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Elizabeth Whiteside March 28, 2024

Uncovering Menopause in Tufted Capuchin Monkeys (*Sapajus apella*): Analyzing the Relationship between Estradiol, Aging, and Behavioral Estrus in a Captive Population

by

Elizabeth Whiteside

Marcela Benítez
Adviser

Anthropology

Marcela Benítez
Adviser

Elizabeth Lonsdorf

Committee Member

Megan Massa

Committee Member

Gita Gnanadesikan

Committee Member

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Abstract

Uncovering Menopause in Tufted Capuchin Monkeys (*Sapajus apella*): Analyzing the Relationship between Estradiol, Aging, and Behavioral Estrus in a Captive Population

By Elizabeth Whiteside

Menopause – the end of reproductive function – is a critical phase in a human female's life; humans can live up to a third of their lives in a post-reproductive stage which is highly unusual among primates. As research on menopause in non-human primates expands, evidence of an age-related cessation of hormone production and fertility is most likely to be seen in other species of non-human primates with extended lifespans. In this study, I examine age-related effects on the concentration of estradiol in fecal samples from female tufted capuchin monkeys (*Sapajus apella*) to provide evidence for the occurrence of menopause in the oldest members of a captive population. I also validated and employed a human estradiol assay as an effective measurement of the menopausal transition in tufted capuchin females. In addition to investigating age-related hormone changes, I investigate how aging affects the frequency of behavioral estrus in individuals over the age of 30 to further explore the occurrence of menopause in tufted capuchins. I also examine the relationship between estradiol and estrus behaviors in normally cycling individuals to analyze how hormonal changes during the menstrual cycle may influence sexual soliciting behavior in tufted capuchins. Comparing adult (<30 years old) with old-age (over 30-year-old) individuals, I found that fecal estradiol concentration declines significantly with age. Additionally, in a biological validation with a female who had undergone an ovariectomy, I found a significant difference in the pre- versus post-operative estradiol concentration but no significant difference between the ovariectomized condition and the old-age individuals. In terms of behavior, I found that sexual soliciting, or estrus, behaviors decline with age. Unlike previous studies using progesterone, I did not find a strong correlation between high concentrations of fecal estradiol and the onset of estrus suggesting a decoupling of behavioral estrus from ovulation in capuchin monkeys and the potential for deceptive estrus to be occurring. Overall, these results suggest the occurrence of menopause in old-age, captive tufted capuchins and have implications for the capacity to use estradiol to explore menopause in other captively housed, long-lived primates.

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Uncovering Menopause in Tufted Capuchin Monkeys (*Sapajus apella*): Analyzing the Relationship between Estradiol, Aging, and Behavioral Estrus in a Captive Population

Overview

Menopause – the end of reproductive function – is a critical phase in a human female's life; humans can live up to a third of their lives in a post-reproductive stage which is highly unusual among primates. Historically, this post-reproductive period has been seen as a human-specific phenomenon with competing hypotheses related to the evolutionary advantages of having grandmother support in child-rearing (Williams, 1957; Hawkes et al., 1998), benefits gained from the reduction of intergenerational reproductive strife (Cant and Johnstone, 2008), or because of the extended lifespans present in humans (Takahashi, 2017). Recent evidence, however, suggests that other long-lived social animals, like killer whales, may also experience a post-reproductive period (Nattrass et al., 2019). In primates, evidence of menopause is relatively rare. A recent study in wild chimpanzees found that fertility decreased in females above the age of 30 with estrogens and progestins decreasing as individuals aged (Wood et al., 2023). Whether other primates may exhibit similar age-related declines in reproductive hormones remains unknown. The integration of hormone analyses into continued research on menopause is critical for understanding the evolutionary and biological factors which shape reproductive strategies in female non-human primates.

As research on menopause in non-human primates expands, evidence of an age-related cessation of hormone production and fertility is most likely to be seen in other species of non-human primates with extended lifespans. In this study, I examine age-related effects on the

concentration of estradiol in fecal samples from female tufted capuchin monkeys (*Sapajus apella*) to provide evidence for the occurrence of menopause in the oldest members of a captive population. Capuchin monkeys are highly social, relatively long-lived, small-bodied New World monkeys. They share many similarities with humans and chimpanzees, including an extraordinarily large brains for their body-size and a long period of juvenile development, which makes them strong comparative models for understanding primate life history (Fragaszy et al., 2004). Cross-species analysis on the occurrence of menopause has potential implications for determining the adaptive significance of post-reproductive lifespans and the socioecological influences on female life history patterns, contributing to a broader understanding of human evolutionary biology.

