Exploring the Endocrine Profile of a Geriatric Female Chimpanzee (*Pan troglodytes*)

by

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A Thesis Submitted to the Faculty of

The Dorothy F. Schmidt College of Arts and Letters

in Partial Fulfillment of the Requirements for the Degree of

Master of Arts

Florida Atlantic University

Boca Raton, FL

May 2010

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This thesis was prepared under the direction of the candidate's thesis advisor, Dr. Douglas C. Broadfield, Department of Anthropology, and has been approved by the members of her supervisory committee. It was submitted to the faculty of the Dorothy F. Schmidt College of Arts and Letters and was accepted in partial fulfillment of the requirements for the degree of Master of Arts.

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ACKNOWLEDGEMENTS

The author wishes to express her sincere thanks to a number of people involved in the writing of this manuscript, either indirectly or directly. First, thank you to the committee associated with this project, especially to Doctors Broadfield and Halloran for their invaluable advice and constructive criticisms throughout all of the phases of this project. The author is further grateful to Dr. Melissa Emery Thompson and the Hominoid Reproductive Ecology Laboratory at the University of New Mexico for the gracious guidance and assistance concerning the samples necessary for this project. Also, thank you to the chimpanzee keepers at Lion Country Safari during collection periods, for taking on the additional work load of having chimps in the house for months at a time. Finally, the author would like to thank her family for lending their support and encouragement throughout the entirety of this project.

ABSTRACT

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Title: Exploring the Endocrine Profile of a Geriatric Female Chimpanzee (*Pan troglodytes*)

Thesis Advisor: Dr. Douglas C. Broadfield

Degree: Master of Arts

Year: 2010

In light of exceptionally delayed reproductive senescence exhibited by a 64 year old female chimpanzee (*Pan troglodytes*) housed in Florida, endocrinal analyses meant to determine the state of her current reproductive viability were conducted. Urine was collected from the study subject for a period of 88 days spaced within an interim of roughly 6 months and the specimens were sent to the Hominoid Reproductive Ecology Laboratory for assessment. Additional data was collected from three control females in order to provide a basis of comparison against the hormonal markers present in the geriatric study animal. Results indicate that the geriatric female does not presently appear to be cycling, but nor does she exhibit signs of complete reproductive cessation. This could signify that *Pan troglodytes* adheres to a pattern of reproductive aging not necessarily shared by *Homo sapiens*, which has further implications for the evolutionary trajectory of menopause in the human female.

DEDICATION

To Mom and Dad, for being proud of me even though you still don't know exactly what it is I do. To Chaz, for missing me while I'm away. And to Cindy, for quietly sitting close to me for no good reason at all.

Exploring the Endocrine Profile of a Geriatric Female Chimpanzee
(*Pan troglodytes*)

TABLES

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I. The Great Biological Riddle

1.1 *Introduction*

The mechanics of menopause have been examined in one form or another for decades. Its underlying principles—such as the shift in hormone levels of estrogen, progesterone, luteinizing hormone [LH] and follicle stimulating hormone [FSH]—have been recorded by many researchers in various fields throughout the years (Cramer et al. 1995; Faddy et al. 1992; Faddy & Gosden 1996; King et al. 1988; Leidy 1994). Additionally, the significance of reproductive cessation within the parameters of life history theory has been argued for quite some time. After all menopause, or the "final reproductive event [that] marks the definite exhaustion of the primordial follicle pool" (Velde & Pearson 147) seems at first glance fundamentally contrary to the natural drive to disperse one's genetic blueprint indefinitely. It is only by delving deeper into the evolutionary conundrum—through a combination of aspects derived from both physiological explorations and life history theory—that a satisfactory conclusion may someday be revealed. Biological expertise helps researchers to better understand the physical characteristics that denote menopause, whereas its role within life history parameters is fundamental in tracing the overall trajectory of human evolution.

However, only recently have alternative members of the hominoid line been considered in attempts to reach a satisfactory conclusion regarding the phenomenon of menopause. In previous years, the reproductive ecologies of the Great Apes were largely ignored—both in terms

of the general parameters governing the fertility of the species, as well as what these parameters could tell us about the origins of the modern human female's premature decline in fecundity. This is unfortunate, as chimpanzees in particular have long been recognized for their unique ability to contribute immensely towards understanding the nuances of human evolution. Resulting in large part from the lack of documentation concerning the reproductive ecology of man's closest living relative, researchers have yet to reach an adequate conclusion to the evolutionary question posed by menopause; it is impossible to compare the thoroughly investigated human reproductive profile against that of a chimpanzee without a first achieving a more complete understanding of the limitations governing *Pan troglodytes*.

In an effort to better isolate the characteristics specific to reproductive aging in the female chimpanzee, the following study therefore incorporates aspects of the species' life history as well as those of the biological constraints within which these characteristics must operate. In the wild, a chimpanzee will achieve perhaps forty years of age before succumbing to death. In captivity, on the other hand, member of this species have been documented to reach over seventy years of age. The research presented herein involves one such member of the captive female population of *Pan troglodytes*.

The endocrine profile of a seventy-two year old chimpanzee in a semi-free ranging zoological park is examined in order to better understand the reproductive capabilities of the species when allowed to reach more advanced years than typically achieved in the wild. the setting in which this study takes place is highly significant, as recent research (Videan et al. 2006; Videan et al. 2008; Atsalis & Videan 2009) suggests that chimpanzees in a captive situation will experience menopause at approximately thirty-five to forty years of age. However, the study subjects utilized in reaching such a conclusion were housed in laboratory settings, which can severely affect the ability of an animal to operate in a natural biological manner. In order to simulate the ecology of a chimpanzee in the wild, the research presented here utilizes study subjects which maintain an extremely naturalistic existence within the confines of a captive

setting. The endocrinal aspect of this research will consider cortisol, estrogen, progesterone and luteinizing hormone levels in relation to reproductive viability. In addition, the anogenital markers considered to be indicative of fertility—such as labial height and width—will also be noted, as will the reproductive histories of the females examined.

1.2 *Life History Theory*

Life history theory is conducive in the organization of data into the various evolutionary forces that influence the timing of life events, with particular focus placed upon fertility, mortality and growth patterns. Fundamental to the composition of life history is the principle that time is the most precious resource available to somatic organisms, as it will dictate in large part how energetic reserves are spent. In an effort to better understand energy allocation in the context of natural selection, Gadgil and Bossert (1970) described the three-pronged relationship between reproduction, maintenance, and growth as the leading factors shaping an organism's life history patterns. They also identified this tripartite relationship as representing an exchange between energy to be put forth for current reproductive ability and that to be utilized for later reproductive endeavors. The loss of future reproductive and somatic longevity due to energy being allocated to current reproduction is often referred to as the "cost of reproduction." It is this trade-off with which evolutionary biologists investigating the difficult issue of menopause are most concerned.

1.3 *The Grandmother Hypothesis*

Of the research utilizing the various facets of life history theory, George C. Williams produced perhaps the most widely recognizable anthropological work (1957). The conjectures presented—based upon the efforts made by closely related women to better ensure the success

of their particular genetic line—have since directed the course of much of the research within the field of life history theory. Williams' tenants were eventually expounded upon (Hawkes et al. 1997); utilizing data from the Hazda population of Tanzania, they concluded that the division of labor operating within current hunter-gatherer populations likely evolved in tandem with the distinctively human long postreproductive lifespan inherent in our life history.

The Grandmother Hypothesis was soon after revisited (Hawkes et al. 1998). However, here the authors approached the theory in conjunction with Charnov's Model [CM] regarding the timing of life history events. In essence, Charnov's Model provides a general layout of rules which serve to predict mammalian growth patterns, while also identifying those tradeoffs necessary to shape such life histories. By examining the structure of the Grandmother Hypothesis in concurrence with those parameters provided by Charnov's Model, the authors were able to better substantiate the claim that the Grandmother Hypothesis "can account for long lifespans after menopause, late age at maturity, early weaning, and high fertility" (1338), but only when the original question is reversed. As opposed to deliberating the reasons for the premature cessation of human female reproduction, they instead speculated that perhaps our human longevity was a product of natural selection and was extended beyond our reproductive capabilities.

The Grandmother Hypothesis was once again the focus of debate in 1999, when a publication revisited the role of life history theory in the context of reproductive senescence (Hill & Kaplan 1999). Given the available evidence from the Ache foragers of Paraguay, the authors arrived at the conclusion that "the genetic contribution grandmothers make by investing in their close kin is not large enough to overcome the loss of genetic contribution through reproduction, without assuming that fertility drops to almost zero (for other reasons) in pre-menopausal women" (414). Based upon such factors as time spent collecting food and directly caring for related individuals, this study established that any child-rearing assistance provided by grandmothers in and of itself was not enough to compensate for the loss of genetic output upon forgoing

reproductive ability. Additionally, Hill and Kaplan call attention to the fact that grandfathers, who had previously been unrepresented in the literature, have been proven to have as much impact on the fertility of daughters and survival of grandchildren as do grandmothers. Therefore, the effects of grandmothering alone, the authors argue, cannot be said to be substantial enough to have had a significant bearing on the evolution of menopause.

1.4 *An Alternative to the Grandmother Hypothesis*

Soon thereafter an alternate life history theory was proposed (Kaplan et al. 2000) in hopes of fully explaining the evolutionary problem posed by menopause. The four distinctive characteristics that define the human life span—the exceptional longevity, the relatively exaggerated period of juvenile dependency, a reproductive ability supported by postreproductive individuals, and the provisioning of females and offspring by males in an effort to assist in the specifics of reproduction—were posited to be co-evolved responses to a dietary shift toward foods that were much more nutrient-dense and difficult to acquire. This theory deviated from the Grandmother Hypothesis in a number of significant ways. Most notably, the former theory has been thought to overlook the magnitude of the costs attributed to the expanding and more complex human brain, whereas the theory under question specifically links high degrees of reproductive ability later in life to those evolutionary investments necessary to the development of a substantial brain. Life history theory argues that such a high degree of energetic allotment would directly affect reproduction and, ultimately, survival; the costs of the expanding human brain must therefore be accounted for in any attempt to reconcile the contributing factors of reproductive cessation with those of the species' life history. Additionally, a variety of other evolutionary quandaries—such as the questions posed by the male role in a woman's reproductive ability, as well as those created by a man's average life expectancy being on par with that of a woman's—were addressed in this report. Still, it holds to similar perceptions as its

precursor in at least one extremely significant way: both hypotheses directly spoke to the issue of female reproductive senescence and its association with life history theory.

In an additional effort to provide a viable explanation for male roles in evolutionary selection pressures resulting in postreproductive lifespans in females, the "Patriarch Hypothesis" was later introduced and illustrated (Marlowe 2000). While it was once again agreed upon that prolonged lifespans are the unique trademark of humans, this scenario postulated that the Y chromosome was not the recipient of selection pressures. Instead, once males were able to maintain status beyond their peak physical condition—consequently gaining extensive access to myriad sexual partners—they experienced extended life expectancies as a natural outcome of this singular set of circumstances. As it is explained by the Patriarch Hypothesis, women were indirectly influenced by this circumstance as well, as they also carry the X chromosome that all men contain, and so any postreproductive lifespan achieved by females is the direct result of male virility. This theory, however, was not met with much accord, and subsequently did not have a large impact on the myriad schools of thought regarding the menopausal state. Still, the innovation of this conjecture could be well-served by a nonhuman primate model of reproduction, as captive settings currently allow for longer-lived study subjects.

1.5 Revisiting *the Grandmother Hypothesis*

Operating under the assertion that longer lifespans, and not shortened reproductive capacity, is likely the trademark of the human animal, a report was published (Alvarez 2000) regarding the corollary claims first established two years prior (Hawkes et al. 1998). An identical suite of guidelines as those from the original study were utilized, with the exception being the inclusion of a much larger, more statistically relevant primate data set. Using sixteen species of primates and Charnov's Model, three of the central criteria of the modified Grandmother

Hypothesis were revisited. Charnov's Model had already demonstrated that age of maturity and average age of adult instantaneous mortality in primates are not only correlated, but their product (aM) and its inverse are invariant (Hawkes et al. 1998). Additionally, the product of age at maturity (a) and annual fecundity (β) are inversely correlated. Alvarez was thus able to plug in the figures from sixteen data sets to ascertain the validity of the Grandmother Hypothesis' claims that (1) human aM values should not vary from those of other pongids, (2) annual fecundity in humans should be higher than in a grandmotherless primate, and (3) higher rates of birth in humans are made possible in large part to the contribution of grandmothers in the foraging of foods and care of related children. Her research found that while the relationship between a and M in humans is within the confidence interval for primates, the human rate of reproduction during the fertile chapter of life falls above the same expected confidence interval. While the first half of that statement provides justification for the working assumption that the current life history tradeoffs and variables present in humans were also found in our ancestral relatives, the second half leads researchers to propose that perhaps grandmothering results in two interdependent periods of adulthood—the initial portion consisting of higher than expected fecundity because of the fact that the latter portion consists of a complete lack of reproductive ability.

Utilizing these results, two consequences of the Grandmother Hypothesis—that our genus began to undergo inherited age-specific fertility declines, while the rate of decline of all other somatic systems regressed—were soon thereafter explored in greater detail (Hawkes 2003). Using the limited data available on female chimpanzee endocrine profiles at the time, Hawkes expounded upon the assertion that similarities in age-specific fertility deterioration and differences in somatic durability of humans and chimpanzees were essentially powered by the role of grandmothers in reproductive undertakings by their kin. The results of Alvarez's study of two years prior provide evidence for the claim that "members of our genus began living longer *not* because they were kept alive when frail…[but because] a change in selection pressures that favored increased allocation to somatic durability resulted in slower aging in our genus" (Hawkes

392-3). That is, while other theories have attributed late maturity in humans at least in part to the requirements demanded by large-brained juveniles (Kaplan et al. 2000) the Grandmother Hypothesis contends that the genus' late maturity and subsequent menopausal state is derived from our design for somatic longevity. It is the general consensus among the field that this extended lifespan, and not the premature discontinuation of reproductive ability, that great apes seem to lack, therefore making it the evolutionarily assigned characteristic of the human animal.

1.6 *The Physiological Phenomenon of Menopause*

Following the assertion that nonhuman primates merely lack the extended lifespans that their human counterparts are prescribed, nonhuman primate models of reproductive senescence were promptly regarded as potentially valuable in unraveling the specifics of human menopause. The original data concerning reproductive aging in chimpanzees specifically was recorded by Graham and Gould in 1979 and 1981, respectively (Graham 1979; Gould & Graham 1981). The former research focused on the physiological markers of chimpanzee menstrual cycling in the form of visible cues that would indicate such had taken pace, by way of degree of anogenital tumescence as well as the manifestation of menstrual blood. The work, however, was based upon a small sample size of females. While the resulting information regarding menstrual cycles in nonhuman primates is important, it would not be fully understood until years later how limited it was, as visual cues of reproductive cycling can be deceptive in *Pan troglodytes* (Dahl 1999; Deschner & Boesch 2007; Wallis 1997; Nishida et al. 2003). Therefore, it is not surprising that the author seems to imply at the conclusion of his work that chimpanzee females are not adequate tools toward achieving a more cohesive model of human reproductive evolution, as his estimations of chimpanzee reproductive ability are "in striking contrast to the human female in which menopause occurs in the fifth decade and death is often postponed for several more decades" (Graham 291). This opinion, however, would soon change.

The latter study (Gould & Graham 1981), on the other hand, concerned itself with the radioimmunoassay of urine samples in order to properly understand the hormonal reactions to aging undergone in the female chimpanzee. This is infinitely more valuable information than visibly evident cues have proven themselves to be, as anogenital swellings have since been discovered to be unreliable indicators of reproductive ability. In fact, in seemingly direct opposition to the final implications of the previous report, this data led the researchers to the conclusion that, "as [the symptoms of] menopause becomes more fully documented in chimpanzees, chimpanzees may become a more attractive model for the study of human reproductive senescence" (Gould & Graham 1981, p.165). Still, this particular study only considered two common chimpanzees and one bonobo, all of which were just slightly over 40 years of age. This is fairly telling, as chimpanzees in captivity are now occasionally living well into their sixth decades of life. The truly exceptional female chimpanzee can live even beyond even that, as is the case of the focus animal presented in the study at hand.

1.7 *An Early Understanding of the Biomechanics of Menopause*

The first clear attempt at a comprehensive understanding of the biological phenomenon of reproductive cessation began with M.J. Faddy *et al*. in his medical article entitled, "Accelerated disappearance of ovarian follicles in mid-life: implications for forecasting menopause" (1992). Faddy was successful in formulating a mathematical blueprint for determining when in life the onset of reproductive cessation in woman would occur by developing a better understanding of the exact mechanisms behind follicular depletion in human women. Perhaps most importantly, he uncovered the fact that follicles are lost at exponential rates throughout life; while a fetus contains millions of follicles, only a few hundred thousand are left at birth, and that number is drastically reduced as they age. Faddy estimated that by age 37.5, human woman are reduced

to only twenty five thousand eggs, and at age 51 only approximately one thousand remain. It is this severe decline in available oocytes which prompts the onset of menopause by the fifth decade of life.

Faddy's initial investigation of the biomechanics behind the inception of menopause proved to need slight revaluation, however. A second article regarding female reproductive senescence (Faddy & Gosden 1996) was published four years later and endeavored to incorporate a larger number of American study subjects into its data set while simultaneously utilizing the figures from the original study. The results indicated once again that primary ovarian aging is responsible for menopause, and while the cell biology that guides the timing of this systematic reproductive failure was still unknown, Faddy took a clear step towards understanding the mechanics of follicular depletion. With this additional knowledge, one can begin to anticipate the inception of menopause based on residual follicle numbers, and the ages at which women typically encounter those numbers.

1.8 *The Reproductive Cycles of Chimpanzees and Humans: Distinctions and Correlations*

In the four year interim, two additional articles concerning reproduction had been published. The first, "Serum Concentrations of Relaxin, Chorionic Gonadotropin, Estradiol-17β, and Progesterone during the Reproductive Cycle of the Chimpanzee (*Pan troglodytes*)" (Steinetz et al. 1992), called much-needed attention to the myriad similarities between human and chimpanzee female reproductive cycles. Still, the authors discovered slight discrepancies between the relaxin levels—a luteal hormone found in the blood throughout pregnancy—evident in humans and P. *troglodytes*. Results indicated that, "the pattern of secretion of relaxin in the chimpanzee is nearly identical to that of women during pregnancy, and also, that relaxin appears

in the serum of non-pregnant chimpanzees during the late luteal phase of the cycle, but in greater abundance than is found in women at that time in the cycle" (Steinetz et al. 1992, p.3602). Even so, this study reconfirmed that the chimpanzee—more than any other primate—is best suited as a tool for better understanding the evolution of human menopause, as their ovarian steroid function closely mirrors that of a human. The corollary of this close endocrinal association between species holds that chimpanzees may reasonably be held up to the human hormonal standards of reproductive cessation.

