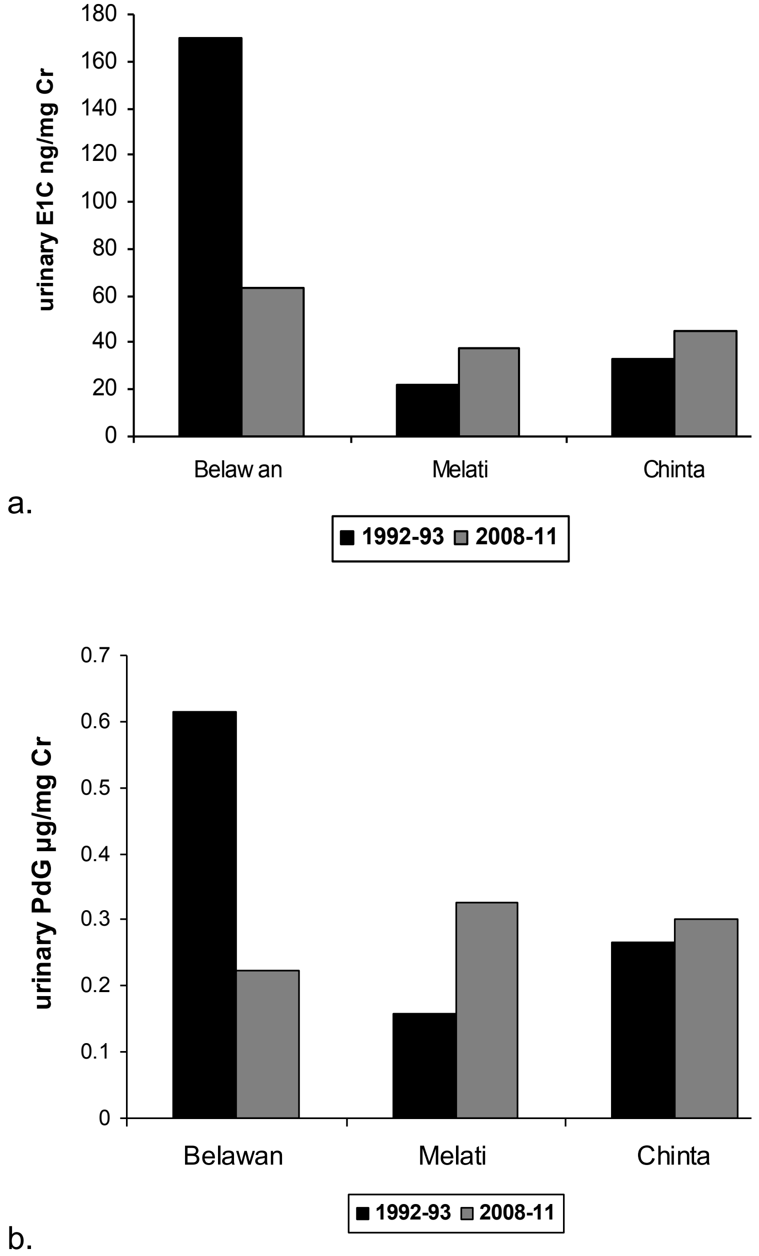

Figure 2-2. Average E_1C (a) and PdG (b) Concentrations in Samples for Belawan, Melati, and Chinta, 1992-1993 vs. 2008-2011.

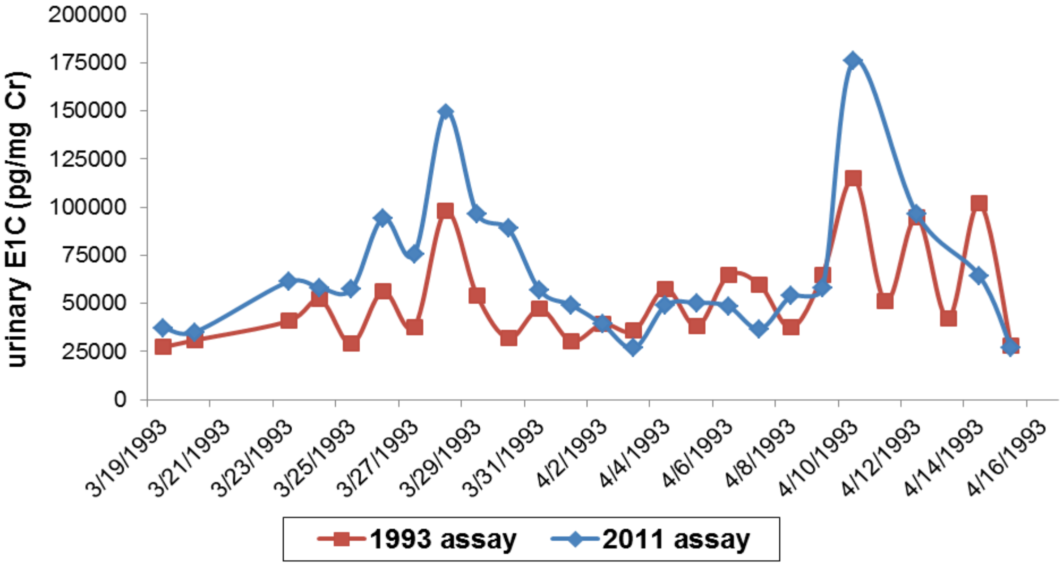

Figure 2-3. Urinary E_1C Levels During Chinta's March-April 1993 Cycle; A Comparison of Assay Results from 1993 and 2011. See text for details.

Identifying Cycle Onset and Phases

Information about the dates of cycles corresponding to the 1992-1993 Woodland Park Zoo urine samples was provided by Dr. Cheryl Knott (personal communication) based on her previous work with these samples. For the more recent samples (2007-2011), I identified the beginning and end of each menstrual cycle based on Hemastix charts provided by Woodland Park Zoo and Great Ape Trust staff. In both facilities, Hemastix are used to test each urine sample for blood, and the results are recorded on the chart. This method of identifying menstrual cycles is especially useful when studying female orangutans because they do not exhibit any visible estrus swelling to indicate the timing of ovulation.

In most cases, I was able to easily identify the onset of a menstrual cycle by visually examining the charts. The beginning of the cycle is characterized by between 2 and 8 days of positive Hemastix results (i.e. blood in the urine), and cycles occur approximately every 29 days. In the few instances in which Hemastix records were incomplete or inconclusive (N=3, excluding Knobi), I estimated the day of cycle onset based on the average duration of all cycles together with a single recorded day of blood in the urine. I followed the same guidelines to determine cycle parameters for Knobi (N=6), whose Hemastix data were more difficult to interpret due to frequent urinary tract infections.

In total my analyses include 98 cycles, as summarized in Table 2-3. The number of days sampled per cycle varies, with hormonal data available for 25-93% of days per cycle (mean= 57%). As previous studies of ovarian cycles in female orangutans have measured a maximum of 6 cycles from an individual female and cycles from less than a dozen females total (Collins et al. 1975; Inaba 1983; Masters and Markham 1991; Asa et al. 1994; Shimizu et al. 2003), these results substantially increase the number of cycles that have been quantified for female orangutans.

Once the parameters of each cycle were identified, I looked for evidence of the transition from the follicular to luteal phase by visually examining graphs of E_1C and PdG levels across individual cycles. Published research has

Table 2-3. Overview of All Cycles

Female	Dates Represented	Age	Number of Cycles	Average Cycle Length
Belawan	Feb. 1992-April 1993	10-11	5	29.6 days
Kelly	Feb. 1992-Dec. 1992	11-12	10	29.1 days
Allie	Feb. 2007-March 2009	12-14	20	32.9 days
Katy	July 2008-March 2009	19-20	4	29 days
Melati	Dec. 1992-April 1993	20-21	9	25.8 days
Chinta	June 1992-April 1993	24-25	12	30.2 days
Knobi	Aug. 2005-March 2006	25-26	6	30.8 days
Belawan	Sept. 2008-March 2011	27-29	13	27.5 days
Melati	Sept. 2008-Feb. 2011	36-39	10	28.2 days
Chinta	Nov. 2008-March 2011	40-43	9	31.8 days
			98	**29.7 days**[1]

[1] Because of her history of unusual cycles, Knobi's cycle lengths were not included in this calculation.

established that among apes, an estradiol surge in the late follicular phase precedes the release of the ovum and a sustained rise in estrogen and progesterone levels indicates the beginning of the luteal phase (Shimizu et al. 2003). In theory, the timing of ovulation can be estimated as the date between the estrogen and progesterone peaks, and the periovulatory period can be defined as ±6 days from ovulation (Stumpf et al. 2008). In practice, however, I found that many cycle graphs did not neatly conform to the expected pattern, or lacked sufficient data points to isolate the ovulation date using this method. As a result, I defined the periovulatory period as the mid-cycle day ±6 days. Using this definition, a clear ovulatory peak in estrogen values is encompassed within the POP in 75% of the cycles I examined.[2] In 84% of cycles, the sustained rise in progesterone levels associated with the onset of the luteal phase also begins within this period.

Composite Cycle and Indices of Cycle Quality

Composite Cycle

The composite cycle shown in Figure 2-4 illustrates the average urinary E_1C and PdG levels of a female orangutan across the ovarian cycle. I created this composite by combining data from 44 cycles representing 6 individuals, ranging in age from 10 to 43 years old. Belawan, Chinta, and Melati each contributed 2 sets of cycles, separated by 19 years, to the composite data. Allie, Katy, and Kelly are each represented by one set of cycles that spans 2 years or less. Due to unusual characteristics of her reproductive history (see above), I did not use any hormonal values from Knobi in calculations for the composite cycle. Details of the cycles used to create the composite are given in Table 2-4.

For each female, I selected the most complete available cycles to include in the composite calculations. In most cases, I was able to choose cycles

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[2] Of the remaining cycles, 87% did not exhibit any pronounced estradiol peak indicative of ovulation.

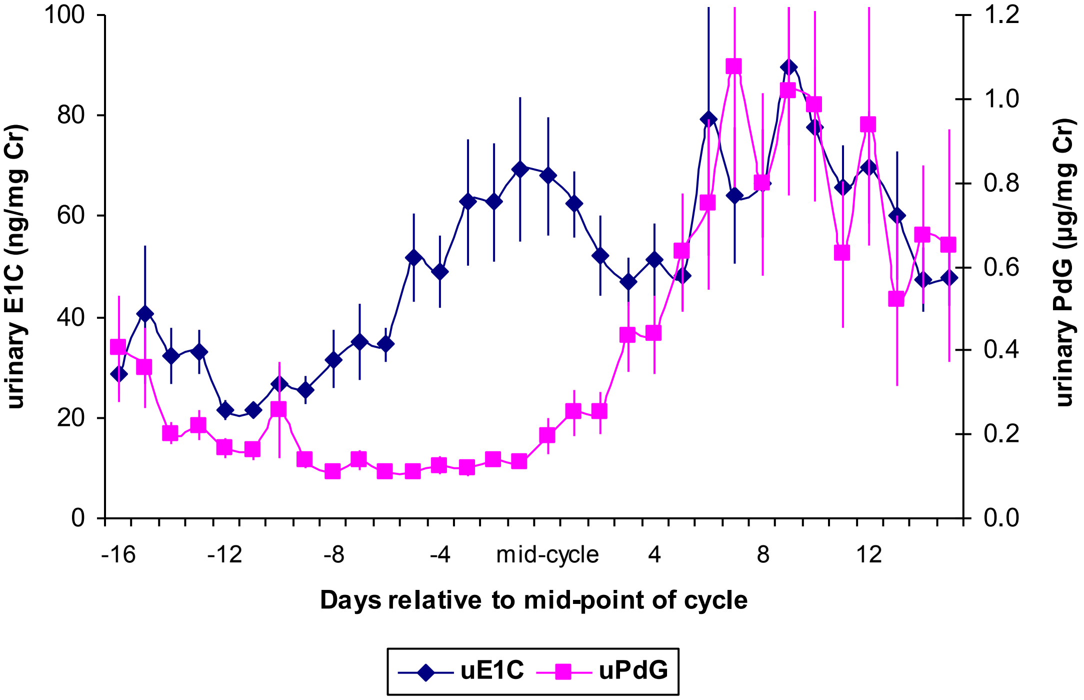

Figure 2-4. Composite Female Orangutan Cycle (Daily Means ± SE; N=7-33 samples per day)

missing no more than four consecutive days of hormonal data, but this was not always possible due to the absence or inviability of some samples. Of the cycles included in the composite, 9% are missing E_1C values for 5 or more consecutive days of the cycle and 29.5% are missing PdG values for 5 or more consecutive days. I was, however, able to limit this subset of cycles to those in which the missing data represents days toward the beginning or end of the cycle. This methodology prioritizes the mid-cycle (i.e. periovulatory) hormonal values. As a

Table 2-4. Overview of Cycles Included in the Composite Cycle

Female	Dates Represented	Age	Number of Cycles	Average Cycle Length
Belawan	Feb. 1992-April 1993	10-11	4	29 days
Kelly	Feb. 1992-Oct. 1992	11-12	5	29.2 days
Allie	Feb. 2007-Jan. 2009	12-14	5	31.8 days
Katy	July 2008-March 2009	19-20	4	29 days
Melati	Dec. 1992-April 1993	20-21	4	26.8 days
Chinta	June 1992-April 1993	24-25	6	30.7 days
Belawan	Nov. 2008-Feb. 2011	27-29	5	27.4 days
Melati	Sept. 2008-Feb. 2011	36-39	6	27.2 days
Chinta	Nov. 2008-March 2011	40-43	5	34.8 days
			44	**29.6 days**

result, only one cycle included in the composite is missing more than 4 consecutive days of E_1C or PdG results within the periovulatory period, defined as the mid-cycle day ± 6 days. I included this cycle, which represents data from 11-year-old Belawan, so that the composite would incorporate at least 4 cycles for each individual female in a given time frame.

I determined hormone values for each day of the composite cycle by aligning the 44 individual cycles at the mid-cycle day and calculating the mean E_1C and PdG value of each day. The maximum length of a cycle included in the composite was 39 days, and the average length of all 44 cycles was 29.6 days.

The days illustrated in Figure 2-4 are those for which more than 5 of the individual cycles included in the composite calculations yielded hormone values.

Several features of the composite cycle are worth noting. First, there are two estrogen peaks visible, the initial one occurring mid-cycle prior to ovulation, and the second occurring during the luteal phase. This is in accordance with previous descriptions of hormonal activity in the great apes (Czekala et al. 1988; Shimizu et al. 2003; see Chapter 1), although the results presented here emphasize that in orangutans, unlike humans and chimpanzees, the values of the luteal estrogen peak exceed those of the mid-cycle peak (Collins et al. 1975; Masters and Markham 1991). Second, the pre-ovulatory estrogen peak generally occurs just prior to the mid-cycle day, supporting the validity of this marker as an indicator of the periovulatory period. The timing of the peak luteal progesterone value is more variable, usually occurring between 8 and 11 days after the mid-cycle estrogen surge. Lastly, the maximum luteal progesterone values of 0.8-1.2µg/mg Cr illustrated here are comparable to those reported by Czekala *et al*. (1988), but far exceed the maximum value of 0.3µg/mg Cr more recently reported by Shimizu *et al.* (2003). This discrepancy may be due to the use of different assays and standards across studies. However, it is also noteworthy that both of these previous descriptions report on a single "representative example" of an orangutan cycle rather than the composite cycle presented here.

Indices of Cycle Quality

Throughout this dissertation, I use the term "quality" to refer to the fecundability of individual cycles (i.e. the monthly probability of conception) (Weinstein et al. 1990; Vitzthum 2008). This can be assessed in several ways. Previous research with humans (Lipson and Ellison 1996) and non-human primates (Masters and Markham 1991; Nadler and Collins 1991; Wasser 1996; Emery Thompson 2005b) has demonstrated that estrogen and progesterone levels are significant predictors of conception. Measuring absolute hormone concentrations thus offers one avenue by which to evaluate relative quality. In this study, I used several hormone measurements, some indicative of overall ovarian activity during the cycle and others focused specifically on the mid-cycle ovulatory stage, to compare cycles. A complete list of hormone values calculated for each cycle is provided in Table 2-5, along with a brief explanation of how estrogen and progesterone levels affect fecundability.

Because estrogen and progesterone must act synergistically in order for conception to occur, quality is also reflected in the overall shape of the curves that are produced by graphing daily E_1C and PdG values. I therefore used the shape of the curves in the composite cycle, which represents average ovarian function in a female orangutan, as a baseline to determine characteristics of cycle shape that can be used to differentiate higher-quality from lower-quality cycles. The resulting checklist that I used to assign a numeric "quality score" to each cycle is illustrated in Table 2-6. Scores ranged from 0-7, and provided

Table 2-5. Hormone Measurements Used to Assess Cycle Quality

Hormone Measurement	*Effect on Fecundability*
➢ Average E_1C	Estradiol concentrations are crucial to follicular development and affect both the probability of ovulation and the chance that an ovum will be fertilized (Eissa et al. 1986; Yoshimura and Wallach 1987). Luteal estrogen also plays a significant role in priming endometrial receptors to respond to luteal progesterone activity (Fritz et al. 1987; de Ziegler et al. 1998; Clancy 2009).
➢ E_1C 3-day average	
➢ E_1C 1st quarter	
➢ E_1C 2nd quarter	
➢ Periovulatory E_1C (mid-cycle day ± 6 days)	
➢ E_1C 2nd half	
➢ E_1C maximum and minimum	
➢ Average PdG	Progesterone levels in the luteal phase determine the thickness and secretory capacity of the endometrial lining and thus the probability of successful implantation (Lenton et al. 1988).
➢ PdG 3-day average	
➢ PdG 1st half	
➢ PdG 2nd half	
➢ PdG maximum and minimum	

Table 2-6. Characteristics of Cycle Shape Used to Assess Quality

Cycle Attribute	*Yes/No*
1. Ovulatory E_1C peak occurs ± 6 days from mid-cycle day	
2. Ovulatory E_1C peak >1 standard deviation above individual median value	
3. Ovulatory E_1C peak >2 standard deviations above individual median value	
4. Luteal E_1C peak > 1 standard deviation above individual median value	
5. Luteal PdG peak occurs 6-12 days after mid-cycle E_1C peak	
6. Luteal PdG peak >1 standard deviation above individual median value	
7. Luteal PdG peak >2 standard deviations above individual median value	

an additional framework for cycle comparisons.

Behavioral Data

Great Ape Trust records provide behavioral data for Knobi and Allie from 2005-2009. From the Woodland Park Zoo, behavioral records representing the 2008-2011 data collection period are available for Chinta, Melati, and Belawan, but there are very few behavioral observations from 1992-1993. No behavioral data are available from Katy, who arrived at the Great Ape Trust relatively late in the period of data collection, or Kelly, who was physically separated from the other orangutans during her stay at the Woodland Park Zoo.

At both sites, behavioral data were collected opportunistically by keeper staff. While this methodology allows for the possibility that some of the orangutans' sexual behaviors and/or mating encounters were not recorded or described, the results presented here are likely to be comparably robust to data collected at orangutan field sites, where ecological constraints (e.g. difficulty locating or identifying individuals, limited dyadic interactions, problems of visibility in the rainforest) place limits on behavioral observations. With captive animals, observers have the advantage of knowing subject identity and age, and individuals' interactions can be monitored more consistently over long periods of time, at a shorter observation distance and without visual obstruction.

Records of the orangutans' mating behaviors at the Great Ape Trust include observations made by a total of four keepers. Although no formal observation schedule was maintained, copulations that occurred between the hours of 8am and 5pm were very likely to have been noted (S. Wich, personal communication). There is, however, significant variation in the degree to which reproductive behaviors were described in detail. On some days, records indicate only that one or more copulations took place between specific individuals. On other days, recorded observations include a thorough description of male long calls, displays, and coercive behaviors, as well as information about female proceptive and prosexual behaviors. Table 2-7 provides examples of some of the more robust observations from the Great Ape Trust records.

Table 2-7. Examples of Behavioral Notes from Great Ape Trust Records

Date	Behavioral Notes
9/15/2005	One copulation between Azy and Knobi following Azy long call. Knobi approached and presented. Relatively long episode including oral and genital copulation.
12/24/2005	One AM copulation (approx. 30 minutes) between Azy and Knobi. Very mutual, several position changes (V-V, D-V). Second copulation later in the day after Azy displayed to [male researcher].
1/28/2006	Possible copulation between Azy and Allie before keepers arrived (based on behaviors, locations, wounds on Allie's hands). Copulation between Azy and Knobi following long call.
8/5/2006	Azy approaches Allie and Knobi interferes and initiates contact (nipple against penis) -- no copulation results. Later in the day Azy displays and heads for Allie; Knobi interrupts and replaces Allie.

Data in the 2008-2011 records from the Woodland Park Zoo were collected by a total of five keepers, although the majority of observations were made by three people. As with the Great Ape Trust records, the amount of detail provided about particular reproductive encounters varies (Table 2-8). The probability that copulations occurred unobserved by keepers is also higher at the Woodland Park Zoo than the Great Ape Trust (L. Lawson, personal communication), largely because keepers are responsible for the maintenance and observation of other animals in addition to the orangutans.

Table 2-8. Examples of Behavioral Notes from Woodland Park Zoo Records

Date	Behavioral Notes
9/12/2008	Heran forced copulation with Chinta today at 10:00. 2 forced copulations yesterday at 11:00 and 17:00.
4/5/2009	Melati and Heran copulate (forced copulation).
1/13/2010	Bela solicitous toward Towan from mid-day on. No copulations observed.
4/6/2010	Bela solicited Towan for copulation. In general Bela was very excited, active, and alert today. She encouraged Towan to display and would watch Heran's reaction. She presented her genitals to Towan. She was acting threatening to female keepers and aides. Towan observed copulating with Bela.

To enable a systematic analysis of the behavioral records from both institutions, I created a database that includes, for each individual female, a binary measure of each day's mating outcome (Y/N). This provided the foundation for all investigations of the relationship between mating and hormone values and/or cycle phase. When appropriate information was available, I also noted (a) days on which multiple copulations took place, (b) instances of inspection or oral stimulation by males or females, (c) the presence or absence of proceptive or resistant behaviors from females, (d) instances of forced copulation, and (e) information about the occurrence of male displays and long calls. These additional criteria are especially relevant in the case of Knobi, who mates at a higher frequency than any of the other females.

Discussion

This chapter has detailed the laboratory and analytical methods that I apply in the remainder of this dissertation to examine ovarian function and reproductive behaviors at different points throughout the life cycle of captive female orangutans. The results reveal that urinary immunoassays provide consistent and accurate estimates of ovarian steroid production in this sample of females, and that hormonal characteristics can be used to identify cycle phase and compare the fecundability of individual cycles. The behavioral analyses in this study benefit from the fact that the reproductive and social histories of each orangutan are known in detail, as this allows for a better understanding of individual idiosyncrasies and dyadic relationships.

Chapter 3: Ovarian Function and Reproductive Behaviors of Adolescent Female Orangutans

Introduction

The developmental process of puberty involves a combination of neuroendocrine, morphological, and behavioral changes that culminate in reproductive capability (DiVall and Radovick 2008). Studies of puberty in humans are common and have examined factors including the genes underlying pubertal timing (Palmert and Boepple 2001) and the impact of morphological, nutritional, and other environmental factors on the initiation and progression of reproductive development (Kirkwood et al. 1987; Vizmanos and Marti-Henneberg 2000; Palmert and Boepple 2001; Ebling 2005; Gluckman and Hanson 2006; DiVall and Radovick 2008). International demographic data, meanwhile, have established an average human age at menarche of 13.5-13.6 years (Marshall and Tanner 1968; Thomas et al. 2001).

Less is known about the timing and pace of reproductive maturation in other ape species. When research is conducted in natural habitats, observers of female chimpanzees can identify the onset of adolescence based on the earliest development of anogenital swellings, which indicate an increase in circulating ovarian steroid hormones surrounding ovulation and may precede the earliest menstrual cycles by several months (Ashley-Montagu 1939a; Pusey 1990; Wallis 1997; Nishida et al. 2003; Mori et al. 2007). However, this methodology does not work when observing female orangutans, who exhibit no sexual swelling (Galdikas 1981; Schürmann 1982) or visible signs of menstruation (i.e. vaginal bleeding) (Profet 1993; Galdikas 1995; Shumaker et al. 2008). As a result, studies of female orangutans in the wild have relied largely on behavioral observations to identify the transition from juvenility to adolescence, focusing on the emergence of proceptive sexual behaviors and copulatory events (Galdikas 1995).

The onset and normalization of ovulatory cycling is in theory easier to study in captive animals, either by testing urine for the presence of menstrual blood or by directly examining fluctuations in ovarian hormone levels. To date, however, information about the average age at menarche in captive chimpanzees, gorillas, and orangutans is drawn from only a handful of studies representing a small number of subjects (Young and Yerkes 1943; Coe et al. 1979; Dixson 1981; Markham 1995). Most descriptions of adolescent development in captive apes therefore echo field studies, drawing heavily on observations of emerging sexual behaviors and obvious milestones such as the age at first parturition (Coe et al. 1979; Courtenay 1987; Sievert et al. 1991; Bellem et al. 1995; Markham 1995; Anderson et al. 2008). A summary of published information about pubertal markers in captive orangutans is provided in Table 3-1, alongside data from field studies.

Table 3-1. Reported Ages at Menarche, First Sexual Behavior, and First Parturition in Captive and Wild Orangutan Populations

	Menarche	First Sexual Behavior	First Parturition
Captive [unspecified]	7.7 (4.5-11.1, *n*=8)[1]	(5.5-9)[1]	11.25 (mode=9-9.9, *n*=372)[2]
Captive [Bornean]			15.5 (*n*=88)[3]
Captive [Sumatran]			16.4 (*n*=101)[3]
Wild [unspecified]	(9-11)[2]		(12-15)[2]
Wild [Bornean]		11 (*n*=1)[4]	15 (*n*=1)[4] 15.7 (15-16, *n*=3)[5]
Wild [Sumatran]			14.7 (12-16, *n*=3)[5] 15.4 (13-18, *n*=7)[6]
Rehabilitant Bornean			11.6 (8-15, *n*=13)[7]

Table adapted from (Knott 2001). Values represent mean ages. If available, ranges and sample sizes are provided in parentheses.
1. (Knott 2001)
2. (Markham 1995)
3. (Anderson et al. 2008)
4. (Galdikas 1981)
5. (Tilson et al. 1993)
6. (Wich et al. 2004)
7. (Kuze et al. 2008)

More detailed records of the hormonal changes that female apes experience during adolescence are necessary to fully elucidate each species' life history pattern and the selective pressures that have shaped it. In particular, quantified ovarian cycles from pubertal females will help characterize and demarcate inferred periods of adolescent subfecundity (Galdikas 1981). Such an endocrinological period is often used as an explanation for why males reject proceptive sexual behaviors from adolescent females, but has not been hormonally verified either in the wild or in captivity (Shumaker et al. 2008).