In addition to investigating age-related hormone changes, I investigate how aging affects the frequency of behavioral estrus in individuals over the age of 30 to further explore the occurrence of menopause in tufted capuchins. Female sexual behavior has been historically understudied; however, this is a burgeoning field which can provide key insights into female mate choice and adaptive strategies and may expand the breadth of knowledge about menopause in non-human primates. I also examine the relationship between estradiol and estrus behaviors in normally cycling individuals to analyze how hormonal changes during the menstrual cycle may influence sexual soliciting behavior in tufted capuchins. Capuchins are one of the few genera of New World Primates with a menstrual cycle, where the uterine lining is expelled rather than reabsorbed, making them an ideal candidate for this study (Mayor et al., 2019). The continued study of female sexual behavior in primates in crucial for understanding

species-specific reproductive strategies, mate choice and sexual selection, and the evolutionarily adaptive function of sexual behaviors.

Menopause: Physiology & Expected Hormonal Profile

Menopause, or reproductive senescence, marks the end of the reproductive period for female animals. The transition is characterized by a decline in circulating estradiol, the primary ovarian hormone, and the cessation of menstrual cycling. As oocytes, or eggs, decline in number, ovarian activity ceases and fertility drops (Talaulikar, 2022). Levels of estradiol fluctuate during perimenopause and drop significantly post-menopause (Santoro and Randolph, 2011). In humans, menopause is diagnosed when the menstrual cycle has not been active for at least a year barring other pathological explanations (Talaulikar, 2022). For non-human animals, the distinction of post-reproductive is defined as a female who has lived longer than her last birth by at least the mean length of interbirth interval plus two standard deviations of interbirth intervals for their population (Caro et al., 1995). In contrast, the category of post-menopausal relies on measurements of decreased hormone concentration as an indicator of ovarian function which has not been as frequently studied in wild non-human animals. Because of this, reproductive senescence may be under documented in species with longer interbirth intervals when only demographic data is available for study.

Recent advances in non-invasive techniques for examining reproductive hormones provides researchers with more accurate tools to examine the menstrual cycle and monitor the onset of reproductive senescence**.** Estradiol, or 17-beta-estradiol, is a type of estrogen released from the ovaries and a significant regulatory hormone in the ovarian cycle. In females primates that are normally cycling and have a menstrual cycle, estradiol is an important hormone that

aids in the maturation of a follicle during the follicular phase, ovulation, the development of the corpus luteum in the luteal phase, and the degeneration of the corpus luteum followed by menstruation (Weinbauer et al., 2008). This process has been seen in Old World Primates that undergo a menstrual cycle, like rhesus macaques and chimpanzees (Weinbauer et al., 2008; Nadler et al., 1985), and in a few genera of New World Primates, including nocturnal monkeys and capuchins (Mayor et al., 2019). In these species, the follicular phase is often evident by the rise in estrogen detectable in circulation (Nagle et al., 1980; Weinbauer et al., 2008); ovulation generally follows this peak in estradiol concentration, but the length of time between the estradiol spike and ovulation depends on both the species of study and the method of hormone collection (Shimizu, 2005; Lima et al., 2019). In terms of aging, almost 95% of estradiol is produced in the ovaries which ceases following menopause. Therefore, in normal cycling females, estradiol would be expected to be highest preceding ovulation and lowest during post-ovulation. If females stop cycling, estradiol should plummet (Santoro and Randolph, 2011).

A recent study on chimpanzees utilized non-invasive hormone methods combined with reproductive data to examine evidence of menopause in 66 wild chimpanzees (Wood et al., 2023). Wood and colleagues found that fertility decreased in females above the age of 30 with estrogens and progestins decreasing as individuals aged (Wood et al., 2023). Females over 40 exhibited the lowest concentrations of ovarian hormones, a pattern also observed in human females. In addition to recent research in chimpanzees, evidence for menopause has been observed in a handful of cetacean species. In female killer whales, menopause occurs in their late 30s to early 40s and they have an approximate post-reproductive lifespan of 15.78 years (Nattrass et al., 2019). Additionally, using age-specific ovarian corpora counts as a measure of

ovarian function, evidence has been found for menopause and the presence of post-reproductive lifespans in beluga whales, narwhals, and short-finned pilot whales (Ellis et al., 2018). While documented menopause in animals is rare, we may expect to see similar patterns of hormonal and reproductive senescence in some other long-lived, highly social species.