In an article that followed shortly thereafter, "Does Incessant Ovulation Increase Risk for Early Menopause?" (Cramer et al. 1995) the authors attempted to understand the correlations between specific variables and age at the onset of menopause in human women. Those variables were either based in menstrual history or reproductive history. Age at first menarche, cycle length, and pain associated with a woman's menstrual cycle fell into the former category, while such factors as the number of live births, ovulatory cycles, and pregnancies fell into the latter. This article was invaluable in demonstrating that factors aside from ovarian age may contribute in some way to the senescence of reproductive ability in women. Still, it remained unresolved as to whether this discovery in humans has similar connotations in chimpanzees or in other nonhuman primates. If so, then research must look to environmental as well as biological factors affecting the nonhuman primate's reproductive cycles when using chimpanzees as a basis of comparison for the inherent human termination of fertility.

1.9 *Exploring Alternative Models of Menopause: Lions, Monkeys and Elephants*

It is noteworthy that, although *P*. *troglodytes* is man's closest living relative, exploring the similarities between the two species was only considered significant very recently. In fact, aside

from the initial efforts of Graham and Gould it wasn't until 1997 that a primate's reproductive ability in the context of senility was again seriously investigated; however, the newest subject was to be the Japanese macaque, as an alternative to the chimpanzee (Nozaki et al.). The first serious study of nonhuman primate reproductive capacity in decades found that "the number of primordial follicles per section [of the ovary] decreases exponentially with advancing postnatal age" (Nozaki et al. 97) in the Japanese monkey. These results parallel those found years earlier (Faddy 1996) in an analysis of human female reproductive organs, alluding to the fact that nonhuman primates may be reasonably held up to the same standards of reproductive senescence as humans.

One year later, a separate species of the same genus was under scrutiny for what they could reveal about the occurrence of menopause (Johnson & Kapsalis 1998). Here, the rhesus macaque (*M. mulatta*) was examined for its suitability as a model of human reproductive cessation, and while found to be an interesting subject the results of the study seem inconclusive. For instance, the monkeys' endocrine profiles were not screened, as the authors instead chose to rely upon behavioral and visual cues to reexamine the state of reproductive ability; as has been previously stated, visual cues are not fully reliable as a method for comprehending reproductive ability. Also, M. *mulatta* experience seasonality in their breeding cycles, which is inherently different from the reproductive cycles of humans. Still, the study had rather momentous implications for the evolution of reproductive cessation in primates, as its purpose was to determine whether the rhesus monkey follows the timing and prevalence parameters of human menopause, in addition to relating this information to the physical condition of the females in question. It was determined by the authors that while the macaques seem to experience an occasional state of reproductive cessation, it is not a significant enough occurrence to warrant the argument that the monkeys provide a good basis of comparison for human menopause; this conclusion was founded in the significant correlation between a rhesus' physical state and reproductive ability. Poor health seemed a good indicator of an inability to maintain reproductive capacity, whereas excellent physical conditioning allowed the female to reproduce later in life.

It was soon thereafter decided that, while macaques may not provide a suitable model for menopause, perhaps there was a discernable pattern within a wider variety of mammals. Based on the available data on lions, elephants and a number of other primates, the authors determined that there are in fact many animals that experience a slowing of reproductive ability with the advancement of years. In the face of this evidence, the argument that human menopause is likely a byproduct of extended somatic aging can be more fully corroborate (Packer et al. 1998).

1.10 *The Role of Somatic Aging in Female Reproductive Cessation*

Once this revelation became the consensus among the academic faction, researchers turned their attention to unveiling the particulars of menopause for a time. To do so, it was apparent that one should look to chimpanzees, the closest living relatives of humans—and therefore the best available model for the progression of human life histories—for answers. For instance, age at reproductive senescence was addressed, as were the precise elements that make up the technicalities of reproductive cessation in nonhuman primates. In rapid succession, a pair of absolutely essential elements for solving this riddle was presented to the scholarly community. Firstly, the age at which chimpanzees are considered to be elderly, a necessary component of understanding the process of senescence, was discussed. It was established that chimps in the wild reach old age in their fourth decade of life, while those in captivity are considered to be geriatric by approximately age fifty (Baker 2000). Additionally, the methodology whereby an estimation of age for wild and captive primates alike may be determined was instituted (Hill et al. 2001; Sugiyama 2004). Distinctive attributes, such as sleek black hair, unworn and generally intact dentition, sure and forceful mobility patterns, and light facial folds were correlated with young adults aged fifteen to twenty years. On the other hand, aged individuals (those 35 years old and higher) will display features such as thinning, dull hair of a

lighter hue, as well as broken, worn teeth and less vigorous movements. Additionally, more facial wrinkles are to be expected in aged chimps. Moreover, this study on mortality rates in chimpanzees attested to the fact that both wild and captive populations of these primates senesce at the same rate, suggesting that the mechanisms whereby aging occurs have a foundation in genetic, biologically inherent systems, as opposed to a basis solely in environmental factors.

A portion of the remaining deficiency in the literature regarding the biological processes of reproductive aging in humans was soon thereafter addressed (O'Connor et al. 2001; Park et al. 2002) in two articles in which female endocrine profiles were utilized to form a basis of reproductive senescence upon which more information could be compounded; this proved to be invaluable in the quest for understanding chimpanzee reproduction, and the evolutionary implications of such insight. Specifically, these papers spoke to the various changes that *Homo sapiens* undergo upon reaching the cessation of their reproductive ability, documenting the fact that this period of follicular inactivity occurs when there are approximately 100-1,000 oocytes left in each ovary; this transitional period can generally be expected to last between 5 and 6 years (O'Connor et al. 2001, p.466).

1.11 *A More Comprehensive Understanding of the Biomechanics of Menopause*

Further enhancing the field's understanding of menopause (O'Connor et al. 2001; Park et al. 2002), recent research has uncovered much of the workings of the hypothalamic-pituitary-ovarian [HPO] axis as well, documenting the role of gonadotropin-releasing hormone [GnRH] in the secretion of luteinizing hormone [LH] and follicle-stimulating hormone [FSH], in addition to explaining the cyclical relationship between the aforementioned hormones and those ovarian

steroids, estradiol and progesterone. In sum, “feedback relationships between the ovarian steroids, on the one hand, and the hypothalamic and pituitary gonadotropins, on the other, maintain the highly regular pattern of ovarian cycles in the adult female” (O’Connor et al. 2001, p.467). In a typical menstrual sequence, ovarian steroid levels are exceptionally low at the start of cycling, which in turn prompts the gonadotropin hormones to stimulate follicular growth via the release of FSH and LH. Once a prepared follicle is stimulated, it begins to emit ovarian steroid levels of significant intensity to induce the recession of those gonadotropin hormones. Ovulation occurs around day 14 of the cycle, when estrogen has achieved peak levels, triggering a surge of LH which ruptures the follicle. As the ovarian steroid levels decline towards the conclusion of a menstrual cycle, the gonadotropin hormones slowly rise once again, and the sequence is recommenced. While this biological configuration is preserved within the fertile years of life, it is interrupted by the depletion of follicles within the ovary that accompanies the climacteric and eventual menopause (Cramer et al. 1995; Faddy et al. 1992; Faddy & Gosden 1996; Velde & Pearson 2002), resulting in a significant lessening of estrogen and progesterone; this in turn leads to sustained elevation of LH and FSH levels, as the negative feedback loop between the pituitary and ovarian hormones is permanently broken. Notably, the articles also elucidated the characteristics of perimenopausal endocrine profiles as encompassing elements from both the fertile and menopausal periods of life. This study also successfully provided a human female model of reproductive function through endocrinal monitoring against which chimpanzee hormonal levels could be validly compared.

Soon thereafter, an additional study was published on the variability of female reproductive aging (Velde & Pearson 2002) to expound upon the available data from years prior (Cramer et al. 1995; Faddy & Gosden 1996; O’Connor et al. 2001). Here, it was discovered that there is a high degree of heritability in reproductive senescence—with approximately 85% of the phenotype variability genetically determined—and also that there is a significant correlation between environment and reproductive decline. What is more noteworthy was the realization that, while age at menopause itself can vary by upwards of 20 years, the rate of reproductive

decline seems to exhibit similar age variations as menopause, “such that the commencement of menopause at an earlier age is associated with an earlier induction of subfertility, sterility and transition to cycle irregularity and vice versa” (Velde and Pearson 2002, p.147). It would appear that once reproductive decline has been initiated there is a six to seven year window in which the body endures relatively sporadic bouts of fertility, until finally complete reproductive senescence occurs. This detail served to shed more light on the unique circumstances presented by reproductive senescence in that it definitively highlighted the fact that the chronological age of a female is not the most crucial trait that defines menopausal timing. Instead, it appears that the period of time between the onset of a decline in fertility and the complete termination of procreative ability is what most contributes to the overall longevity of a woman’s reproductive phase. This additional minor degree of understanding further allows for extrapolations into the biomechanics of menopause; again, geriatric female chimpanzee subjects can provide supporting evidence of this trend via a precise study of the timing of their fertility declines.

1.12 *Visual, Behavioral and Hormonal Markers of Reproduction in* Pan troglodytes

By 2003, there were numerous primate research sites in various locations within Africa at which much data could be collected in regards to both behavioral and physiological facets of chimpanzee life cycles. Mahale in particular broke new ground when research concentrated on studying the life history parameters of the female population. Studies conducted on the premises (Nishida et al. 2003) attempted to infer—from previously recorded data—the percentage of the female population that had ceased to cycle; this was the first of many to turn attention toward such an imperative aspect of demographic parameters. Additionally, they added to the available literature with their information regarding life history variables such as females’ age at first swelling and birth, age at last birth, and interbirth intervals. Unfortunately, the means by which to

detect an absolute end to fertility in chimpanzees in the field were not yet solidified and as a result the findings rely solely upon sexual swellings as a means of interpreting whether reproductive cessation had begun. The article acknowledged that this was in no way a comprehensive methodology for determining the reproductive state of a primate, and attempted to compensate for that lack by subtracting 5 years from the time of last birth to the time of death. Adhering to these parameters, approximately 25% of the female population of Mahale would be classified as having some degree of a post-reproductive lifespan. Still, Nishida and collaborators are careful to reiterate that reliance upon tumescence alone as an indicator of ovulation is not entirely dependable, as the physical phenomenon has been shown to fluctuate in response to stressful or unfamiliar situations, and so a definitive answer to the question of reproductive cessation in *Pan troglodytes* could not be achieved.

Fortunately, techniques for urine collection and endocrinal analysis within a controlled, laboratory environment were finalized by this time, and several researchers at Kyoto University in Japan published a study on the hormonal profiles of several pregnant chimpanzees (Shimizu et al. 2003). However, to properly understand the hormonally altered state of a pregnant female, one must first have a basis of comparison with which to hold it up against. Therefore, within this single study researchers distinguished between endocrine profiles for both infertile and fertile chimpanzees, which are invaluable in establishing a standardized baseline for the chimpanzee menstrual cycle. Moreover, the study validated the assumption that urinary hormones are a dependable method for calculating reproductive function in this species.

Simultaneously, a workshop was held in which nonhuman models of menopause were a central topic. Soon thereafter, a report detailing the goings on of the proceedings was released; while most of the information within the pages was a dedicated recap of the findings within the field over the years, there were a few revelations that had not yet been available within the literature (Bellino & Wise 2003). Baboons, especially, provided original statistics to be included within the body of knowledge regarding menopause, in that they do not appear to experience

human-like hormonal changes in the climacteric, nor do they undergo—on average—a change in the length of menstrual cycles. This is telling in the evolutionary sense, as chimpanzees have been documented to experience such changes on both counts, much like their close relative, H. *sapiens*. The lack of reproductive similarities between humans and baboons conceivably indicate that the phenomenon of menopause arose closer to the time at which chimpanzees and humans diverged. In the end, however, the presiding committee members of the workshop were certain of one thing: that "insufficient data exists in many areas regarding the biology of the aging female NHP [nonhuman primate] to allow a critical evaluation of whether these species can provide insights into the human menopause" (Bellino & Wise 2003, p.15). It is a problem which has yet to be entirely rectified, although increasing steps have been taken to do so.

One of such steps came in the form of an endeavor to further unravel the biological specifics of the female Hypothalamic-Pituitary-Ovarian axis. While the study primarily tracked the change in estradiol [E^2] and Follicle Stimulating Hormone across the early menopausal transition, the emphasis was in determining whether age had a forceful impact on these changes (Randolph et al. 2003). The results indicate that there is a marked and independent difference between the serum concentration levels of E^2 and FSH within the various ages studied; these changes were observed to occur during the beginning stages of the menopausal transformation. Whereas serum E^2 concentrations significantly decreased with age, concentrations of the pituitary controlled hormone FSH increased dramatically over time. Perhaps most interestingly, all changes in hormonal elevation were much steeper as study subject age escalated. These findings suggest that there is statistically significant age-specific variation operating within the HPO axis in *H. sapiens* which, when considered alongside findings in the rates of follicular depletion (Faddy et al. 1992; Faddy & Gosden 1996) and environmental factors influencing the velocity of reproductive cessation (O'Connor et al. 2001), carries interesting implications for the timing of menopause during the course of human evolution which chimpanzee endocrine profiles may help to sort out.

By 2004, the study site of Bossou published a report on the demographic parameters and life history patterns of the female population residing in the area, much like that published earlier (Nishida et al.), adding much-needed information to the pool of available data which had at that time been fragmented and sparse (Sugiyama 2004). This particular study was appealing in that it encompassed data from P. *troglodytes verus*, or West African chimps, which have been documented to exhibit somewhat peculiar characteristics when compared to their relatives in the east and central regions of Africa (Boesch & Boesch 2000; Emery Thompson et al. 2007). Additionally, with the distinct reduction of natural predators in Bossou and more readily available food sources, many aspects of their life were found to mirror those found in captive situations more closely than that of east or central African chimps. Some of these similarities, such as a relatively young age at primiparity, interbirth intervals, and age at death, were discussed at length. Without exception, the age at first birth for females fell below 11 years, time between births was averaged to be 4 years, and females may live to 50 or more years. Perhaps most importantly, the published data allowed researchers a more in-depth look into the numerous variables which can affect the life history of female chimpanzees, both in and out of captivity. While these environmentally influencing factors had been previously discussed in connection to human women (Cramer et al. 1995), it was the first time that the same could be definitively said to be true regarding chimpanzee life history traits.

The following year, the reproductive capacity of captive chimpanzees was once again a topic of careful study (Roof et al. 2005). In this particular course of research, the birthing outcome of 1,255 P. *troglodytes* pregnancies was examined in relation to maternal age and overall parity. Rather than relying solely upon anogenital tumescence to indicate sexual receptivity, this study undertook a more comprehensive methodology. The authors determined to focus on recent publications—for instance, the demographic parameters from Bossou—which indicated that other measures, such as interbirth intervals, age at primiparity, birth outcome, and birth weight influence reproductive deterioration. In this longitudinal analysis of reproductive outcome in correlation with the maternal age and parity in chimpanzees, evidence suggests that

not only does reproductive ability decline in aged females, but complete cessation of said ability will eventually occur as well, albeit perhaps at different rates and possibly due to alternate mechanisms than those apparent in human populations. Additionally, “maternal age, rather than parity, was found to be the most important predictor of negative birth outcome” (Roof et al. 2005, p.199). This is remarkable, as it can be taken to signify a comparable reproductive timeline between humans and chimpanzees, whereby viability decreases at approximately 40 years of age while negative birth outcomes—such as Down’s syndrome, birth defects, and spontaneous abortions—increase.

1.13 *The Great Debate: Does* Pan troglodytes *Undergo Complete Reproductive Cessation?*

Soon thereafter, a report (Emery Thompson 2005) was published which allowed the progressive trend of explorations into chimpanzee reproduction to continue. In an essential requisite for sorting through available facts, this article—while specifically meant to address the relationship between ovarian hormone levels and the probability of conception—simultaneously served to fill numerous gaps in the established methodology for deciphering the hormonal cues of female chimpanzees. The endocrine profiles of eighty-one wild East African chimpanzees (*Pan troglodytes schweinfurthii*) were evaluated in relation to sexual tumescence, and the findings corroborated previous studies that suggested that the fertile window for a female chimpanzee is restricted to the final week of anogenital swelling. Noninvasive sampling methods were explored and storing methods were subsequently evaluated for effectiveness; it was determined that ovarian steroid levels could be successfully assayed based upon small, readily available amounts of urine and fecal matter, independent of the time of day at which the waste was collected. In addition, the publication assessed storage techniques for keeping the integrity of the urine and feces intact and concluded that the former may be frozen to retain reliability, whereas the latter

may be baked or frozen. The multitude of advancements made in this particular study was absolutely critical in achieving headway towards a more comprehensive understanding of the mechanics and implications of female chimpanzee reproduction.

By the following year, a collaborative effort intent upon reviewing the demographic and life-history parameters of the population of the Chimpanzee Rehabilitation Project [CRP] at the River Gambia National Park in Africa was published (Marsden et al. 2006). This paper is made especially appealing by the unusual circumstances of the subject matter at hand; these chimpanzees were first generation released or wild-born, and so retained many of the reproductive limitations of their wild counterparts. Also, they were provisioned, and therefore preserve many reproductive parallels between the inhabitants of CRP and first generation or wild-caught chimpanzees living in captive situations. The most ardent of similarities may be found between the primates at CRP and those found living in "semi-captive" situations—such as that provided by Lion Country Safari in Loxahatchee, Florida—whereby both sets of animals are confined by a water barrier, residing on islands where they spend their time foraging for foods, and interacting with their environment in general and each other in particular, set a relatively significant degree apart from human influence. While the study spoke to the analogous aspects of captive and wild reproductive parameters—such as the inevitable results of the lack of predation and episodes of provisioning—it also cautions against believing this seeming surfeit of similarities to be true of all captive populations. Age at first swelling and first birth, as well as interbirth intervals, were discussed at length; the first full tumescence was observed at approximately 11.8 years old, while age at primiparity was estimated to be 13.5 years and interbirth intervals were calculated at five to six years, which is substantially longer than the average 3.5-4.5 years in captivity. They do, however, concede that there is not much available literature on captive chimpanzees against which to compare their statistics. Nevertheless, a wild-caught individual living in a habitat equivalent to that described at the CRP could be argued most likely operating under the same reproductive boundaries of many populations of wild chimpanzees.