In this chapter, I test several hypotheses involving the ovarian function and reproductive behaviors of adolescent female orangutans. I begin by examining ovarian hormone activity from three females between the ages of 10 and 12 and looking for evidence of menarche and the normalization of menstrual cycling. I then look at behavioral data from two of these females to examine adolescent proceptivity and assess the relationship between ovarian steroid concentrations, cycle phase, and mating behaviors. Finally, I compare the estrogen and progesterone levels of these adolescent females to those of cycling adult females to determine whether a significant difference in ovarian function exists between these two age groups. If the observed reluctance of adult males to mate with young females is the result of a period of adolescent subfecundity, significant differences are expected.

Methods

Urine and behavioral data were collected from female orangutans at the Woodland Park Zoo and the Great Ape Trust, together with behavioral data from males at these locations (see Chapter 2). The hormone data in this chapter derive from approximately 1,250 urine samples representing 66 cycles (Table 3-

2). Endocrine sampling and assay methods are described in Chapter 2 and Appendix B.

Table 3-2. Overview of Cycles Described and Analyzed in Chapter 3.

Female	Dates Represented	Age	Number of Cycles
Belawan	Feb. 1992-Apr. 1993	10-11	5
Kelly	Feb.-Dec. 1992	11-12	10
Allie	Aug. 2006-Jan. 2008	11-12	11
Allie	Jan.-Mar. 2009	14	2
Katy	July 2008-Mar. 2009	19-20	4
Melati	Dec. 1992-Apr. 1993	20-21	9
Chinta	June 1992-Apr. 1993	24-25	12
Belawan	Sept. 2008-Mar. 2011	27-29	13
			66

To categorize individuals by age group, I began with Galdikas' demarcation of 9-15 years as the adolescent period in female orangutans (Galdikas 1981, 1995). However, because subsequent examination of hormonal data revealed that by January 2009 Allie had been cycling normally for two years (see below), I elected to classify Allie as an adult at age 14.

As detailed in Chapter 2, I evaluated the quality of individual cycles by comparing absolute measurements of ovarian activity, and by calculating a broad "quality score" for each cycle based on the pattern of E_1C and PdG values.

Statistical Methods

In all statistical analyses involving comparisons of adolescent and adult females' hormone values, Group A includes females age 10-12 and Group B includes females age 14-29 (See Table 3-2).

Because the hormonal data under consideration represent multiple cycles from a small number of individuals, two of whom are represented in both age group categories, I used a linear mixed model to compare ovarian function across Groups A and B. Linear mixed modeling is a useful statistical tool in cases involving non-independent data points because it allows for repeated measures and unequal sample sizes and controls for individual identity (i.e. potential correlations within subjects across time) (Heistermann et al. 2007; Garson 2012). In this case, I separately modeled the relationship of cycle length and log-transformed ovarian hormone values to age group, controlling for subject effects.

In a separate analysis of the longitudinal data from Belawan, I compared her adolescent and adult hormone values using a Mann-Whitney U-test. I conducted non-parametric analyses on these data because of the particularly small sample size, and because hormone data rarely have a normal distribution.

The probability that hormone concentrations and cycle phase had predictable effects on Allie's mating outcomes was analyzed using binary logistic regression.

All statistical analyses were performed using IBM SPSS v.20.

Results

Ovarian Hormone Production during Puberty

Allie

Allie was the only one of the three adolescent females examined to exhibit a clear hormonal transition indicative of the onset of menses and the normalization of ovulatory cycles during the period of urine collection reported above. Shumaker *et al*. (2008) have previously discussed some details of Allie's pubertal development, but their results were derived only from an analysis of blood in her urine samples. The hormonal assays I have conducted serve to confirm Allie's developmental timeline as reported by Shumaker *et al*. (2008), but also provide additional details about Allie's earliest cycles. Table 3-3 summarizes these results.

Figure 3-1 displays several months of hormonal data from Allie just prior to her 11th birthday. Although urinalysis revealed the presence of blood in 29% of samples represented on this graph, those days were not distributed in a recognizable pattern. Likewise, the hormonal data reveal no definable follicular or luteal phases, and no estrogen or progesterone peaks indicative of ovulatory events. During this time period, urinary estrone conjugate values did not exceed 5 ng/mg Cr (N=57 samples) and urinary PdG values remained below 0.7 µg/mg Cr (N=59 samples)[3].

Table 3-3. Summary of Information Regarding the Pubertal Maturation of Allie, Ages 10-12.

Dates	Age	Cycle[a]	Duration[b]
November 2005-July 2006	10-11	no evidence of cycles or menses	
August-October 2006	11	A	73 days
October 2006-February 2007	11-12	B	109 days
February-March 2007	12	1	33 days
March-April 2007	12	2	33 days

a. Numbering of cycles differs from Shumaker *et al*. (2008). I use letter designations A and B for Allie's initial cycles to denote that each is significantly longer than the average orangutan cycle. Subsequent cycles of species-typical length are numerically labeled.
b. Interval from first day of menstrual bleeding to onset of next menses.

Figure 3-2 (a and b), by contrast, illustrates the earliest ovulatory cycles for Allie, which were identified by Shumaker *et al*. (2008) and confirmed during this study. Each of these cycles significantly exceeds the average cycle length of 29.7 days calculated during this project (see Table 3-3), a pattern also observed in human girls' early cycles (Treloar et al. 1967). As is the case in humans

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[3] Progesterone concentrations of 0.7µg/mg Cr would be indicative of increased luteal-phase activity in most female orangutans (see Chapter 2), but Allie consistently displays above-average progesterone concentrations prior to and after the onset of cycles. See below for further discussion.

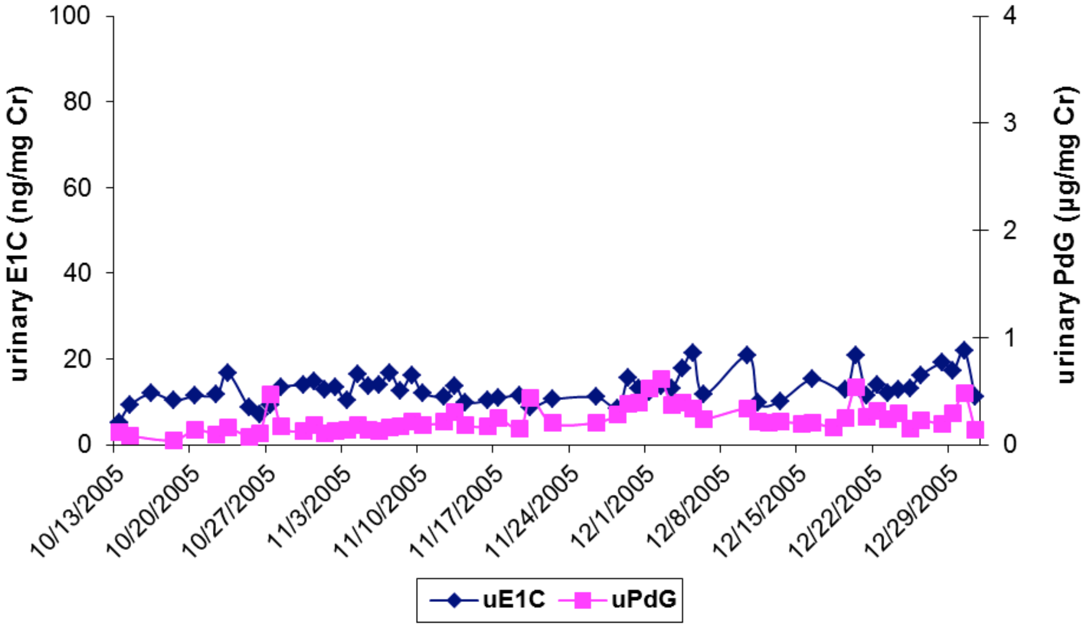
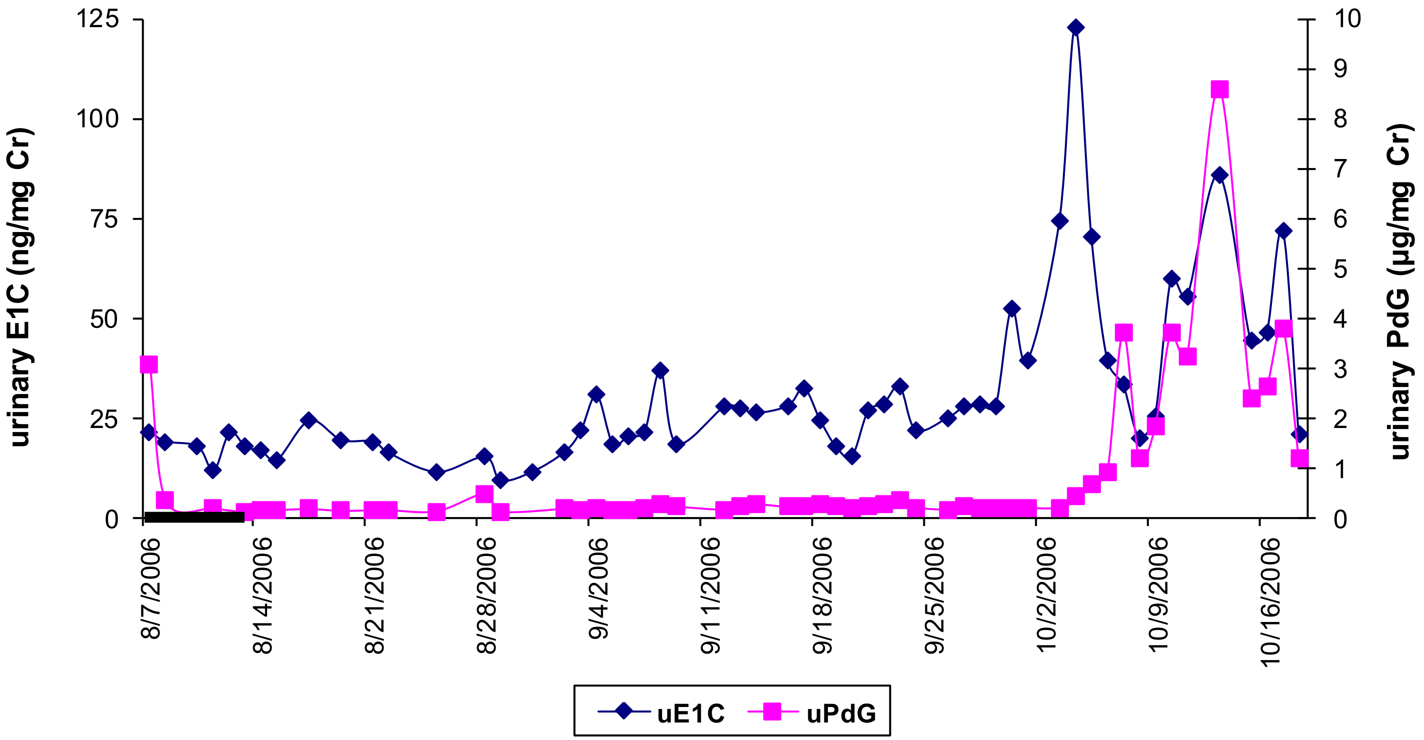


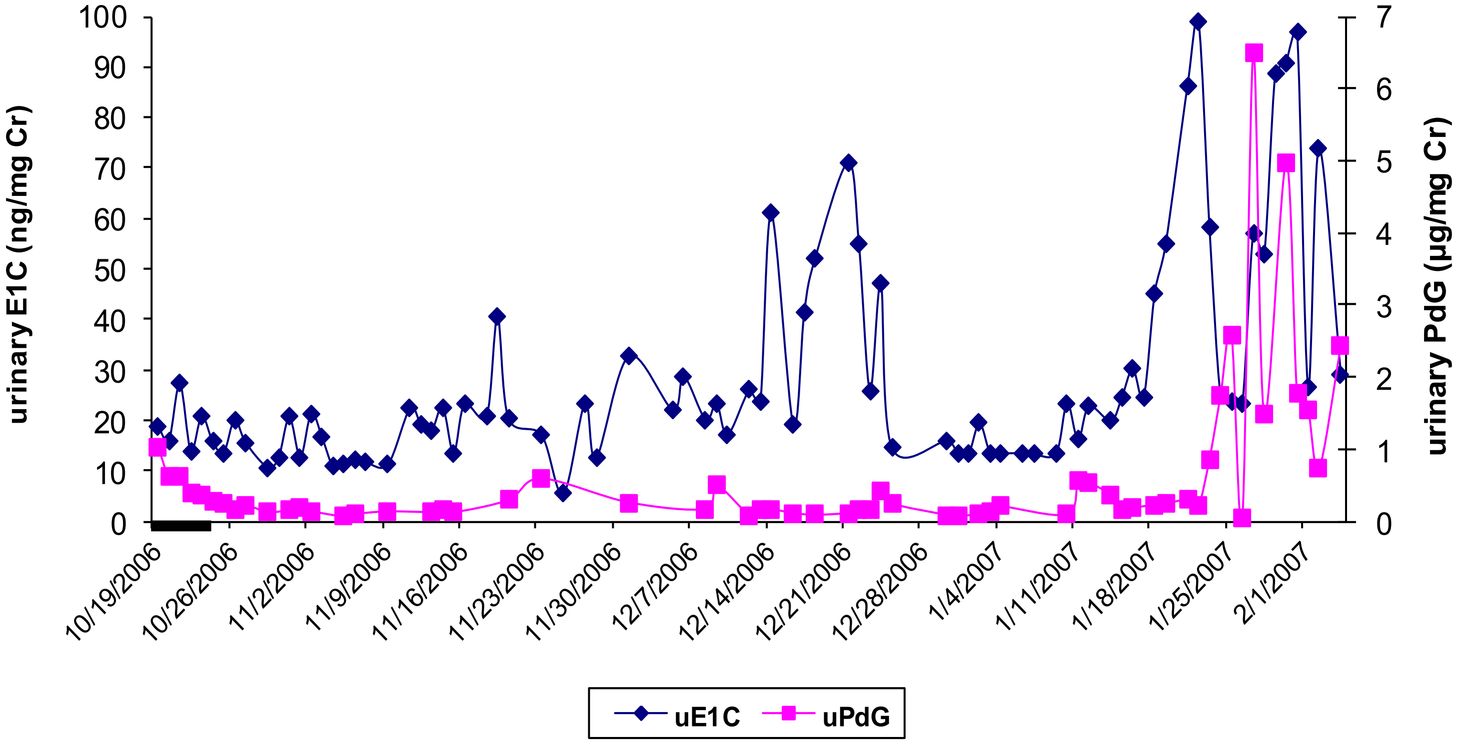
Figure 3-1. Urinary Estrogen and Progesterone Data for 10-year-old Allie, October-December 2005

(Sherman and Korenman 1975), hormonal analyses reveal that the length of these early cycles is primarily the result of an extended follicular phase. Cycle A, for example, has a follicular phase of 59 days, followed by a more species-typical luteal phase of 14 days. Cycle B follows the same pattern, with a follicular phase lasting 95 days and a 14-day luteal phase. There is, however, a mid-December estradiol peak during cycle B that is not followed by the sustained rise in progesterone levels characteristic of the luteal phase. This type of asynchronous hormonal activity without subsequent menstrual bleeding was not evident in the



a.

Figure 3-2. Earliest Cycles of Adolescent Allie (age 11-12): August-October 2006 (a) and October 2006-February 2007 (b). Black bar indicates menstrual period as determined by Hemastix results.



b.

Figure 3-2. Earliest Cycles of Adolescent Allie (age 11-12): August-October 2006 (a) and October 2006-February 2007 (b). Black bar indicates menstrual period as determined by Hemastix results.

analysis of Shumaker *et al*. (2008), but may be characteristic of the normalization of ovarian cycling during puberty.

The peak urinary E_1C and PdG values in cycles A and B, which are significantly higher than those illustrated in Figure 3-1, illustrate that despite their prolonged length, each of these cycles was characterized by ovulation and subsequent endometrial development. E_1C concentrations reach as high as 120 ng/mg Cr in cycle A, and almost crest 100 ng/mg Cr in Cycle B. Peak PdG values are 8.6 and 6.5 µg/mg Cr, respectively, and average PdG concentrations during the 14-day luteal phase of each cycle remain above 2.2 µg/mg Cr.

By February-March 2007 Allie's ovarian hormone and menstrual cycle patterns had normalized to those of a typical female orangutan, albeit with a longer than average follicular phase and consequently later ovulatory estradiol peak (Figure 3-3). It is worth noting, however, that she continued to display very high levels of progesterone, especially in the second half (i.e. luteal phase) of the cycle (Table 3-6). From February 2007 through March 2009, Allie's peak urinary PdG values exceeded 4 µg/mg Cr in 80% of cycles. This is in stark contrast to the other females whose progesterone values were analyzed in this chapter: of 49 cycles from those 4 females, the peak urinary PdG concentration was above 3 µg/mg Cr in only 1 cycle, and the peak remained below 1µg/mg Cr in 36 cycles.

As mentioned in Chapter 1, anovulatory cycles are often thought to be a major cause of adolescent subfecundity. Resko *et al*. (1982) documented that the frequency of anovulatory cycles in adolescent rhesus monkeys was highest immediately following menarche, and decreased with time. I thus examined Allie's data for evidence of anovulatory cycles, but did not find a similar pattern of change over time: of the nine cycles she exhibited from February-December 2007, only one might be classified as anovulatory. All other cycles during this time had a quality score of 6 or 7 which, together with robust ovarian steroid concentrations, suggests that Allie experienced only 6 months of subfecundity following the onset of menses. This is much less prolonged than the primate subfecund period of 1-5 years that is estimated in many references (Schurmann and van Hooff 1986; Markham 1990; Pusey 1990; Galdikas 1995; Wallis 1997; Knott 2001; Saltzman et al. 2010).

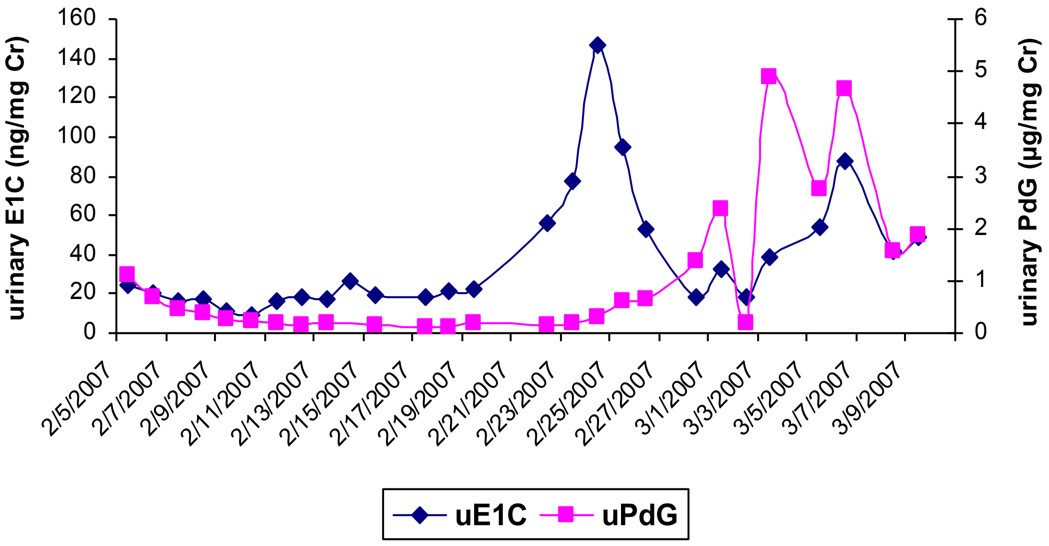
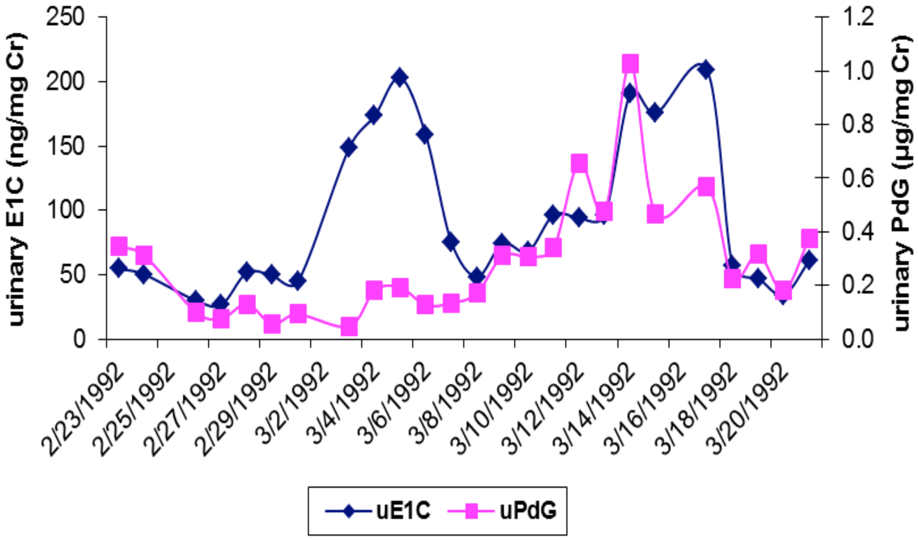


Figure 3-3. Urinary Estrogen and Progesterone Data for Allie, February-March 2007.

Belawan and Kelly

Belawan and Kelly were 10 and 11 years old, respectively, at the time urine collection began, and Hemastix data indicate that each already exhibited regular menstrual cycles (Belawan mean cycle length=29.6 days; Kelly mean cycle length = 29.1 days). The E_1C and PdG assays that I conducted revealed no instances of anovulatory cycles in either female during the time considered. However, variation in the quality of cycles is apparent when hormone values and the shape of the ovarian steroid curves are compared across cycles. Figure 3-4 illustrates an example of a higher-quality and lower-quality cycle for Kelly; although each is ovulatory, maximum hormone concentrations are roughly twice as large in the former.

Due to the relatively low number of samples that are available to characterize each of Belawan's adolescent cycles, the shape of ovarian steroid curves is difficult to assess. However, E_1C concentrations are unusually high in all five of the cycles examined (Table 3-6). Belawan's PdG values during these cycles are also above-average, but do not reach concentrations comparable to those seen in Allie's cycles.



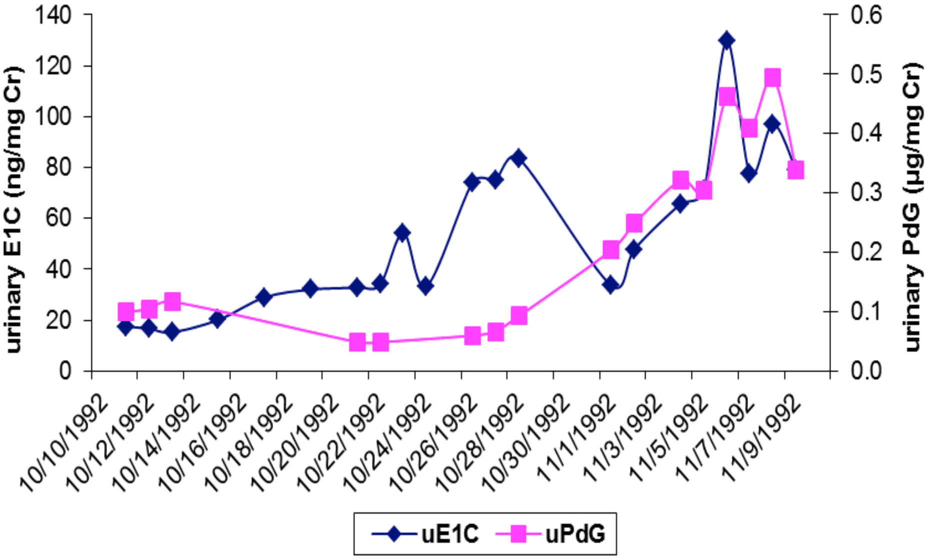


Figure 3-4. Representative Higher-Quality (top) and Lower-Quality (bottom) Cycles of Adolescent Kelly.

Mating Data

Allie

Behavioral records from November 2005 to January 2007, prior to the onset of regular ovulatory cycling, denote copulations between Allie and Azy as follows (Figure 3-5):

- November 2005-July 2006: Copulations were observed on eight days. None of these mating encounters involved proceptive behaviors; rather, Allie resisted copulation in four of the encounters.
- August-October 2006 (Cycle A): One copulation was observed.
- October 2006-January 2007 (Cycle B): Copulations were observed on six days. Two of these were during a period of menstrual discharge (as indicated by Hemastix results). Allie exhibited no proceptive behaviors.

These data indicate that all mating encounters during this time were initiated by Azy, and reveal a distinct lack of proceptivity by Allie. This is in sharp contrast to Belawan's behavioral data, as I will discuss below.

During nine ovulatory cycles from February 2007 to January 2008, copulations between Allie and Azy were observed on 25 days (Figures 3-5 and 3-6). The present data suggest that average E1C values were higher on mating than non-mating days, but the difference was not statistically significant (Figure 3-7; Mann-Whitney U, z=-.553, two-tailed p=.581). The results of logistic regression analyses examining the effects of hormonal variables and cycle phase on the probability of mating during these cycles are presented in Table 3-4 and

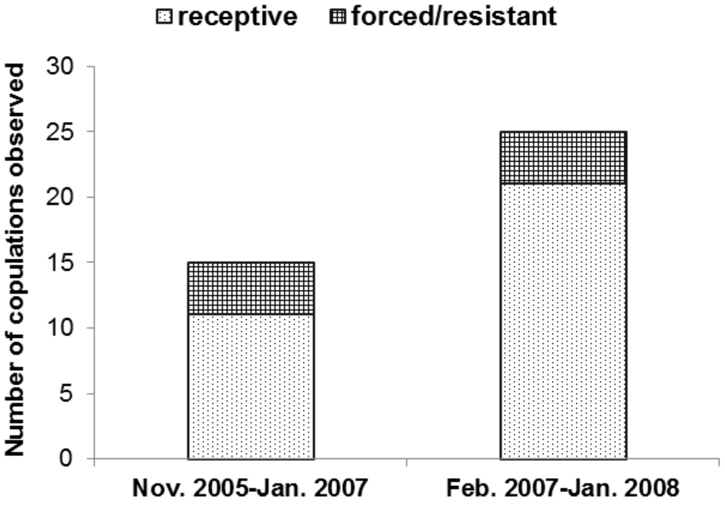


Figure 3-5. Copulations Observed Between Allie and Azy from November 2005-January 2008, by Type.