Capuchins are small-bodied, gregarious primates that live in large, multi-sex social groups (Fragaszy et al., 2004). Capuchins stand out amongst other primates as having relatively large brains and long lifespans (Fragaszy et al., 2004). In captivity, capuchins can live to 55 years of age (Hakeem et al. 1996). We know considerably less about maximum lifespan in the wild; however, it is not uncommon for female capuchins to live into their mid 30s (Perry et al., 2012). Given these extraordinarily long lives – especially for a relatively small species – capuchin females may exhibit a period of post-reproductive senescence but whether they exhibit menopause remains unknown.

The first goal of this thesis was to examine whether female tufted capuchins (*Sapajus apella*) exhibit age-related changes in estradiol that may signify a menopausal transition and potential of a post-reproductive lifespan in this species. My research focused on a population of 31 captive tufted capuchins at the Language Research Center where we have hormonal and behavioral data since 2017. Over the course of the study, these capuchins span from age 7 to an estimated age of 50 years. By using non-invasive methods for examining age-related changes in estradiol in this captive population, my goal was to investigate a potential pattern of estradiol decline and, therefore, assess the age in which menopause may occurs in this population. Based on the patterns observed in humans (Santoro and Randolph, 2011) and

chimpanzees (Wood et al., 2023), I predict that if capuchins females undergo menopause, then their estradiol concentration will drop dramatically (Santoro and Randolph, 2011).

In addition to age-related changes, one of the females in this population, Irene (age 22), underwent a complete ovariectomy during the study period due to the presence of an unusual growth on her ovaries. Irene serves as important biological validation of what estradiol decline looks like in capuchins that are no longer cycling. In humans, the removal of ovaries without hormonal intervention results in early-onset menopause, as hormones such as estrogen and progesterone cease to be produced in the ovaries (Rodriguez and Shoupe, 2015). I therefore predict that Irene will exhibit a drastic decline in estradiol following her ovariectomy. If older capuchins exhibit menopause, then I predict that older capuchin females will exhibit similar estradiol concentrations to Irene. Estradiol is also produced in a small proportion of other tissues, like body fat, in addition to being primarily produced in the ovaries. Thus, estradiol concentrations may not drop to zero in non-cycling individuals, making the need for a direct comparison between the older female samples and post-ovariectomy samples significant.

Estradiol & Estrus Behaviors: Honest or Deceptive Signaling?

The second goal of my thesis is to examine the correlation between estrus behaviors, age, and estradiol concentration in capuchin monkeys. Female mammal sexual behavior can be characterized by three categories: sexual attractivity, proceptivity, and receptivity (Beach, 1976). Attractivity denotes a female stimulus which promotes a male response, proceptivity involves female action to initiate sexual interaction with a male, and receptivity refers to the necessary female behaviors which lead to successful copulation. Estrus can be defined as a period of sexual receptivity in which females engage in proceptive behaviors to attract mates.

For most primates, *estrus behaviors,* sexual behaviors used to attract mates, seem to be correlated with an increase in estradiol in the periovulatory phase, such that estrus behaviors signal that a female is ovulating. However, some primates exhibit *deceptive estrus* behaviors, behaviors displayed discordantly with the menstrual cycle phase and that are not closely correlated with the periovulatory phase as might be expected for proceptive behaviors.

Tufted capuchin monkeys have exaggerated proceptivity and concealed ovulation. Proceptive behavior in tufted capuchins is generally directed to an alpha male first, at the beginning of the periovulatory phase (Linn et al., 1995; Welker et al., 1990). The extreme proceptive behaviors of capuchin females are preceded by a period of skittishness following which females may grin, raise their eyebrows exaggeratedly, touch-and-run from males, and vocalize with a high pitch whistle-to-whine sound specific to this behavioral estrus period (Janson, 1984, as cited in Carosi et al., 2005; Phillips, 1994; Carosi, 1999). In captive tufted capuchins, there is evidence of decreased social behavior like grooming and proximity in older individuals (Schino and Pinzaglia, 2018). Given this age-related decrease, it seems likely that sexual behavior and specific estrus behaviors may also decrease in the eldest female tufted capuchins if their ovarian function ceases and circulating estradiol declines.