Shortly thereafter, a long-term exploration of the endocrine profiles of a number of laboratory-housed chimpanzees commenced, using the newly developed methods for accurately monitoring reproductive hormone levels (Videan et al. 2006). Here, researchers provided the exact concentration of follicle-stimulating hormonal levels necessary to be considered menopausal in human women as exceeding 20 IU/I or 30 IU/I, whereas LH levels—while lacking a precise number—are reported to have typically more than doubled by the menopausal transition. Additionally, the researchers evaluated estradiol [E^2] stages. They opted to apply these relatively fixed levels in women to female chimpanzees as a means of indicating that the cessation of reproductive ability had occurred; this has been the accepted methodology since *P. troglodytes* was interpreted to provide an adequate model for the evolution of human reproductive ecology.

The goal of the research was to document the long-term, age-related physiological changes undergone in female chimpanzees as they reach the projected fulfillment of their reproductive capacity. The study detailed the cycle length and endocrine levels of 14 individuals, ranging in age from 31 to 50 years old, the results of which suggested that menstrual cycle length—as indicated by anogenital swelling—was drastically altered upon reaching thirty-five to forty years of age. Moreover, the percentage of the cycle at which the maximal swelling is present decreases at this age, whereas the percentage of maximal detumescence increases, signifying significant shifts in the females' pituitary hormone levels and cycles. "Analysis of hormonal data revealed a bell-shaped curvilinear relationship between age and LH, with LH peaking around age 30-35 years" (Videan et al. 2006, p.293), and E^2 declining with age, although not considerably enough to be regarded as especially significant. This is in direct opposition to the findings of years prior (Randolph et al. 2004), whereby E^2 showed a sharp decline with age. The study concluded that FSH, on the other hand, peaked at approximately forty years, with a sharp rise beginning at around thirty-five; all of this data taken together mirrors anogenital

markers which suggest that reproductive viability in chimpanzees begins diminishing after age thirty.

The overall report points toward the conclusion that female chimpanzees included in the data set were exhibiting indications that they were undergoing the same patterns of reproductive cessation that their human counterparts display. However, the levels of gonadotropins used to signify reproductive senescence in these specific chimpanzees were not permanently sustained at high intensity—instead subsiding again after a period of time—which is unlike the pattern found in human menopausal females. Therefore, to ascertain, based upon unclear data, that *P. troglodytes* undergoes menopause by the fortieth year is difficult. Still, the publication has fostered much debate regarding the potential genetic composition of *P. troglodytes* reproductive aging, as the possibility that chimpanzees are reproductively senescing at the exact same rate as human females would effectively put to rest the discussion originally posed by life history theorists: that *H. sapiens* experience extended somatic longevity rather than a lengthened reproductive capability.

Shortly thereafter, research (Jones et al. 2007) provided evidence further making the case for chimpanzee similarities to human processes of reproductive decline in a study on follicular depletion rates among the two species. Through examination of nineteen specimens, ranging in age from three months to forty-seven years, it was determined that chimpanzee rates of ovarian follicular decline were virtually synonymous with those found in human females. With this improved awareness regarding the rate of follicular decline in chimpanzees, one can begin to anticipate the species-specific inception of menopause based on residual follicle numbers, and the ages at which females would typically encounter those numbers, as was proven a number of years earlier (Faddy 1992; Faddy & Gosden 1996). Seeing as how the primordial follicular count between humans and chimpanzees are reportedly very similar, one could logically anticipate that menopause in the latter would occur at approximately the same time that menopause in the former seems to regularly transpire—in approximately the fifth decade of life. This data,

combined with adult mortality rates in wild chimpanzees, once again suggests that humans may be unique in their innate ability to live well beyond their reproductive lifespan, whereas the reproductive viability of P. *troglodytes* apparently fails in tandem with the remnants of their physiological systems. Assuming then that somatic longevity deteriorates at equal rates as is evident in reproductive cessation, a female chimpanzee surviving well beyond the projected time line should conceivably have undergone the menopausal transition by her fiftieth year; the validity of this assumption would be revealed through exploration of geriatric animal's endocrine profile.

Later that same year, further investigations into chimpanzee reproductive aging were carried out in a demographic study on six populations of chimpanzees in Africa, whereby the question of whether chimpanzee reproductive senescence can be attributed to general somatic aging or if it is more closely linked with that of human reproductive aging was once again assessed (Emery Thompson et al. 2007). The similarities between human and chimpanzee declines in birth rates associated with age are recounted by the current data, as is the belief that this points to a conservation of physiological processes occurring throughout human evolution. However, it is made apparent that—at least in the chimpanzee—this can be attributed to the correlation between overall health declines and the declines in fertility, which reinforces the conclusion that it is likely somatic aging which prompts reproductive senescence in the species. Humans, on the other hand, exhibit marked declines in fertility rates while mortality rates also remain extremely low, which leads to the long post-reproductive lifespan apparent in human populations but not found in chimpanzees. This data corroborates prior findings (Jones et al. 2007) in that both data sets strongly suggest that age-related rates of fertility decline are similar between these two very closely related species, which could lend to a more comprehensive understanding of the mechanics of menopause operating along the course of human evolution.

By 2008, further inquiries regarding nonhuman primate reproductive senescent patterns were commenced (Videan et al. 2008). This research once again contributed to the body of knowledge regarding chimpanzee reproductive senescence through use of a number of

laboratory-housed female specimens aged thirty-two to fifty years. Utilizing data collected biannually from these fourteen captive female chimpanzees over a period of 14.1 years on average, the authors established that there exists a bell shaped curvilinear relationship between age and serum concentrations of LH and FSH, although the correlation in the latter was rather poor. However, the authors rely upon human hormonal levels of both ovarian steroids as well as gonadotropins to indicate the onset of menopause, but do not subsequently adhere to the rules established by these human assays. For example, the spike in LH occurring around age thirty-five to forty years of age in chimpanzees is documented, but this increase is soon after followed by a subsiding of gonadotropin levels. This does not occur in human menopausal females, and should therefore not be present in postreproductive chimpanzees, as available levels of LH and FSH reach a sustained high without surcease upon the permanent interruption of the negative feedback loop inherent in the HPO axis which accompanies the menopausal transition. Additionally, it should be noted that anogenital swellings occurred seemingly without disruption during the 14.1 years of data collection, even upon the reported cessation of reproductive viability in these individuals. These inconsistencies have since lead to a number of rebuttals regarding the proposed age at reproductive termination in P. *troglodytes* (Lacreuse et al. 2008).

1.14 *The Contribution of Environmental Factors to Potential Reproductive Stasis in the Nonhuman Primate*

One year later, research (Atsalis and Videan 2009) concerning reproductive aging of the common chimpanzee female proposed that the differences between the breeding agenda of captive and wild populations of *Pan troglodytes* could potentially lead to the markedly dissimilar results in age at menopause between the two. The study addresses the accelerated maturation within all reproductive parameters experienced by chimpanzee females in captivity, and attribute the ensuing oocyte loss to the higher overall reproductive success than is found in wild

populations; it is, however, acknowledged that the estimated lifetime reproductive success in wild chimpanzees may very well be underrated. It is the ensuing high rate of follicular depletion in captive apes—due to the lack of reproductive amenorrhea reported in captive or provisioned settings—which would potentially result in the premature age of reproductive cessation. While this proposal necessitates reference to previous works (Cramer et al. 1995) in which incessant ovulation is indeed shown to have an effect on the timing of menopause in women, it is not necessarily fair to utilize that data in such a manner as to explain away the obvious discrepancies between suggested captive and wild chimpanzee timing of reproductive senescence, as the calculated difference is more than a full decade. Additionally, the captive populations studied here were once again housed in laboratories, the conditions of which may have had a great influence over the animals' state of reproductive ability. Finally, the parameters used to indicate menopause in these chimpanzees were the same from prior research design (Videan et al. 2006; Videan et al. 2008), and the anomalies involved in estimating reproductive cessation in such a manner have already been recounted.

A study published shortly prior in which thirty captive western gorillas were scrutinized for both behavioral and endocrinal markers used to communicate the animals' state of reproductive viability lends further data to the debate regarding nonhuman primate models of reproductive senescence (Atsalis & Margulis 2006). Twenty-two of these gorillas were geriatric, or over thirty years of age, and findings suggested that aged female gorillas may experience a post-reproductive lifespan lasting more than twenty-five percent of their overall lifespan. This is especially interesting, as these results coincide with those found in relation to aging wild chimpanzee populations (Nishida et al. 2003); however, it should once again be noted that there the article was quick to communicate the unreliable portions of the demographic parameters necessitated. This study also points out somewhat erratic features in methodology, but the occasional lack in the data set—such as the inclusion of only progesterone in the data set, to the detriment of estradiol or either pituitary gonadotopin— is justified by referring to the benefits derived from utilizing fecal samples. For instance, estradiol was discounted due to low readings.

Ultimately, results do indicate that along with numerous behavioral aspects that coincide with reproductive senescence in gorillas, there also exist hormonal changes in geriatric females. Nevertheless, large portions of the data were discarded in order to reach these conclusions, perhaps the most pertinent of which being the complete rejection of all data related to estrogen levels, leaving the authors with only progestogen concentrations with which to glean information. Additionally, available gonadotropin levels—which have since proven to be a more reliable marker of fertility than behavioral or ovarian steroid data—were not at all considered in relation to reproductive cessation. Gorilla timelines of fecundity can, of course, offer additional insight into the trajectory of menopausal onset within human evolution; this is especially true when parallels with chimpanzees are discovered.

Later that same year, records from thirty-seven years of wild mountain gorilla observations were examined in an attempt to isolate correlations between the various reproductive markers occurring along the lifespan and age (Robbins et al. 2006). Total birth rates, interbirth intervals, offspring survival, and reproductive termination were all scrutinized for any implications they may hold regarding the processes involved in nonhuman primate senescence, as well as the inferences that may be reached regarding the homologous processes operating across related species. While this publication allowed for a closer insight into the life cycles of gorillas, however, no hormonal data was utilized in relation to the reproductive progression of this species. Still, based on interbirth intervals as well as age at last birth, the findings suggested that no more than perhaps 1% of the total lifespan of wild mountain gorilla females may be potentially said to be post-reproductive. These results are in striking contrast from the findings of a year before (Atsalis & Margulis 2006), where results suggested that gorilla lifespans may include a post-reproductive period that would encompass roughly twenty-five percent of their lives. However, whereas this study examined gorillas living in the wild, previous research scrutinized gorillas in a captive setting; and the two studies altogether demonstrated that while captive situations are artificial in that they extend the lifespan of animals, they are relatively

uniform in this extension, which provides a unique opportunity to view the underlying genetic potential available within species.

II. Monitoring the Endocrine Profile of a 72 year old Female Chimpanzee (*Pan troglodytes*).

2.1 *The Significance of the Project*

In 2000, a chimpanzee in Florida estimated to be over 60 years of age gave birth (Erwin et al. 2002; Cloutier et al. 2009). The infant died four months later. The following year, at the approximate age of sixty-four, this particular female chimpanzee gave birth once more. Again, the offspring did not survive infancy. The exceptional female who gave birth so unexpectedly was "Little Mama", the study subject of the current research project.

News of this unusual occurrence is still met with interest within the field of anthropology, as female chimpanzees in captivity have recently been estimated to experience complete reproductive cessation well before their sixtieth year (Videan et al. 2006; Videan et al. 2008) and were also shown—through follicular depletion data—to undergo similar rates of fertility decline as those displayed in humans (Jones et al. 2007). Therefore, parity beyond the sixth decade of life is unanticipated, due to the decline in fertility that accompanies advanced somatic aging in *Pan troglodytes*.

However, additional previous work did much to dispel the belief that chimpanzee females were rendered completely infertile so prematurely, stating that there was, "little support in

the wild data for the recent conclusion from captive studies that menopause occurs in chimpanzees at the age of thirty-five to forty years" (Emery Thompson et al. 2007, p. 2153). Instead, results reaffirmed that somatic aging was virtually synonymous with reproductive aging in the common chimpanzee. This data—in conjunction with the findings of previous research (Jones et al. 2007)—suggests that the pattern of reproductive decline in both *Homo sapiens* and *Pan troglodytes* had its origin in the last common ancestor between the species; therefore, the trajectory of human evolution likely underwent selection pressures which would extend overall somatic durability rather than cut reproductive capacity short.

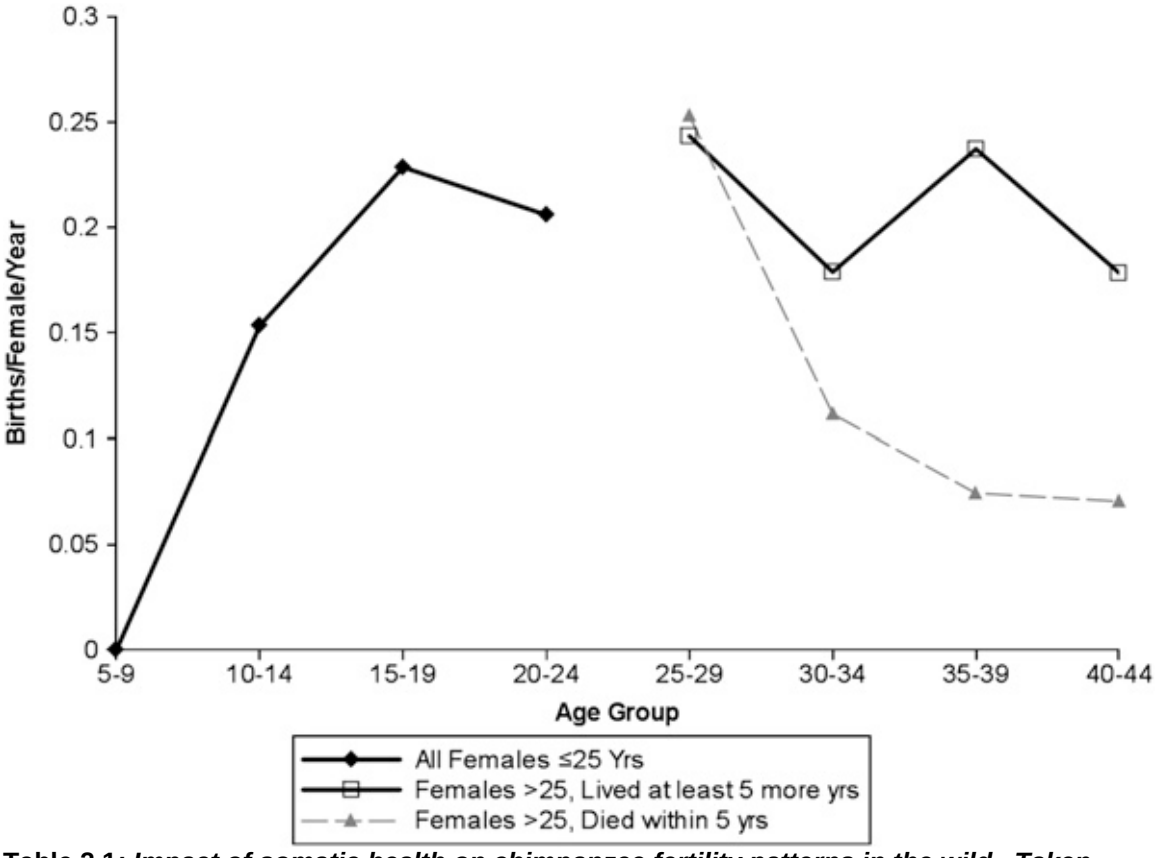

Table 2.1*: Impact of somatic health on chimpanzee fertility patterns in the wild. Taken from Emery Thompson et al. 2007.*

Still, there is much information missing from the popular concept of human and nonhuman primate evolution in reference to reproductive capacity that would be better served by additional data. For instance, the fact that the most recent research on captive chimpanzee females suggests that they experience menopause by their fortieth year can be well disputed by the case of Little Mama: she was still producing offspring well into her sixties. Additionally, Little Mama provides an example of a chimpanzee who has achieved the longevity of which they were not necessarily believed capable; alongside that extended lifespan, the question remains: is a healthy, seventy-two year old female chimpanzee hormonally capable of producing offspring? There is a pressing need for further research on female chimpanzees that have reached these highest levels of somatic existence, so that more concrete conclusions regarding the trajectory of human and nonhuman primate evolution may be achieved. Currently, the case can be made that available data sets are too small and varied to conclusively lend evidence to the claim that chimpanzee females do or do not experience menopause.

Various analyses have successfully linked reproductive aging with somatic aging in populations of *Pan troglodytes* (Emery Thompson 2007). That is, patterns of reproductive senescence seem to parallel the overall health of the individual animal sampled. However, data from reportedly healthy females in captivity asserts that these animals, for various reasons, attain a state of complete reproductive cessation by the age of thirty-five or forty (Atsalis & Videan 2009; Videan et al. 2006; Videan et al. 2008). Therefore, a comprehensive screening of Little Mama's endocrine profile is integral to reaching a better understanding of the reproductive capacity of *Pan troglodytes*, as the state of her health is known, as are her reproductive history and her housing and social situation, all of which may be said to have a direct effect on the overall wellbeing of the animal. Information on the current reproductive state of a healthy, socially housed chimpanzee in her seventy-second year will contribute greatly to the available body of knowledge regarding both human and nonhuman primate reproductive aging.

Figure 2.1*: Little Mama at 72 years of age.*

Perhaps most importantly, this study provides a foundation upon which more extensive research can be built. Future studies may focus on the patterns of geriatric female chimpanzee reproductive aging over time; with the established methodology in place, endocrine profiles may be routinely assessed for females—young and old—housed with Little Mama in a semi-free ranging and inherently social environment. This would allow greater insight into the progression of reproductive aging in the species, as captive animals provide the advantage of being regularly accessible, while the semi-free ranging environment insures that the animals retain as naturalistic a lifestyle as possible. Additionally, captive chimpanzees have been proven to extend their somatic existence well beyond what their wild counterparts are generally capable of, thereby providing more extensive information on patterns of reproductive aging in the species.

2.2 *Contributions to Anthropological Theory*

Before any theoretical conclusions regarding the trajectory of human reproductive evolution may successfully be reached, one must first cement an understanding of the reproductive processes presenting within *Pan troglodytes*. Anthropologists have long asserted that, as the closest living relative to humans, the chimpanzee provides a useful tool to understanding many of the behavioral and physiological traits attributed to modern man. Specifically, *Pan troglodytes* has been used as a model for better understanding human menopause for quite some time (Alvarez 2000; Bellino & Wise 2003; Gould & Graham 1981; Graham 1979; Jones et al. 2007; King et al. 1998; Leidy 1994; Hawkes 2003; Hawkes et al. 1998. Kaplan et al. 2000). However, postulating on the potentially homologous processes operating across related primate species is better served with a comprehensive perception of the capabilities and limitations of the various species in question.