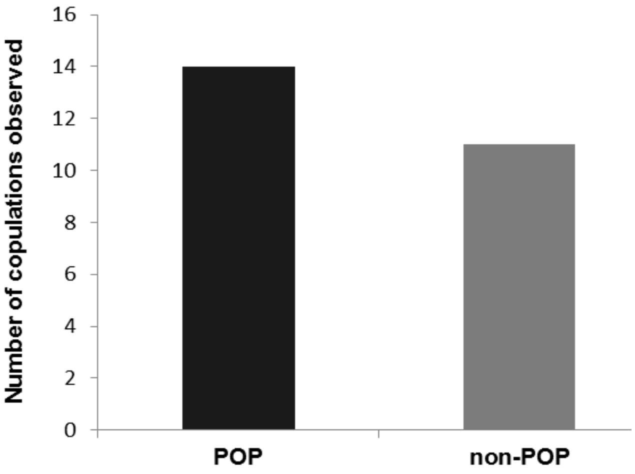


Figure 3-6. Number of Copulations by Ovulatory Status for Allie, February 2007-January 2008. POP= periovulatory period. No more than one copulation observed per day.

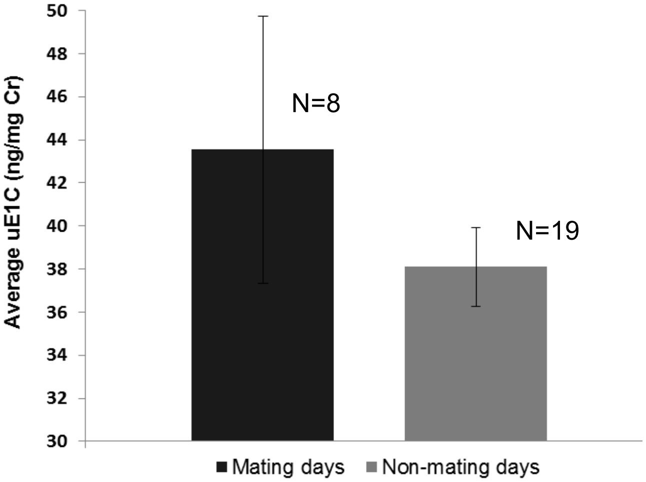


Figure 3-7. Average Urinary E_1C (mean ± SE) for Allie on Mating vs. Non-Mating Days, February 2007-January 2008. No significant difference (Mann-Whitney U, z=-.553, p>.05)

Table 3-5. No significant relationships were found, although the effect of ovulatory status on mating probability approached significance (p=.067). Once again, Allie exhibited no proceptive behaviors during this time.

Table 3-4. Hormonal Predictors of Mating for Allie, February 2007-January 2008 (N=22 mating days)

	df	Wald-Z	p-value
Daily E_1C value	1	.855	.355
E_1C 3-day average	1	1.104	.293
E_1C: PdG ratio	1	.687	.407

Table 3-5. Result of Logistic Regression Analysis of the Relationship Between Periovulatory Phase and Mating Probability for Allie, February 2007-January 2008

	β±SE	Wald-Z	p-value	OR (95%CI)
Periovulatory period	-.771+.422	3.346	.067	.462 (.202:1.057)

β±SE shows the coefficient and its associated SEs in logits and OR shows the transformed odds ratios with an associated 95% confidence interval.

Belawan

I could not conduct logistic regression analyses with Belawan's behavioral data because she was observed copulating only once during the five cycles that I was able to characterize with hormonal data. However, behavioral records spanning February 1992-July 1993 indicate a high degree of proceptivity. Belawan pursued and presented to Towan on at least 12 days on which urine samples were unavailable, and copulations resulted on at least three of these days. Keepers' notes further indicate at least five days on which Towan initiated sexual encounters with Belawan.

Adolescent vs. Adult Ovarian Cycle Profiles

Table 3-6 provides an overview of the ovarian indices measured for each adolescent and adult female.

Table 3-6. Urinary Indices of Ovarian Function in Adolescent and Adult Female Orangutans

	Average E_1C (ng/mg Cr)	Average PdG (µg/mg Cr)	Periovulatory E_1C (ng/mg Cr)	PdG, 2nd half (µg/mg Cr)	Maximum E_1C (ng/mg Cr)	Minimum E_1C (ng/mg Cr)	Maximum PdG (µg/mg Cr)	Minimum PdG (µg/mg Cr)
Belawan [10-11] (*n*=5)	170.1 ± 24.8	0.6 ± 0.1	187.2 ± 63.4	0.8 ± 0.1	380.7 ± 72.0	70.1 ± 15.3	1.7 ± 0.5	0.1 ± 0.0
Kelly [11-12] (*n*=10)	66.3 ± 6.4	0.3 ± 0.0	67.6 ± 7.2	0.4 ± 0.0	185.4 ± 25.7	18.0 ± 1.9	1.0 ± 0.2	0.0 ± 0.0
Allie [11-12] (*n*=11)	36.6 ± 2.0	1.1 ± 0.1	48.3 ± 4.3 (*n*=9)[a]	2.0 ± 0.2 (*n*=9)[a]	111.4 ± 10.6	12.7 ± 1.3	5.4 ± 0.6	0.1 ± 0.0
Allie [14] (*n*=2)	59.9 ± 16.1	1.2 ± 0.4	63.8 ± 17.9	1.9 ± 0.7	125.6 ± 41.2	13.3 ± 4.4	4.8 ± 2.6	0.2 ± 0.0
Katy [19-20] (*n*=4)	47.2 ± 8.5	n/a[b]	52.8 ± 7.5	n/a[b]	127.6 ± 24.4	10.8 ± 1.5	n/a[b]	n/a[b]
Melati [20-21] (*n*=9)	21.6 ± 3.9	0.2 ± 0.0	21.7 ± 4.9	0.2 ± 0.0	55.8 ± 25.5	11.4 ± 0.9	0.4 ± 0.1	0.1 ± 0.0
Chinta [24-25] (*n*=12)	33.0 ± 3.8	0.3 ± 0.0	34.9 ± 4.9	0.4 ± 0.1	80.9 ± 13.4	14.3 ± 1.4	1.0 ± 0.2	0.1 ± 0.0
Belawan [27-29] (*n*=13)	63.3 ± 3.2	0.2 ± 0.0	67.7 ± 3.2	0.3 ± 0.0	127.5 ± 9.2	21.5 ± 1.9	0.5 ± 0.0	0.1 ± 0.0

Steroid levels are reported as mean ± standard error. Age is given in brackets. Sample sizes (in parentheses) match the number of cycles given in Table 3-2 unless otherwise noted.

a. Cycles A and B were omitted from these calculations due to their unusual length, which prevented me from applying the previously described definition of the periovulatory period and ensured that examination of PdG values in the 2nd half of the cycle would not accurately represent the post-ovulatory progesterone rise.

b. PdG results for Katy were inconsistent, and have not been used in cycle analyses.

A linear mixed model analysis revealed that all measures of ovarian function except minimum PdG levels differ significantly between the two age groups. However, contrary to the pattern expected if the period of adolescence is characterized by subfecund measures of ovarian activity, steroid concentrations were consistently higher in Group A (adolescent females) than in Group B. Individual identity was not a significant factor in any of the analyses. The results of the linear mixed modeling analysis are presented in Table 3-7 and Figure 3-8.

Table 3-7. Results of Linear Mixed Modeling Analysis Comparing Hormone Values by Age Group

	Parameter Estimate	**t-value**	**Significance**
Average E_1C	.231	3.640	**.001**
Average PdG	.291	4.226	**.000**
Periovulatory (POP) E_1C	.225	3.153	**.003**
PdG, 2nd half of cycle	.291	3.626	**.001**
Max E_1C	.308	3.539	**.001**
Min E_1C	.317	4.227	**.000**
Max PdG	.368	4.042	**.000**
Min PdG	.069	1.054	.297

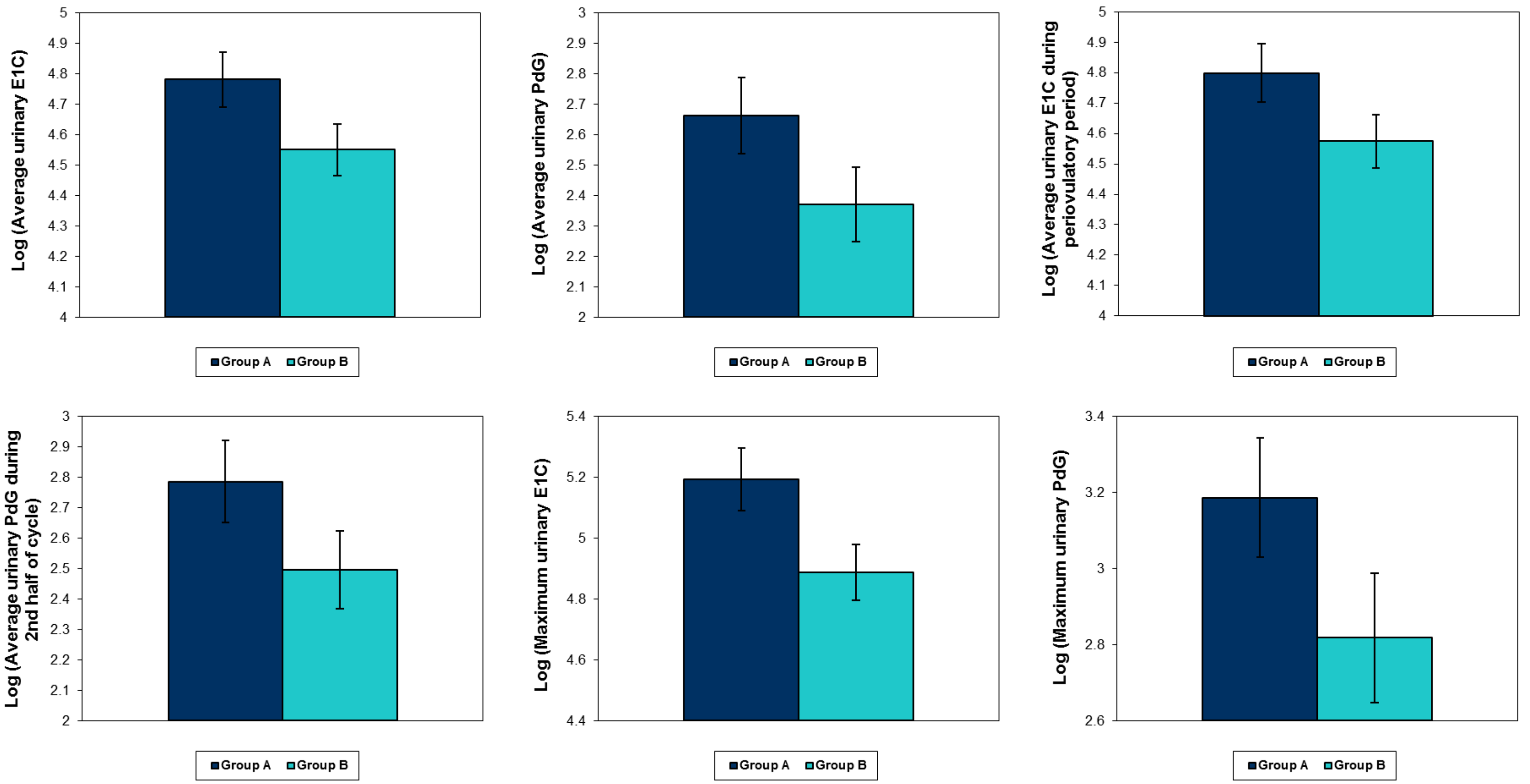


Figure 3-8. Ovarian Hormone Indices of Females Age 10-12 (Group A) vs. Females Age 14-29 (Group B). Graphs illustrate estimated mean ± SE predicted by mixed model, controlling for subject. $p \leq .003$ for all comparisons shown. Comparisons of minimum E_1C ($p=.000$) and PdG ($p>.05$) values are not shown.

A separate longitudinal comparison of Belawan’s samples showed the same relationship between adolescent and adult hormone values. All measures of ovarian function decreased from 1992-1993 (N= 5 cycles) to 2008-2011 (N= 13 cycles) (Table 3-8).

Table 3-8. Longitudinal Comparison of Hormone Values for Belawan’s Samples, 1992-1993 vs. 2008-2011 (Mann-Whitney U-test, 2-tailed p-values)

	z-value	**Significance**	**Direction of Change**
Average E_1C	-3.204	**.001**	Decrease
Average PdG	-3.204	**.001**	Decrease
Periovulatory (POP) E_1C	-3.204	**.001**	Decrease
PdG, 2nd half of cycle	-3.204	**.001**	Decrease
Max E_1C	-3.204	**.001**	Decrease
Min E_1C	-2.908	**.004**	Decrease
Max PdG	-3.204	**.001**	Decrease
Min PdG	-2.316	**.021**	Decrease

Discussion

Great ape researchers have long observed that adolescent females and mature males are not equally motivated to unite as mating partners (Muller et al. 2006). Galdikas and others, for example, have reported that while free-ranging adolescent female orangutans commonly display proceptive behaviors and frequently attempt to initiate and maintain consort relationships with flanged males, they are often ignored (Schürmann 1982; Galdikas 1995). This pattern is contrary to the predictions of evolutionary theory, which posits that males in species without paternal investment can maximize their reproductive success by copulating with every potentially fecund female (Bateman 1948). Within this framework, male orangutans, constrained by the species' 6-8 year average interbirth interval and the consequent fact that few cycling females are available at any given time, would be expected to take advantage of the additional reproductive opportunities provided by adolescent females. So why don't they?

Based on observations of a lag time between the onset of sexual activity and first parturition among wild apes, adolescent subfecundity is frequently offered as a "just so" explanation for males' disinterest. In other words, primatologists hypothesize that males reject mating opportunities that are unlikely to result in the production of offspring. However, hormonal data verifying subfecundity during female reproductive maturation are lacking. In addition, no hypotheses have been proposed regarding the possible costs that could have selected for males that mate discriminately.

This chapter has quantified several endocrinological characteristics of cycles from three adolescent females to examine how the pubertal development of female orangutans is reflected in ovarian function. Results from one female offer some indication that early cycles may be characterized by a prolonged follicular phase and/or ovarian steroid activity that is unlikely to result in ovulation or implantation, but cycles from two other females aged 10-11 exhibit no signs of anovulation or subfecund ovarian function at the time of investigation. Instead, cross-sectional and longitudinal comparisons of the hormone values of adolescent and adult females reveal that the younger females have higher concentrations of estrogen and progesterone, and presumably greater fecundability across most cycles.

I interpret these results cautiously because, in addition to the small sample size, the data derive from captive subjects whose environment and individual histories make life history comparisons with wild populations challenging. On the basis of behavioral and morphological characteristics, Galdikas delineated the adolescent period for female orangutans as between 9 and 15 years old (Galdikas 1981, 1995). The three females that are the focus of this chapter fall within these limits, and have thus been categorized as adolescent. However, previous studies have established that the pace of reproductive scheduling can substantially differ between captive and wild populations of a species (Knott 2001). The fact that Belawan and Kelly each appear to have transitioned through menarche prior to the age of 10 or 11 is in agreement with observations of earlier maturation in captive animals (see Table 3-1), and highlights that a more formal definition of adolescence is required for comparative studies and broad life history analyses.

Additional data regarding the proximate triggers of menarche will be especially useful in this regard. Early analyses of female development in humans concluded that weight is a key determinant of the onset of menarche, and thus produced models emphasizing the importance of reaching certain growth thresholds. Frisch, for example, suggested that a minimum ratio of fat to lean body mass is necessary to initiate and maintain normal ovulatory cycles (Frisch et al. 1970; Frisch 1972, 1996; Vizmanos and Marti-Henneberg 2000). This is based on the hypothesis that adipose tissue is a source of stored energy that is mobilized for reproductive processes, and that reproductive efforts will not be initiated if energy stores are insufficient to support pregnancy and lactation (Frisch et al. 1974; Ellison 1990). This model has since been criticized, however (review: Ellison 1990), most notably due to the lack of correlation between age at menarche and weight at menarche.

Today, researchers considering metabolic constraints on reproduction argue that energy balance impacts age at menarche and ovarian function more significantly than weight or nutritional status (Bronson and Manning 1991; Bronson 2000; Lipson 2001; Thomas et al. 2001; Ellison 2008; see Chapter 4 for further discussion of reproductive ecology). As a result it has been proposed that leptin, a hormone that regulates energy intake and expenditure, functions either as a signaling trigger for puberty or as a metabolic gateway that enables reproductive maturation when resources are sufficient. Studies have found that leptin is related to the onset of reproduction in rodents (Cheung et al. 1997; Urbanski 2001), and some suggest it plays a similar role in humans (Matkovic et al. 1997). If so, leptin may serve as an important indicator of reproductive development in non-human apes as well (Bribiescas and Anestis 2010).

Peter Ellison, a proponent of the hypothesis that energy balance affects ovarian function, also emphasizes that girls must pass certain skeletal growth thresholds before energetic constraints on reproduction become relevant. He notes that in humans, the adolescent height spurt is more closely correlated to reproductive parameters than are body mass changes (Ellison 1981a, 1981b), and argues that pelvic maturation in particular is a crucial precursor to female reproductive success (Ellison 1990).

These proposed proximate determinants of human reproductive maturation are relevant to considerations of orangutan adolescence not only because of the species' similarities in reproductive physiology, but also because the dichotomy of energetic conditions in which free-ranging and captive orangutans live resembles the variability of human environments. The recent historical trend toward an earlier age at menarche in well-nourished human populations is consistent with predictions that conditions of energy surplus will accelerate growth rates and expedite the onset of fecundity (Marshall and Tanner 1968; Bentley 1999; Lipson 2001; Gluckman and Hanson 2006; Ellison 2008). We may therefore expect that female orangutans in captivity, who face minimal energetic stress, will grow and mature more quickly than those in the wild, who may experience significant periods of negative energy balance (Knott 1998; Emery Thompson and Knott 2008). In fact, the impact of positive energy balance on reproductive maturation may be more significant in orangutans than in humans: a smaller cranium at birth combined with a pelvic outlet that has not been reshaped by bipedalism frees female orangutans from the skeletal constraints that Ellison has emphasized (but see Dunsworth et al. 2012), and likely enables them to reproduce relatively earlier under favorable conditions.

The occurrence of menarche at an earlier age may have further effects on lifetime ovarian function in captive female orangutans. In a study of adolescent human girls, Vihko and Apter (1984) found that girls who experienced early menarche had higher circulating levels of estradiol pre- and post-menarche, and exhibited fewer instances of anovulation during early cycles. This pattern may explain the relatively short period of asynchronous hormonal activity that I have reported for Allie, as well as the apparent early maturation of Belawan and Kelly, and Belawan's particularly high estrogen values. If evidence of this pattern is found in additional captive females, it will suggest that the conditions of surplus energy that accelerate reproductive maturation in captivity also shorten or eliminate the period of adolescent subfecundity that has been inferred in wild populations, and contribute to long-term differences in ovarian steroid production (Ellison 1996).

On the basis of these data and the results I have presented, I propose that adolescence in orangutans and other apes should be defined based on a *combination* of hormonal and behavioral criteria that can be comparably assessed in free-ranging and captive individuals. Because sexual behaviors can serve developmental and social as well as reproductive functions (Hanby and Brown 1974; Hashimoto 1997; Li et al. 2007), it is problematic to presume that the sociosexual interactions that field researchers have previously used to identify adolescence represent underlying hormonal changes. Endocrinological analyses of urine samples from wild females are thus necessary to evaluate how the timing of behavioral and physiological changes coincides. On the other hand, several lines of evidence suggest that hormone measurements alone are insufficient to identify adolescence in captive females. First, there is a high degree of variability in individual ovarian steroid concentrations (e.g. Allie's consistently high PdG levels), which precludes the simple application of "threshold" ovarian hormone levels to define this life history stage (Gesquiere et al. 2005). Second, as an analysis of humans has pointed out, accelerated maturation of the HPO axis can produce an adolescent period that, instead of subfecundity, is characterized by a pattern in which physiological maturation precedes psychosocial reproductive competence (Gluckman and Hanson 2006). This disjunction has been shown to affect reproductive dynamics and female choice in provisioned ring-tailed lemurs by increasing instances of sexual coercion against fecund females who have not yet achieved social dominance over males (Parga and Henry 2008). I therefore argue that the adolescent period of captive female apes should be evaluated by collecting behavioral evidence of sexual development along with hormonal data.

From a methodological perspective, captive institutions can improve the quality of data available for developmental comparisons with free-ranging populations by keeping more detailed records of individual females' transitions through menarche. A collaborative effort to collect daily urine samples that can be tested for the presence of blood and assayed for ovarian steroid concentrations would allow for a better understanding of physiological variability among young females, and could confirm whether development in a captive environment systematically shortens or eliminates any subfecund phase. Unfortunately, management practices frequently present significant challenges to data collection. For example, females may be subject to precautionary birth control measures prior to any signs of reproductive maturation. Of the females considered in this chapter, both Allie and Belawan were maintained on birth control pills for periods of time predating data collection; it is unclear whether such artificial manipulation of hormones impacts the timing of menarche or has long-term effects on ovarian function.

The second major goal of this chapter has been to investigate the degree to which hormone concentrations and cycle phase affect adolescent females' receptivity, attractivity, and proceptivity. An investigation of Allie's data revealed that neither ovulatory status nor ovarian steroid levels significantly predicted the occurrence or frequency of copulations. Moreover, contrary to expectations derived from descriptions of adolescent female behavior in the wild, Allie did not exhibit any proceptive behaviors throughout the multi-year period of data collection; all copulations were initiated by the flanged male Azy. Together, these results suggest that Allie's estrogen and progesterone levels did not significantly affect her mating motivation, or her desirability as a partner.

However, it is unclear whether Allie's ovulatory status was truly concealed from Azy, or whether he was simply indifferent to any indicators of cycle phase given the limited reproductive opportunities available in captivity.

Several other factors must also be considered when interpreting the reproductive dynamics of the Great Ape Trust orangutans. At the age of six, Allie became ill, and was later diagnosed with chronic inflammatory demyelinating polyneuropathy (R. Shumaker, personal communication). This condition has caused Allie to lose significant voluntary control over her lower body, and I suspect that her reproductive motivation has been curtailed as a result of this disability. In addition, as mentioned in Chapter 2, Allie shared her enclosure at the Great Ape Trust with both Azy and Knobi, a reproductively mature female. I will discuss Knobi's mating behaviors extensively in the next chapter, but she is notable among the orangutans discussed in this dissertation for her extensive receptivity and high mating frequency and I suspect that her presence also affected interactions between Allie and Azy.

The Woodland Park Zoo records show that Belawan displayed proceptive behaviors more frequently than Allie and that, in line with the predictions outlined at the beginning of this chapter, these did not always result in copulation. Once again, however, these data must be considered in terms of how the captive environment limits behavioral options. In this case, Belawan's only option for a mating partner was her father Towan. Mate choice is similarly restricted for most captive orangutans, though not necessarily to family members, and individual females' proceptive behaviors may increase or decrease as a result.

I was unable to examine the effects of ovarian activity on Belawan's adolescent behavior due to inadequate data, but will provide an analysis of her reproductive behaviors as an adult in the next chapter.

Conclusions

The results I have presented in this chapter clearly demonstrate that any comparative life history analysis of adolescent endocrinology must involve careful consideration of the pace of reproductive maturation. The data show that female orangutans reach full HPO axis functionality at an earlier age in captivity than has been estimated in the wild, and exhibit high-quality ovulatory cycles soon after the initiation of menses. However, until researchers collect additional hormonal data from free-ranging female orangutans it will be challenging to draw general conclusions about whether the occurrence and duration of a period of adolescent subfecundity differs significantly between wild and captive individuals, or if its ubiquity and length have been overestimated in wild populations.

Chapter 4: Intra- and Inter-Individual Variability of Ovarian Function and Reproductive Behaviors in Female Orangutans

Introduction

Although clinical literature often considers female reproductive physiology within a dichotomous framework, classifying the ovarian function of individual women simply as ‘normal’ or ‘pathological’ (Erickson 1978), evidence indicates that ovarian hormone production and ovulatory frequency vary significantly among individuals and populations, and across the cycles of individual females (Ellison et al. 1993; Ellison 1994a; Jasienska and Jasienski 2008; Vitzthum 2008, 2009). Researchers investigating the sources and consequences of this variation have determined that nutrition, energy expenditure, and energy balance are among the important factors underlying differences in ovarian function (see below). Questions remain, however, about additional intrinsic and environmental sources of variation in ovarian function, and especially about the causal link between ovarian hormone activity and reproductive behaviors in humans and other apes.

Over the past 25 years, reproductive ecology has emerged as the primary explanatory paradigm for variation in ovarian function. Evidence has demonstrated not only that chronic differences in nutritional status lead traditional populations to exhibit systematically lower ovarian hormone levels than modernized nations (Ellison et al. 1989; Ellison et al. 1993), but also that moderate changes in diet and energetic status affect short-term ovarian function regardless of an individual woman's starting point (Bentley 1999). Ellison and Lager, for example, showed that among women in industrialized nations, slight restrictions in caloric intake (Lager and Ellison 1990) and moderate levels of aerobic exercise (Ellison et al. 1986) independently caused luteal progesterone concentrations to decrease. Increased energy expenditure has also been shown to lower progesterone levels among non-nutritionally stressed rural Polish women (Jasienska and Ellison 2004) and among Nepalese women during periods of insufficient energy intake (Panter-Brick et al. 1993), reinforcing the conclusion that workload and energy balance can alter hormone production separately or in tandem (Ellison 1990). Changes in ovarian function that result from reversible alterations to diet or energetic status are likewise reversible (Bullen et al. 1985), but nonetheless illustrate how potential and actual fecundity are shaped by a woman's interactions with her environment.

Figure 4-1 illustrates the spectrum of possible ovarian functionality derived from these and other studies of reproductive ecology. From an evolutionary perspective, the flexibility of this physiological system reflects the ability of human women to respond adaptively to environmental fluctuations (Ellison 1990, 1994b, 2008; Vitzthum 2009). Natural selection, in other words, has shaped female reproductive physiology to downgrade ovarian activity and limit investment in reproduction when inadequate energetic resources diminish the probability of successful offspring production.