Examining whether estrus behaviors are linked with ovarian cycle in capuchins has been challenging. Initial studies on the reproductive behavior of capuchin monkeys relied on a suite of invasive measures to establish the menstrual cycle and determine the hormonal and cytological changes associated with each phase. Hamlett (1939) pioneered the use of vaginal lavage to determine cellular components of the menstrual cycle phases for Azara's capuchins (*Sapajus cay*). More modern studies have employed the use of vaginal swabbing for menstrual

bleeding detection and characterizing the cellular components of each menstrual phase (Nagle, 1979; Linn et al., 1995; Ortiz et al., 2005; Lahoz et al., 2007). However, although menstrual bleeding has been observed in capuchins, it is not a reliable indicator of the menstrual phase or changes in hormones due to the inability to observe vaginal bleeding in a non-invasive manner (Carosi et al., 2005). Modern techniques have been employed which provide precise, quantitative data on menstrual phase changes, but these methods are limited because they often require restraints and general anesthesia which may stress subject animals (e.g., ultrasonography, Ortiz et al., 2005).

More recently, non-invasive techniques have been established for the collection of estradiol and other ovarian hormones and have been validated in tufted capuchins (Lima et al., 2019) and other non-human primates (Shimizu, 2005). Though significant for their stress-free approach to hormone collection, fecal samples may exhibit time lags in hormone detection. In capuchins, there may be a delay of up to six days in the detection of estradiol and progesterone from fecal samples (Lima et al., 2019). Integrating these non-invasive methods into behavioral studies have shed light on how estrus behaviors relate to progesterone concentrations in female tufted capuchins. Although the findings of these studies may be complicated by the delay in the detection of fecal progesterone, certain proceptive behaviors have been found to be more tightly associated with the ovarian cycle than others (Linn et al., 1995; Carosi et al., 1999). Raised eyebrows with vocalization, touching and running, and nuzzling (Carosi et al., 1999; Carosi and Visalberghi, 2002) along with grimaces or grins with teeth (Linn et al., 1995) have been found to be correlated with the periovulatory phase. These behaviors have been found to exhibit menstrual phase differences in tufted capuchin females with minimal incidence

of such behaviors outside of the periovulatory phase; however, other behaviors typically associated with estrus – such as body touching and mutual gaze – have not been found to display strong correlations with menstrual cycle phase. This indicates a degree of flexibility for the timing of proceptive behaviors in tufted capuchin females which could be explained by an adaptive strategy of deceptive estrus where behavior is discordant with hormonal cycles. My research expands on these matched behavioral and hormone findings but is unique in its use of estradiol as a marker of ovarian function and a transition into menopause in old-aged female tufted capuchin monkeys.

My study focuses on further examining how these estrus behaviors are related to estradiol changes over a capuchin's ovarian cycle. I examine whether capuchins exhibit deceptive estrus behaviors by examining if these behaviors are linked to peaks in estradiol. If these behaviors are honest indicators of a "fertility window", I would expect that behavioral estrus would be exhibited near the peak of estradiol concentration which would immediately precede ovulation (Nagle et al., 1979), roughly around day 8 of a 20-day menstrual cycle in this species (Nagle et al., 1979; Linn et al., 1995; Carosi et al., 1999; Ortiz et al., 2005; Lima et al., 2019). Given that fecal estradiol may experience delay in detection, behavioral estrus may not immediately precede peak estradiol concentration in my results. However, if behavioral estrus is frequently exhibited when estradiol concentrations are low, this would support the hypothesis that tufted capuchin females display deceptive estrus.

RESEARCH QUESTIONS

My research examines the relationship between estradiol, age, and behavioral estrus in a captive population of capuchin monkeys at the Georgia State University Language Research Center. To do this, I first validated a method for analyzing fecal estradiol from this population. Utilizing this method, I examined whether tufted capuchin females undergo a menopausal transition. In addition, my goal was to gain a better understanding of the individual variation in behavioral estrus and further determine the correlation of specific estrus behaviors to menstrual cycle phase.

My research questions include the following:

1. What is the relationship between age and estradiol concentration in female capuchins?
2. How does the concentration of estradiol in older females compare to a female that has undergone an ovariectomy?
3. How does old age affect the occurrence of behavioral estrus compared to cycling females?
4. How are estradiol concentrations related to the occurrence of behavioral estrus in cycling females?