The role that menopause plays within the context of chimpanzee reproduction sheds light on the major anthropological puzzle of human uniqueness in their patterns of reproductive senescence. If humans are alone in attaining a significant postreproductive lifespan, what is the reason? This study helps to allow that question to be asked, as the endocrine profile of a chimpanzee past her 70th year of life can help to provide a clear answer to the debate regarding whether or not the species experiences reproductive senescence apart from rates of somatic aging. Such information is critical in any attempt to better understand the evolutionary processes operating along the hominoid line. Currently, incomplete or diminutive primate data sets are routinely plugged into formulae designed to provide better insight into the homologous processes inherent between related species. This lack in the available primate record, however, can lead to potentially uncertain or imprecise views of the course of human reproductive evolution over time.

Additionally, the validity of literature which determines that chimpanzee females undergo menopause by age thirty-five to forty in captivity is ascertained by careful review of the current hormonal levels associated with reproductive function in Little Mama (Atsalis & Videan 2009; Videan et al. 2006; Videan et al. 2008). As she has produced three offspring well past the proposed menopausal interval in captive chimpanzees, the veracity of this claim can already said to be in question. Still, if endocrinal data reveals that she falls outside of the parameters of a menopausal female—and is therefore clinically capable of reproduction—recent publications which maintain that non-wild populations of female chimpanzees achieve a significant postreproductive existence will necessitate re-evaluation.

2.3 *Additional Contributions*

As increasing populations of captive chimpanzees reach old age, it will become progressively more important to provide proper health care for these animals. The species as a whole is experiencing rapid population decline in the wild, taking with them an unparalleled contribution into understanding the origins of human evolution. This is due in large part to the growing rates of deforestation apparent in all areas of Africa. For example, a recent census detailing the approximate numbers of *Pan troglodytes* in Côte d'Ivoire revealed an alarming extent of decline. It is estimated that local chimpanzee populations have experienced a 90% drop, with perhaps only 800 to 1,200 animals remaining (BBC 2009). This is a far cry from the 2003 estimate of 8,000 to 12,000 members of the species in the region (Butynski 2003). Soon, chimpanzees may only flourish within the confines of captive settings.

As a result, there is a pressing need for monitoring the endocrine profiles of the geriatric members of *Pan troglodytes*. The estrogen deficiency which presents during menopause in humans is potentially afflicting the health of aged female chimpanzees as well, although this has yet to be determined. Further research into the patterns of reproductive aging in nonhuman primates will shed light on the various physiological changes of which we know astonishingly little, but which potentially wield great influence over the overall health and longevity of geriatric female chimpanzees. This would allow caretakers to administer to the animals under their supervision with greater efficiency.

Figure 2.2*: 0.1 chimpanzee "Gin" and her infant, 0.1chimpanzee "Olive". Gin is 38.*

Table 2.2: ***Estimated number of robust (common) chimpanzee Pan troglodytes in 2003 by subspecies and country. Taken from Butynski 2003.***

Subspecies and country	Number of chimpanzees	
	Low	High
Western Chimpanzee (*P. t. verus*)	**21,300**	**55,600**
Benin	0	0
Togo	0	0
Nigeria	0	?a
Burkina Faso	0	Few?
Senegal	200	400
Ghana	300	500
Guinea-Bissau	600	1,000
Sierra Leone	1,500	2,500
Liberia	1,000	5,000
Mali	1,600	5,200
Coˆ te d'Ivoire	8,000	12,000
Guinea	8,100	29,000
Nigeria Chimpanzee (*P. t. vellerosus*)	**5,000**	**8,000**
Nigeria	2,000	3,000[a]
Cameroon	3,000	5,000[b]
Central Chimpanzee (*P. t . troglodytes*)	**70,000**	**116,500**
Democratic Republic of Congo (DRC)	?	?
Angola (Cabinda)	200	500
Central African Republic (CAR)	800	1,000
Equatorial Guinea (Rio Muni/Mbini)	1,000	2,000
People's Republic of Congo (PRC)	10,000	10,000
Cameroon	31,000	39,000[b]
Gabon	27,000	64,000
Eastern Chimpanzee (*P. t. schweinfurthii*)	**76,400**	**119,600**
Central African Republic (CAR)	?	?
Sudan	200	400
Burundi	200	500
Rwanda	500	500
Tanzania	1,500	2,500
Uganda	4,000	5,700
Democratic Republic of Congo (DRC)	70,000	110,000
TOTAL	**172,700**	**299,700**

Note: All data from Teleki (1991), except as follows: Burundi (Teleki 1991; Nishida 1994); Cameroon (Usongo 2001); Coˆ te d'Ivoire (Marchesi *et al.* 1995; Herbinger *et al.* 2003, Chapter 12); Equatorial Guinea (Teleki 1991; J. Sabater-Pi, pers. comm. quoted in Nishida 1994); Gabon (Tutin and Fernandez 1984; Blom *et al.* 1992; L. White, pers. comm. quoted in Stevens 1997); Guinea (Ham 1998; Kormos, Humle *et al.* 2003, Chapter 9); Guinea-Bissau (Gippoliti *et al.* 2003, Chapter 8); Liberia (Teleki 1991; Nisbett *et al.* 2003, Chapter 11); Mali (Duvall *et al.* 2003, Chapter 6); Nigeria (Oates *et al.* 2003, Chapter 17); PRC (S. Kuroda, pers. comm. quoted in Nishida 1994); Rwanda (Nishida 1994); Senegal (Galat-Luong *et al.* 2000); Uganda (Plumptre *et al.* 2003). Teleki (1991) does not provide references for the sources of his estimates, but most of these can be found in Lee *et al.* (1988).

a The chimpanzee in Nigeria west of the Niger River may belong to the subspecies *P. t. verus.*

b An unknown number of the approximately 35,000 chimpanzees in Cameroon in 1988 (Usongo 2001) belonged to the subspecies *P. t. vellerosus* (Gonder *et al.* 1997). This table assumes that 3,000–5,000 are *P. t. vellerosus* (E. Gadsby, P. Jenkins, J. Oates and J. Groves, pers. comm.).

For instance, estrogen replacement therapy may be desirable in the oldest of the captive female primates to assist in achieving good health and longevity. Unfortunately, decisions of this nature are often left unresolved, as captive breeding policies—which generally adhere to a strict regimen of chemical contraceptives for the females—make reaching a better understanding of chimpanzee reproductive senescence logistically difficult. However, there are a small number of animal facilities which opt to vasectomize the males of the group rather than place the females on contraceptives. The facility at which Little Mama is housed is one of the few that practice male vasectomization, allowing her—as well as the remaining females of the facility—to be used as models for research in endocrinal patterns of reproductive aging.

Finally, human populations are annually reaching longer periods of somatic longevity (Riley 2001). The advent of modern science, which has allowed for significant medical breakthroughs, has ensured that the human species in general currently achieves longer lifespans than ever before.

Geographic Distribution	***1950-1955***	***1995-2000***	***2045-2050***
World	46.5	65.4	76.3
Africa	37.8	51.4	70.4
Asia	41.3	66.3	77.2
Europe	66.2	73.3	80.3
Latin America and the Caribbean	51.4	69.2	77.6
North America	69	76.9	81.9
Oceania	60.9	73.8	80.7
Spread from Highest to Lowest	31.2	25.5	11.5

Table 2.3: ***Human Life Expectancy for Both Sexes (in years).***
(Riley, 2001)

As this forward trend progresses, any increased knowledge of latter life history phases in geriatric individuals will prove to be of tantamount importance. This study will increase the understanding of reproductive aging within nonhuman primate species, which can in turn aid the perception of senescence in humans. With a more solid foundation of knowledge regarding the trajectory of human reproductive evolution, medical innovations aimed at extending reproductive capacity can possibly be designed.

III. Methods

3.1 *The Reproductive Capacity of* Pan troglodytes

For the purposes of this study, the endocrine profile of a geriatric common chimpanzee (*Pan troglodytes*) female was examined in order to ascertain the state of her current reproductive ability. The results of this data will lend conclusive evidence to the debate regarding the capacity of the nonhuman primate reproductive life cycle: if allotted a lengthy enough somatic lifetime, is *Pan troglodytes* capable of achieving an end to reproductive viability prior to death? Additionally, the knowledge gained by charting the hormonal levels of a chimpanzee at such an advanced stage of life—well beyond what a chimpanzee in the wild could attain, and outside even what most primates in captivity reach—can potentially serve to provide insight into the evolutionary trajectory of the human species in regards to menopause: are human females alone possessed of the phenomenon of complete reproductive cessation while maintaining good health, or do other species of primate have the ability to share this trait? If so, the question must be further expanded to encompass by what means and for what reason this would be so.

As control animals against which to compare the hormonal results of Little Mama's testing, the endocrine profiles of three separate chimpanzee females housed at Lion Country Safari were also examined. All three of these females are in various stages of their life, ranging from fourteen to upwards of forty years of age. The eldest of these females, "Cindy", currently

displays somewhat irregular anogenital swellings, and was chosen as a variable in order to better understand the timeline of the aging female chimpanzee reproductive cycle. "Tonic" and "Janice", the two remaining sample individuals, regularly display monthly perineal tumescence, and were twenty-four and fourteen years of age at the time of this study, respectively. They were chosen as variables in order to ascertain a baseline hormonal profile against which to compare that of Little Mama, as both of these females are well within the age interval of a reproductively viable chimpanzee, in addition to exhibiting visual cues that are indicative of monthly cycling.

3.2 *The Significance of Anogenital Tumescence in Female Chimpanzees (Pan troglodytes)*

The functional significance of anogenital tumescence—or the swelling of the perineal skin during portions of the menstrual cycle—in females of the genus *Pan* has long been debated. While viewed as a visible marker of sexual fecundability for decades, recent research indicates that this is perhaps a simplified account of the true nature of these ovulatory cues. For instance, pregnant or lactating females are well documented to continue to produce monthly, though anovulatory, genital swellings (Anderson & Bielert 1994; Stanford 1998; Wallis 1992; Wallis & Goodall 1993). Still, as tumescence is generally correlated with reproductive function in chimpanzees, much research has been formulated around its occurrence.

Recent exploration has determined that age is a determining factor in swelling size only insomuch as it correlates with a decline in labial width. Other characteristics of anogenital tumescence, such as: pink height, full height, labial depth, anal width, area and volume, were not evidenced to be associated with age. Swelling size was, however, closely correlated with those aspects of ovarian function previously found to have been indicative of fecundity in human and non-human primate trials; significant positive correlation between hormonal readings and swelling

size was demonstrated to be present within three key phases of ovarian function in chimpanzees. These were early follicular estradiol levels, early luteal progesterone levels, and the duration of luteal progesterone elevation (Emery Thompson & Whitten 2003).

The menstrual cycle of *Pan troglodytes* is much like that of a human's, with the exception of overall duration. Whereas human female menses last 28 days, the chimpanzee course lasts an average of 35.5 days (Emery Thompson & Whitten 2003). Still, there are three principle phases of menstruation: follicular, ovulatory, and luteal. The follicular phase is characterized by increased levels of pituitary-produced hormones relative to baseline and significantly decreased levels of ovarian steroids. Menstruation occurs at the beginning of the follicular phase. Halfway through this phase, however, the negative feedback on the part of the pituitary is lost, and there is a rather abrupt rise in both estrogens and progestrogens, while LH and FSH decreases; this eventually culminates in the rupturing of the follicle by a sudden surge in LH, whereby the ovulatory stage is reached. Immediately following ovulation, the pituitary-policed hormones decline whereas the ovarian steroid hormones increase. This transition marks the beginning of the luteal phase. In the absence of fertilization, this cycle will recommence shortly after the negative feedback on FSH and LH is once again removed, which allows the follicular phase to begin anew (Emery Thompson 2005). The corresponding tumescence levels displayed by female chimpanzees are typically as follows: twelve to sixteen days of initial swelling, followed by approximately four days of maximal tumescence. Ovulation typically occurs on the first day of detumescence, but ranges between four days prior to detumescence to three days after detumescence. Anogenital reduction continues for approximately 16 days following ovulation, after which swelling recommences (Emery Thompson & Whitten 2003). Therefore, the chimpanzee luteal phase is indicated by detumescence, while the follicular phase is displayed via perineal swelling.

3.3 *Little Mama*

The primary study subject utilized in this research was “Little Mama.” Born in approximately 1937, in what was most likely Sierra Leone, Africa, Little Mama was imported into the United States at a very young age. While available records on this chimpanzee are very sparse, it has been conjectured that she was initially sold to the entertainment industry, where she performed on ice skates in public forums. She was then thought to have been sold to a circus owned by the Hunt family, and resided there until she was 30 years old (Personal Communication, Terry Wolf). What is definitively known about Little Mama is that she was retired to Lion Country Safari in 1968 as a sexually mature female.

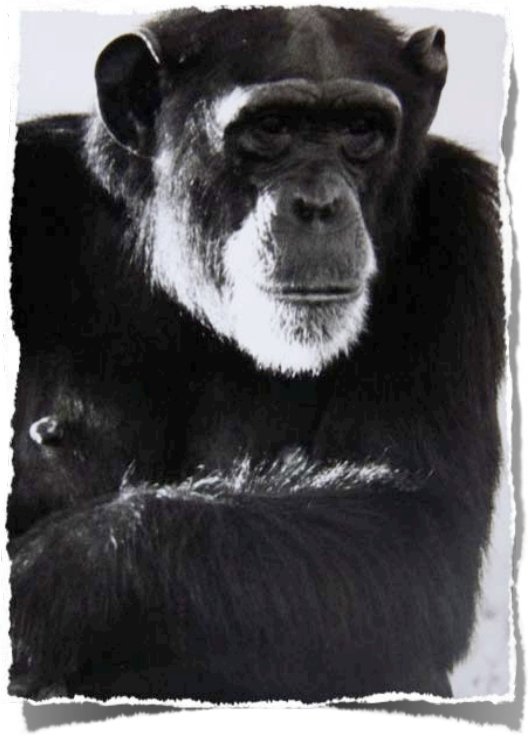

Figure 3.1*: Little Mama in 1968, at the age of 30.*

Five years after her arrival at Lion Country Safari, Little Mama was examined by Jane Goodall in an effort to discern the age of the chimpanzee. Dr. Goodall studied the quality of Little Mama's coat and teeth, as well as her mobility patterns and the condition of her eyes and skin and arrived at an age estimate of 35 years in 1972 (Jane Goodall Institute Newsletter 2008). This age estimate is sympatric with her appearance in photographs taken in 1968, as well as those captured in 1972. Today, Little Mama is a visibly geriatric chimpanzee who is presumed to be 72 years of age and still maintains an important social niche within her group.

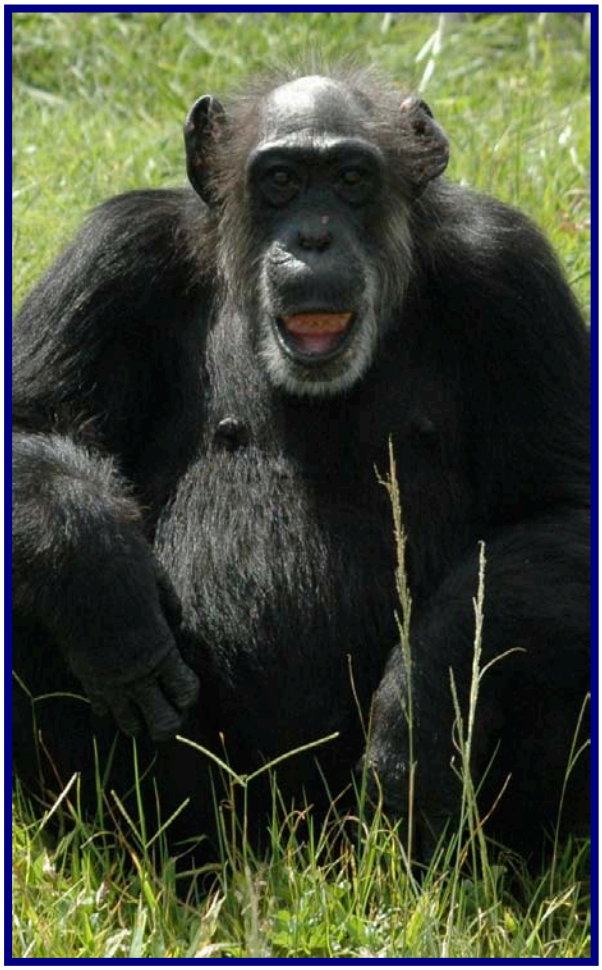

Figure 3.2*: Little Mama in 2008, at the age of 72.*

Records of this particular chimpanzee's reproductive history indicate that she has birthed at least six offspring during her time at Lion Country Safari. The first documented death of an infant occurred in the mid-1970s. The sex of the offspring was unknown, as were the age at and cause of death. In 1973, Little Mama produced female (0.1) infant "Katie Belle", who died approximately 16 months after birth; the cause of death in this case is officially listed as anemia. Male chimpanzee "Jason" (1.0) was born in 1979, and was reportedly dispersed to Monkey Jungle in Miami in 1980. The current state of this primate is currently unknown, as officials at Monkey Jungle were unable to corroborate this accounting. This is unsurprising, as zoo records during that time period were especially lax. After an extensive lull in births, Little Mama produced an offspring (1.0 chimpanzee, "Grant") in 1998. However, Grant survived only one week; his cause of death is also unknown.

The two most extraordinary births credited to Little Mama occurred in 2000 and 2001, respectively. The death of the first infant, "Miracle" (1.0), allowed Little Mama to produce another offspring rather quickly, and there is consequently only a one-year interval between the birth dates of the two. Miracle was born on the 29th of April in 2000 and expired on the 24th of August of the same year, whereas the most recent infant (1.0 chimpanzee "Ezra") was born on the 1st of May in 2001 and died shortly thereafter, on the 16th of August. The cause of death for Miracle was definitively listed to be conspecific trauma, however a postmortem necropsy revealed that respiratory disease was also present at the time of death. The cause of death for Ezra is unknown.

Name	*Sex*	*Date of Birth*	*Date of Death*
Unnamed	Unknown	Circa 1970	Circa 1970
Katie Belle	Female	06/08/73	10/24/74
Jason	Male	1/30/98	Dispersed 09/03/80 Health Unknown
Grant	Male	01/30/98	02/06/98
Miracle	Male	04/29/00	08/24/00
Ezra	Male	05/01/01	08/16/01

Table 3.1: ***The Reproductive History of Little Mama***

Interestingly, Little Mama has not had a visible menstrual cycle since May of 2006 according to reproductive charts maintained by the primate keepers at Lion Country Safari. This is pertinent, as the prolonged lack of sexual swellings and menstrual blood are potentially indicative of cyclical stasis. However, a more complete understanding of the particulars of Little Mama's current reproductive function will be provided by close examination of her endocrine profile.