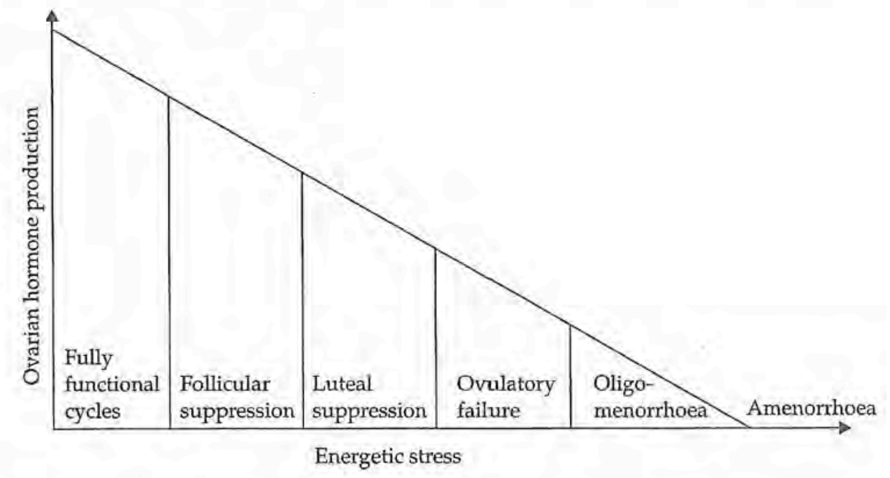


Figure 4-1. The "graded continuum of ovarian response" described by Ellison (Ellison 1990)**. Image reproduced from** (Knott et al. 2009).

The same adaptive complex is also apparent in other apes. Female chimpanzees with access to a high quality core area exhibit higher ovarian hormone concentrations, shorter interbirth intervals, and higher rates of offspring survival than counterparts feeding in lower quality areas (Emery Thompson et al. 2007; Emery Thompson and Wrangham 2008). Orangutan estrogen concentrations and rates of copulation likewise increase during times of greater fruit availability (Knott 1997; Knott et al. 2009), although direct measures of fitness consequences are not available for this species. Finally, studies indicating that ovarian steroid levels are higher in captive than wild ape populations resemble the results of comparisons between human populations that are nutritionally or energetically stable and those that are stressed (Knott 1999; Emery Thompson 2005a).

In this chapter, I investigate inter- and intra-individual variation from a slightly different perspective by examining ovarian function in adult female orangutans living in nutritionally and energetically stable captive environments. Because the traditional constraints of reproductive ecology are not present under these circumstances, I consider potential alternative sources of variation. I also examine the within-cycle relationship between estrogen and progesterone levels, and the implications these measures have for broad assessments of cycle quality. Finally, I use records of mating behavior from 3 adult female orangutans to test the hypotheses that (1) orangutan ovulation is 'concealed,' and (2) variation in ovarian function directly affects receptivity, attractivity, and/or proceptivity.

Methods

Urine and behavioral data were collected from female orangutans at the Woodland Park Zoo and the Great Ape Trust, together with behavioral data from males at these locations (see Chapter 2). The hormone data derive from approximately 820 urine samples. These represent 46 cycles from 6 adult females (Table 4-1), as well as additional days of sampling from Knobi during June and July 2005. Endocrine sampling and assay methods are described in

Chapter 2 and Appendix B. Analyses of mating data from Knobi also include information from post-ovariectomy records.

Table 4-1. Overview of Cycles Described and Analyzed in Chapter 4.

Female	Dates Represented	Age	Number of Cycles
Allie	January-March 2009	14	2
Katy	July 2008-March 2009	19-20	4
Melati	Dec. 1992-April 1993	20-21	9
Chinta	June 1992-April 1993	24-25	12
Knobi	Aug. 2005-March 2006	25-26	6
Belawan	Sept. 2008-March 2011	27-29	13
			46

I evaluated the degree of intra-individual variability in ovarian function by comparing daily ovarian steroid concentrations and indices of overall cycle quality across multiple cycles for each female. I then compared the results of different females to one another to assess inter-individual variability. Using behavioral records for Allie, Belawan, and Knobi, I also examined variation in reproductive behaviors, and assessed the degree to which ovarian hormones and cycle phase influenced mating.

Statistical Methods

I used Spearman's rank correlation analyses to test the significance of the relationship between estrogen and progesterone within cycles. Because the sample size of mating days was small and the hormone data were unlikely to have a normal distribution, I also used a non-parametric analysis (the Mann-Whitney U-test) to compare the average urinary E_1C levels of individual females on mating vs. non-mating days.

To test the probability that cycle phase and/or hormone levels had predictable effects on mating outcomes involving Allie and Belawan, I applied a generalized linear mixed model analysis. GLMM, like linear mixed modeling, controls for subject effects by including individual identity as a random factor in the model, but is more appropriate in cases with a binary outcome variable. In this case, I examined whether mating was more likely to occur during the periovulatory period for each of these females, and whether daily estradiol concentrations, 3-day estradiol averages, or daily estrogen to progesterone ratios affected the probability of mating. I chose to use these particular ovarian hormone measurements based on the previously mentioned evidence that estradiol and progesterone have stimulatory and inihibitory effects, respectively, on female primates' motivation to mate (Baum et al. 1977; Nadler et al. 1983; Takahashi 1991; Nadler 1995; Zehr et al. 1998; see Chapter 1).

Finally, I used binary logistic regression analyses to test whether Knobi's mating outcomes were predictably affected by (1) her ovulatory status, or (2) her daily estradiol concentration, 3-day estradiol average, or daily estrogen to progesterone ratio.

All statistical analyses were performed using IBM SPSS v.20.

Results

Intra- and Inter-Individual Variability in Ovarian Function

Among adult females aged 14-29, daily urinary E1C concentrations ranged from a low of 2.5 ng/mg Cr (Belawan, age 27) to a high of 257.7 ng/mg Cr (Melati, age 20), and the average E1C concentration of individual cycles ranged from a low of 13.1 ng/mg Cr (Melati, age 20) to a high of 85.6 ng/mg Cr (Belawan, age 29). Daily urinary PdG levels ranged from a low of 31.0 ng/mg Cr (Chinta, age 24) to a high of 7386.1 ng/mg Cr (Allie, age 14)[4], and the average PdG concentration of individual cycles ranged from a low of 71.0 ng/mg Cr (Melati, age 21) to a high of 1631.0 ng/mg Cr (Allie, age 14). A complete overview of daily and cyclic hormonal variation is provided in Table 4-2, and Figures 4-2 and 4-3.

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[4] If Allie's data are excluded due to her age, the highest daily PdG value exhibited by an adult female is 2277.1 ng/mg Cr (Chinta, age 24).

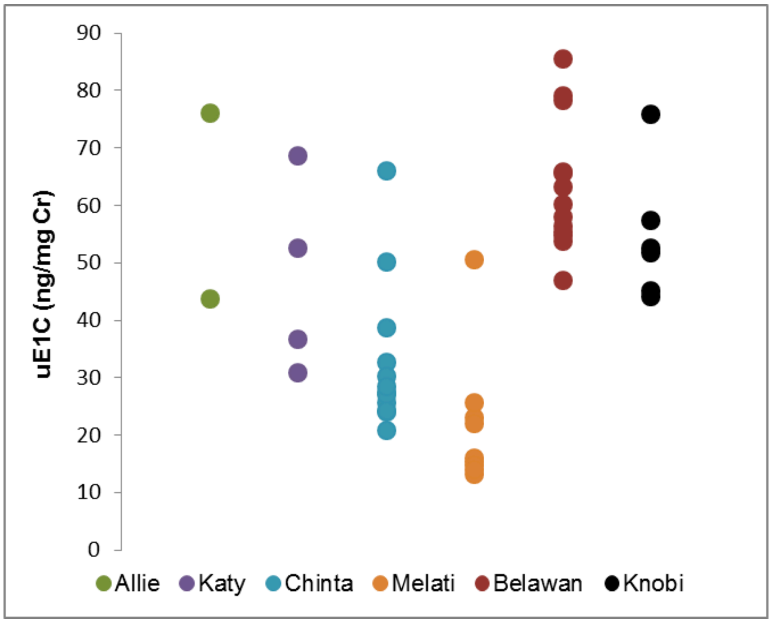
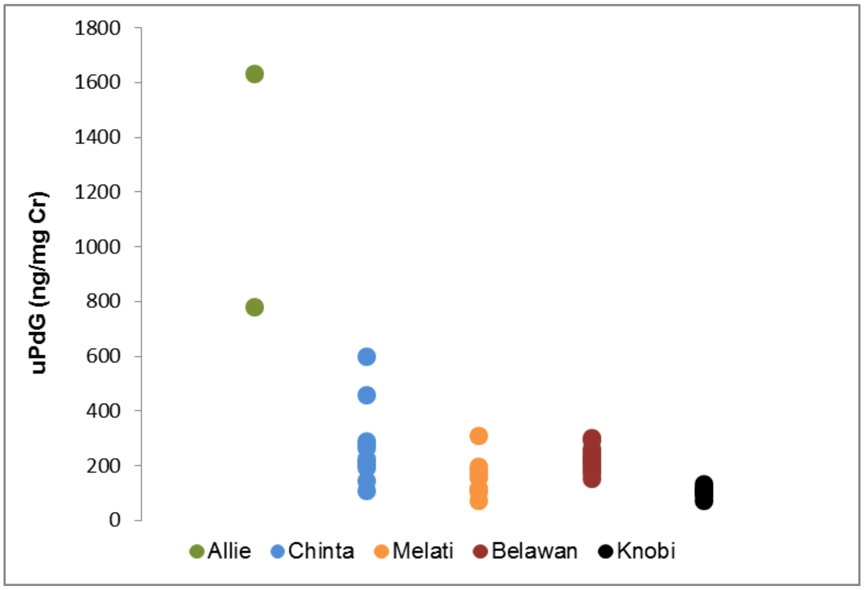


Figure 4-2. Average Urinary E1C Value per Cycle, by Individual. Cycles Shown for Individuals Age 14-29 only. N=2-13 cycles per individual.



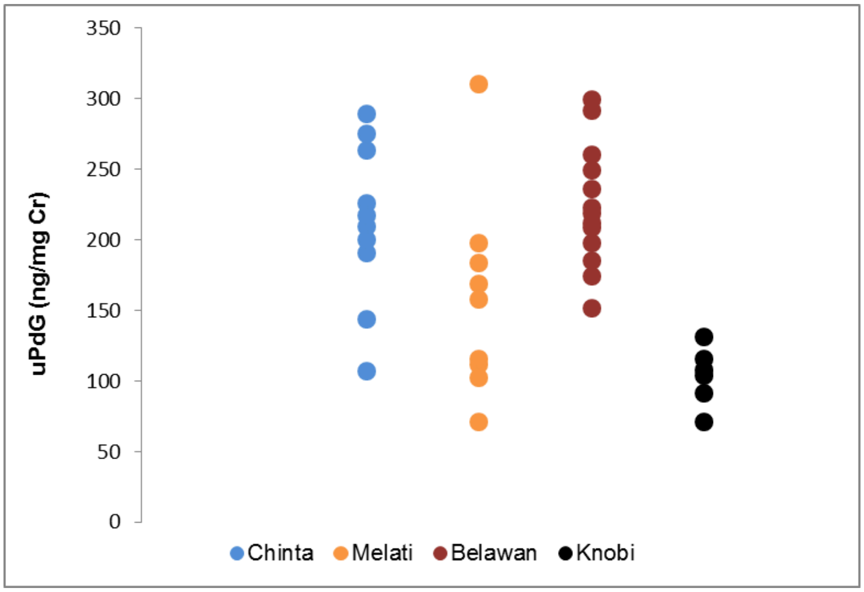


Figure 4-3. Average urinary PdG value per cycle, by individual. Cycles shown for individuals age 14-29 only. N=2-13 cycles per individual. Top: All cycles. Bottom: Cycles with uPdG < 350 ng/mg Cr.

Table 4-2. Overview of Intra- and Inter-Individual Variation in Ovarian Function Among Captive Female Orangutans

	Belawan[a] (*n*=5) [10-11]	**Kelly**[a] (*n*=10) [11-12]	**Allie**[a] (*n*=9) [11-12]	**Allie** (*n*=2) [14]	**Katy** (*n*=4) [19-20]	**Melati** (*n*=9) [20-21]	**Chinta** (*n*=12) [24-25]	**Belawan** (*n*=13) [27-29]	**Melati**[b] (*n*=10) [36-39]	**Chinta**[b] (*n*=9) [40-43]	**Knobi**[c] (*n*=6) [25-26]
Urine samples assayed	*50*	*231*	*218*	*34*	*82*	*140*	*224*	*135*	*111*	*117*	*119*
Maximum daily uE_1C (ng/mg Cr)	666.9	362.1	157.5	166.8	162.5	257.7	176.0	190.5	134.5	99.3	253.2
Minimum daily uE_1C (ng/mg Cr)	25.1	10.6	9.3	3.8	6.9	4.9	9.7	2.5	12.7	21.2	10.0
Maximum E_1C cycle average (ng/mg Cr)	265.5	92.7	46.4	75.9	68.6	50.6	66.1	85.6	55.6	56.0	75.9
Minimum E_1C cycle average (ng/mg Cr)	130.8	43.5	27.7	30.3	30.8	13.1	20.9	47.0	25.8	34.5	44.1
Maximum daily uPdG (ng/mg Cr)	3435.6	2140.8	8053.1	7386.1	750.7	658.3	2277.1	821.7	1747.8	2072.7	512.8
Minimum daily uPdG (ng/mg Cr)	80.3	29.2	22.7	89.7	32.8	32.7	31.0	49.3	47.9	67.5	27.7
Maximum PdG cycle average (ng/mg Cr)	1028.4	401.9	1780.1	1631.0	n/a	310.2	597.5	299.4	655.0	478.6	131.5
Minimum PdG cycle average (ng/mg Cr)	417.2	192.7	678.2	349.1	n/a	71.0	107.2	151.7	165.6	134.0	71.0

Sample sizes (in parentheses) match the number of cycles given in Table 2-3. The age of each individual is noted in brackets.

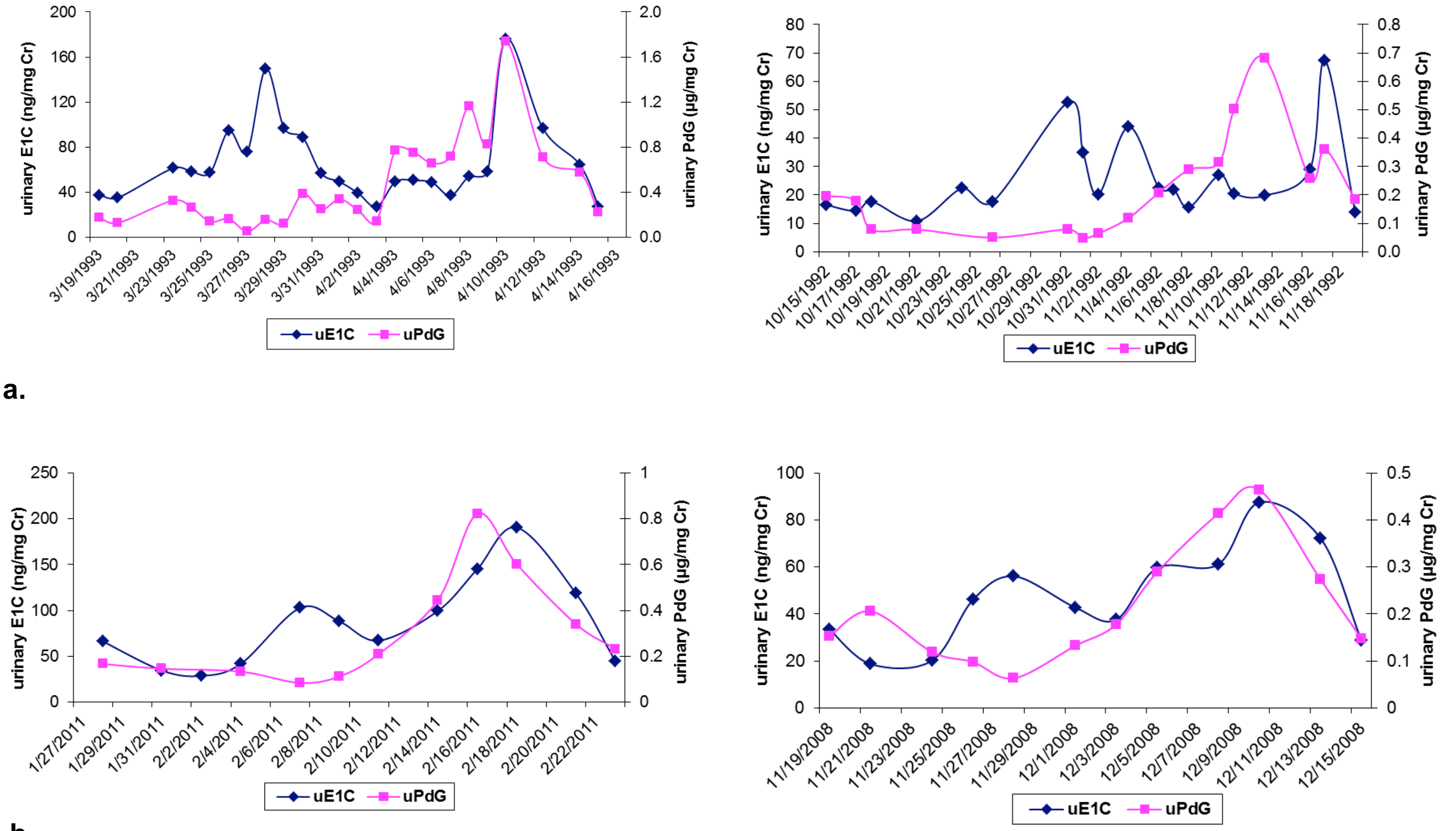
a. See chapter 3 for discussion of adolescent females

b. See chapter 5 for discussion of ‘older’ adult females

c. Data from Knobi is considered separately for reasons I have previously described (see Chapter 2)

Further evaluation of cycle quality using the criteria described in Chapter 2 revealed that all individuals experienced both high and low quality cycles during the period of data collection (Figure 4-4). The factors underlying lower-quality cycle scores varied, but most often included E1C values at the ovulatory peak that did not reach one or more standard deviations above an individual's median value. Katy, Chinta, and Melati each exhibited one or more anovulatory cycles as well (Figure 4-5). Individuals' luteal progesterone activity remained more stable across cycles, which is in accordance with predictions that follicular suppression precedes luteal suppression on the continuum of ovarian response to stress (see Figure 4-1).

Despite this evidence of intra and inter-individual variation in ovarian hormone production, I found significant positive correlations between the average E1C and PdG values of each cycle (Spearman's rho = .583, p=.000; Figure 4-6) and between the periovulatory E1C and luteal PdG values of each cycle (Spearman's rho = .521, p = .001; Figure 4-7) when data from all adult cycles were combined. The strength of these correlations diminished when the cycles of adolescent and 'older' adult females were included in the analysis, but remained significant (average E1C and PdG: rho = .307, p=.004, N=90 cycles; periovulatory E1C and luteal PdG: rho = .345, p=.001, N=88 cycles). This demonstrates the validity of using either estrogen or progesterone measurements as an indicator of the overall hormonal quality of a given cycle.



a.

b.

Figure 4-4. Representative Higher-Quality (left) and Lower-Quality (right) Cycles of Chinta (a) and Belawan (b)

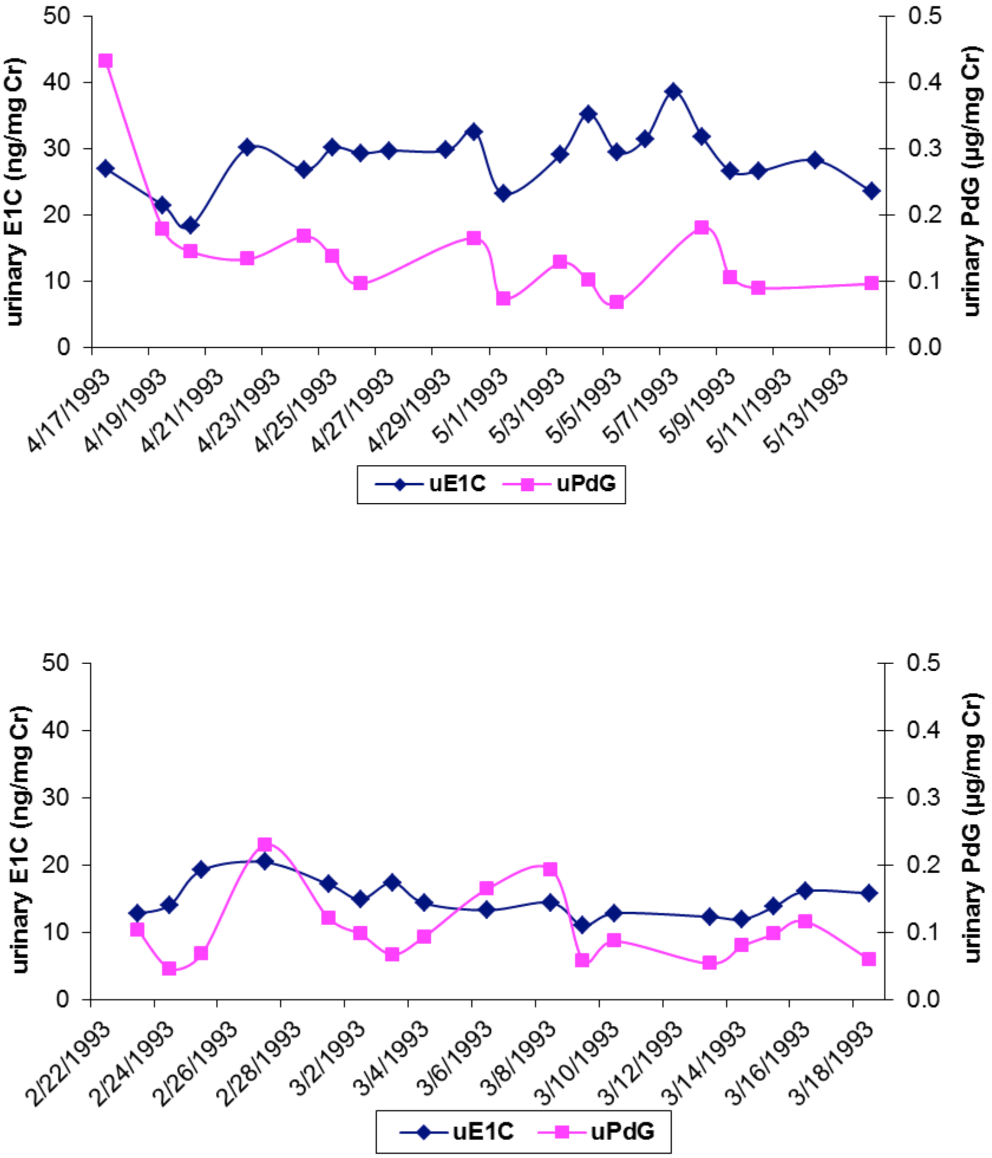


Figure 4-5. Anovulatory Cycles from Chinta at Age 25 (top) and Melati at Age 21 (bottom).

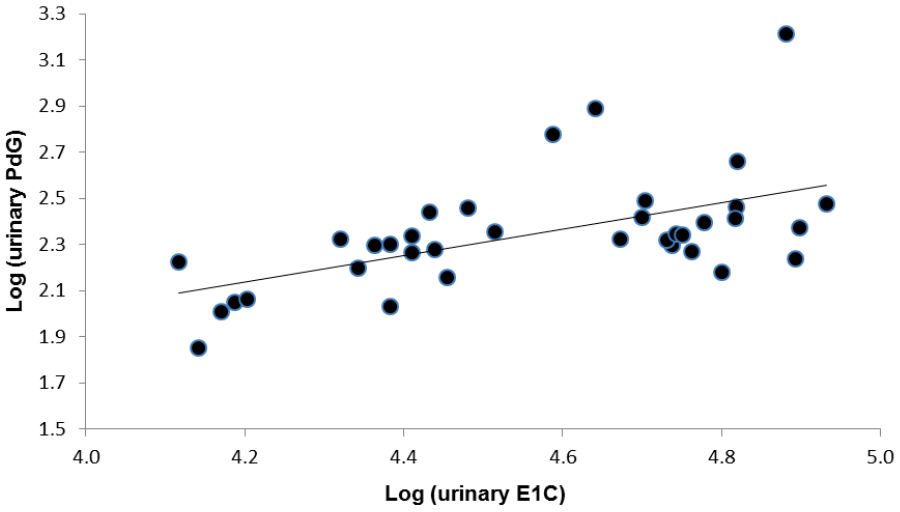


Figure 4-6. Correlation of Average Urinary E_1C and PdG Values. Each marker represents one cycle. N=36 cycles. Spearman's rho = .583, p=000.

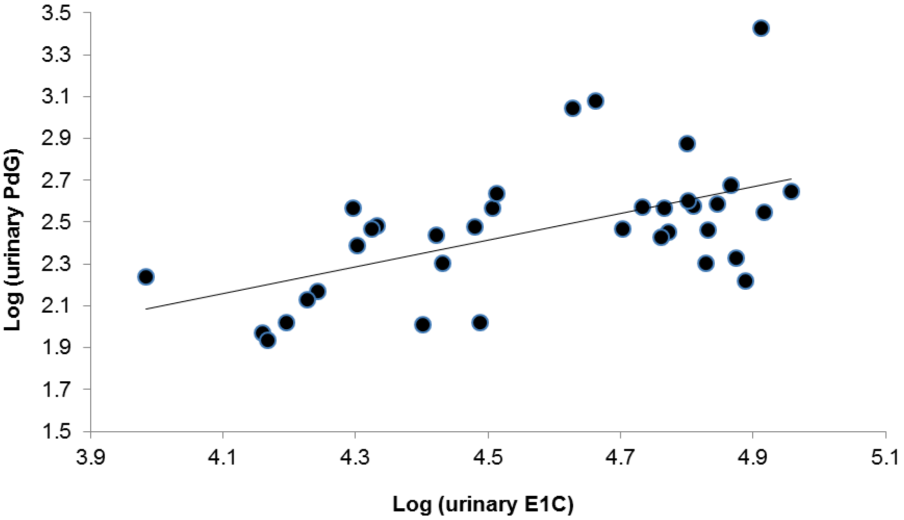


Figure 4-7. Correlation of Periovulatory E_1C and Luteal PdG Values. Each marker represents one cycle. N=36 cycles. Spearman's rho = .521, p=.001.