METHODS

Study Site and Subjects

The study subjects were 22 captive-born female tufted capuchins (aged 7 – estimated 50 years) living in five multi-male, multi-female social groups at the Georgia State University Language Research Center. Given the longitudinal nature of fecal samples and behavioral observations available for this study, two deceased females were included in the data set. Ages were determined by estimation for the eldest individuals based on their acquisition dates from other institutions. Younger individuals who were born in captivity have precise ages using their dates of birth. Social groups were housed in large indoor-outdoor enclosures. In the morning, subjects were given the opportunity to voluntarily enter individual testing enclosures attached to the side of their primary indoor housing. Monkeys were fed multiple times per day with fresh fruit and vegetables and primate chow regardless of the testing schedule. Water was available *ad libitum* to all monkeys including those in testing boxes.

Hormones Collection & Extraction

I collected fecal samples opportunistically from all subjects between 9:30 am- 11:00 am while they were separated in their individual testing chambers. I checked samples for potential contamination with urine or other matter and, if uncontaminated, placed them into collection cups using wooden collection sticks within 30 minutes of defecation. Samples I deemed suitable for collection were labeled with monkey ID, time of collection, and date and were transferred to a frozen freezer cooler (FlexiFreeze) and kept cool until samples were transferred to the

Language Research Center Hormone Laboratory within 2 hours of collection. Samples were immediately placed in a -20 °C freezer until thawed for extraction.

To extract the steroid hormones from the sample, I homogenized the entire sample and then weighed 0.20 ± 0.01 grams of fecal material, recorded this *wet* weight, and added the weighed fecal material to a Falcon tube with 2 mL of 80% ethanol. The sample was then vortexed for 3 minutes and the process was repeated for all samples. After being vortexed, samples were centrifuged for 10 minutes at 2700 rpm. Being careful to avoid the pellet of solid material, I transferred 500 µL to a screw top tube for each sample in duplicate. Extracted samples were placed in cryogen freezer boxes and into a -20 °C freezer until transferred in a cooler to the Social Cognition and Primate Behavior Lab at Emory and stored in -80 °C freezer until assaying.

After extraction, I left the Falcon tubes with fecal pellets uncapped on a drying rack. I weighed the dried fecal pellets approximately two weeks after extraction and recorded this *dry* weight for later standardization against the initial *wet* weight. Prior to 2022, all samples had the additional step of being dried down with a lyopholizer before being kept in the -80 °C freezer for assaying. This step was later removed as it did not affect hormone concentrations (Benítez, unpublished data).

Estradiol Assay Validation

An important step was to validate a commercially available ELISA assay for use with capuchin monkey fecal samples. The Arbor DetectX® Estradiol ELISA kit (Catalog #: K030-H) measures 17β-estradiol in a competitive binding assay. Plates come pre-coated with goat anti-rabbit IgG antibody which binds an estradiol rabbit antibody which in turn binds with either the

kit estradiol peroxidase conjugate solution or the estradiol from the sample. As the concentration of estradiol in the sample increases, it is expected that the bound estradiol peroxidase conjugate decreases which causes a decrease in the optical density measurable by the plate reader. The Arbor Assays DetectX® Estradiol ELISA assay kit is validated for human use and, as such, there were several additional analytical validation steps required to ensure this kit analytically measured fecal estradiol reliably in capuchins. This validation included measuring serial dilution, parallelism, accuracy, and precision.

Serial Dilution. I conducted a serial dilution validation to determine if analytes above the normal upper limit of detection could be measured reliably after dilution. I first pooled samples to create a pool which was used for all estradiol assays. From the neat pool, I started at 1:2 dilution and serially diluted the solution until I reached a 1:512 dilution; I ran all samples in duplicate and reported percent binding. I completed this protocol first to determine at which concentration samples were bound 20%, 50%, and 80% so that I could select the appropriate dilution factor to run samples nearest to 50% binding.