3.4 *Cindy*

Data was also collected on Cindy, a forty year old female chimpanzee housed in the same social group as Little Mama. At the time of this study, this particular female exhibited sporadic visual markers of ovulation, as both perineal swelling and menstrual blood were only present once every few months. This interruption in reproductive cycles, when combined with the advanced age of the study subject and available literature (Atsalis & Margulis 2006; Videan et al. 2006; Videan et al. 2008; Atsalis & Videan 2009) , could be construed to be potentially indicative of perimenopausal endocrine activity. Therefore, Cindy's hormonal levels were recorded as a

potential means of establishing the temporal progression of the reproductive parameters governing *Pan troglodytes*.

Figure 3.3: ***0.1 chimpanzee "Cindy".***

Although born in the wild, Cindy was raised as a pet until she was four years of age. Prior to her arrival at Lion Country Safari in 1973**,** she was housed solitarily and was not aged enough to reproduce. Since then, she has had a total of five offspring. The first four were born on December of 1979, May of 1981, August of 1982, and May of 1989. All expired within five months, with one of the infants expiring on the same day of birth. Cause of Death was generally conspecific trauma, likely due to the fact that Cindy was unaware how to properly mother and protect her progeny, as she was not afforded the opportunity to learn how to do so as a pet. 1.0 chimpanzee, "Hank", was born to Cindy at Lion Country Safari on November 30th, 1990. Hank is her sole surviving offspring; he is currently healthy and residing at Chicago's Lincoln Park Zoo as the acting alpha male of his social group.

Name	*Sex*	*Date of Birth*	*Current Condition*
Unknown	Unknown	12/8/1979	Deceased, 12/16/1979
Unknown	Unknown	5/1981	Deceased, 10/1981
Unknown	Unknown	8/1982	Deceased, 8/1982 Same day as birth
Smiley	Female	5/15/1989	Deceased, 7/22/1989
Hank	Male	11/30/1990	Healthy, Dispersed to LPZ

Table 3.2: ***The Reproductive History of Cindy***

3.5 *Tonic*

0.1 chimpanzee "Tonic", born at Lion Country Safari on April 26, 1985, was also chosen to be a part of this study. As she was 23 years of age at the time of data collection, Tonic was able to provide an ideal representation of the baseline parameters for a healthy, reproductively viable female chimpanzee. In addition to falling directly within the appropriate demographic group, Tonic also has a highly successful and well-documented reproductive history.

Figure 3.4*: 0.1 chimpanzee "Tonic".*

Tonic has produced a total of three offspring. Her first birth occurred in 1999, at the age of 14. This first male (1.0), "Optimus Prime", was born on the 9th of February; he is currently healthy and residing at Lincoln Park Zoo as the beta male of Hank's group. The second of Tonic's offspring is "Janice" (0.1). Janice has also provided constructive data to this study and will be discussed further in the following paragraphs. "Bamboo" (1.0)—Tonic's final and most recent issue—was born on the 10th of January, 2003. He currently resides with his mother at Lion Country Safari, and provides a dynamic component to the group: as an adolescent, he incessantly mimics the group's alpha male, "Higgy", and is virtually the only option to succeed Higgy as alpha male. Also, Bamboo plays with and supervises the two infants (0.2) of the group.

Name	*Sex*	*Date of Birth*	*Current Condition*
Optimus Prime	Male	2/9/1999	Dispersed to LPZ; Healthy
Janice	Female	06/12/95	Healthy
Bamboo	Male	01/10/03	Healthy

Table 3.3: ***The Reproductive History of Tonic***

Tonic, housed with both Little Mama and Cindy, contributes a vital component to her social group. As the mother of two young, integral members, she maintains a significant political role, and is often seen in the company of Higgy. Additionally, Tonic is the daughter of the group's most dominant female, "Gin".

3.6 *Janice*

The final female chosen to provide baseline comparisons of Little Mama's endocrine levels was "Janice". Like Tonic, Janice is well within the established reproductive parameters of a female chimpanzee. As she was fourteen years of age during the time of data collection, Janice was more mature than the reported age at first full swelling in both wild and captive populations. Additionally, her age fell roughly within the same interval as wild female chimpanzees often become parous. Moreover, Janice was well beyond the year at which most captive females first give birth, which is generally a full three years prior to that of their wild counterparts (Nishida et al. 2003; Sugiyama 2004; Marsden et al. 2006).

Figure 3.5*: 0.1 chimpanzee, "Janice".*

Janice does not have a documented reproductive history, as she has never given birth. This, however, can not be construed as a reflection upon her ability to do so, as the only reproductively viable male in her group was vasectomized in 2005**.** Janice would have been eleven years of age when this occurred, and while she could have potentially had an offspring in that interim, it is not unusual for her not to have done so either. Additionally, Janice was not

reported by the chimpanzee keepers to achieve full sexual tumescence until her twelfth year. Still, Janice currently displays large monthly swellings and copulates frequently during her period of maximal tumescence. Therefore, it is unknown whether—given the proper circumstances—Janice would have become parous prior to the time of data collection.

3.7 *Lion Country Safari*

Lion Country Safari is a semi-free ranging animal park located in Loxahatchee, Florida. All of the animals in the preserve section of the park—from the giraffe to the rhinoceros, and all manner of hoofstock in between—are housed in extremely large territories where they are allotted the freedom to roam within significantly sized social groups. This allows for social dynamics rarely seen in captivity to evolve within the confines of a closed setting.

The chimpanzees especially develop complex socio-political ties within this atmosphere of relative freedom, much like those seen in the wild. Housed on a total of five islands, there are three separate chimpanzee groups all living well within vocal and visual distance of one another. While Lion Country Safari has 25 chimpanzees in total, Higgy's Group in particular features 12 members.

NAME	SEX	DATE OF BIRTH	GROUP NUMBER
Higgy	M	5/15/1973	1
Elgin	M	5/13/1981	1
Gin	F	~1971	1
Tonic	F	4/26/1985	1
Janice	F	6/12/1995	1
Juniper	F	3/24/1997	1
Irene	F	10/14/1999	1
Bamboo	M	1/10/2003	1
Little Mama	F	~1937	1
Cindy	F	12/28/1969	1
Noel	F	12/02/2005	1
Olive	F	8/17/2005	1
Whitey	M	~1963	2
Ian	M	2/26/1982	2
Peter	M	1/10/1974	2
Melody	F	3/10/1968	2
Sabrina	F	10/28/1982	2
Luna	F	9/28/1961	2
Orbit	F	1/28/1995	2
Cooper	M	~1970	3
Bashful	M	~1970	3
Figment	M	7/07/1990	3
Doll	F	~1968	3
Swing	F	~1968	3
Dandy	F	7/30/1990	3

Table 3.4*: Chimpanzee Group Composition and Demographic at Lion Country Safari. The highlighted rows represent individuals utilized in the current study.*

The chimpanzees are moved to a new island daily, where they spend their time engaging in naturalistic behaviors, such as foraging for food and playing. Here, the chimpanzees can often be seen ripping up the soil or turning over rocks in order to locate insect larvae, or digging for turtle eggs buried on the island. They learn social collaboration while chasing alligators, fish and birds. There is an established social hierarchy that must simultaneously be adhered to and periodically assessed.

Figure 3.6*: One of the primate islands at Lion Country Safari.*

Guests to the park must observe these behaviors from within their vehicles, as the park maintains a distance between humans and animals rarely duplicated in captive settings. This separation between animals and humans is extended to the primate caretakers as well. The chimpanzees are kept on the islands and away from human interference at all times, eating and sleeping outdoors. Mothers raise their offspring, and social and political ties are established and strengthened or weakened daily. The violation of this general rule occurs only when the chimpanzees must be taken into the indoor housing facility for temporary observation or medical attention.

The chimpanzee house was built in 2000 and is accessible to the animals by means of a bridge. The keepers will open the bridge to bring the primates into the facility for occasional monitoring. The building consists of concrete flooring and a combination of mesh wire and

concrete walls. Keeper-controlled shift doors separate the six holding areas, all of which provide extensive views of the islands. Each room contains a number of benches and ropes for the chimpanzees' use. The dimensions of the holding areas range from 20'x30' to 12'x20'; all rooms are 10' in height.

Figure 3.7: ***The Chimpanzee House at Lion Country Safari***

3.8 *Data Collection*

In order to ascertain the hormonal levels present in the four study chimpanzees (0.4), urine was collected from them in a non-invasive manner. Data collection of Little Mama lasted a period of eighty-eight days altogether, as her endocrine profile was more pertinent to this study in addition to being visually undetectable. The roughly three months of research was broken up into

two separate periods of collection: the first was from July 21st to August 21st of 2008, while the second lasted from the 6th of October to the 2nd of December, 2008. During the first stage of collection, Little Mama and control female Cindy were brought into the housing facility for the entirety of the collection period. Attempts were made to collect from both females daily, however keeper schedules rendered this task not always possible. Still, a relatively complete endocrine profile of both females was successfully assembled. Immediately following the necessary period of collection, Little Mama and Cindy were released back onto the island to rejoin the rest of their group.

The second stage of collection lasted from October 6th to December 2nd of 2008. As the second stage of collection was considerably longer than the first, it was deemed unnecessary to have the chimpanzees remain in the holding area for the duration. Instead, the remaining control females—Tonic and Janice—were periodically brought into the indoor housing facility alongside Little Mama for a total period of fifty-seven days. The time length of the second stage of collection was increased in order to definitively collect data indicative of at least one full reproductive cycle, in the event that Little Mama's cycles were slightly irregular. During this data set, attempts were made to collect samples from Little Mama every two to three days; however, due to unforeseen and periodic construction to the facility, this was not always possible. In addition, urine was to be collected from Tonic and Janice every three to four days; again, this was not always possible. Still, relatively complete endocrine profiles were once again assembled for all three females.

In order to safely collect urine samples, the chimpanzees were individually moved into a single holding area of the indoor facility via the shift door mechanisms. They were then passively observed until they produced urine waste. A new, sterile syringe was used to pipette samples from the sterilized concrete floors of the facility within ten minutes after the waste was produced. A minimum of three cubic centimeters [cc] of urine was collected per instance, and the sample was subsequently stored in clear, sterile polystyrene tubes at temperatures at or below zero

degrees Fahrenheit, as per the methodological suggestions prepared in 2005 (Emery Thompson).

The flooring of the holding facility was sterilized using a mixture of bleach and water, which was scrubbed into the concrete. The mixture was completely washed away with pressurized water and the floors were allowed to dry completely prior to the animals being placed into the holding area for collection. Additionally, adequate time was allowed for the bleach mixture to completely evaporate before a chimpanzee was moved into the collection room.

3.9 *Statistical Methods*

As per the available literature (Graham 1979; Gould & Graham 1981; Steinetz et al. 1992; Leidy 1994, Park et al. 2002; Shimizu et al. 2003; Emery Thompson 2005; Videan 2006; Emery Thompson et al. 2007; Lacreuse et al. 2008; Videan et al. 2008; Atsalis & Videan 2009) hormonal levels of cortisol, estradiol, progesterone, and luteinizing hormone [LH] within the system were assayed. The relationship between the ovarian steroid hormones—or estradiol and progesterone—and the gonadotropins regulated by the hypothalamus and pituitary—such as LH—evident in the results served to definitively illustrate the state of Little Mama's reproductive viability in that they were predicted to follow one of three patterns: (1) Endocrine levels are indicative of a fully reproductively receptive female chimpanzee, with levels of ovarian steroid hormones following the accepted patterns alongside those of the gonadotropins. (2) Endocrine levels are indicative of a female chimpanzee not necessarily cycling at the moment, nor in a state of complete reproductive cessation. This can potentially result from an animal experiencing the climacteric. Levels of ovarian steroid hormones may be lower than average, whereas levels of gonadotropins are not higher than average. Alternatively, estrogen and progesterone may be high while pituitary gonadotropins are repressed. (3) Endocrine levels are indicative of a female

chimpanzee in a complete state of reproductive cessation, with ovarian steroid hormone levels exceedingly low and gonadotropins exceptionally high. Results did indeed follow one of these prescribed patterns, the details of which will be discussed in further detail in the following chapter.

Upon completion of collection, the samples were sent to the Hominoid Reproductive Ecology Laboratory [HREL] for assessment. The Hominoid Reproductive Ecology Laboratory is located in New Mexico, and is an established research wing of the University of New Mexico. HREL is run by Drs. Martin Muller and Melissa Emery Thompson, and is fully equipped to assay the urine samples for chimpanzee hormonal levels of LH, estrogens, progesterones, and cortisol.

Linear Regression was used, as were two dependent variables: Little Mama served as the single representative for the group of chimpanzees over forty-five years of age tested, while the remaining females—Cindy, Tonic and Janice—were together placed into the group of chimpanzees under the age of forty-five. Binomial representation was then assigned to each group. Little Mama is represented by a "1" while the others are distinguished by a "0". Regressions were conducted in order to determine whether Little Mama fell within the same hormonal range as that of her group-mates. If this had been so, the case could have been made that she is not in a state of reproductive stasis.

However, as Little Mama's hormonal levels have fallen significantly outside of the projected regression line constructed by Cindy, Tonic and Janice, an alternative conclusion was reached. Were her endocrine profile completely concurrent with those of the control females, it could have been argued that she is not experiencing menopause. However, as her hormonal levels are relatively unparalleled by those of the females under the age of forty-five, more evidence is lent to the case that Little Mama may be heading towards a state of complete reproductive cessation. Again, this matter in discussed in further detail within the "Results" section of the manuscript.

Additionally, analyses were individually performed between the hormonal readings of Little Mama and those of her group-mates. Such further investigation allowed for closer insight into the progression of the reproductive processes presenting within aged populations of chimpanzees as compared to those of younger, more fecund individuals. It is hopeful that this data will be further useful in illustrating the similarities and differences between the senescing patterns of human and chimpanzee females within future works.

IV. Implications for Reproductive Cessation in Nonhuman and Human Primates

4.1 *Results*

Urine specimens were sent to the Hominoid Reproductive Ecology Laboratory [HREL] at the University of New Mexico in sealed plastic tubes. Prior to shipment, they were frozen and placed in a sealed cooler which also contained significant amounts of dry ice to keep the specimens frozen until receipt in New Mexico. The cooler was then placed within a shipping package, and mailed express for overnight delivery.

At the Hominoid Reproductive Ecology Laboratory, the urine was first standardized for creatinine in order to certify the integrity of the sample; overly diluted urine—that which contained creatinine lower than 0.1 mg/ml—was excluded from the data set and therefore warranted no further analysis. Following protocols firmly established within the literature (Czekala et al. 1986; Shideler et al. 1990), but which were adjusted for expected chimpanzee concentration ranges and current reagent stocks, cortisol and metabolites of estradiol (estrone conjugates, E_1C) and progesterone (pregnanediol-3-glucuronide, PdG) were then quantified using ELISA, or enzyme-linked immunosorbent assay. Assay sensitivities were approximated to be 300 pg/ml for cortisol, 175 pg/ml for E_1C, and 5 ng/ml for PdG. The CV—or interassay coefficients of variation—for cortisol, E_1C and PdG were 13%, 11% and 13%, respectively. Replicate determinations of CV

averaged 9%, 5%, and 7% for cortisol, E_1C and PdG. Reagents were provided by the University of California-Davis' Department of Population Health and Reproduction.

Also using an ELISA kit, concentration levels of luteinizing hormone [LH] were then assessed. Applicability of this particular assay for urine was evaluated based on assay recoveries for a low-dose sample (1.3 mIU/ml). It was determined that these recoveries were high and consistent across the range of measurement (84.7$\pm$ 2.3%), and were subsequently appropriate determinants of LH levels within the urine. Also, it was confirmed that urine samples measured with no dilution produced results highly uniform with those reduced 1:2 in a serum-based zero standard (R^2 = 0.980, N = 20, y = 1.02x – 0.03). Assay sensitivity is approximated at 1 mIU/ml. While there was a single assay of these samples, CVs of replicate determinants averaged 8%; manufacturer estimates of interassay variation are 6-8%.

It is especially significant that cortisol levels from the study animals are shown to be within the range of those of their wild counterparts; this indicates that no amount of undue stress was placed upon the subjects during the collection period. Extremely high levels of the stress-detecting hormone could potentially be said to alter the endocrinal balance occurring within each animal, whereas the observed levels—which were within the normal range—indicate that endocrinal function was as usual.

Hormonal results from the four females evaluated show the animals to be operating within the parameters of very different states of reproductive viability. This assessment was determined by logistic regression, which was used to statistically quantify the reported data. This particular statistical model is widely used for determining the probability of an event occurring; in this case, the mathematical model was invested in the likelihood of the eventual cessation of reproductive ability within each animal evaluated. The current endocrine levels of those hormones most closely associated with reproductive function were assessed in order to

determine the slope between the variable female—Little Mama—and that of each individual control animal. Also, Little Mama's endocrine profile was compared to that of the remaining group in its entirety. This was accomplished by first assigning a numeric binomial to the two categories: those animals over forty-five years of age (Little Mama) and those animals less than forty-five years of age (Cindy, Tonic and Janice). Next, the slope was determined through use of Smith's Statistical Package. The results are listed in the tables below, and show that there is a significant difference between the hormonal levels of Little Mama and those of her entire control group. The null hypothesis—that the endocrine profile of Little Mama will not vary significantly from that of visible cycling females—can be rejected on all three counts: her E_1C, PdG and LH levels demonstrate marked variation from those of Cindy, Tonic and Janice. The P-Values for these three hormones are 0.0000, 0.0002 and 0.0123 respectively, and the confidence intervals are 100% for estrogen, 99.98% for progesterone, and 98.8% for luteinizing hormone.