As mentioned previously (see Chapter 1), this measure of quality has been shown to be associated with fecundability in a variety of primate species.

Mating Data

Allie and Belawan

From January 2008 through February 2009, a total of 15 copulations were observed between Allie and Azy (Figure 4-8). Thirteen of these took place on days during the periovulatory period, and Allie behaved receptively in each case. Allie was resistant, however, to the two copulations that occurred outside of the periovulatory period. At no point during these months of data collection did Allie exhibit proceptive behaviors.

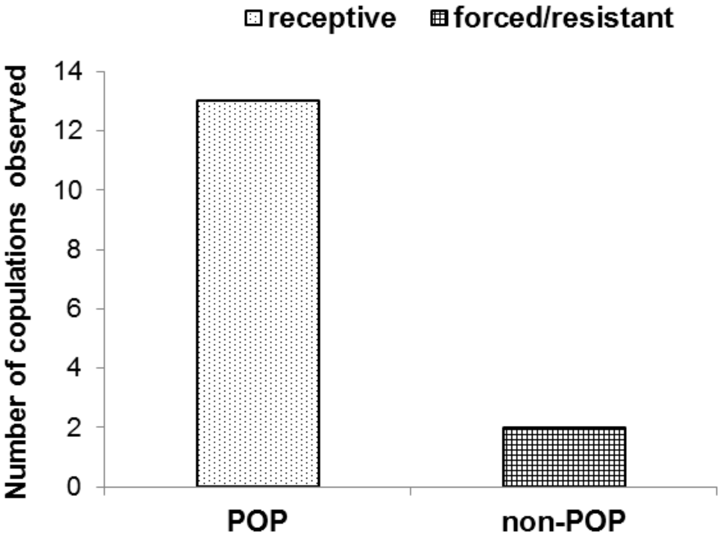


Figure 4-8. Copulations Observed Between Allie and Azy from January 2008-February 2009, by Ovulatory Status and Type.

Records of Belawan's mating behaviors offer a stark contrast. From August 2008 to March 2011, 84% of copulations between Belawan and Towan were initiated by proceptive behaviors from Belawan (N=27; Figure 4-9). Belawan was also receptive to 5 copulations initiated by Towan, and exhibited proceptive behaviors that did not result in copulation on an additional 22 days. No forced copulations were observed.

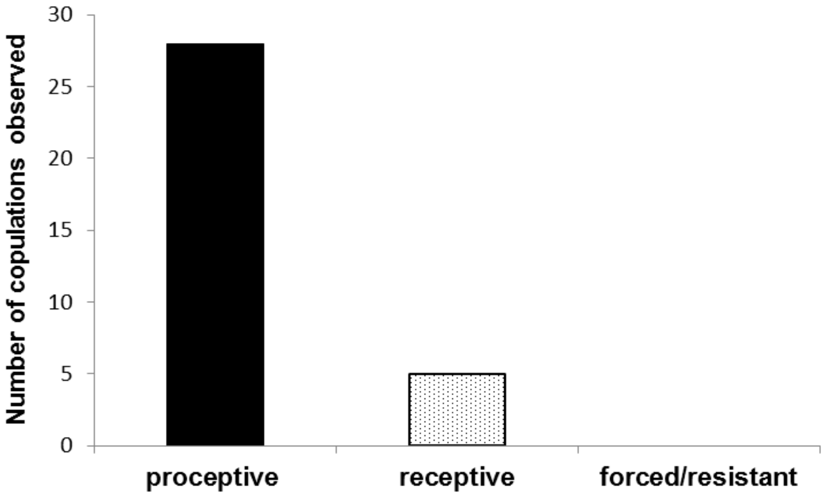


Figure 4-9. Copulations Observed Between Belawan and Towan from August 2008-March 2011, by Type.

Hormonal data are not available for all of the days on which copulations between Belawan and Towan occurred, but of 16 mating days for which I was able to determine cycle phase, 15 occurred during the periovulatory period. Combining these data with Allie's results using a generalized linear mixed model analysis reveals that ovulatory status is a significant predictor of mating outcome for these two females (p=.016; Figure 4-10). There was, however, no significant difference in the average urinary E_1C values of mating vs. non-mating days for either female (Allie: Mann-Whitney U, z=-1.201, p=.230; Belawan: Mann-Whitney U, z=-.757, p=.449; Figures 4-11 and 4-12), and no significant effect of daily E_1C concentration (p=.466) or 3-day E_1C average (p=.543) on the probability of mating. The influence of the daily estrogen to progesterone ratio approached but did not reach significance (p=.057).

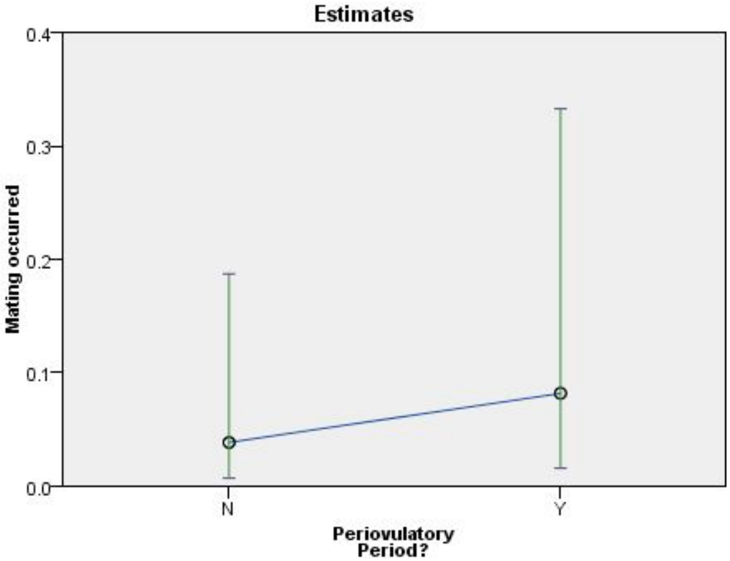


Figure 4-10. GLMM-Estimated Mean Probability of Mating by Ovulatory Status (with 95% CI; N=11 cycles from Allie and 13 cycles from Belawan, p=.016)

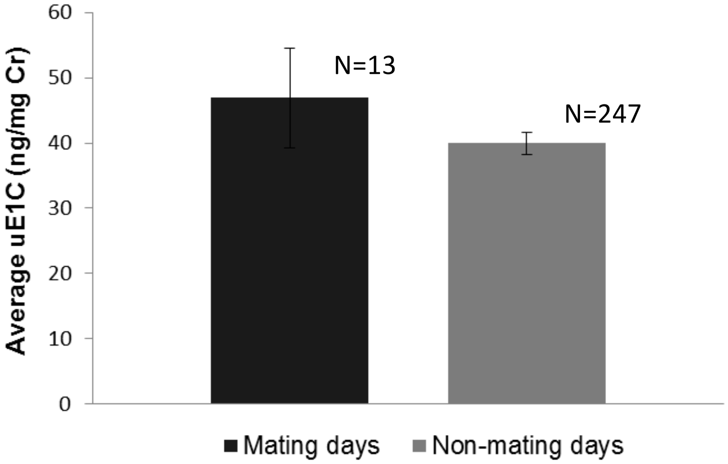


Figure 4-11. Average Urinary E$_1$C (mean ± SE) for Allie on Mating vs. Non-Mating Days, January 2008-February 2009. No significant difference (Mann-Whitney U, z=-1.201, two-tailed p=.230).

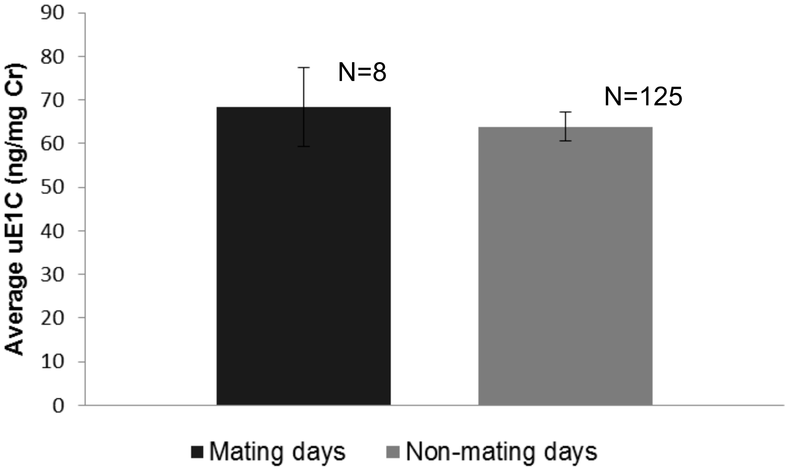


Figure 4-12. Average Urinary E$_1$C (mean ± SE) for Belawan on Mating vs. Non-Mating Days, October 2008-May 2009 and December 2010-March 2011. No significant difference (Mann-Whitney U, z=-.757, two-tailed p=.449).

In a separate analysis of Belawan's data, I also tested whether the daily E_1C concentration and/or the daily estrogen to progesterone ratio significantly affected the likelihood that she exhibited proceptive behaviors. Results of a binary logistic regression were non-significant for daily E_1C values (N=11 proceptive days, Wald-Z=.153, p=.696), but daily E_1C to PdG ratio was a significant predictor of proceptivity (N=10 proceptive days, Wald-Z=4.454, p=.035).

Knobi

As mentioned in Chapter 2, Knobi underwent an ovariectomy in August 2007 due to an unusual history of menstrual bleeding. In Figure 4-13, the number of days on which Knobi and Azy were observed mating and the total number of copulations that took place are shown for both pre-ovariectomy and post-ovariectomy periods of data collection. A total of 146 copulations were observed on 115 days during the pre-ovariectomy period. On five of these days Knobi resisted mating, but she was receptive to the majority of copulations. Knobi also displayed proceptive behaviors on 21 mating days, and exhibited proceptivity on 6 additional days on which copulations were not observed. Fewer copulations occurred post-ovariectomy, although the number of mating days (N=57) and copulations (N=63) remained high relative to the other females discussed in this dissertation. Equally notable, the proportion of copulations that were proceptive (18%), receptive (77%), and resisted (5%) remained identical to those observed pre-ovariectomy.

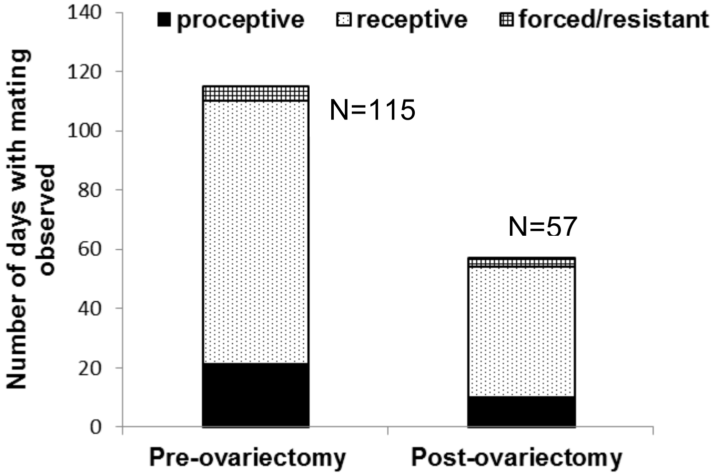


Figure 4-13. Number of Days with Observed Mating Between Knobi and Azy, by Type. Pre-ovariectomy period = 301 days total; total of 146 copulations observed. Post-ovariectomy period = 562 days total; total of 63 copulations observed.

As with Belawan, hormonal data are not available for all of Knobi's pre-ovariectomy mating days, but of 88 mating days for which I was able to determine cycle phase, 48 occurred during the periovulatory period (Figure 4-14). Further investigation of the relationship between Knobi's ovulatory status and mating likelihood using a binary logistic regression analysis yielded a result bordering on significance (p=.051; Table 4-3).

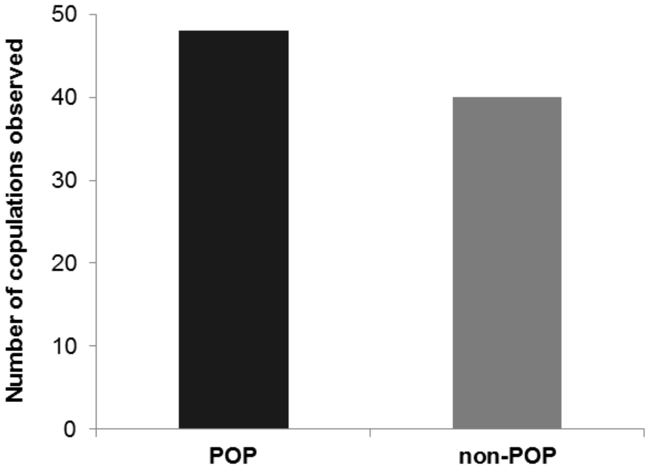


Figure 4-14. Observed Copulations Between Knobi and Azy by Ovulatory Status, August 2005-March 2006

Table 4-3. Result of Logistic Regression Analysis of the Relationship Between Periovulatory Phase and Mating Probability for Knobi, August 2005-March 2006

	β±SE	Wald-Z	p-value	OR (95%CI)
Periovulatory period	-.539±.276	3.813	.051	.583 (.340:1.002)

β±SE shows the coefficient and its associated SEs in logits and OR shows the transformed odds ratios with an associated 95% confidence interval.

While this result is similar to that described for Allie and Belawan above, additional analyses of the relationship between mating and ovarian hormones revealed that unlike Allie or Belawan, Knobi's average urinary E_1C values differed significantly on mating and non-mating days (Mann-Whitney U, z=-4.16, p=.000; Figure 4-15). Logistic regression also demonstrated that copulations were significantly more likely to occur on days with higher estradiol concentrations (Wald-Z=10.174, p=.001), 3-day estradiol averages (Wald-Z=4.682, p=.030), and estrogen to progesterone ratios (Wald-Z=4.955, p=.026; Table 4-4). However, these hormonal measurements did not significantly predict proceptivity by Knobi (daily E_1C: N=19 proceptive days, Wald-Z=.331, p=.565; 3-day E_1C: N= 13 proceptive days, Wald-Z=.011, p=.917; E_1C to PdG ratio: N=12 proceptive days, Wald-Z=.049, p=.825) or the occurrence of long calls and/or display behaviors by Azy (p>.05 for all analyses).

Table 4-4. Hormonal Predictors of Mating for Knobi, June 2005-March 2006

	N (mating days)	**df**	**Wald-Z**	**p-value**
Daily E_1C value	74	1	10.174	**.001**
E_1C 3-day average	28	1	4.682	**.030**
E_1C: PdG ratio	53	1	4.955	**.026**

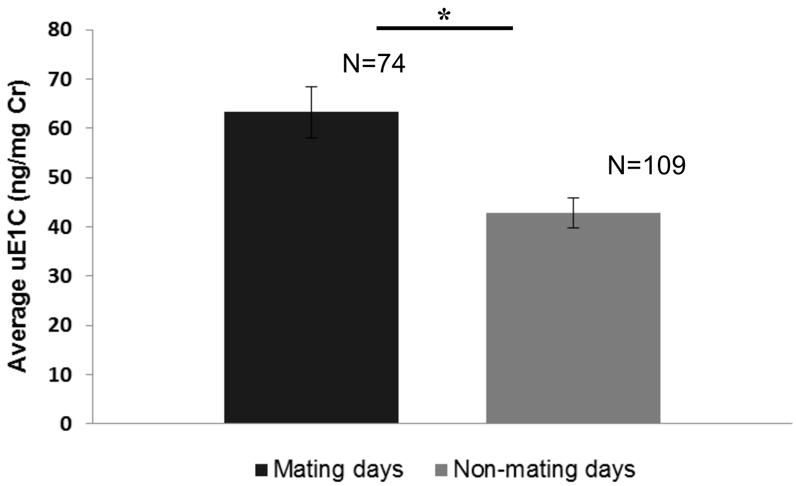


Figure 4-15. Average Urinary E_1C (mean ± SE) for Knobi on Mating vs. Non-Mating Days, June 2005-March 2006. (Mann-Whitney U, z=-4.16, two-tailed p=.000)

Discussion

The data in this chapter demonstrate that ovarian function varies considerably among captive female orangutans. As I discuss in Chapters 3 and 5 of this dissertation, age may be one factor underlying this variation. The controlled conditions of captivity, however, make it unlikely that ovarian hormone production in reproductively mature females is significantly affected by nutritional or energetic status.

If the observed inter- and intra-individual variability is not the result of adaptive responses to ecological fluctuations, how can it be explained? I propose that among primates in captivity, differences in fecundity and fecundability are due primarily to (1) genetic variation, and (2) variation in the presence of and reaction to psychological and social stressors. Each of these factors is known to affect ovarian activity in humans, but has not yet been examined as a source of variation in orangutans or other apes.

Intrinsic Sources of Variation in Ovarian Function

Analyses of genetic and hormonal data from regularly cycling and postmenopausal women have revealed several genetic polymorphisms that influence estrogen and progesterone production in humans (Feigelson et al. 1998; Sharp et al. 2004; Dunning et al. 2004; Small et al. 2005; Haiman et al. 2007; Vitzthum 2009). Jasienska *et al*. (2006), for example, examined variation at the *CYP17* locus, which is involved in the production of enzymes necessary for estrogen synthesis, and found that women with the *A2/A2* genotype had average salivary estradiol levels that were 37% higher than those of women with the *A1/A2* genotype and 54% higher than those of women with the *A1/A1* genotype. Based on the considerable overlap of the orangutan and human genomes (Locke et al. 2011) and the many similarities in female reproductive physiology between the two species (see Chapter 1), I hypothesize that future research will find similar genetic polymorphisms underlying baseline variation in orangutan ovarian function.

Captive populations, due to the ease with which they can be sampled, may offer the earliest opportunity to conduct such genetic investigations. If so, it will be particularly interesting to discover how allele frequencies among females of Sumatran or Bornean descent compare to those of females with a hybrid genetic background.

Environmental Sources of Variation in Ovarian Function

As mentioned above, physical and energetic stressors are rare in captive institutions such as the Woodland Park Zoo and Great Ape Trust, which specifically aim to maximize the welfare of inhabitants. Abundant literature suggests, however, that aspects of captivity including restricted space, artificial surroundings, caretaker actions, and visitor presence are a significant source of stress in a variety of species (Hosey 2005; Peel et al. 2005; Skurski 2006; Morgan and Tromborg 2007). This may be especially true in primates due to their social complexity, cognitive sophistication, and ability to react to both existing and anticipated stressors (Honess and Marin 2006). A consideration of the effects of psychological and social stress on the ovarian function of captive orangutans is thus warranted.

Physiological and behavioral responses to either physical or psychological stressors are initiated and coordinated by the hypothalamic-pituitary-adrenal (HPA) axis (Sapolsky 1994), a feedback loop functionally similar to the HPO-axis that controls female reproduction (see Chapter 1). The effects of HPA-axis activity on reproductive physiology have been investigated in non-human primates and humans, and data indicate that its hormonal secretions, including corticotropin-releasing factor (CRF; a peptide hormone) and cortisol (a steroid hormone), impact HPO-axis function on multiple levels (Rivier and Rivest 1991; Xiao and Ferin 1997; Tilbrook et al. 2000). Most notably, CRF inhibits hypothalamic secretion of gonadotropin-releasing hormone (GnRH), while elevated cortisol concentrations suppress pituitary production of luteinizing hormone and follicle-stimulating hormone (Chrousos et al. 1998; Tilbrook et al. 2002; Wingfield and Sapolsky 2003; Tamashiro et al. 2005). The cascading effects of these disruptions can include decreased ovarian steroid production, inferior folliculogenesis and corpus luteum formation (Rivier and Rivest 1991; Xiao and Ferin 1997; Chrousos et al. 1998), and the stunting of proceptive and receptive behaviors (Wingfield and Sapolsky 2003). As is the case with energetic stressors (see Figure 4-1), however, evidence suggests that psychological stressors affect ovarian function along a graded continuum: stressors of different type, duration, and perceived intensity result in responses ranging from a modest decrease in ovarian hormone production to anovulation and/or amenorrhea (Xiao and Ferin 1997; Ferin 1999; Bethea et al. 2008).

On the basis of these data, I propose that temporal fluctuations in the presence of stressors, and consequent increases or decreases in HPA-axis activity, contribute significantly to intra-individual variation in ovarian hormone production and cycle quality in captive female orangutans. Inter-individual variability may similarly reflect differences in the relative sensitivity of each female’s stress response (Bethea et al. 2008; Herod et al. 2011), which is known to be influenced by temperament and personality factors (Sapolsky 1994; Anestis et al. 2006; Carere et al. 2010). Additional hormonal analyses, particularly the measurement of cortisol concentrations during lower quality and anovulatory cycles, will be necessary to test these hypotheses in the future.

‘Concealed’ Ovulation?

The second major goal of this chapter has been to quantify the effects that ovarian hormone concentrations have on the receptivity, attractivity, and proceptivity of reproductively mature female orangutans. To my knowledge, this is the first study to directly measure these relationships, and the results provide an important complement to data regarding the effects of orangutan ovulatory status on the frequency and type of copulations (Nadler 1977, 1982, 1995; Knott et al. 2010; see Chapter 1). By providing new insight into the relationship between hormones and mating motivation in orangutans, these data also contribute to a growing literature about the ways in which male and female reproductive strategies interact.

The results of analyses quantifying hormone-behavior relationships in Allie, Belawan, and Knobi are compared in Table 4-5. While the sample size of individuals is small, these data suggest the following preliminary conclusions:

- Receptivity in female orangutans is not restricted to the periovulatory phase of the cycle.

- Although female orangutans exhibit extended receptivity, mating occurs more frequently during than outside of the periovulatory period.
- Ovarian steroid concentrations do not consistently predict mating outcomes, and their influence on reproductive behaviors is limited. I draw this conclusion based on the fact that the relationship suggested by Belawan's data (i.e. estrogen levels primarily affect proceptivity) is opposite that suggested by Knobi's data (i.e. estrogen levels primarily affect attractivity).

These data suggest, in other words, that while the frequency of orangutan copulations varies in conjunction with cycle phase, female and male mating motivation are not exclusively or strongly influenced by elevated estrogen levels near ovulation. I suspect that in captivity in particular, mating patterns are more strongly influenced by social dynamics, individual history and temperament, and imposed restrictions on mate choice.

Knobi's post-ovariectomy behavioral records, a resource unique to this study, offer further support for this hypothesis. Knobi continued to exhibit receptive and proceptive mating behaviors in the absence of ovarian functionality, and remained attractive to Azy despite the presence of the younger and more fecund Allie. This indicates that ovarian steroid production is neither necessary nor sufficient to explain variability in receptivity, attractivity, or proceptivity. The possibility remains, however, that testosterone or other androgenic

Table 4-5. Hormone-Behavior Relationships Compared Across Allie, Belawan, and Knobi

	Allie	Belawan	Knobi
Receptive matings occur outside the POP	No	Yes	Yes
Mating is more common during the POP	Yes	Yes	Yes
E_1C concentrations on mating days are significantly higher than concentrations on non-mating days	No	No	Yes
Daily E_1C concentration is a significant predictor of whether mating will occur	No	No	Yes
3-day E_1C average is a significant predictor of whether mating will occur	No	No	Yes
Daily E_1C:PdG ratio is a significant predictor of whether mating will occur	No	No	Yes
Instances of proceptivity are more frequent during the POP		Yes[1]	No[2]
Daily E_1C concentration is a significant predictor of proceptivity		No	No
3-day E_1C average is a significant predictor of proceptivity			No
Daily E_1C:PdG ratio is a significant predictor of proceptivity		Yes	No

Boxes left blank in cases of insufficient data

1. All instances of proceptivity by Belawan that could be associated with cycle data occurred during the periovulatory period

2. Of 20 instances of proceptivity by Knobi that could be associated with cycle data, 11 occurred during the periovulatory period

hormones are important proximate stimulators of female mating motivation (Beach 1976; Baum et al. 1977; van Goozen et al. 1997).

As for the implications that these data have regarding the phylogenetic distribution and adaptive significance of 'concealed' ovulation, I emphasize that the lack of a periovulatory swelling (a trait shared by orangutans and humans) must be considered as part of a larger suite of traits that characterize female reproductive physiology. Based on the strictest definition, evidence of behavioral and olfactory changes mid-cycle suggest that ovulation is not truly concealed in either orangutans or humans, but females of both species clearly exhibit mate preferences and have evolved additional mechanisms (e.g. an extended mating period, facultative association) that help obscure ovulatory timing.

Conclusions

1. Captive female orangutans exhibit both “high quality” and “low quality” cycles, including instances of anovulation, in the absence of fluctuating dietary and environmental conditions. Intra- and inter-individual variation in ovarian function among these females is likely shaped by a combination of genetic factors and variability in HPA-axis activity.
2. Social dynamics and individual temperament are important variables affecting reproduction, and likely influence orangutan mating behaviors as strongly as or more strongly than ovarian hormones. This may be especially true among captive orangutans due to the limits captivity imposes on mate choice.
3. Hypotheses suggesting that concealed ovulation is a uniquely human trait associated with the evolution of pair bonds and/or paternal investment (e.g. Benshoof and Thornhill 1979; Lovejoy 2009) should be reconsidered in

light of evidence that many primate species, including orangutans, exhibit extended periods of receptivity. Variable selective pressures may have favored the presence or absence of sexual swellings and other reproductive ‘strategies’ (e.g. facultative association) in different primate lineages.

Chapter 5: Ovarian Function and Reproductive Behaviors of Female Orangutans in Later Adulthood

Introduction

Among humans, males and females who survive into old age experience a marked and unavoidable decline in reproductive function. In females especially, the transition to infecundity, marked by the cessation of ovulation and menstrual cycling around the age of 50 (Wu et al. 2005; Walker and Herndon 2008), occurs significantly earlier than other signs of senescence (Fedigan and Pavelka 2011). The fact that women may have a post-reproductive lifespan of three or more decades is frequently interpreted as evidence that this is an important life history phase in *Homo sapiens*, and has elicited hypotheses suggesting adaptive explanations (Hawkes et al. 1997; Sear et al. 2000; Hawkes 2003; Lahdenperä et al. 2004). However, a more thorough understanding of female reproductive senescence across the primate order is necessary to properly evaluate the selective significance of human menopause.