Parallelism. I carried out a parallelism validation to determine if samples with high estradiol concentration would be detectable by the same degree on the standard curve after being diluted. I serially diluted pool samples starting at a 1:8 dilution which were in the 10 – 20% binding range to match the dilution factors of the estradiol standards. I ran these five diluted pool samples with a set of standards and found the percent binding for each concentration. The pool sample closest to the 50% binding range was given an assigned concentration that matched the standard sample closest to 50% binding. I then assigned all pool samples an assigned concentration that mimicked the standard concentration at that

percent binding (e.g. standard sample 2 ran at 14.3% binding with a concentration of 2500 pg./mL and pool sample 1 ran at a 13.1% binding; therefore, pool sample 1 was assigned the concentration of 2500 pg./mL). The logarithm of the assigned concentrations for the pool samples and the logarithm of the standard concentrations were plotted against percent binding and checked for parallelism.

Accuracy. I conducted an accuracy protocol to determine the percent recovery difference between samples and standards. I used 25 uL of a 50% binding solution of pool sample and added 25 uL of standards for each standard dilution to create 'spikes'. I ran the spikes and a set of standards at a 25 uL volume. I then calculated the expected values for the spikes using known concentrations of medium pool or the 50% binding solution plus halved standards. I calculated percent recovery and plotted the spiked concentrations with the expectation that adjusted spiked values and expected values should have a high correlation.

Precision. I used a precision validation to calculate how precisely the estradiol assay can measure sample concentration values. I used low, medium, and high pool samples at 1:256, 1:64, and 1:16 dilutions in 3 sets of duplicates. I calculated the inter- and intra-assay variation and reported the coefficient of variation (CV).

Estradiol Concentrations

I conducted estradiol assays using the Arbor Assays DetectX® Estradiol ELISA assay kit, according to the manufacturer's protocol. The assay kit reagents and samples were allowed to thaw for at least 30 minutes before creating pools, standards, and sample dilutions. I ran samples initially at a 1:64 dilution and all standards and samples were run within 2 hours of preparation. Samples which had been dried down were reconstituted with 250µL of 80%

ethanol and vortexed on a multi-tube vortexer for 10 minutes before dilution. The high, medium, and low pools were created from a single sample pool run at dilutions of 1:16, 1:64, and 1:256, respectively. The assay standard curve ranged from 39.06 pg./mL to 10,000 pg./mL; samples with measured estradiol concentrations below 39.06 pg./mL were rerun at a 1:16 dilution. The manufacturer reports the lower limit of detect for this kit is 26.5 pg./mL and the sensitivity is 39.6 pg./mL. I read the estradiol assay plates at 450 nm on an Epoch plate reader and the output was recorded using Gen5 software.

Biological Validation: Ovariectomy

During the study, one female (Irene, age 22) had both ovaries removed due to a benign cyst; I considered Irene's fecal samples from prior to her operation as active cycling and compared these samples to her post-operation samples. I further compared these two categories of Irene samples to concentrations from older females to assess their similarity.

Behavioral Observations

Behavioral observations were conducted daily by researchers in the morning and/or afternoon over the same period as fecal collection. Focal animal sampling was carried out across five groups using group proximity scans and individual focal recording techniques (Altmann, 1970); individual focal scans were conducted for 10 minutes per individual in a group. All instances of affiliative, aggressive, and sexual behavior from the focal individual to any member(s) of the group were recorded using the software Animal Observer on a tablet. An ethogram of estrus behaviors was established for the tufted capuchin females and these behaviors were recorded when performed during an individual focal or group scan (see Table

1). In addition, based on daily morning observations, researchers used the estrus ethogram to note whether an individual in the group was in estrus, the identity of the individual, and the start date of the onset of estrus.

Table 1.

Ethogram of Female Tufted Capuchins Estrus Behaviors (adapted from Carosi et al., 1999)

Behavior	Definition
Body touching	Female touches male and maintains contact for several seconds.
Eyebrow raising	Female's eyebrows are raised up and lowered rapidly drawing the tufts backwards over the crown of the head.
Eyebrow raising with grin & vocalization	As above with drawing open of mouth to expose teeth and distinct whistle and whining vocalization.
Extended arm(s)	Female spreads arm(s) towards male but does not contact.
Head cocking	Female's head is tilted (45º) towards one side and then the other repeatedly.
Hold body	Females holds or slowly rubs own genitals or chest.
Grin (grimace)	Female's mouth drawn open to bare teeth with jaw closed.
Mounting attempt	Male tries to mount female, but female retreats.
Mounting	Male mounts female and copulates, usually with thrusting. May be isolated or in a sequence.
Mutual gaze	Female and male maintain eye contact for at least 3 seconds, with eyebrow raising.
Nuzzling	Female contacts male with face.
Touching and running	Female contacts male repeatedly and retreats rapidly.
Vocalization	Female emits a continuous whistle sound which leads into a whine.