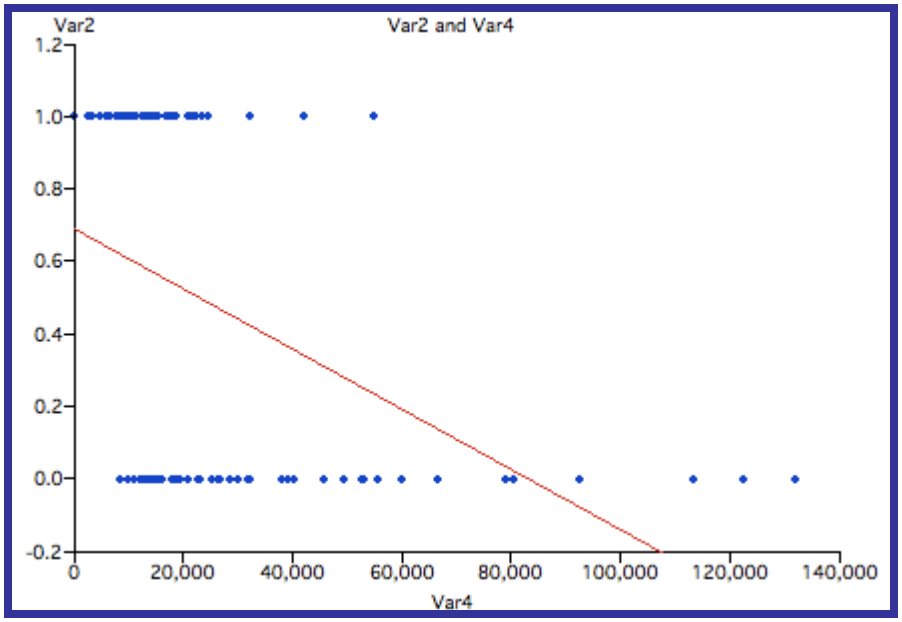

Table 4.1*: Little Mama's E_1C levels compared to those of the Control Group*

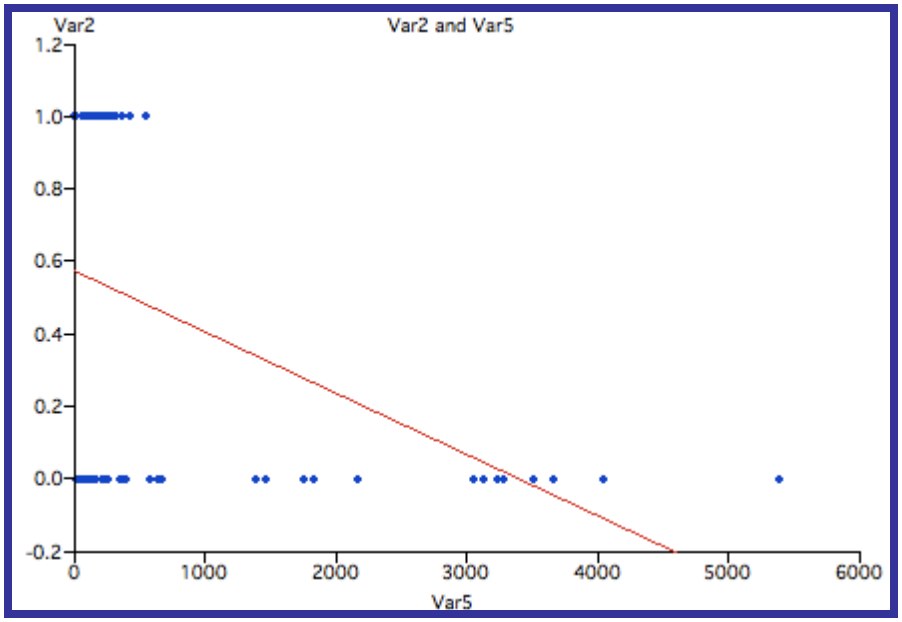

Table 4.2*: Little Mama's PdG levels compared to those of the Control Group*

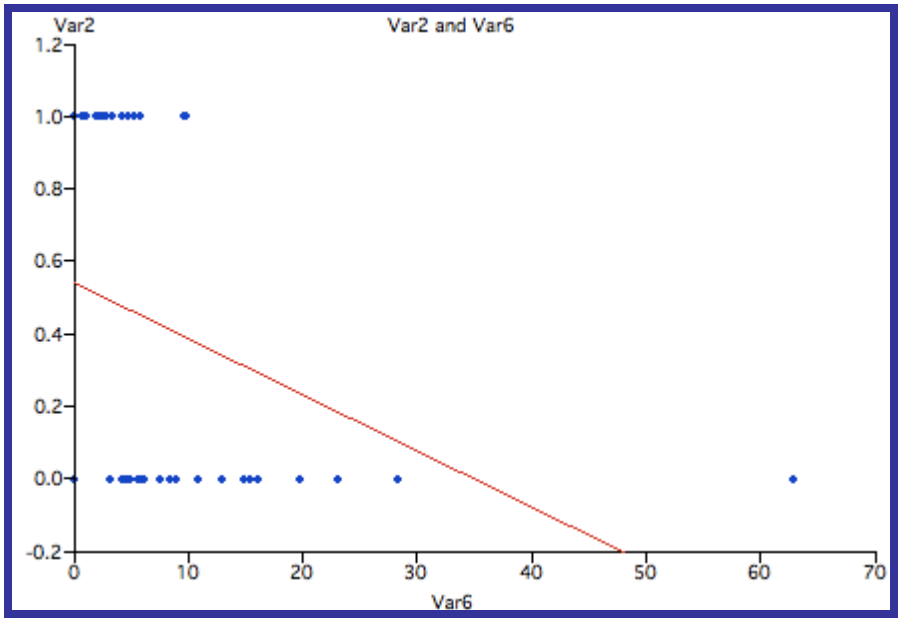

Table 4.3*: Little Mama's luteinizing hormone levels compared to those of the Control Group*

When logistic regression was performed on each individual animal and subsequently compared to Little Mama results were similar, with nearly all of the null hypotheses being rejected. The exception to this, however, was Cindy: the estrogen levels presenting during the month of data collection were not considerably different from those of Little Mama. Here, the P-Value was only .2 and the confidence interval 80%. However, the progesterone levels recorded for Cindy differed considerably from those of Little Mama in a remarkable way, as they were lower than those of the more geriatric female (P<.0001, N= 25). Progesterone was found to have a P-Value of 0.0001 and a confidence interval of 100%. LH was unable to be evaluated for Cindy's sample, which is presumed to be due to undetectable levels of the hormone in her urine.

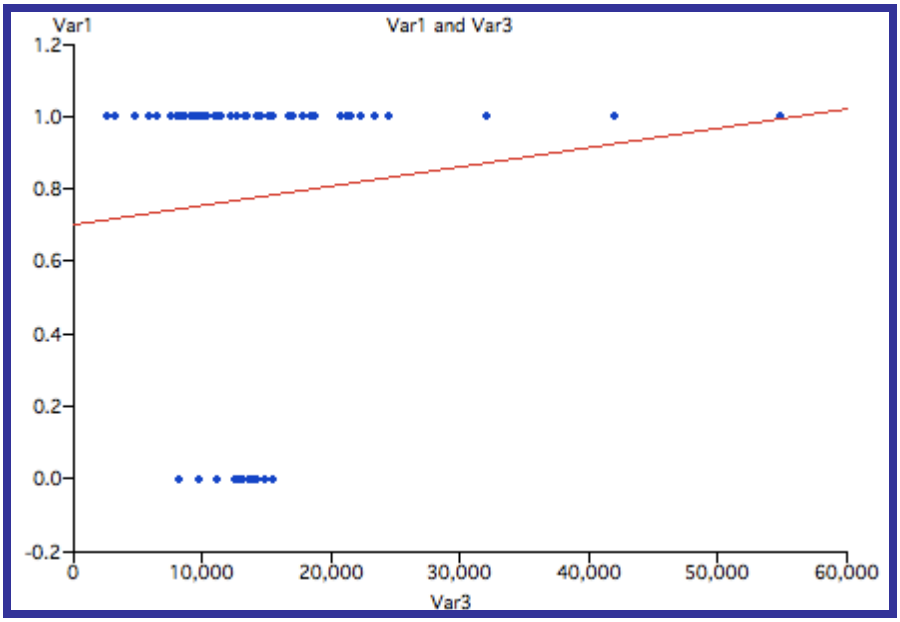

Table 4.4*: E_1C (Little Mama vs. Cindy)*

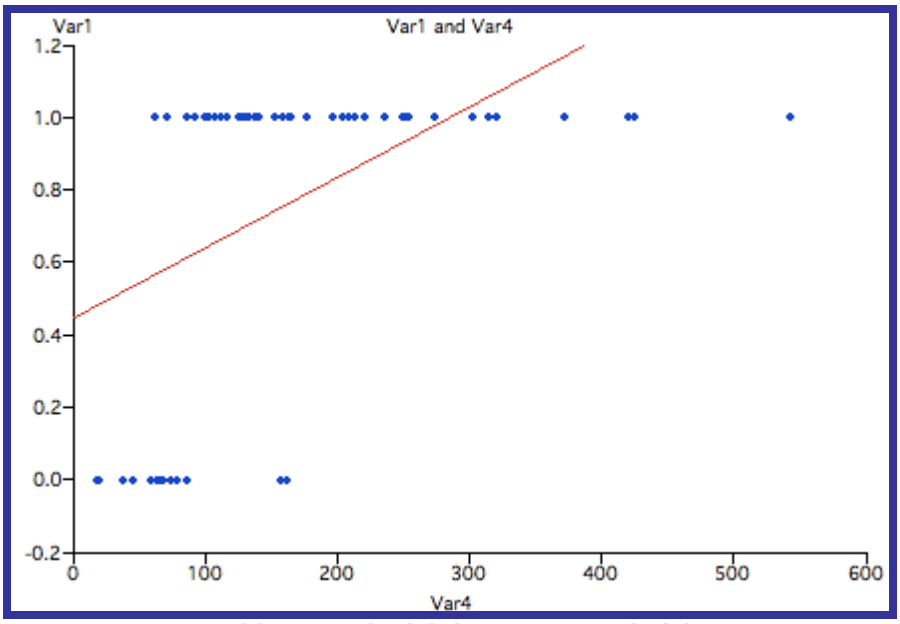

Table 4.5*: PdG (Little Mama vs. Cindy)*

Tonic's P-Values for E_1C, PdG and LH were 0.0000, 0.0000 and 0.003 respectively; her confidence intervals for the ovarian steroid hormones were 100%, and the luteinizing hormone was within 99.7%. Janice displayed like results, with her P-Values for E_1C and PdG at 0.0000 and the confidence interval at 100%. Her LH levels presented a P-Value of 0.0128 and a confidence interval of 98.82%.

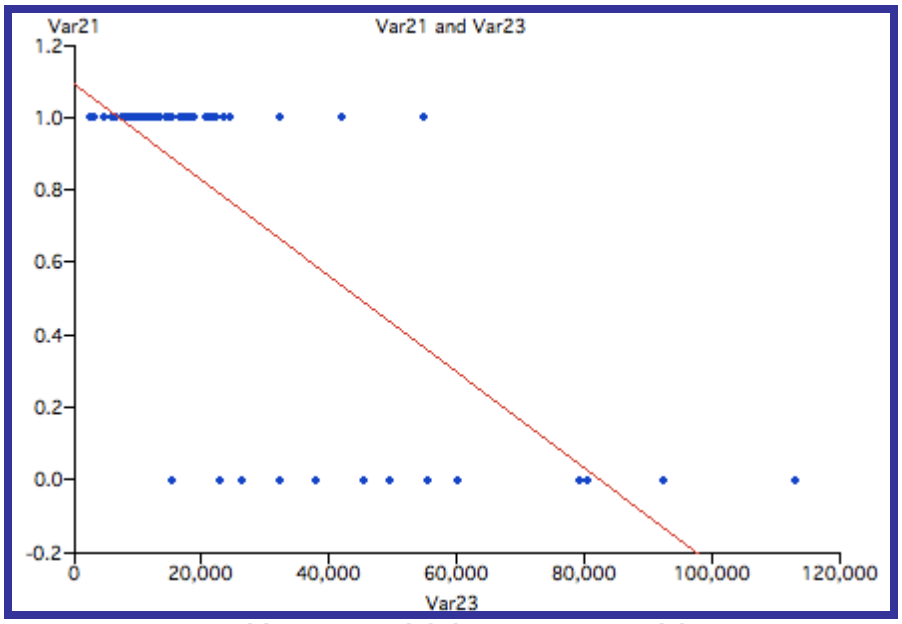

Table 4.6*: E_1C (Little Mama vs. Tonic)*

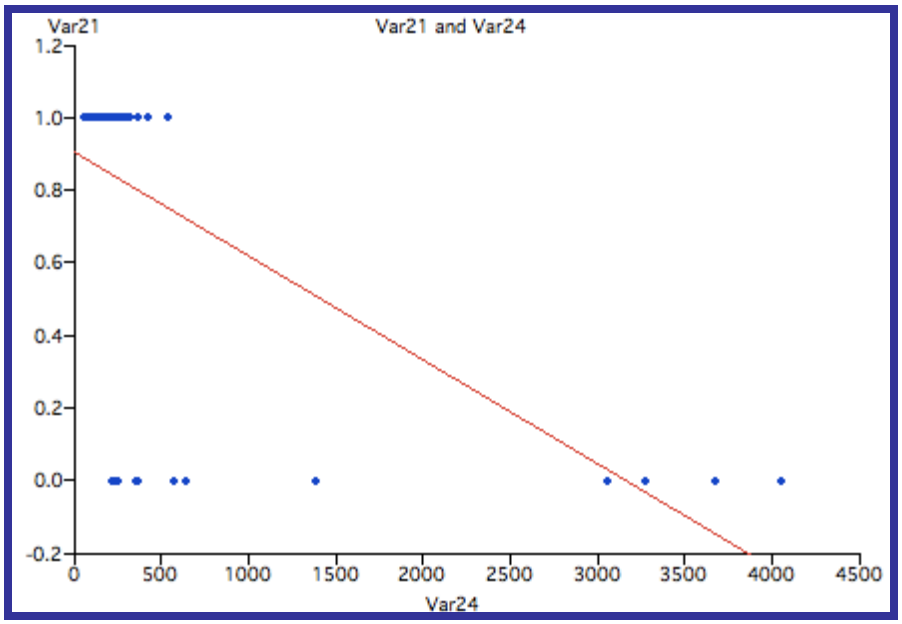

Table 4.7*: PdG (Little Mama vs. Tonic)*

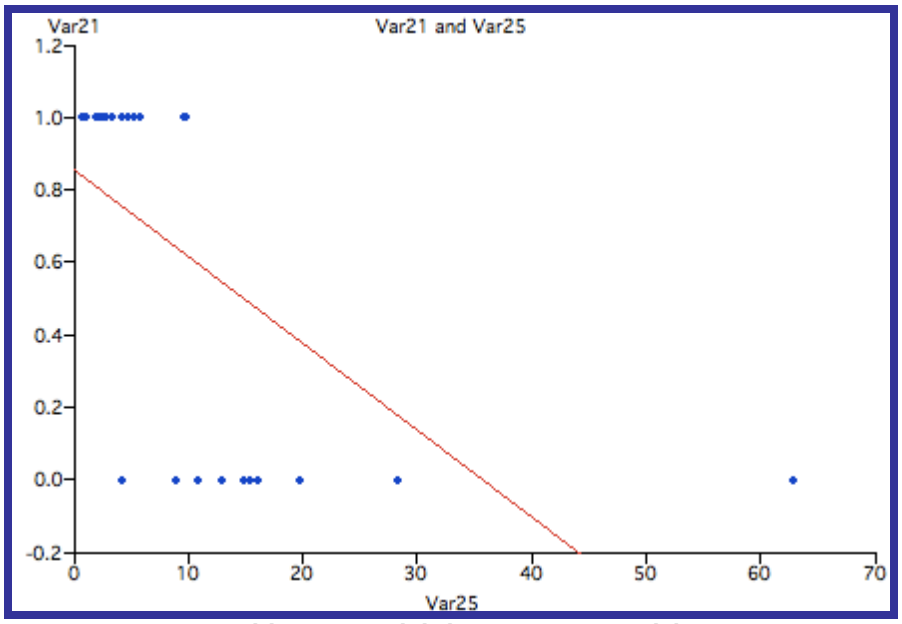

Table 4.8*: LH (Little Mama vs. Tonic)*

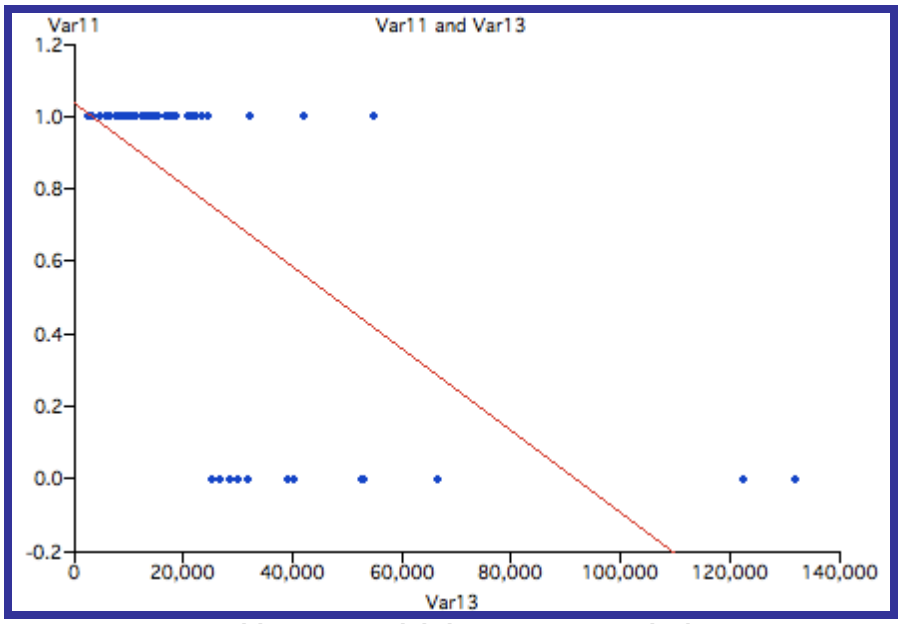

Table 4.9*: E_1C (Little Mama vs. Janice)*

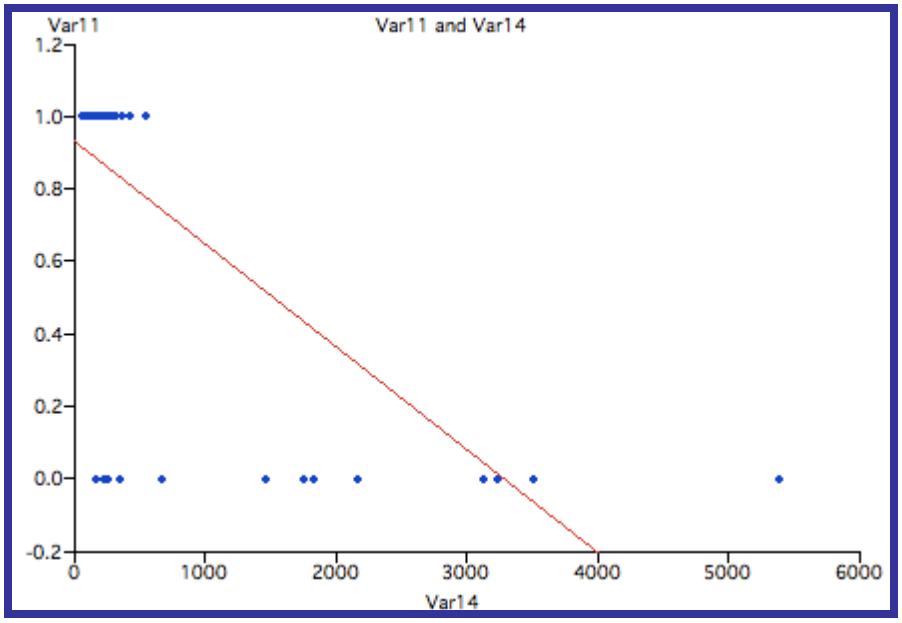

Table 4.10*: PdG (Little Mama vs. Janice)*

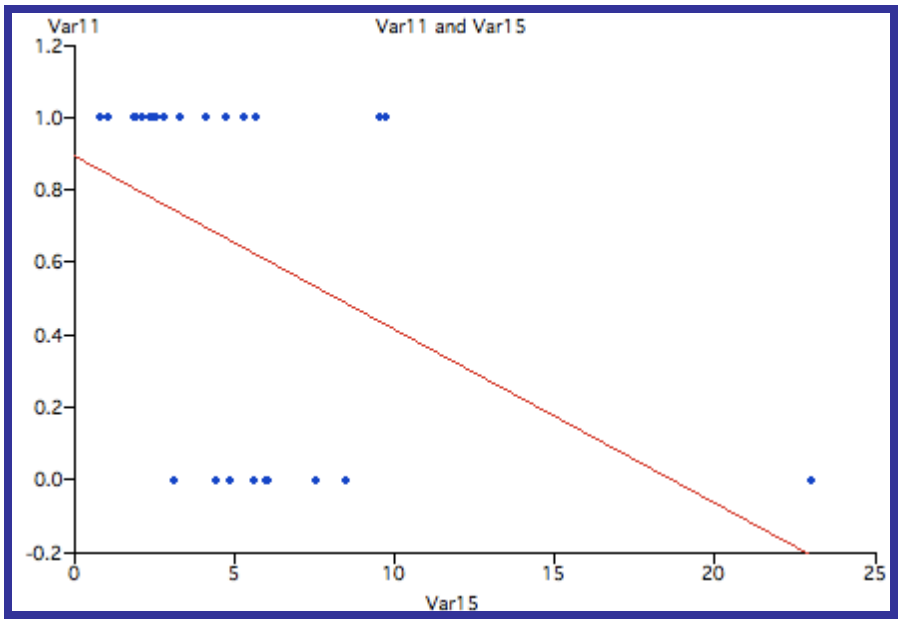

Table 4.11*: LH (Little Mama vs. Janice)*

4.2 *Discussion*

The hormonal results acquired from the Hominoid Reproductive Ecology Laboratory, in addition to the statistical tests run, are indicative of several things related to reproductive function and cessation in nonhuman primates. First, this study calls into question to validity of reports which find menopause to be presenting within captive chimpanzee populations by the fortieth year of age. It also identifies a potential pattern of reproductive aging in the species, while establishing possible incongruities between endocrinal markers of human and nonhuman primate reproductive ecology. Nevertheless, the research presented here has supported claims that there is in fact a correlation between aging and reproductive viability in chimpanzees.