At present, the existence or degree of reproductive decline in females of other primates species remains disputed. The few data that are available from lemurs suggest no decrease in fertility at older ages, although infants born to older mothers may have lower rates of survival during climatic extremes (Wright et al. 2008). Among New World monkeys, some older female tamarins exhibit anovulatory cycles, but nonetheless maintain moderate concentrations of ovarian steroid hormones (Tardif and Ziegler 1992). Among Old World monkeys, reproductive termination appears to be strongly tied to overall senescence. Data from Japanese macaques and rhesus macaques reveal that females remain fertile into late adulthood (Johnson and Kapsalis 1998; Pavelka and Fedigan 1999; Fedigan and Pavelka 2011; but see Gilardi et al. 1997), a pattern also observed among baboons (Altmann et al. 2010). It is worth noting, however, that similar data sets have been interpreted differently. Packer *et al*. (1998) and Altmann *et al*. (2010) both note that female baboons, who have a maximum lifespan of approximately 27 years, continue to produce offspring into their early twenties, but they offer different conclusions as to the comparability of the baboon and human life history patterns. Packer *et al*. (1998) suggest that reproductive cessation is similarly meaningful across species, and emphasize declining survival rates of infants born to older mothers along with increasingly irregular menstrual cycles with age. Altmann *et al*. (2010), however, see a pronounced difference, noting both that baboon estrogen concentrations remain relatively high into late adulthood and that only the last 20% of the female baboon's lifespan can potentially be described as post-reproductive, compared to over 50% in human women with a maximal lifespan of 120 years.

Results from non-human apes have also yielded disparate interpretations. Atsalis and Margulis (2006) conducted fecal steroid analyses on samples from 22 captive female western lowland gorillas over the age of 30 and found that approximately one-third of 'geriatric' individuals showed an age-related decline in progesterone levels analogous to that of perimenopausal human women. A further 25% of older females exhibited no signs of ovulatory cycling and were classified as menopausal, and the authors argue that over 25% of the female gorilla lifespan may be post-reproductive. Robbins *et al.* (2006), however, found no demographic evidence of a post-reproductive lifespan among mountain gorillas in the Virunga region. A similar dichotomy is found between captive female chimpanzees, who show poorer maternal outcome (Roof et al. 2005), increased concentrations of circulating gonadotropins (Videan et al. 2006), and follicular depletion with age (Jones et al. 2007) comparable to perimenopausal human women, and those in the wild, who despite some evidence of reproductive aging (Nishida et al. 2003; Jones et al. 2007), exhibit declines in fertility parallel to declines in survivorship (Emery Thompson et al. 2007).

To date, few data are available from which to draw conclusions about orangutan reproductive aging. Demographic evidence from a wild Sumatran population suggests that females do not undergo menopause (Wich et al. 2004), but there have been no hormonal investigations of the aging process undertaken at sites on either Borneo or Sumatra. In captivity, the oldest female to give birth was 41 years old, but the likelihood of older females giving birth may be altered in captivity by management practices (Shumaker et al. 2008). Hormonal examination of one older female showed that she retained the normal pattern of estrogen peaks in at least some cycles at ages 46-47, although her cycles were shorter and more irregular than those of younger females (Masters and Markham 1991).

In this chapter I take a multi-faceted approach to test the hypothesis that female orangutans experience reproductive aging and have a post-reproductive life history stage analogous to that of human women. First, I use cross-sectional data to compare the hormonal values and cycle data of the two oldest females in the dataset – Chinta (43) and Melati (39) -- with values from younger female orangutans to determine whether a significant difference in ovarian function exists between these two age groups. I then use longitudinal data to view Chinta and Melati as individual case studies, and test whether either female shows more irregular cycling or exhibits a decline in ovarian function with age. Lastly, I evaluate whether these two past-prime females continue to exhibit receptive and proceptive behaviors, and whether they remain attractive to males. If females lose fecundity later in life, both sexes are expected to display little motivation to mate.

Methods

Urine and behavioral data were collected from female orangutans at the Woodland Park Zoo and the Great Ape Trust, together with behavioral data from males at these locations (see Chapter 2). As I have excluded adolescent females from the analyses in this chapter, the hormone data derive from approximately 860 urine samples representing 59 cycles (Table 5-1). Endocrine sampling and assay methods are described in Chapter 2 and Appendix B.

Table 5-1. Overview of Cycles Described and Analyzed in Chapter 5.

Female	Dates Represented	Age	Number of Cycles
Allie	January-March 2009	14	2
Katy	July 2008-March 2009	19-20	4
Melati	Dec. 1992-April 1993	20-21	9
Chinta	June 1992-April 1993	24-25	12
Belawan	Sept. 2008-March 2011	27-29	13
Melati	Sept. 2008-Feb. 2011	36-39	10
Chinta	Nov. 2008-March 2011	40-43	9
			59

In both the cross-sectional and longitudinal comparisons of physiology, I assessed age-related reproductive decline using measures of cycle length and variance as well as the hormonal indices of ovarian function (i.e. cycle quality) described in Chapter 2. I place a strong emphasis on cycle length and variance because these are among the most commonly examined indicators of reproductive aging in human women (O'Connor et al. 2001; Weinstein et al. 2003; Ferrell et al. 2005), and have been used as indicators of reproductive aging in previous studies of non-human primates (Videan et al. 2006; Atsalis and

Margulis 2006; Margulis et al. 2007; Lacreuse et al. 2008). In this chapter I look at both the average length and variance of cycles to determine whether cycle irregularity increases with age.

Statistical Methods

For all statistical analyses involving group comparisons, Group A includes females age 14-29 and Group B includes females age 36-43 (See Table 5-1).

Because the cross-sectional data in this study represent multiple cycles from a small number of individuals, some of whom are represented in both age group categories, I used a linear mixed model to compare ovarian function across Groups A and B. Linear mixed modeling is a useful statistical tool in cases involving non-independent data points because it allows for repeated measures and unequal sample sizes and controls for individual identity (i.e. potential correlations within subjects across time) (Heistermann et al. 2007; Garson 2012). In this case, I separately modeled the relationship of cycle length and log-transformed ovarian hormone values to age group, controlling for subject effects.

For the analysis of longitudinal data from Chinta and Melati, I used non-parametric statistics to compare the ovarian steroid concentrations of the older and more recent samples. Differences in cycle length and variance in these two females across time were measured using independent-sample t-tests and Levene's test for equality of variance.

To examine the degree to which hormone concentrations and cycle phase predicted the mating outcomes of Chinta and Melati, I used a generalized linear mixed model analysis. GLMM, like linear mixed modeling, controls for subject effects by including individual identity as a random factor in the model, but is more appropriate in cases with a binary outcome variable (e.g. mating did or did not occur). As in the previous chapters, I focused on measurements of daily estradiol and the estrogen to progesterone ratio.

All statistical analyses were performed using IBM SPSS v.20.

Results

Cross-Sectional Comparison: Ovarian Cycle Length and Variance

Figure 5-1 illustrates the average cycle length calculated for Groups A (N=40 cycles, mean =28.23 days) and B (N=19 cycles, mean =29.89 days). A t-test revealed that cycle length does not significantly differ between the two groups (t=-1.653, p=.104). To ensure that the presence of Chinta and Melati in both age groups did not affect the outcome, I also compared cycle length using a linear mixed model, which confirmed that cycles do not grow significantly longer or shorter with increasing age (t=-1.828, p=.073; Figure 5-2). Cycle length variance also does not differ between the two groups (Levene’s test F=2.887, p>.05).

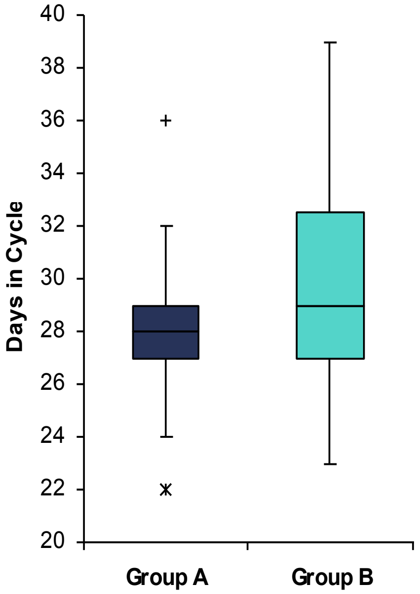


Figure 5-1. Average Cycle Length of Females Age 14-29 (Group A) vs. Females Age 36-43 (Group B). Maximum and minimum outliers are designated by + and x.

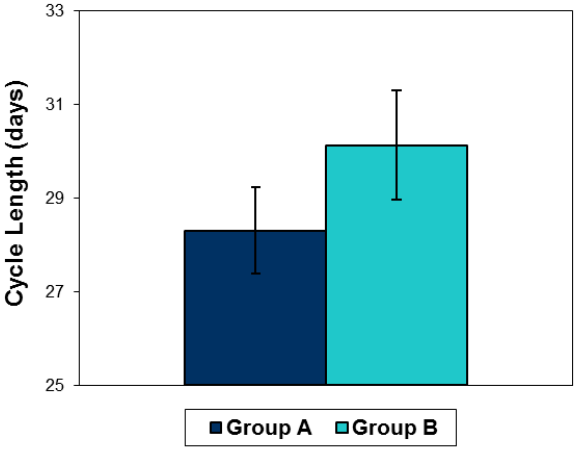


Figure 5-2. Estimated Mean Cycle Length (± SE) of Females Age 14-29 (Group A) and Females Age 36-43 (Group B) Predicted by Mixed Model, Controlling for Subject. p>.05.

Table 5-2. Urinary Indices of Ovarian Function in Prime and Older Adult Females.

	Average E_1C (ng/mg Cr)	**Average PdG (µg/mg Cr)**	**Periovulatory E_1C (ng/mg Cr)**	**PdG, 2nd half (µg/mg Cr)**	**Maximum E_1C (ng/mg Cr)**	**Minimum E_1C (ng/mg Cr)**	**Maximum PdG (µg/mg Cr)**	**Minimum PdG (µg/mg Cr)**
Allie (*n*=2)	59.9 ± 16.1	1.2 ± 0.4	63.8 ± 17.9	1.9 ± 0.7	125.6 ± 41.2	13.3 ± 4.4	4.8 ± 2.6	0.2 ± 0.0
Katy (*n*=4)	47.2 ± 8.5	n/a[a]	52.8 ± 7.5	n/a[a]	127.6 ± 24.4	10.8 ± 1.5	n/a[a]	n/a[a]
Melati [20-21] (*n*=9)	21.6 ± 3.9	0.2 ± 0.0	21.7 ± 4.9	0.2 ± 0.0	55.8 ± 25.5	11.4 ± 0.9	0.4 ± 0.1	0.1 ± 0.0
Chinta [24-25] (*n*=12)	33.0 ± 3.8	0.3 ± 0.0	34.9 ± 4.9	0.4 ± 0.1	80.9 ± 13.4	14.3 ± 1.4	1.0 ± 0.2	0.1 ± 0.0
Belawan [27-29] (*n*=13)	63.3 ± 3.2	0.2 ± 0.0	67.7 ± 3.2	0.3 ± 0.0	127.5 ± 9.2	21.5 ± 1.9	0.5 ± 0.0	0.1 ± 0.0
Melati [36-39] (*n*=10)	38.1 ± 2.4	0.3 ± 0.1	39.0 ± 1.5	0.5 ± 0.1	73.3 ± 8.2	16.8 ± 0.9	0.9 ± 0.2	0.1 ± 0.0
Chinta [40-43] (*n*=9)	45.0 ± 2.2	0.3 ± 0.0	48.4 ± 3.7	0.4 ± 0.1	76.9 ± 7.3	27.3 ± 1.4	0.7 ± 0.2	0.1 ± 0.0

Steroid levels are reported as mean ± standard error. Age is given in brackets. Sample sizes (in parentheses) match the number of cycles given in Table 5-1.

a. PdG results for Katy were inconsistent, and have not been used in cycle analyses.

Cross-Sectional Comparison: Ovarian Cycle Hormone Profiles

Table 5-2 provides an overview of the ovarian indices measured for each female. In general, E1C values vary more obviously across females than do PdG values, although Allie exhibits unusually high average and maximal PdG concentrations (see Chapter 3 for discussion).

A linear mixed model analysis revealed that all measures of ovarian function except maximum E1C and PdG levels differed significantly between the two age groups. However, the results do not support the hypothesis that female orangutans exhibit lower indices of fecundability as they age. Instead, ovarian steroid concentrations were consistently higher in older females (Table 5-3 and Figure 5-3). This suggests that ovarian hormone activity does not systematically decrease in orangutans as they reach their late thirties and early forties. Once again, individual identity was not a significant factor in any of the analyses.

Table 5-3. Results of Linear Mixed Modeling Analysis Comparing Hormone Values by Age Group

	Parameter Estimate	t-value	Significance
Average E_1C	-.208	-5.323	**.000**
Average PdG	-.175	-2.974	**.005**
Periovulatory (POP) E_1C	-.229	-5.129	**.000**
PdG, 2^{nd} half of cycle	-.196	-2.528	**.015**
Max E_1C	-.119	-1.811	.076
Min E_1C	-.228	-4.583	**.000**
Max PdG	-.057	-.676	.502
Min PdG	-.226	-5.252	**.000**

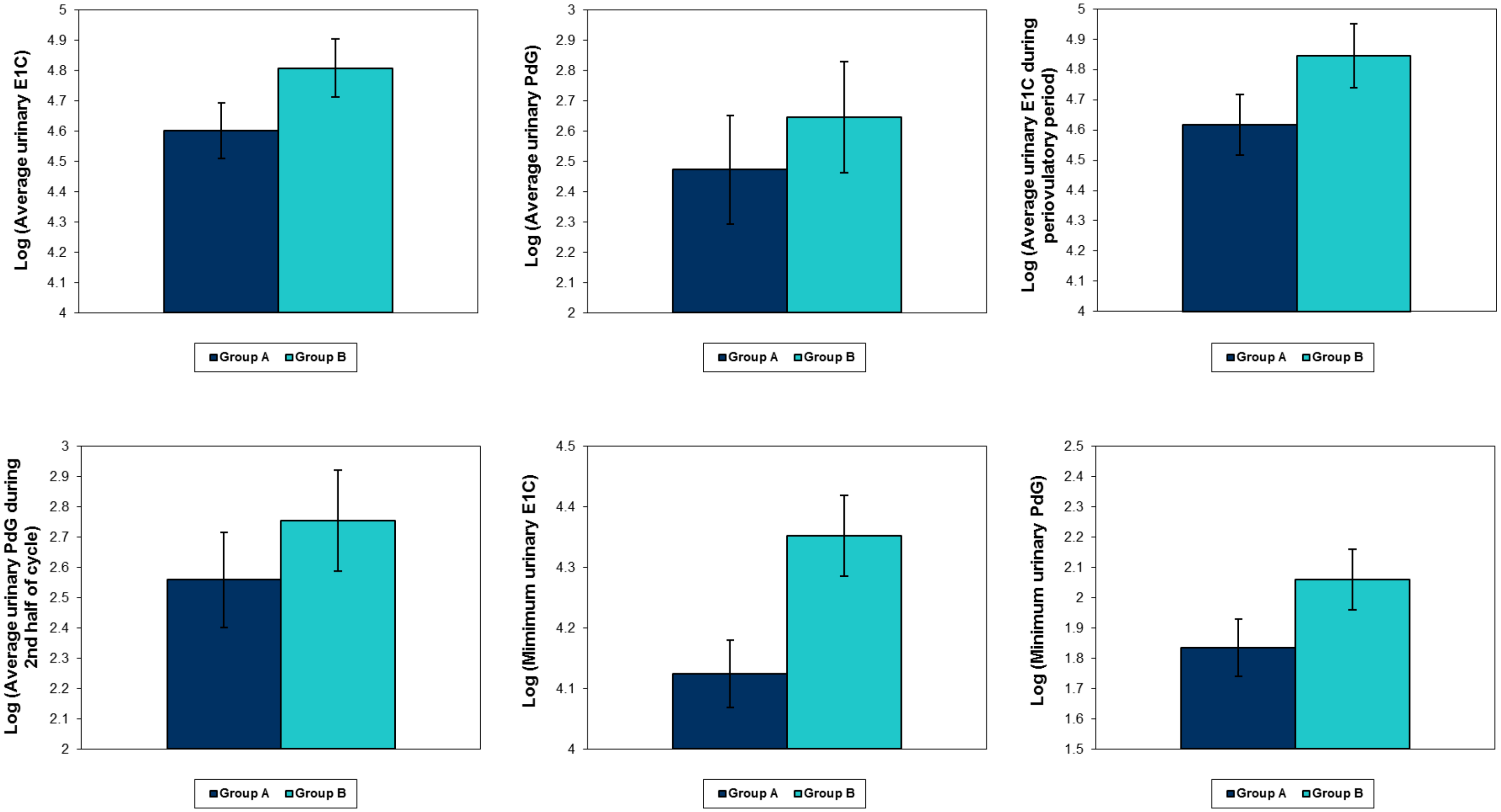


Figure 5-3. Ovarian Hormone Indices of Females Age 14-29 (Group A) vs. Females Age 36-43 (Group B). Graphs illustrate estimated mean ± SE predicted by mixed model, controlling for subject. $p<.02$ for all comparisons shown. Comparisons of maximum E1C and PdG values ($p>.05$) are not shown.

Longitudinal Comparison: Ovarian Cycle Length and Variance

Neither Chinta nor Melati displayed a significant difference in average cycle length when the 1992-1993 samples were compared to the 2008-2011 samples (Chinta: 1992-93 N=12, mean=30.25; 2008-11 N=9, mean =31.78; t=-.779, p > .05; Melati: 1992-93 N=9, mean =25.78; 2008-11 N=10, mean = 28.2; t=-1.587, p>.05), indicating not only that female orangutans continue to exhibit menstrual cycles past the age of 35 or 40 but also that cycle length remains consistent at older ages. During both time periods, the average cycle length of each female remained within one standard deviation of the species average of 29.7 days (Figure 5-4). I also found that cycle variability did not increase: Chinta's cycle length variance did not change from 1992-93 to 2008-09 (Levene's test F=2.149, p>.05) and Melati's cycle length variance actually decreased across the represented time span (Levene's test F=4.804, p=.043), although this result became non-significant if one unusually long cycle from 1992 was removed from the analysis (Levene's test F=1.719, p>.05). These results are in opposition to several studies of aging gorillas and chimpanzees in captivity that have noted either increases in cycle length and variability or the complete cessation of menstrual cycling in some individuals (Atsalis and Margulis 2006; Videan et al. 2006; Margulis et al. 2007; Atsalis and Videan 2009a).

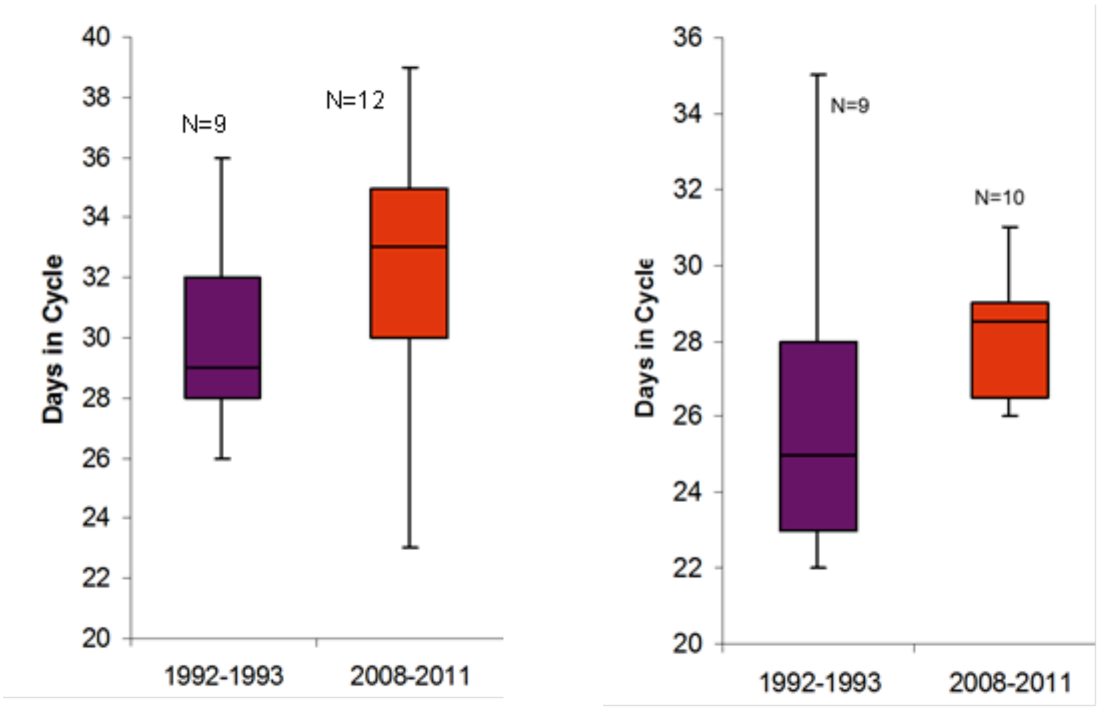


Figure 5-4. Cycle Lengths for Chinta (left) and Melati (right), 1992-93 and 2008-11

Longitudinal Comparison: Ovarian Cycle Hormone Profiles

Figure 5-5 shows cycles from Chinta from just after her 25th birthday (a) and from just before her 43rd birthday (b). Although the later cycle is longer and has a prolonged follicular phase, both cycles are high-quality and show a clear ovulatory estrogen peak as well as luteal progesterone activity indicative of endometrial development. Hormone concentrations are higher in the earlier cycle, representing the upper limits of Chinta’s ovarian activity during the 1992-

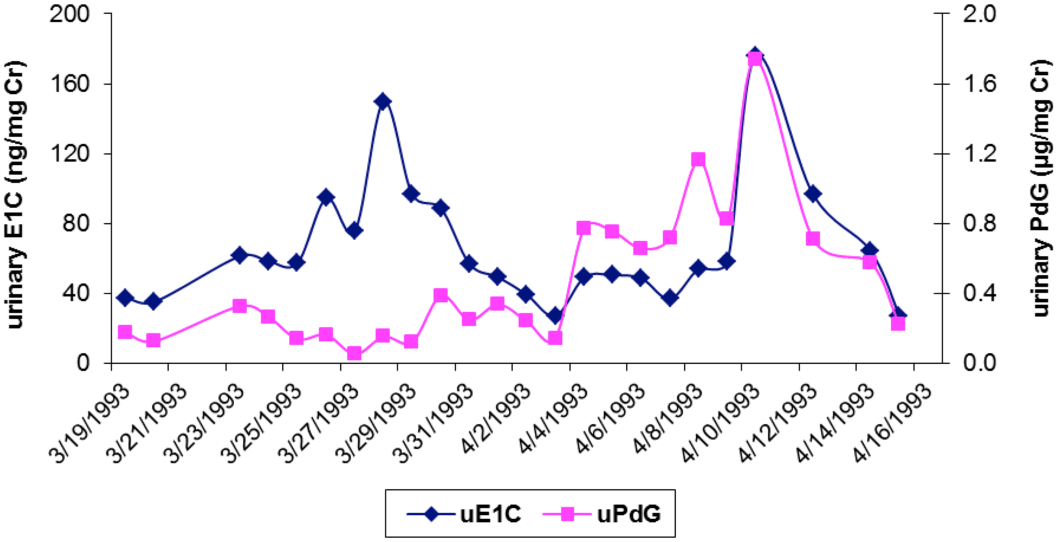
1993 period of data collection (see Table 5-2). In the 2011 cycle steroid values more closely resemble those seen in the composite cycle (see Figure 2-4).

Figure 5-6 shows cycles from Melati at age 20 (a) and age 37 (b). Again, both are relatively high-quality cycles, although estradiol levels were higher in the 1992 cycle and progesterone levels were higher in the 2009 cycle. Of the two, the later cycle is again longer, but at 29 days it is in accord with the species average.

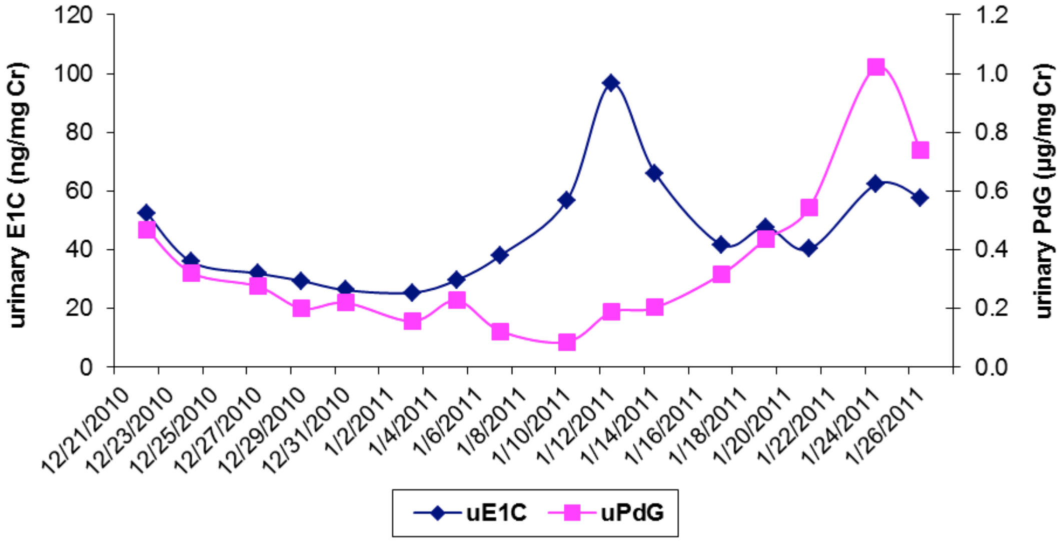
Table 5-4 summarizes the results of all longitudinal hormonal comparisons for Chinta and Melati. Both females evince significant change in several measures of fecundity, but in all of these cases the 2008-2011 samples yield higher hormone levels than the 1992-1993 samples.