DATA ANALYSES

Validation

To examine how well the assay performed across the four analytical validations, I calculated a percent mean recovery by comparing the expected values to observed values in the serial dilution, parallelism, accuracy, and precision. In the serial dilution, I conducted a paired t-test to examine if there was a significant difference between observed and expected values. To determine parallelism, a linear regression was plotted comparing known standard concentrations of estradiol and serially diluted pool samples. In the analysis of kit accuracy, I created a simple linear regression to determine any significant difference in the observed and expected values of estradiol concentration. For precision, I compared the extent of variation for each duplicate pair on the same plate (intra-assay) and on different plates (inter-assay). This was reported as a coefficient of variation [CV = (standard deviation / mean) x 100%].

Estradiol Assays and Statistical Modeling

To calculate estradiol concentrations per sample, each fecal sample was standardized using the recorded dry weights to determine the final weight of samples post-extraction; I then log-transformed the concentration of estradiol to reach an approximately normal distribution prior to running the statistical analysis. For models containing age, age was considered a continuous variable as recorded at the time of fecal sample collection; however, depending on the model age may be presented graphically as categorical. I conducted all statistical model analyses in R v.4.3.3 (R Core Team, 2015) using the “lmer” function in the lme4 packages v.1.1-35.1 (Bates et al., 2015).

First, to examine the relationship between age and estradiol concentration I constructed a linear mixed model (LMM) with the logarithm of estradiol as the dependent variable, age as a fixed effect, and monkey ID as a random effect. Age was determined based on the age-at-collection for each sample and 30 years of age was used as the cut-off between the adult and old-age groups. A total of 375 adult and 129 old-age samples were used in this analysis. I then compared this LMM to a null model that just contained the random effect of monkey ID.

Second, to explore how an ovariectomy influences estradiol concentration, I constructed a linear model for Irene's samples with the logarithm of estradiol concentration as the dependent variable and whether the sample was collected pre- or post-ovariectomy procedure date (October 11th, 2023) as a categorical fixed effect. To compare the hormone profile of the old-age females to Irene post-ovariectomy, I constructed a model that included Irene pre-operation samples (n = 73), Irene post-operation samples (n = 4), and samples from the over 30-year-old individuals: Bias, Star, Isabelle, and Mango. The logarithm of estradiol concentration was the dependent variable and monkey ID was a fixed effect.

Third, to assess how old age was related to the occurrence of behavioral estrus in cycling females I ran a negative binomial generalized linear model with age as the fixed variable and count as the dependent variable. The count of estrus was reported as the number of times a female was reported to be in estrus using the ethogram of estrus behaviors I established (Table 1); a new estrus 'bout' was reported if the start date of estrus behaviors was at least 7 days past the previous start date. I then calculated the estrus rate based on the count of estrus bouts per individual, normalized by the number of months in which the individual was under observation.

Lastly, to investigate the relationship between estradiol and estrus behaviors, I focused my analysis on a subset of individuals for whom we had at least 10 samples (N = 9 females) and excluded samples which were farther than 21 days away from the nearest recorded estrus date as this indicated that we had missed the previous estrus cycle. I created a linear mixed model with the logarithm of estradiol concentration as the dependent variable and the amount of time prior to or after a recorded estrus date in units of weeks as a fixed effect.

RESULTS

To look at estradiol concentrations, I ran 15 estradiol assay plates between the fall of 2022 and spring of 2024. I analyzed 504 fecal samples from 22 female capuchin monkeys collected between 2017 and 2023. I ran extractions on samples from 2021 to 2024 and the subset of older samples from 2017 to 2019 had been previously extracted by other researchers at the Language Research Center. For the estradiol assay plates, I found the mean inter-assay coefficients of variation for the medium and high pools as 23.69% and 13.37%, respectively. The mean intra-assay coefficient of variation was 15.26%.