Recent articles (Videan et al. 2006, Videan et al. 2008) illustrate the complete termination of reproductive viability in female members of *Pan troglodytes* by the time they reach thirty-five to forty years of age. Interestingly, the raw data published within this study seem to be similar to that of the present account on Little Mama. Whereas the test subjects of the aforementioned report also demonstrated decreased levels of estrogen and progesterone, the luteinizing hormone levels presenting within the geriatric females were reported to spike and then decrease; Little Mama's LH levels never spiked, but as she is considerably older than the test subjects used by Videan et al., she may have been demonstrating only to latter part of the pattern documented within their report of menopause. However, as menopause is clinically identified by the presence of low ovarian steroid hormones accompanied by extremely elevated pituitary hormone levels, this study cannot be said to reflect the conclusions drawn by the aforementioned research (Videan et al. 2006; Videan et al. 2008).

Instead, results from this study would appear to indicate that, while there does seem to be a process of reproductive aging apparent in *Pan troglodytes*, it does not necessarily parallel

the senescing pattern of the human female. It has shown that the extremely geriatric female chimpanzee will exhibit ovarian steroid hormones on par with those of a lactating or acyclical female in the wild while simultaneously maintaining low luteinizing hormone levels. This is interesting in that it could potentially indicate that the "break" in the HPO axis which causes humans to display low levels of estrogen and progesterone while elevated levels of LH are present is not adhered to in *Pan troglodytes*. If this is so, it is plausible to assume that chimpanzees may experience an entirely distinct endocrinal mechanism in relation to reproduction. Such a pattern would lend support to claims that the human state of menopause likely arose after the evolutionary divergence between the last common ancestor of humans and chimpanzees had occurred.

Alternatively, this separation from the expected pattern of reproductive aging could plausibly occur during the perimenopausal period. Accounts of the climacteric transition in humans have illustrated endocrinal profiles which embody aspects of both a fecund and menopausal individual (O'Connor et al. 2001). Perhaps, then, the hormonal pattern presenting within Little Mama is indicative of the conversion process to complete reproductive cessation; this is also notable, as a seventy-two year old chimpanzee in the perimenopause is not likely to ever fully complete the transition and can therefore be said to never fully experience reproductive cessation. Further, it is interesting that data on Cindy seems to indicate that she may be entering into this phase of sporadic cycling as well. As she is forty years of age, an inception of perimenopause presently would indicate that, unlike the five to six year transitional period experienced by humans, chimpanzees may undergo a significantly more extensive duration of endocrinal symptoms related to the climacteric.

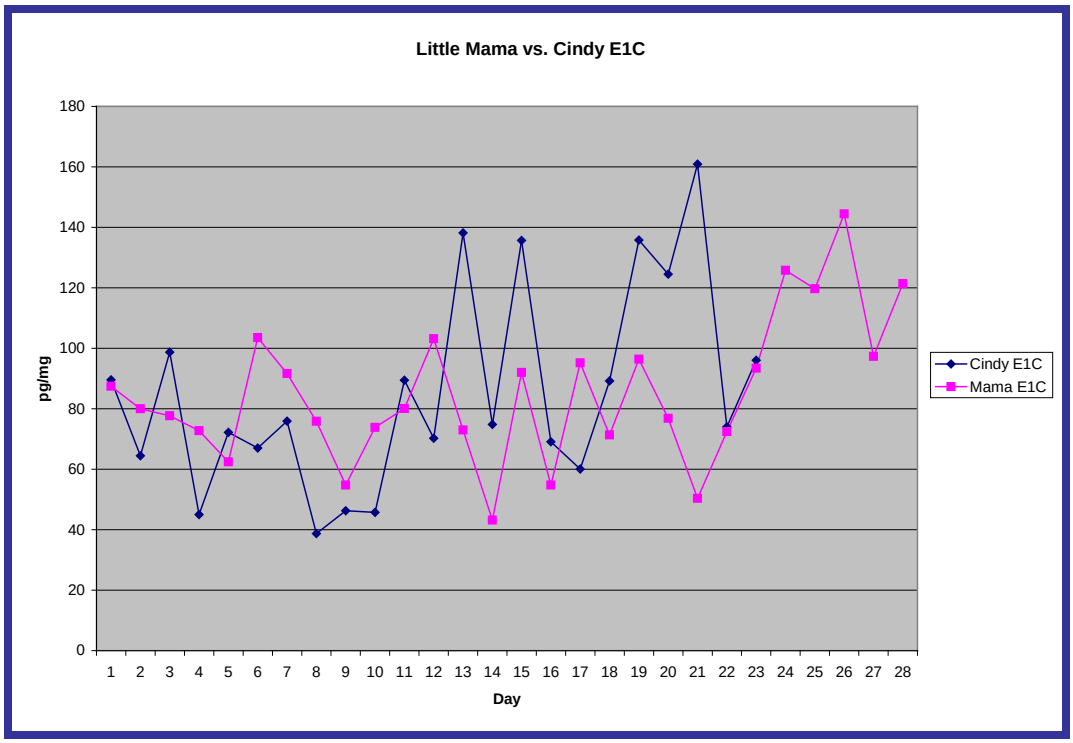

Table 4.12: ***E_1C levels presenting within Little Mama versus those of Cindy***

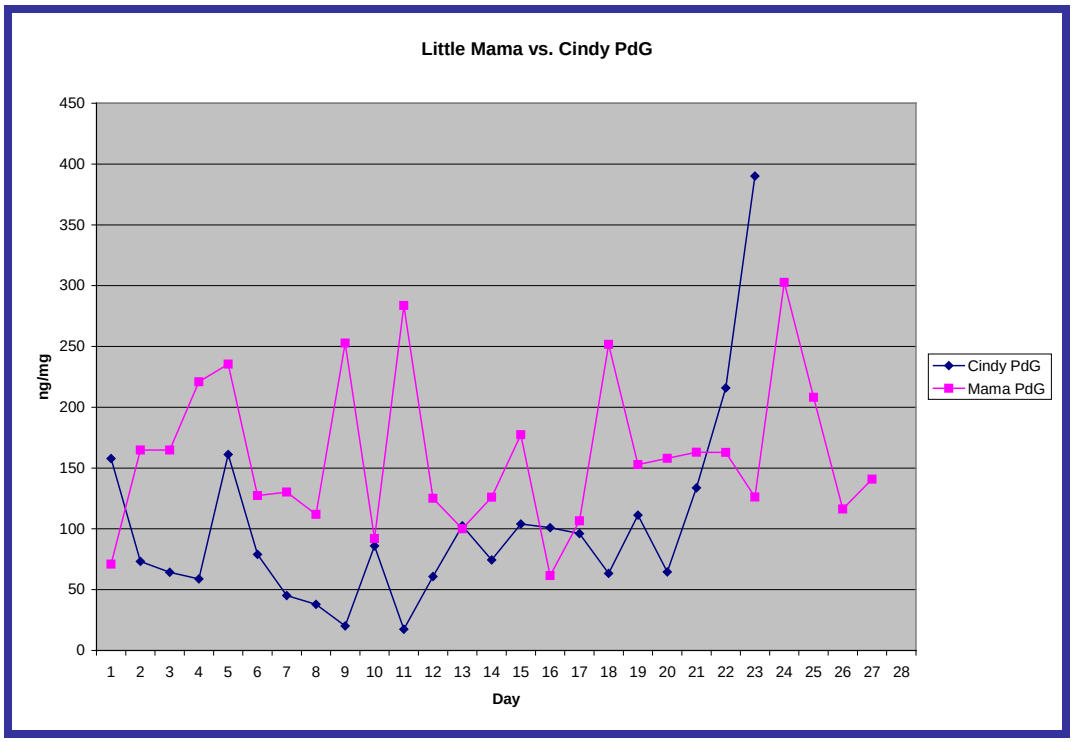

Table 4.13: ***PdG Levels presenting within Little Mama versus those of Cindy***

There were an additional number of striking circumstances which arose during the course of this study that serve to reinforce the need for a more complete grasp of nonhuman primate reproductive ecology and aging, the most notable of which being that statistical evaluations indicate that there is the largest degree of difference between the reproductive viability of Little Mama and Tonic, rather than that of Janice. This is particularly remarkable, as Tonic was twenty-three at the time of collection and had given birth three times, while Janice was fourteen and had never reproduced.

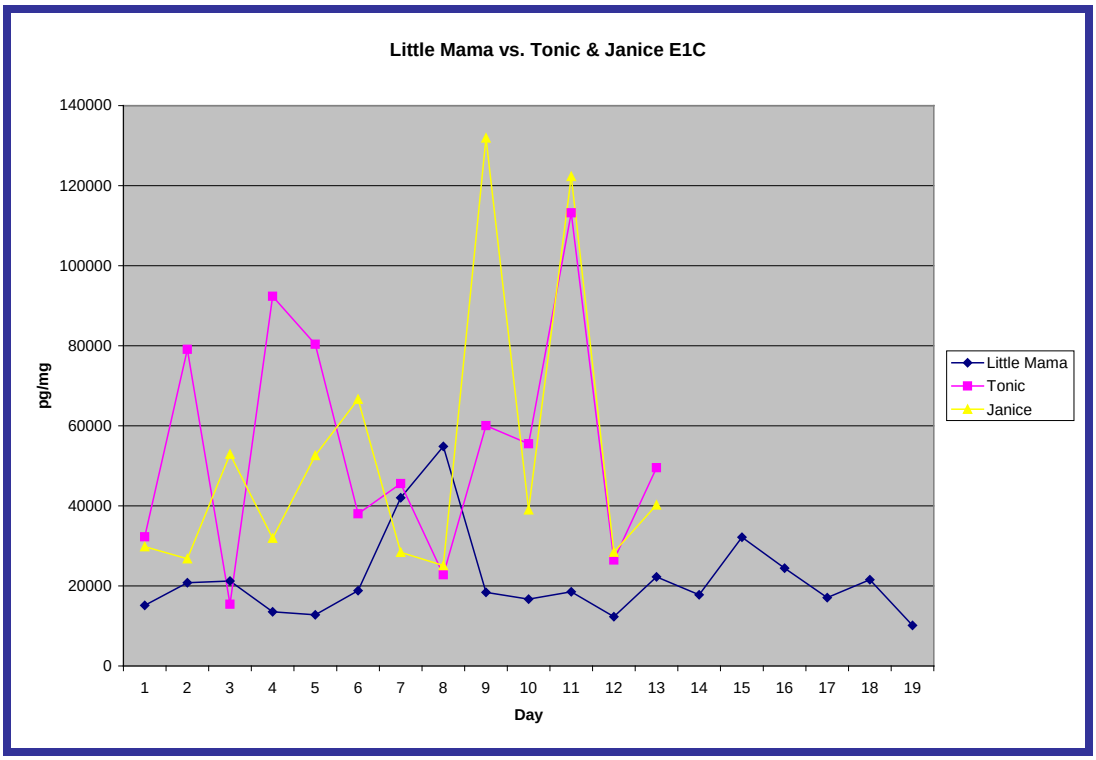

Table 4.14: ***E_1C levels presenting within Little Mama versus those of Tonic and Janice***

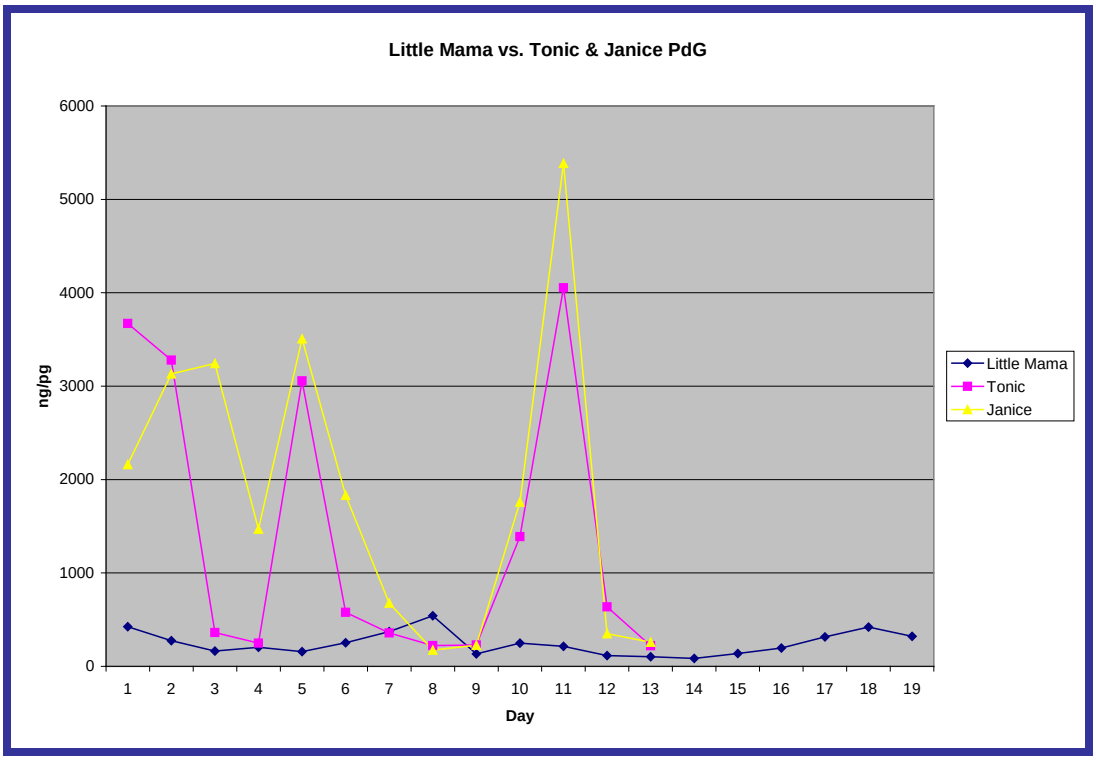

Table 4.15: ***PdG levels presenting within Little Mama versus those of Tonic and Janice***

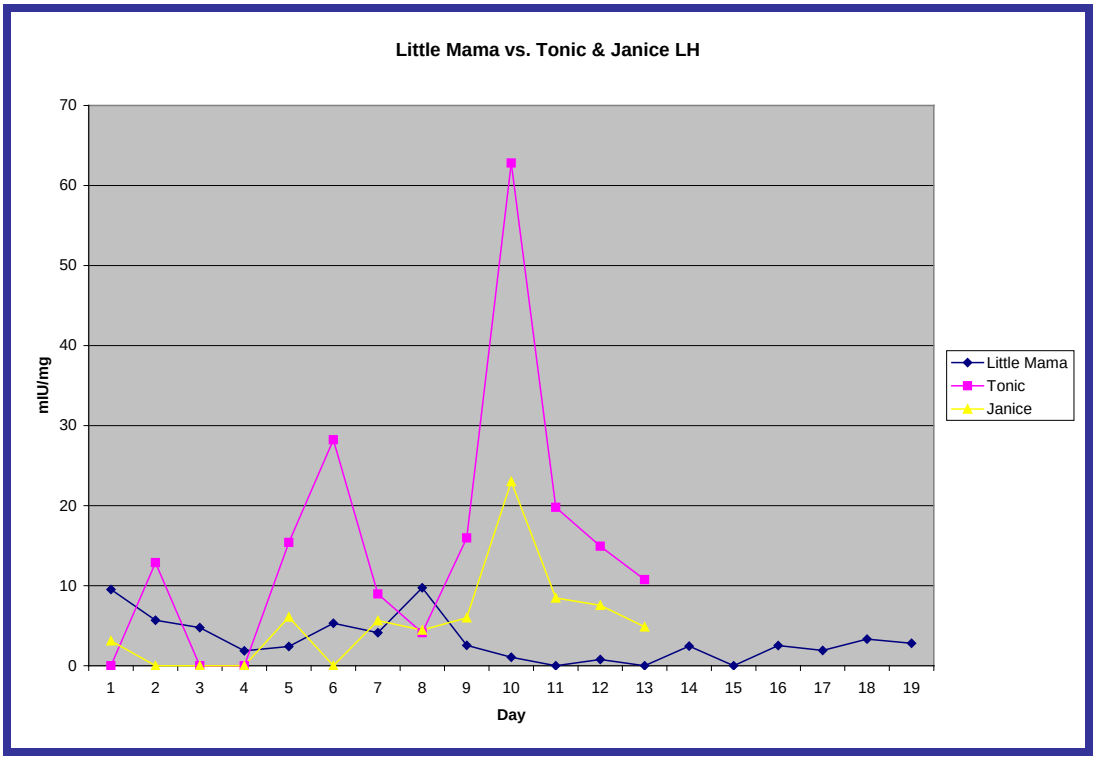

Table 4.16: ***LH levels presenting within Little Mama versus those of Tonic and Janice***

One would assume that there would be the most prevalent degree of difference between the youngest and the eldest study subjects, especially as there is not only the variability between age to consider, but also recent conjecture (Atsalis and Videan 2009), whereby multiple births are proposed to decrease reproductive ability in chimpanzee populations. If such is the case, one would expect to find evidence of a more marked degree of difference between the two subjects which represent not only the broadest ends of the age spectrum available, but also the parous versus the nulliparous chimpanzee female.