Table 5-4. Longitudinal Comparison of Hormone Values for Chinta and Melati's Samples, 1992-1993 vs. 2008-2011 (Mann-Whitney U-test, 2-tailed p-values)

	Chinta		**Melati**	
	z-value	*Significance*	*z-value*	*Significance*
Average E_1C	-2.629	**.009**	-2.939	**.003**
Average PdG	-.640	.522	-2.776	**.006**
Periovulatory (POP) E_1C	-2.416	**.016**	-2.858	**.004**
PdG, 2nd half of cycle	.000	1.00	-3.184	**.001**
Max E_1C	-.497	.619	-2.531	**.011**
Min E_1C	-3.553	**.000**	-3.103	**.002**
Max PdG	-1.208	.227	-1.878	.06
Min PdG	-3.838	**.000**	-1.878	.06

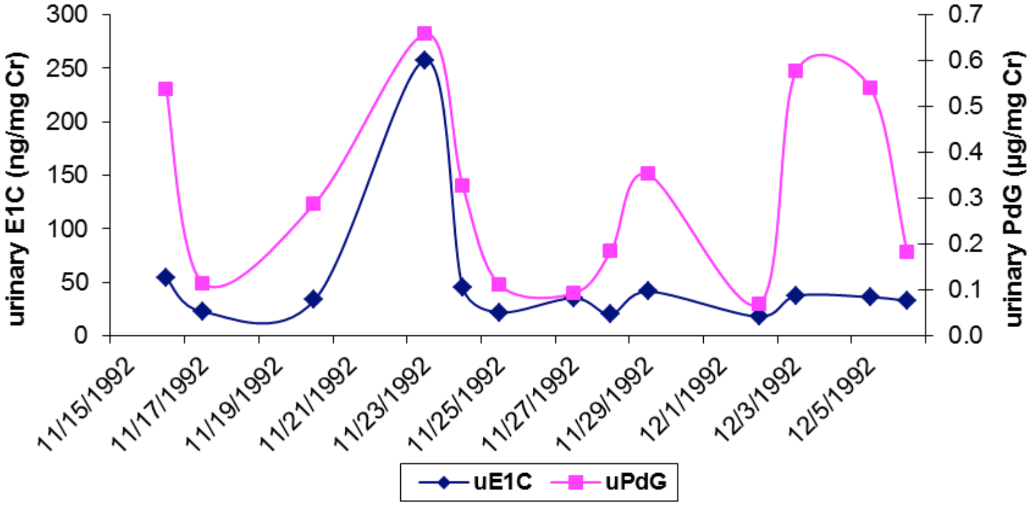


a. Chinta cycle, 1993

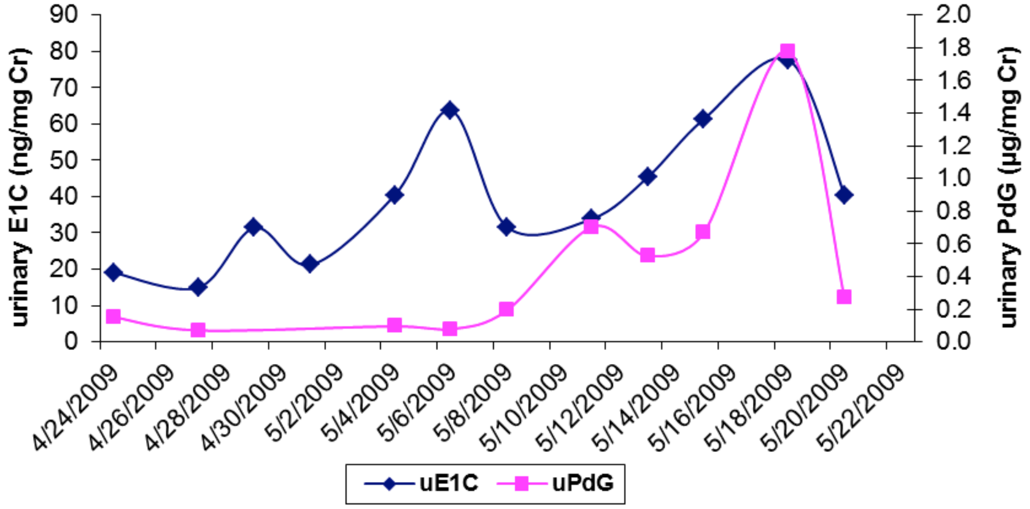


b. Chinta cycle, 2011

Figure 5-5. Chinta Cycles at Age 25 (a) and 42 (b)



a. Melati cycle, 1993



b. Melati cycle, 2009

Figure 5-6. Melati Cycles at Age 20 (a) and 37 (b)

Of the 2008-2011 cycles that I assayed for Melati (N=10 cycles) and Chinta (N=9 cycles), only 2 from each female appeared anovulatory. Figure 5-7 offers an example of a cycle with no discernible E_1C or PdG peak. It is important to emphasize, however, that this cycle is not characteristic of Chinta's ovarian hormone activity past age 40, and that occasional anovulatory cycles can also be identified in younger females (see Chapter 4).

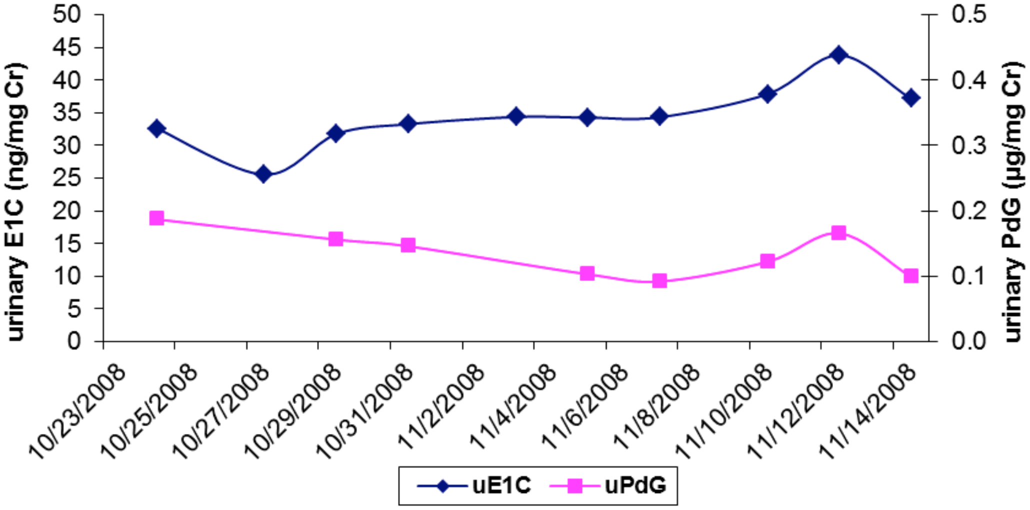


Figure 5-7. Anovulatory Cycle from Chinta, Age 40

Mating Data

Although copulations involving each female were relatively rare, both Chinta and Melati exhibited proceptive and receptive behaviors during the most recent period of data collection, as well as resistance to some matings. Figure 5-

8 summarizes the type and number of copulations observed from August 2008-February 2011.

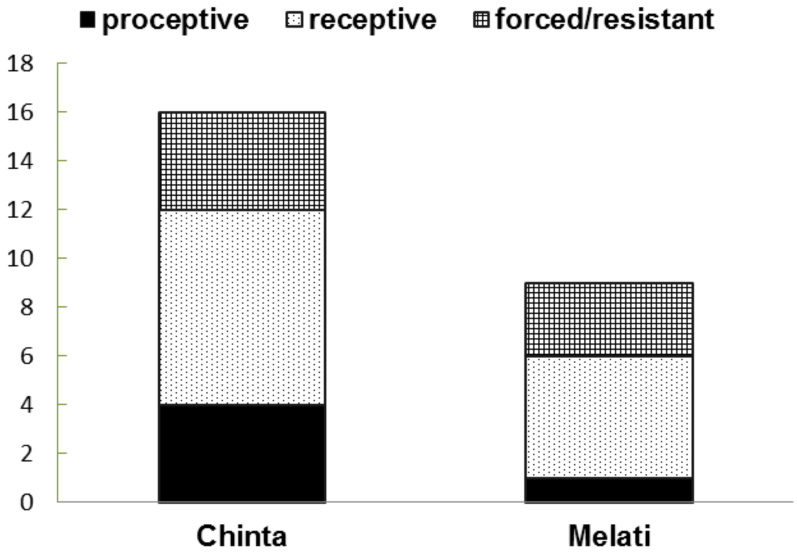


Figure 5-8. Number of Copulations Observed with Heran from August 2008-February 2011, by Type. No more than one copulation observed per day.

Urine samples do not exist for all days on which copulations occurred. However, an examination of available hormone values showed that estrogen concentrations were higher on mating days than non-mating days for both Chinta and Melati (Figure 5-9 and 5-10). The difference was significant only for Chinta (Mann-Whitney U, z=-2.424, p=.015), although sample sizes in both instances were small.

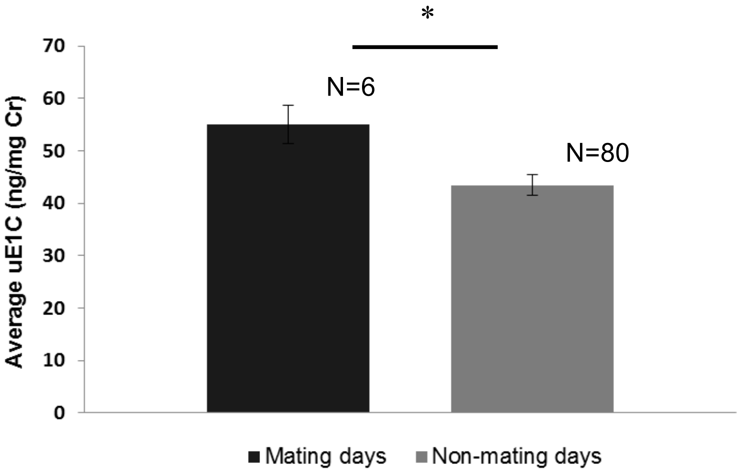


Figure 5-9. Average Urinary E1C (mean ± SE) for Chinta on Mating vs. Non-Mating Days, October 2008-April 2009. (Mann-Whitney U, z=-2.424, two-tailed p=.015.)

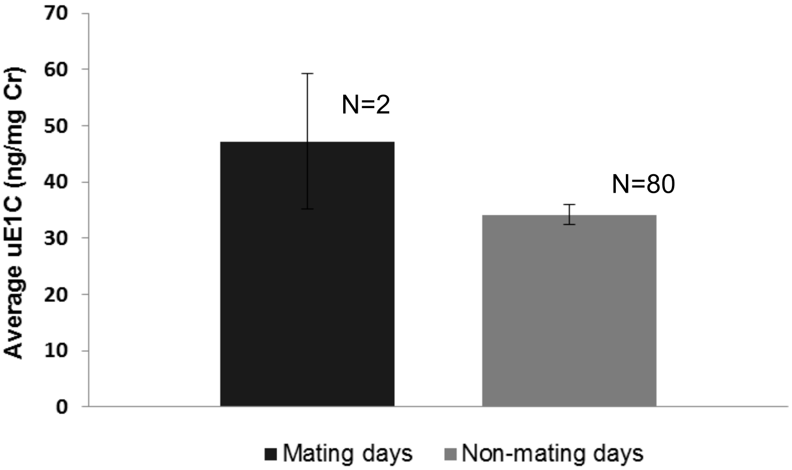


Figure 5-10. Average Urinary E1C (mean ± SE) for Melati on Mating vs. Non-Mating Days, September 2008-April 2009. No significant difference (Mann-Whitney U, z=-1.293, two-tailed p=.196)

A total of seven days on which Chinta mated have associated data regarding ovulatory status. Six of these mating days were within the periovulatory period of a cycle, and Chinta exhibited proceptive behaviors on four of them. Similarly, three of four days on which Melati mated fell within the POP, and she displayed proceptivity on one of these days. While there appears to be a clear trend in the relationship between ovulatory status and mating probability, the difference was not significant when analyzed with a generalized linear mixed model (p=.270; Figure 5-11). Similarly, despite positive trends, the likelihood of copulation did not significantly increase on days with a higher E1C concentration (p=.274; Figure 5-12a) or larger E1C:PdG ratio (p=.296; Figure 5-12b).

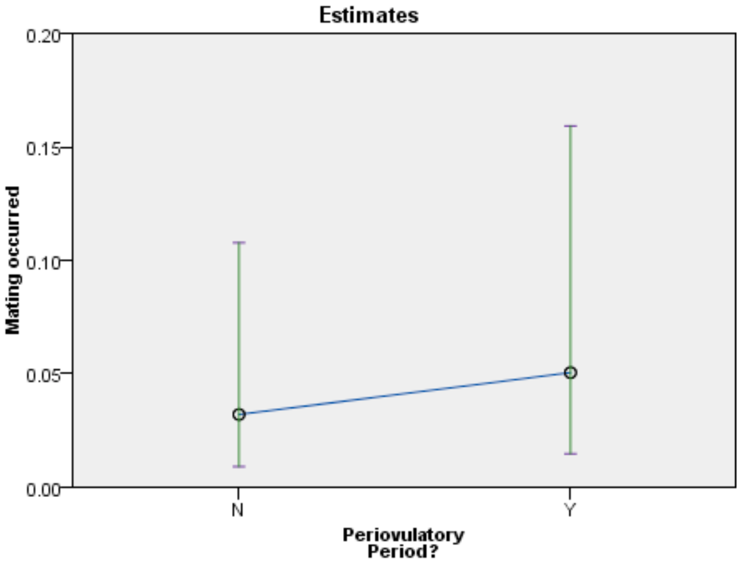
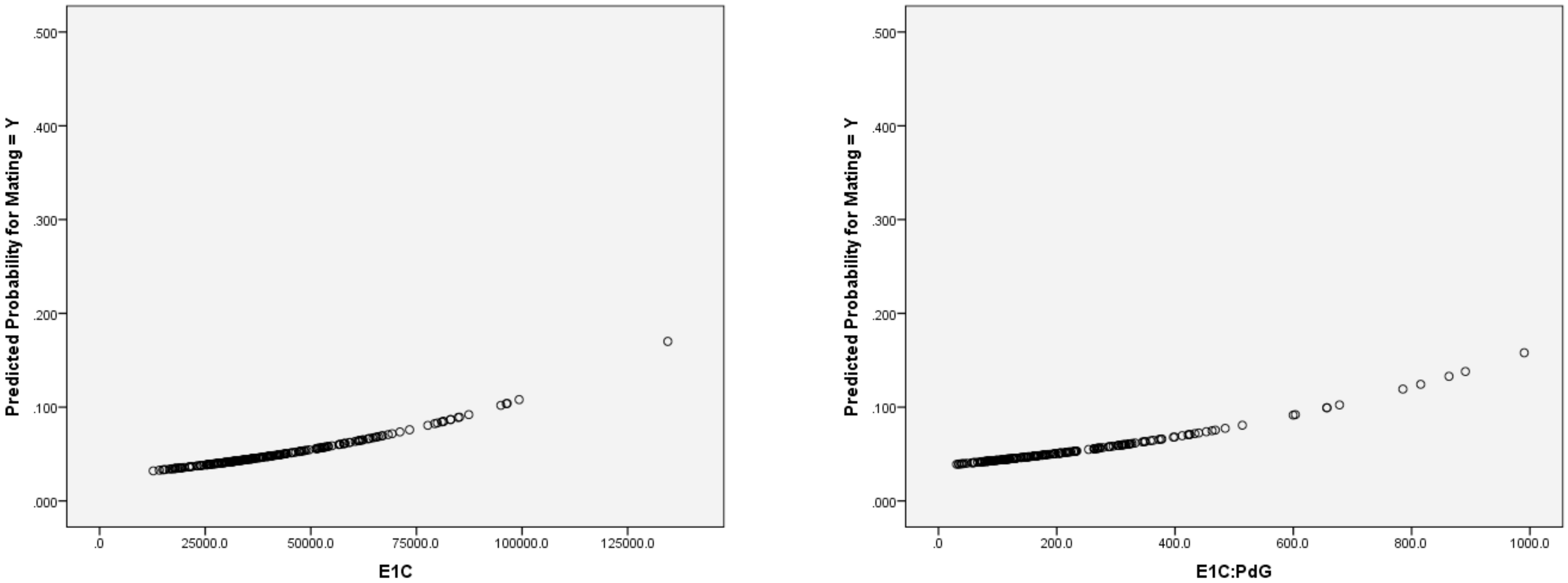


Figure 5-11. GLMM-Estimated Mean Probability of Mating by Ovulatory Status (with 95% CI; N=9 cycles from Chinta and 10 cycles from Melati, p=.270)



a. **b.**

Figure 5-12. GLMM-Estimated Probability of Mating by E1C concentration (a) and E1C:PdG ratio (b). See text for details.

The Woodland Park Zoo behavioral records show no copulations involving Chinta from March 2010 to March 2011, and no copulations involving Melati from July 2010 to March 2011.

Discussion

One of the key tenets of Darwin's theory of evolution by natural selection is that individual fitness increases with continued reproductive success. As such, we predict that selection will shape the life history pattern of each species to maximize reproductive success. Often, this means that the reproductive lifespan of individuals is maximized relative to the total lifespan. The human life history pattern, however, includes a lengthy post-reproductive period, especially in females. What, if any, selective pressure favored early termination of women's reproductive capacity?

This question has been the inspiration for a considerable amount of research. Some anthropologists have argued that menopause is adaptive, a result of the fact that "increased risk of personal reproduction late in life makes it biologically more advantageous to rechannel reproductive energy into helping existing descendants" (Austad 1994). Others have suggested that menopause simply represents the natural limit of a reproductive system constrained by a finite number of follicles (Pavelka and Fedigan 1991; vom Saal et al. 1994), and that it is not the timing of reproductive senescence but the postponement of other senescent effects that makes the human life history pattern unusual (Ellison

2010; Jones 2011). Comparative data regarding the pattern of reproductive senescence in non-human apes are an important resource against which hypotheses about the evolutionary origins and adaptive significance of the human post-reproductive life history phase can be tested. Unfortunately, the use of different definitions of "perimenopause," "menopause," and "post-reproductive" life stages in studies of humans and other primates has generated more confusion than consensus.

Here, I have defined perimenopause, menopause, and a post-reproductive lifespan as follows:

- *Perimenopause* is a period of gradual decline in reproductive functioning that is caused by an age-related decline in follicular activity (vom Saal et al. 1994; Kirkwood and Shanley 2010). Perimenopause may span many years, and can be operationalized using varied criteria such as altered patterns of menstrual bleeding, decreased ovarian steroid concentrations, or increased gonadotropin levels (review: Walker and Herndon 2008; see Chapter 1)
- *Menopause* is the complete cessation of ovulatory cycling. In species that exhibit menstrual bleeding it is identified retrospectively as 12+ months of amenorrhea (Walker and Herndon 2008; Atsalis and Videan 2009b). In other species it can be identified by the near or total absence of ovarian hormone activity, or through histological examination of the ovaries.

- A *post-reproductive lifespan* refers to the time an individual survives beyond menopause. Individuals who have stopped reproducing but remain fecund are not classified as post-reproductive.

I particularly emphasize this last distinction because while a variety of physical, social, or ecological factors can lead individuals to stop reproducing while still fecund, authors frequently use measures of fertility such as the time lag between last parturition and death as a basis to characterize individuals as "post-reproductive" and/or to infer that ovulation has ceased (vom Saal et al. 1994; Pavelka and Fedigan 1999; Cohen 2004; Robbins et al. 2006).

Application of these definitions to the data I have presented in this chapter suggests that reproductive senescence does not affect female orangutans at the same pace (i.e. at the same relative stage of the life cycle) that it does female humans. A cross-sectional comparison between (1) two female orangutans over the age of 35, and (2) a group of younger female orangutans revealed no age-related differences in cycle length or variance, and multiple hormonal measures showed more robust ovarian function in the older females. Analyses of longitudinal samples from Chinta and Melati similarly showed that both females exhibited significantly higher E_1C concentrations in 2008-2011 than in 1992-1993; Melati's PdG levels increased as well. Longitudinal samples are rarely available in studies of reproductive aging in great apes or humans (for exceptions see Landgren et al. 2004; Ferrell et al. 2005), and these data are thus a particularly valuable source of information.

These results notably contradict some previous analyses of non-human apes, which have presented evidence that aging females exhibit markers of lower HPO axis functionality (Masters and Markham 1991; Atsalis and Margulis 2006; Videan et al. 2006; Jones et al. 2007). I suspect that this is due to differences in the way reproductive senescence was operationalized in each study (Walker and Herndon 2008), and to the fact that while Chinta and Melati are relatively old by captive standards, neither is yet approaching the maximum known orangutan lifespan of approximately 60 years (Leighton et al. 1995; Shumaker et al. 2008). Alternatively, it is possible that the elevated E1C levels of the more recent samples do reflect perimenopausal ovarian function: among human women, researchers have sometimes observed that estrogen levels temporarily increase before they begin to steadily decline (Santoro et al. 1996; Ferrell et al. 2005; Hall 2007). The concurrent pattern of increasing or stable progesterone levels in the recent samples suggests that this is not the case, however, as progesterone declines generally precede changes in estrogen levels among humans (Ferrell et al. 2005) .

Because of the small sample size reported here, I hesitate to conclude that female orangutan reproductive physiology is unaffected by the process of follicular depletion. Further analyses of gonadotropin and ovarian steroid levels in female orangutans nearing or older than 50 years may reveal markers of reproductive decline characteristic of perimenopause, or possibly evidence of menopause. The current data, however, clearly imply that orangutans do not have an extended post-reproductive period, and thus add to the growing body of evidence which supports the hypothesis that early menopause and a prolonged post-reproductive life history phase are derived in the human lineage.

Table 5-5 illustrates some of this supporting evidence. Most importantly, while the human female post-reproductive period may comprise more than half of the total lifespan, the post-reproductive period does not represent more than 20% of the maximum lifespan in any non-human primate species in which researchers have documented female survival beyond the cessation of ovulatory cycling. A broader literature search further reveals that, with the possible exception of killer whales and short-finned pilot whales (Foote 2008; Ward et al. 2009; Johnstone et al. 2010), no mammalian species has a post-reproductive period that represents one-third or more of the total lifespan. The results I have presented, among the first to quantify age-related changes in ovarian hormone secretion in female orangutans, indicate that a post-reproductive period, if verified in future studies, will similarly represent no more than 15-20% of the maximum orangutan lifespan. This prediction is bolstered by known instances of reproduction by females as old as 41 years in captivity (Shumaker et al. 2008), and an estimated age at last reproduction >40 years in the wild (Wich et al. 2004).

Table 5-5. The Post-Reproductive Lifespan in Humans and Various Non-Human Primate Species

Species	Average age at menopause (years)	Estimated maximum lifespan (years)	Percentage of lifespan that is post-reproductive
Cotton-top tamarin/ Saddle-back tamarin[1]	17	21	19%
Japanese macaque[2]	27 (*n*=6)	30	10%
Rhesus macaque[3]	29.5 (*n*=2)	35	16%
Hanuman langur[4]	30	32	6%
Gorilla[5]	44 (*n*=5)	52	15%
Orangutan[6]	>40 *(n=2)*	60	<15-20%
Chimpanzee[7, 8]	50 (*n*=3)	60	17%
Human	50[9]	73.5[10] 122[11]	32% 59%

When available, sample sizes (pooled across studies in instances with multiple citations) are provided in parentheses.

1. (Tardif and Ziegler 1992)
2. (Nozaki et al. 1995)
3. (Gilardi et al. 1997)
4. (Sommer et al. 1992)
5. (Atsalis and Margulis 2006)
6. Present study
7. (Lacreuse et al. 2008)
8. (Videan et al. 2006)
9. (O'Connor et al. 2001; Thomas et al. 2001; Wu et al. 2005; Walker and Herndon 2008)
10. Average female lifespan based on a meta-analysis including traditional and industrialized populations (Wang et al. 2012)
11. Maximum known female lifespan (Whitney 1997)

An additional difference between the life history pattern of humans and other species is apparent in the percentage of the population that reaches post-reproductive status. In all non-human primate populations in which this question has been investigated, only a small fraction of females survive to the age of menopause (Pavelka and Fedigan 1991; Johnson and Kapsalis 1998; Pavelka and Fedigan 1999). Human women, by contrast, have an average life expectancy of 73.5 years (Table 5-5), meaning that a large proportion of women will live for several decades following the predictable cessation of ovulation around age 50. Even in populations with a lower life expectancy, a significant proportion of women who survive to the age of menopause are likely to have a significant post-reproductive lifespan (Gurven and Kaplan 2007).

Though I have defined the post-reproductive period in this chapter in physiological terms, the behavioral data that I have presented for Chinta and Melati offer additional insight into the effects aging has on orangutan reproduction. Both females continued to mate in 2008-2011, and the majority of copulations were characterized as proceptive or receptive. This indicates that mating motivation was not entirely absent in either female, and the apparent positive relationship between periovulatory status and proceptivity, similar to that observed among wild females (Knott et al. 2010), suggests that mating motivation was influenced at least in part by reproductive endocrinology. The occurrence of several forced copulations, meanwhile, suggests that both females remained attractive to Heran as potential mating partners. This finding is in accordance with data suggesting that older female chimpanzees not only remain attractive, but are preferred by males as mating partners (Muller et al. 2006).

Once again, these results must be interpreted with caution in light of the limits captivity places on mate choice. Chinta and Melati may have been less attractive mating partners to Heran if a younger female were also present in the enclosure. On the other hand, the infrequency with which Melati and Heran engaged in any sexual interaction may be explained partially by the fact that she is his mother.

While these results accentuate the uniqueness of the extended human post-reproductive period, they do not elucidate the selective pressures that might have favored this life history stage. Currently, the most frequently cited explanation for the evolution of human longevity is the “grandmother hypothesis,” which suggests that selection favored a prolonged post-reproductive period in women because continued investment in children and grandchildren increased inclusive fitness (Hawkes et al. 1998; Alvarez 2000; Hawkes 2003; Caspari and Lee 2004; Shanley et al. 2007). In this scenario, selection for slower senescence acted on somatic chromosomes and men were ‘brought along for the ride.’ An alternative possibility is the “patriarch hypothesis,” which posits that males are the primary targets of selection for delayed senescence. This hypothesis, proposed by Frank Marlowe, suggests that selection favored an extension of the male lifespan “once males became capable of maintaining high status and reproductive access beyond their peak physical condition” (Marlowe 2000), and that female longevity increased as an epiphenomenon.

Attempts to empirically validate the grandmother hypothesis have produced mixed results (Austad 1994; Cohen 2004; Sear and Mace 2008). Hawkes *et al*. (1997) have demonstrated that levels of productivity among postmenopausal Hadza women exceed those of younger women, and contend that the energetic contributions of grandmothers are critical to the reproductive success of their offspring. Studies have also shown that the presence of a maternal grandmother has positive effects on offspring health in the Gambia (Sear et al. 2000; Shanley et al. 2007), and that Finnish and Canadian women with a prolonged post-reproductive period have a larger number of grandchildren (Lahdenperä et al. 2004). Several authors, however, have used theoretical models to argue that inclusive fitness benefits are insufficient to explain the evolution of a post-reproductive period (Hill and Hurtado 1991; Kachel et al. 2011b). As stated previously, the data presented here neither substantiate nor disprove the grandmother hypothesis or other adaptive explanations for the human post-reproductive period, but they do confirm that it is not a life history stage ancestral to the hominoid clade.