Not surprisingly, during the entirety of observation period for Little Mama, she was never evidenced to display swelling of the anogenital region. She had also failed to show perineal tumescence during two years of daily interactions with her prior to data collection, whereas Cindy presented only sporadic swelling cycles. Tonic and Janice regularly underwent visible cycles, including during the period of data collection. This is in accordance with published data on the swelling patterns of aging female chimpanzees (Videan et al. 2006).

4.3 *Summary*

This study examined the endocrine profile of a female chimpanzee (*Pan troglodytes*) estimated to be seventy-two years of age. Hormonal levels of urinary cortisol and metabolites of estradiol (estrone conjugates, E_1C) and progesterone (pregnanediol-3-glucuronide) as well as luteinizing hormone (LH) were assayed in order to determine the reproductive viability of a female chimpanzee well beyond the age at which she would have assumed to have undergone menopause. Results have indicated that she is not in the clinical stage of reproductive cessation typically referred to as menopause in humans. However, it would seem that the common chimpanzee potentially experiences a process of reproductive aging unlike that of human

females, which could then be said to have occurred subsequent to the evolutionary split between the last common ancestor of *Homo sapiens* and *Pan troglodytes*.

Additionally, the endocrine profiles of three control females were assessed in order to provide a basis of comparison against which Little Mama could be held. Interestingly, results indicated that there are a number of incongruities between the published data and the information gleaned by comparison of the control group to the reproductive profile of Little Mama. However, the study did lend additional evidence to the correlations between aging and reproductive viability in the common chimpanzee. Further study is clearly required in order to sort through the available data concerning the topic of reproductive ecology and aging in the nonhuman primate.

4.4 *Future Studies*

Future studies should include longer-term investigations into the reproductive profile of Little Mama, as she is demonstrative of such a small population of available study subjects. There are few geriatric female chimpanzees in captivity, and even fewer that have surpassed the age of 60. Also, it is rare to find a facility which does not practice birth control of their animal populations through oral contraceptives of the female, which drastically alters their cycling patterns. Combined with the fact that Lion Country Safari is a semi-free ranging animal park, there is an extremely rare opportunity in Little Mama to gather ample information about the processes of reproductive aging in nonhuman primates. The knowledge of a study subject's medical, social and reproductive history is also extremely valuable in sorting out the reproductive parameters of the species; Little Mama, as an animal that has resided in the same location for over 40 years and within the same stable social group for over twenty years, is worthy of close and thorough investigation.

Also, there is a large and unstudied population of chimpanzees housed at Lion Country Safari living in excellent conditions for additional hormonal research. In total, there are twenty-five chimpanzees, the vast majority of which are female. All are kept in identical conditions, and are allowed as natural a lifestyle as possible which allows researchers to expect hormonal levels on par with those of wild chimpanzees, while also retaining the ability to locate and sample from the target animal daily. Of this population, five females are over forty years of age and would provide an excellent opportunity for long-term study. Specific fecal samples are easily collected by keepers each day, and as per recent methodology suggests (Atsalis & Margulis 2006) have been demonstrated to be easily distinguished from those of sympatric animals via distribution of food coloring in the diet.

Results of an ongoing study on reproductive function in chimpanzees may assist in a number of contemporary issues in both human and primate societies alike. The biological mechanics of menopause in human females have still not been sufficiently explored, due to a lack of multidisciplinary understanding regarding the integration of the proximate and ultimate causes of the process. This situation can be rectified through a more complete understanding of the potentially homologous processes occurring across related primate species. As larger numbers of humans enjoy more extended longevity, the trend has been to push the age at primiparity in females back; this results in higher risks of birth defects and infant deaths due to more advanced age in the mother. Research is currently being conducted to deflect the impact that time has on a woman's reproductive ability, and a multidisciplinary approach is practically guaranteed to assist in achieving those goals more expediently.

Additionally, future studies should focus on the reproductive ecology and aging in the nonhuman primate for the sake of the animals themselves. Population densities of all extant primates are drastically declining in the wild, and soon these animals will potentially be confined only to zoos. Before that time, achieving a firm grasp on the reproductive parameters of the

animals—as well as the conditions in which they must be kept to ensure fecundity—can serve to elongate the average reproductive life of a captive primate, which would in turn extend the time that the various species have on the planet.

This study has plainly illustrated the need for further investigation into the endocrine profiles and reproductive aging of nonhuman primates while also providing a clearer picture of the processes occurring within the hormonal tract of a geriatric female chimpanzee. As such, future studies should further this knowledge and assist in reaching solutions to the problems present in contemporary culture.

Appendix: Results of Urine Analyses

A. Collection Period 1 (07/21/2008 – 08/21/2008)
The highlighted numbers have been configured for the concentration ranges and reagent stocks of chimpanzees, and are therefore the figures utilized for statistical assessment.

Tube	Sample #	Female	Date	cr mg/ml	C PG/ML	C ng/mg Cr	E1C pg/ml	E1C pg/mg cr	PdG ng/ml	pdg ng/mg Cr
1	C080108	Cindy	39661	0.784	52522	66.99234694	6472.5	8255.739796	61.96	79.03061224
2	C080908	Cindy	39669	0.229	31644	138.1834061	3622.2	15817.46725	23.46	102.4454148
3	C071508	Cindy	39644	0.28	24978	89.20714286	3532.5	12616.07143	17.76	63.42857143
4	C080808	Cindy	39668	0.287	25666	89.42857143	4262.5	14851.91638	5	17.42160279
5	C072308	Cindy	39652	0.276	27254	98.74637681	3951.5	14317.02899	17.76	64.34782609
6	C072708	Cindy	39656	LOW						
7	C081708	Cindy	39677	0.168	20917	124.5059524	3275.5	19497.02381	10.86	64.64285714
8	C081608	Cindy	39676	0.281	38162	135.8078292	4490.5	15980.42705	31.26	111.2455516
9	C081008	Cindy	39670	0.29	21699	74.82413793	3711.5	12798.27586	21.6	74.48275862
10	C080908	Cindy	39669	0.235	16510	70.25531915	3323.5	14142.55319	14.28	60.76595745
11	C081108	Cindy	39671	0.159	10980	69.05660377	2941.5	18500	16.06	101.0062893
12	C072108	Cindy	39650	0.193	17287	89.56994819	2977.5	15427.46114	30.46	157.8238342
13	C072208	Cindy	39651	0.239	15404	64.45188285	3325	13912.13389	17.52	73.30543933
14	C081808	Cindy	39678	0.17	27355	160.9117647	3512	20658.82353	22.74	133.7647059
15	C080408	Cindy	39664	0.305	32265	105.7868852	3937	12908.19672	20.54	67.3442623
16	C072508	Cindy	39654	0.273	12286	45.003663	3578	13106.22711	16.04	58.75457875
17	C080208	Cindy	39662	0.256	19428	75.890625	2853	11144.53125	11.56	45.15625
18	C080308	Cindy	39663	0.422	16350	38.74407583	4142	9815.165877	16.02	37.96208531
19	C080608	Cindy	39666	0.453	20970	46.29139073	4393	9697.571744	9.16	20.22075055
20	C081408	Cindy	39674	0.467	28062	60.08993576	6275	13436.83084	44.92	96.18843683
21	C081208	Cindy	39672	0.182	24694	135.6813187	3280	18021.97802	18.94	104.0659341
22	C072808	Cindy	39657	0.624	45051	72.19711538	8555	13709.9359	100.56	161.1538462
23	C082008	Cindy	39680	0.451	33382	74.01773836	5452.5	12089.80044	97.34	215.8314856
24	C080708	Cindy	39667	0.281	12851	45.73309609	4194	14925.2669	24.14	85.90747331
25	C082108	Cindy	39681	0.158	15174	96.03797468	3631	22981.01266	61.645	390.1582278
26	M081908	LittleMama	39679	0.34	49121	144.4735294	3906.5	11489.70588	70.755	208.1029412
27	M081708	LittleMama	39677	0.191	24028	125.8010471	2952	15455.49738	24.105	126.2041885
28	M080608	LittleMama	39666	0.395	28820	72.96202532	3798	9615.189873	39.53	100.0759494
29	M080208	LittleMama	39662	0.395	29157	73.81518987	3426.5	8674.683544	36.33	91.97468354
30	M072808	LittleMama	39657	0.154	14115	91.65584416	2378	15441.55844	20.063	130.2792208
31	M082108	LittleMama	39681	0.384	46612	121.3854167	3757.5	9785.15625	54.105	140.8984375
32	M072908	LittleMama	39658	0.305	23129	75.83278689	2805	9196.721311	34.145	111.9508197
33	M081608	LittleMama	39676	0.434	40550	93.43317972	4001.5	9220.046083	70.66	162.8110599
34	M072608	LittleMama	39655	0.737	45999	62.41383989	4829.5	6552.917232	173.53	235.4545455
35	M080408	LittleMama	39664	0.702	56193	80.04700855	5354	7626.780627	199.165	283.7108262
36	M081108	LittleMama	39671	0.192	13692	71.3125	2739	14265.625	48.31	251.6145833
37	M080808	LittleMama	39668	0.169	15552	92.02366864	3955.5	23405.32544	29.985	177.4260355

38	M072108	LittleMama	39650	0.413	36113	87.44067797	4099.5	9926.150121	29.335	71.02905569
39	M080508	LittleMama	39665	0.4	41271	103.1775	1273.5	3183.75	50.03	125.075
40	M072708	LittleMama	39656	0.127	13148	103.5275591	750	5905.511811	16.17	127.3228346
41	M082008	LittleMama	39680	0.284	27641	97.32746479	750	2640.84507	33.015	116.25
42	M081208	LittleMama	39672	0.158	15229	96.38607595	750	4746.835443	24.14	152.7848101
43	M081408	LittleMama	39674	0.284	20574	72.44366197	empty		empty	
44	M081308	LittleMama	39673	0.332	25516	76.85542169	3161	9521.084337	52.465	158.0271084
45	M072208	LittleMama	39651	0.24	19199	79.99583333	3195.5	13314.58333	39.57	164.875
46	M072308	LittleMama	39652	0.492	38210	77.66260163	4002.5	8135.162602	81.095	164.8272358
47	M081808	LittleMama	39678	0.258	30886	119.7131783	3275.5	12695.73643	78.075	302.6162791
48	M080108*	LittleMama	39661	0.368	20147	54.74728261	3851	10464.67391	93.015	252.7581522
49	M080708*	LittleMama	39667	0.364	15715	43.17307692	3986.5	10951.92308	45.86	125.989011
50	M081308*	LittleMama	39673	0.337	16983	50.39465875	3792	11252.22552	54.93	162.9970326
51	M072508*	LittleMama	39654	0.646	46975	72.71671827	5426	8399.380805	142.7	220.8978328
52	M081008A*	LittleMama	39670	0.392	21458	54.73979592	3258	8311.22449	24.13	61.55612245
53	M081008B*	LittleMama	39670	0.213	20280	95.21126761	3115.5	14626.76056	22.71	106.6197183

B. Collection Period 2 (10/06/2008 – 12/02/2008)
The highlighted numbers have been configured for the concentration ranges and reagent stocks of chimpanzees, and are therefore the figures utilized for statistical assessment. The red boxes indicate that there was not enough material available for assessment and had to be discounted.

Sample	NAME	DATE	Cr mg/ml	LH mIU/ml	E1C pg/ml	PdG ng/ml	Cort ng/ml	LH mIU/mg Cr	E1C pg/mg Cr	PdG ng/mg Cr	Cort ng/mg Cr
54	Mama	10/6/2008	0.107	1.02	1619	45.52	3.189	9.53271028	15130.8411	425.420561	29.80373832
55	Mama	10/7/2008	0.273	1.55	5675.3	74.86	14.835	5.67765568	20788.6447	274.212454	54.34065934
56	Mama	10/11/2008	0.0075	0.79	lo	37.46					
57	Mama	10/13/2008	0.033	1.15	2046.69	25.03					
58	Mama	10/14/2008	0.231	1.1	4898.58	37.97	6.956	4.76190476	21205.974	164.372294	30.11255411
59	Mama	10/15/2008	0.642	1.2	8679.58	131.15	6.913	1.86915888	13519.595	204.283489	10.76791277
60	Mama	10/16/2008	0.66	1.58	8430.55	104.37	20.329	2.39393939	12773.5606	158.136364	30.80151515
61	Mama	10/18/2008	0.3069	1.63	5770.49	77.86	14.512	5.31117628	18802.509	253.698273	47.28576083
62	Mama	10/19/2008	0.235	0.97	9875.11	87.28	9.517	4.12765957	42021.7447	371.404255	40.49787234
63	Mama	10/20/2008	0	0.64	2557.83	lo					
64	Mama	10/24/2008	0.076	0.74	4168.79	41.23	3.445	9.73684211	54852.5	542.5	45.32894737
65	Mama	10/25/2008	0.009	0	lo	lo					
66	Mama	10/28/2008	0.43	1.1	7909.86	57.32	34.203	2.55813953	18395.0233	133.302326	79.54186047
67	Mama	11/2/2008	0.5995	0.64	10008.52	149.61	55.38	1.0675563	16694.779	249.557965	92.37698082
68	Mama	11/3/2008	0.275		5102.82	58.69	5.09		18555.7091	213.418182	18.50909091
69	Mama	11/4/2008	0	0.84	2955.13	60.97					
70	Mama	11/5/2008	0.028	0.66	2243.84	lo					
71	Mama	11/6/2008	0.904	0.71	11111.22	104.98	39.283	0.78539823	12291.1726	116.128319	43.45464602
72	Mama	11/7/2008	0.268		5970.59	27.46	6.999		22278.3209	102.462687	26.11567164
73	Mama	11/8/2008	0.321	0.79	5713.39	27.46	18.29	2.46105919	17798.7227	85.5451713	56.97819315
74	Mama	11/10/2008	0.143		4597.47	19.72	7.858		32150.1399	137.902098	54.95104895
75	Mama	11/11/2008	0.214	0.54	5227.38	42.08	16.241	2.52336449	24427.0093	196.635514	75.89252336
76	Mama	11/19/2008	1.014	1.94	17318.26	319.04	130.787	1.91321499	17079.1519	314.635108	128.9812623
77	Mama	11/24/2008	0.214	0.71	4617.6	90	3.311	3.31775701	21577.5701	420.560748	15.47196262
78	Mama	12/2/2008	0.309	0.87	3134.36	99.18	20.96	2.81553398	10143.5599	320.970874	67.83171521
79	Tonic	10/10/2008	0.788		25440.34	2893.1	53.749		32284.6954	3671.4467	68.20939086
80	Tonic	10/12/2008	0.282	3.63	22313.37	924.4	28.467	12.8723404	79125.4255	3278.01418	100.9468085
81	Tonic	10/14/2008	0.79		12173.89	285.69	49.602		15409.9873	361.632911	62.78734177
82	Tonic	10/14/2008	0.184		16996.43	45.84	2.52		92371.9022	249.130435	13.69565217
83	Tonic	10/16/2008	1.393	21.45	111977.6	4256.56	101.5	15.3984207	80385.8938	3055.67839	72.86432161
84	Tonic	10/20/2008	0.085	2.4	3232.36	49.08	10.75	28.2352941	38027.7647	577.411765	126.4705882
85	Tonic	10/24/2008	0.125	1.12	5693.33	44.67	4.271	8.96	45546.64	357.36	34.168
86	Tonic	10/26/2008	1.21	4.99	27579.88	269.15	42.622	4.12396694	22793.2893	222.438017	35.22479339
87	Tonic	11/5/2008	0.574	9.17	34455.15	130.68	44.555	15.9756098	60026.3937	227.665505	77.62195122
88	Tonic	11/9/2008	0.075	4.71	4164.83	104.19	5.182	62.8	55531.0667	1389.2	69.09333333
89	Tonic	11/19/2008	0.295	5.84	33400.83	1195.57	30.517	19.7966102	113223.153	4052.77966	103.4474576
90	Tonic	11/24/2008	0.264	3.94	6984.93	168.35	12.29	14.9242424	26458.0682	637.689394	46.5530303
91	Tonic	12/2/2008	0.107	1.15	5299.65	23.67	2.092	10.7476636	49529.4393	221.214953	19.55140187
92	Janice	10/10/2008	0.721	2.24	21482.14	1558.08	44.742	3.10679612	29794.9237	2160.99861	62.0554785
93	Janice	10/11/2008	1.308		35071.86	4096.04	21.136		26813.3486	3131.52905	16.15902141
94	Janice	10/12/2008	0.258		13650.09	836.79	8.742		52907.3256	3243.37209	33.88372093
95	Janice	10/13/2008	0.499		15941.38	733.61	8.01		31946.6533	1470.16032	16.05210421
96	Janice	10/14/2008	0.428	2.6	22516.35	1500.99	7.265	6.07476636	52608.2944	3506.98598	16.97429907
97	Janice	10/14/2008	0		1506.4	23.67	3.368				
98	Janice	10/15/2008	0.745		49652.91	1364.84	26.323		66648.2013	1832	35.33288591
99	Janice	10/19/2008	0.616	3.47	17486.57	417.77	28.217	5.63311688	28387.289	678.198052	45.80681818
100	Janice	10/26/2008	0.377	1.68	9451.69	65.42	15.69	4.45623342	25070.7958	173.527851	41.61803714
101	Janice	10/28/2008	0	0	lo						
102	Janice	11/5/2008	1.408	8.44	185751.2	319.21	153.031	5.99431818	131925.568	226.711648	108.6867898
103	Janice	11/9/2008	0.419	9.64	16354.77	736.63	24.063	23.0071599	39032.864	1758.06683	57.42959427
104	Janice	11/19/2008	1.272	10.76	155550.7	6850.7	128.873	8.4591195	122288.286	5385.77044	101.3152516
105	Janice	11/24/2008	0.464	3.5	13188.76	162.24	15.193	7.54310345	28424.0517	349.655172	32.74353448
106	Janice	12/2/2008	0.6	2.91	24155.48	155.37	16.967	4.85	40259.1333	258.95	28.27833333

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