Conclusions

1. Future comparative studies of reproductive senescence and life history patterns will benefit significantly from the use of more precise terminology (Walker and Herndon 2008). Researchers need to better distinguish menopause as it is defined physiologically by the end of ovulation (e.g.

“operational menopause”; Atsalis and Videan 2009b) from the more easily identifiable variable of age at last birth (alternately referred to as “functional menopause” (Atsalis and Videan 2009b) or “reproductive cessation” (Cohen 2004)). Attempts to operationalize reproductive decline must likewise recognize that “a change in any one [biological parameter] cannot be considered an exclusionary criterion” by which to infer that ovulation has permanently stopped (Walker and Herndon 2008).

2. Menopause may exist in a number of species in which reproductive decline is tied to overall senescence (Pavelka and Fedigan 1991; Johnson and Kapsalis 1998; Pavelka and Fedigan 1999; Emery Thompson et al. 2007; Kirkwood and Shanley 2010), although the present study reveals no evidence of menopause in captive female orangutans. Humans appear to be unique in that menopause clearly precedes a significant post-reproductive life history stage that defies theoretical predictions (Peccei 2001; Blurton Jones et al. 2002).

Chapter 6: Conclusions and Directions for Future Research

The data presented in this thesis substantially increase our knowledge of female orangutan life history, reproductive physiology, and behavioral endocrinology. In this chapter I summarize the most important implications of these results, and suggest avenues by which future research may further elucidate the importance of ovarian hormone variability and hormone-behavior interactions across the life cycle of this and other primate species.

An examination of ovarian function in several adolescent female orangutans in captivity illustrated that ovarian steroid levels are higher than predicted at young ages. Average and periovulatory estrogen and progesterone concentrations in 3 females age 10-12 exceeded those of 5 adult females in a cross-sectional analysis, and longitudinal data from one female exhibited the same pattern. In addition, whereas researchers have estimated that free-ranging female orangutans experience an adolescent subfecund period of 1-5 years (Schurmann and van Hooff 1986; Galdikas 1995), the present results indicate that HPO-axis coordination and ovulatory regularity can be achieved in as little as six months.

It will be necessary to quantify reproductive maturation in a larger sample of captive orangutans to confirm these preliminary results. Because energetic conditions appear to accelerate reproductive maturation in captivity, investigators should focus on individuals ranging from 6-10 years of age. In addition, further efforts must be made to collect samples from free-ranging adolescent females (9-15 years), as well as from adolescent gorillas, chimpanzees, bonobos, and humans. Comparative data from different species as well as from wild and captive populations will provide better estimates of the average age at menarche and the average duration of the subfecund period under varying environmental conditions, and will thus clarify whether the pattern of reproductive maturation and relative timing of this life history transition is conserved in the hominoid clade.

More detailed information about the relative fecundity of adolescent females will also be useful for further testing hypotheses about female proceptivity and attractivity. The results presented here suggest that absolute hormone concentrations and cycle phase have no effect on female mating motivation during adolescence, but these are based on data from only one individual. Indications of a high degree of proceptivity in another of the subjects I have discussed show that additional data are necessary to characterize the relationship between ovulatory status, cycle quality, and male and female mating motivation during this portion of the female life cycle.

Quantifying cycle quality in reproductively mature female orangutans demonstrated significant intra- and inter-individual variability in ovarian hormone production despite the stable nutritional and energetic conditions of captivity.

These results are consistent with evidence that human women exhibit variability independent of reproductive ecology (Vitzthum 2008, 2009), but little information about the source(s) of this variation is available for either species.

There are many opportunities for future research in this area. The significance of genetic variation within and between populations has not been fully investigated in humans, nor have attempts been made to examine genetic variation and its influence on ovarian function in any non-human ape. In addition, studies are needed to further measure the relationship between psychosocial stress, cortisol, and cycle quality, and to look at stress as a possible cause of anovulatory cycles (Ferin 1999). A study in Finland found higher than expected rates of anovulation in a group of energetically robust college-aged women and suggested that stress might be an underlying factor (Vuorento et al. 1989), but no additional research has pursued this possibility. Because captive institutions are particularly well suited to test the effects that known psychosocial stressors have on ovarian steroid levels in other ape species, samples from the Woodland Park Zoo, Great Ape Trust and other zoos should be assayed for cortisol as well as estrogen and progesterone.

The results presented here also highlight the need for additional efforts to measure the relationship between absolute estrogen and progesterone concentrations and the mating motivation of adult males and females. The data from this project are consistent with numerous studies that have demonstrated increased rates of copulation during the periovulatory period, suggesting that elevated ovarian steroid levels have an effect on attractivity or proceptivity (Gordon 1981; Nadler 1982; Murray et al. 1985; Harris and Monfort 2006; Heistermann et al. 2007). However, it remains unclear after these analyses whether the motivation to mate increases in a dose-dependent fashion with ovarian steroids or whether there is a ‘threshold’ hormone level above which mating interest is triggered. It is also unclear whether hormone levels primarily affect attractivity or proceptivity: among both orangutans and humans it appears that hormone levels produce subtle behavioral changes in females, but male awareness of ovulation is questionable.

Testosterone assays offer yet another possibility for future research. To date, studies have produced equivocal results regarding the proximate stimulatory effects adrenal androgens have on female mating behavior (Beach 1976; Baum et al. 1977; Nadler et al. 1983; Wallen et al. 1984). Future protocols could easily measure testosterone alongside ovarian steroids and glucocorticoids to examine this relationship.

Methodologically, the variability of hormone patterns within and across cycles that has been illustrated by this project suggests that researchers may benefit by reassessing the standard methods that are used to establish the timing of ovulation. In particular, further analyses are necessary to establish whether the relative timing of the luteinizing hormone peak, follicular estrogen peak, and luteal progesterone peak are consistent across cycles. If these relationships prove more variable than expected, the periovulatory period should be delineated through the use of gonadotropin as well as ovarian steroid measurements.

Among the most interesting results of this dissertation is the discovery that captive female orangutans reveal no signs of reproductive senescence, and in fact show higher average and periovulatory ovarian steroid concentrations at older ages than during their prime reproductive years. Together with the aforementioned evidence of elevated estrogen and progesterone levels in adolescent females, these data suggest a pattern of ovarian function across the life cycle that contrasts significantly with predictions based on life history theory and principles of energy allocation (Figure 6-1). In the future, I hope to measure ovarian function in additional female orangutans above the age of 40 to determine whether this is a species-specific pattern (and if so, its adaptive significance). I also plan to collect additional behavioral data from older females to examine rates of proceptivity and to test whether, as has been shown in chimpanzees (Muller et al. 2006), male orangutans find older females to be more attractive mating partners.

Finally, I emphasize that while these hormonal data suggest significant intra- and inter-individual differences in fecundity across the reproductive life cycle of female orangutans, the fitness effects of these differences cannot be

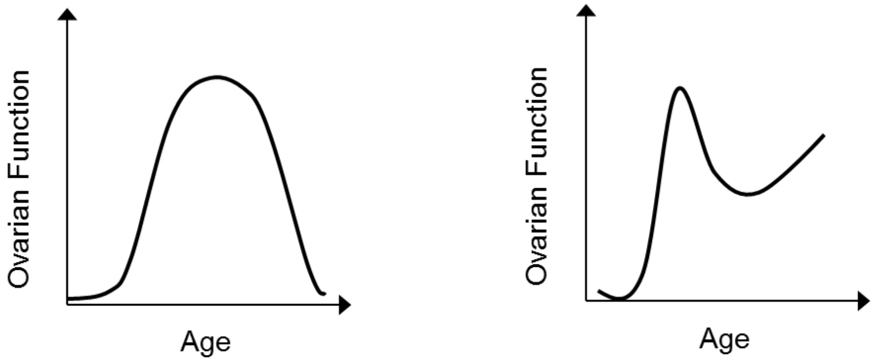


Figure 6-1. A comparison of the expected[5] (left) and observed (right) relationship between ovarian steroid production and age in captive female orangutans.

properly understood without accompanying measures of fertility. Because husbandry practices place artificial restrictions on the reproductive success of captive female orangutans, free-ranging populations will be the best source for this type of data.

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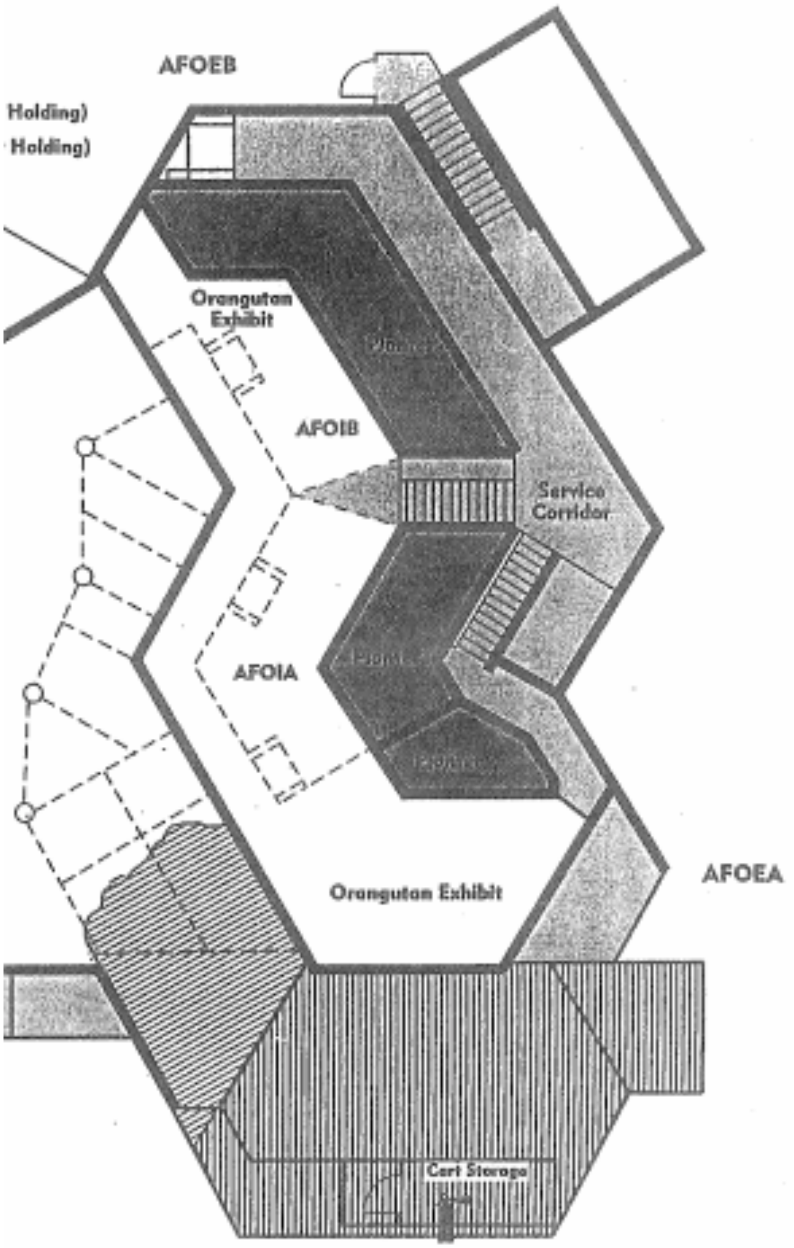
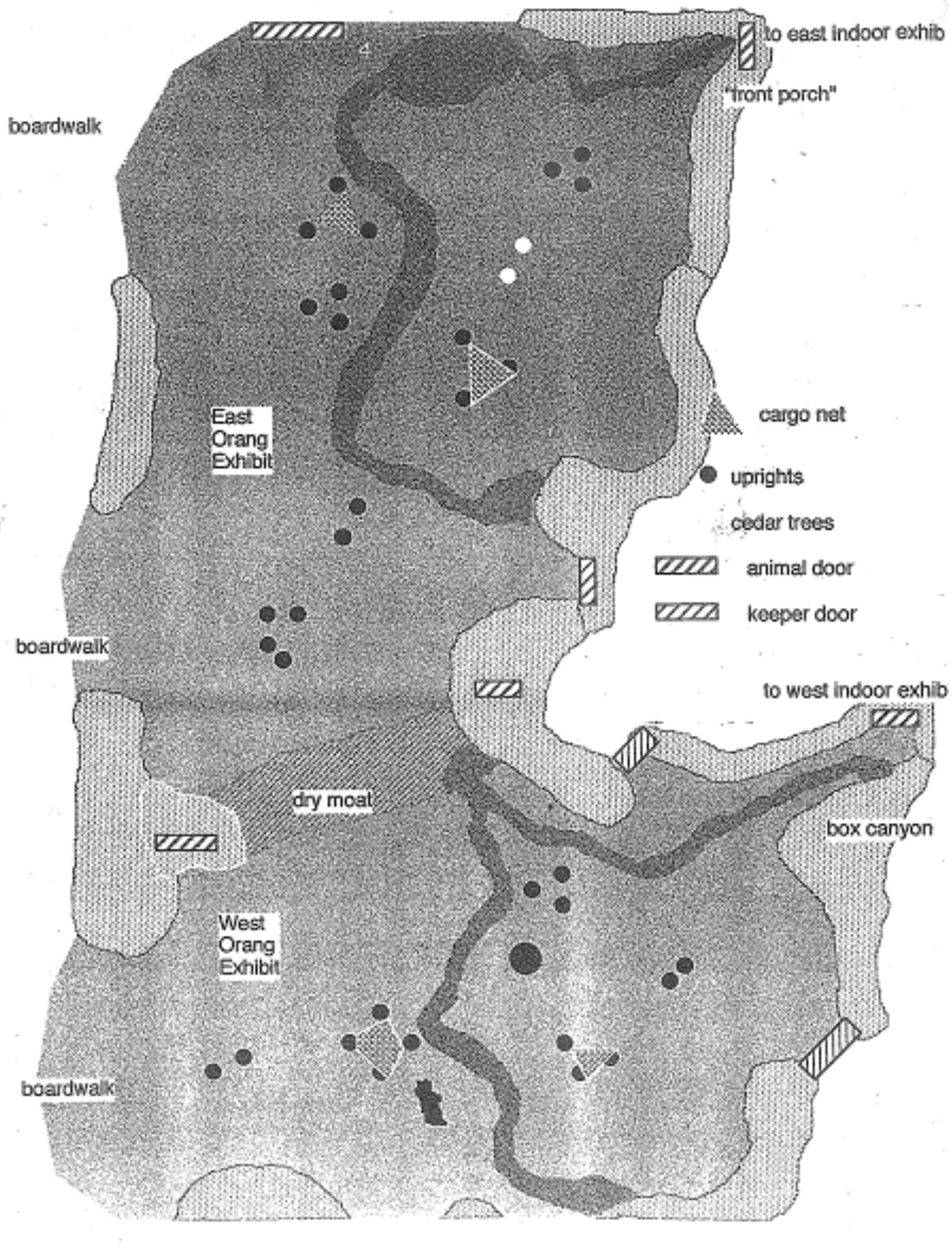
[5] The 'expected' pattern of change in ovarian function with age (i.e. a bell-shaped curve) is derived from predictions based on life history theory, but has not been verified with empirical data from primate populations.

Appendix A(1). Woodland Park Zoo Indoor and Outdoor Orangutan Enclosures

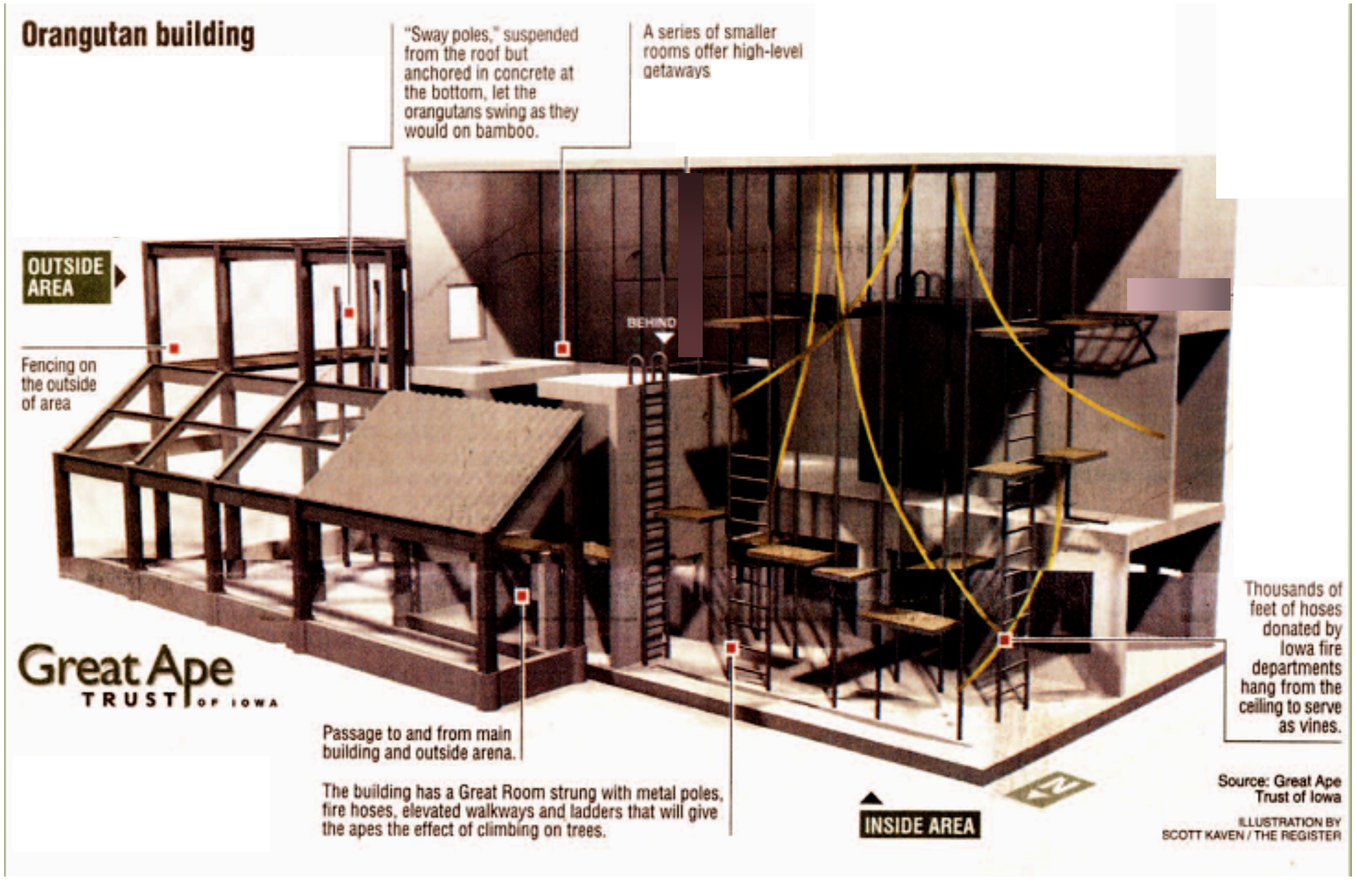
Diagrams courtesy of Libby Lawson.



Tropical Asian Forest
UPPER LEVEL


to east indoor exhib
"front porch"
boardwalk
cargo net
uprights
cedar trees
animal door
keeper door
East Orang Exhibit
boardwalk
to west indoor exhib
dry moat
box canyon
West Orang Exhibit
boardwalk

Appendix A(2). Great Ape Trust Orangutan Enclosure



Modified from graphic provided by the Great Ape Trust and originally published in the Des Moines Register.

Appendix A(3). Woodland Park Zoo Orangutan Diet

Information about daily diet courtesy of Libby Lawson.

	Chinta	Melati	Heran	Belawan	Towan
Morning	102g biscuit 1 apple ½ banana 8 grapes	74g biscuit ½ apple ½ banana 8 grapes	211g biscuit 1 apple ½ banana 10 grapes	102g biscuit 1 apple ½ banana 8 grapes	136g biscuit 1 apple ½ banana 10 grapes
1st snack	¾ orange	¾ orange	1 ¼ orange	¾ orange	1 ¼ orange
Afternoon	11g biscuit	11g biscuit	77g biscuit	11g biscuit	63g biscuit

	East Group (Chinta, Melati, Heran)	West Group (Belawan and Towan)
Morning	1.5 bunches broccoli 1 bunch kale 2 heads romaine 1.5 bunches spinach	1 bunch broccoli 1 bunch kale 2 heads romaine 1 bunch spinach
1st Snack	4 heads romaine	2 heads romaine
2nd Snack	5 heads celery	5 heads celery
Afternoon	300g yam/carrot mix 600g vegetable of the day 1.5 bunches broccoli 1 bunch kale 2 heads romaine 1.5 bunches spinach	225g yam/carrot mix 450g vegetable of the day 1 bunch broccoli 1 bunch kale 2 heads romaine 1 bunch spinach

Vegetable of the Day
Sunday: beets
Monday: onions
Tuesday: turnips
Wednesday: parsnips
Thursday: beets
Friday: onions
Saturday: rutabagas

Each female is allowed 150kcal daily for enrichment, and each male is allowed 300kcal daily. Enrichment items include blueberries, popcorn, sunflower seeds, raisins, ketchup, and Jell-O.

Appendix B. Laboratory Protocols

Creatinine Assay

1. Dilute each urine sample 1:10 in deionized water.
2. Aliquot 100ul of each sample in triplicate into 96-well assay plate.
3. Aliquot 100ul of creatinine standards into plate in triplicate (0.01mg/ml, 0.03mg/ml, 0.10mg/ml; Sigma-Aldrich, St. Louis). Additionally, aliquot 3 replicates of deionized water as 0 standards.
4. Add 50 ul of NaOH (0.75N) to all wells.
5. Add 50 ul of picric acid (0.02N) to all wells.
6. Incubate on plate shaker for 15-20min.
7. Read optical densities using 490nm and 630nm wavelength filters on the Thermo Multiskan plate reader.

Enzyme Immunoassay for Estrone Conjugates (E1C)

Samples, standards, internal controls, stock HRP solutions, and stock and working antibodies should be stored in the freezer. All buffers and working HRP dilutions should be stored at 4 degrees C and allowed to reach room temperature before use in immunoplates.

1. Coat Nunc Maxisorp immunoplates with 50ul of E1C antibody (anti-estrone-β-glucuronide, R522-2, UCDavis, c/o C. Munro) diluted 1:10,000 in coating buffer (0.05M, ph9.6) and cover with film. Allow absorption by refrigerating overnight (or up to 1 week) at 4 degrees C.
2. Prepare samples in minitubes by creating an appropriate dilution with phosphate buffer (0.1M w/0.1% BSA, pH 7.0). Starting dilution for orangutans was 1:10. Prepare standards by serial dilution of estrone-β-glucuronide standard (Sigma Co.) to achieve a range of 156.25-5000pg/40ul. The standard strip should also include 0 wells (phosphate buffer) and internal controls (human urine diluted 1:5, 1:10, 1:25, and 1:50). Samples may be prepared up to 1 week in advance and stored at 4 degrees C.
3. Wash plates with NaCl-Tween20 wash solution and pound plates dry.
4. Add 50ul of phosphate buffer to rows B-G and 100ul to rows A and G which will serve as blanks.
5. Use the multipipetter to deliver 40ul of standards and samples in duplicate rows. Standards should be delivered to row B and then to row G in the reverse direction; this serves to control for differences in conditions across the plate.
6. Add 50ul of horseradish peroxidase (HRP)-labeled E1C (1:125,000 in phosphate buffer) in the order of sample delivery. Reseal plates and store, insulated from light, at room temperature at least two hours or overnight.

7. Wash plates and pound out extra moisture.
8. Add 100ul of substrate solution (hydrogen peroxide 2%, 0.5M; ABTS 40mM, pH6.0; citrate buffer 0.05M, pH4.0) and reseal plates. The HRP on the bound competitor will react with hydrogen peroxide resulting in a green color change in the ABTS substrate. After approximately 45-90 minutes, the 0 wells should reach optimum optical density of 1.00.
9. When 0 wells reach an optical density of 0.9-1.1 or after 90 minutes, read plates using filters 405nm and 630nm wavelengths on the Thermo Multiskan plate reader.
10. Samples should be reassayed if duplicate aliquots produce divergent estimates or if the starting dilution was above or below the standard range.

<u>Enzyme Immunoassay for Pregnanediol Glucuronide (PdG)</u>

1. Coat Nunc Maxisorp immunoplates with 50ul of PdG antibody (anti-pregnanediol-3-glucuronide, R13904, UC Davis, c/o C. Munro) diluted 1:15,000 in coating buffer (0.05M, ph9.6) and cover with film. Allow absorption by refrigerating overnight (or up to 1 week) at 4 degrees C.
2. Prepare samples in minitubes by creating an appropriate dilution with phosphate buffer (0.1M w/0.1% BSA, pH 7.0). Starting dilution for orangutans was 1:5. Prepare standards by serial dilution of pregnane-3α,20α-diol-glucuronide standard (Sigma Co.) to achieve a range of 3.906-500pg/20ul. The standard strip should also include 0 wells (phosphate buffer) and internal controls (human urine diluted 1:5 and 1:25). Samples may be prepared up to 1 week in advance and stored at 4 degrees C.
3. Wash plates with NaCl-Tween20 wash solution and pound plates dry.
4. Add 50ul of phosphate buffer to rows B-G and 100ul to rows A and G which will serve as blanks.
5. Use the multipipetter to deliver 20ul of standards and samples in duplicate rows. Standards should be delivered to row B and then to row G in the reverse direction; this serves to control for differences in conditions across the plate.
6. Add 50ul of horseradish peroxidase (HRP)-labeled PdG (1:75,000 in phosphate buffer) in the order of sample delivery. Reseal plates and store, insulated from light, at room temperature at least two hours or overnight.
7. Wash plates and pound out extra moisture.
8. Add 100ul of substrate solution (hydrogen peroxide 2%, 0.5M; ABTS 40mM, pH6.0; citrate buffer 0.05M, pH4.0) and reseal plates. The HRP on the bound competitor will react with hydrogen peroxide resulting in a green color change in the ABTS substrate. After approximately 30-60 minutes, the 0 wells should reach optimum optical density of 1.00.
9. When 0 wells reach an optical density of 0.9-1.1 or after 60 minutes, read

plates using filters 405nm and 630nm wavelengths on the Thermo Multiskan plate reader.

10. Samples should be reassayed if duplicate aliquots produce divergent estimates or if the starting dilution was above or below the standard range.

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Curriculum Vitae

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