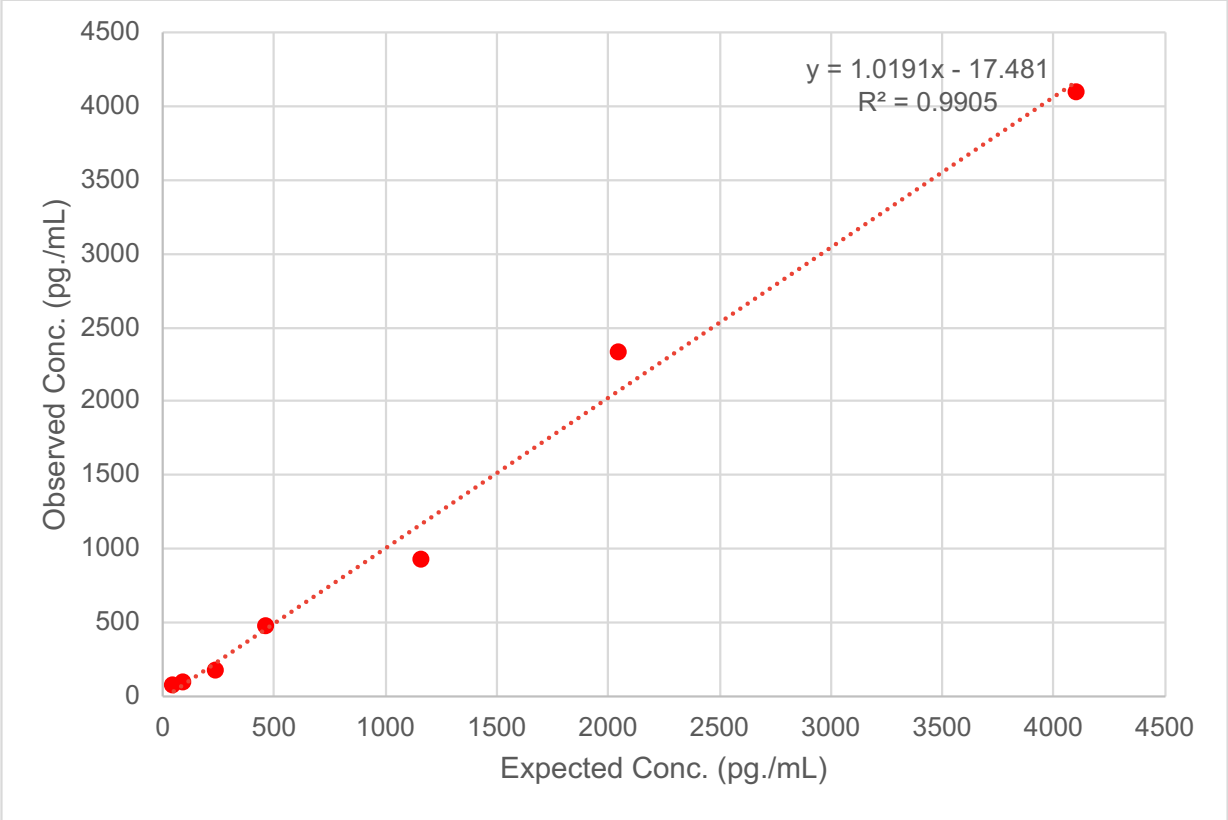
For analyzing estrus and its relationship to estradiol and age, I looked at recorded estrus behavior data for 22 females from 2017 – 2024. This data is preliminary because it relies solely on the estrus logs which only denote the individual which was in estrus and duration of the estrus bout rather than the full focal observation data which includes the specific estrus behaviors recorded. In addition, data from 2020 is included but is not indicative of full counts of estrus or rates for that year because of the facility's emergency lock-down due to the COVID-19 pandemic.

Analytical Validations

Serial Dilution. In the serial dilution, I found a 1:64 dilution to be closest to the 50% binding; low controls were determined to require a 1:256 dilution to attain approximately 80% binding and high controls used a 1:16 dilution to attain approximately 20% binding. Samples at the lowest dilution behaved erratically in terms of mean percent recovery. Samples running at lower than 10% binding or less than a 1:8 dilution had extreme mean recovery values; when these samples were included the mean recovery value was 142%. The samples were diluted by adding the ethanol suspension of fecal hormones into assay buffer; therefore, the high concentration of ethanol in the samples at the lowest dilutions (1:2 and 1:4) likely interfered with the mean recovery of the assay. When I excluded the samples at a 1:2 and 1:4 dilution, I found the observed and expected concentration values were highly correlated and not significantly different (r^2*=0.991; t*(6) = 0.08491, *p* = 0.9351) and the mean recovery was 106% (see Figure 1).

Figure 1.

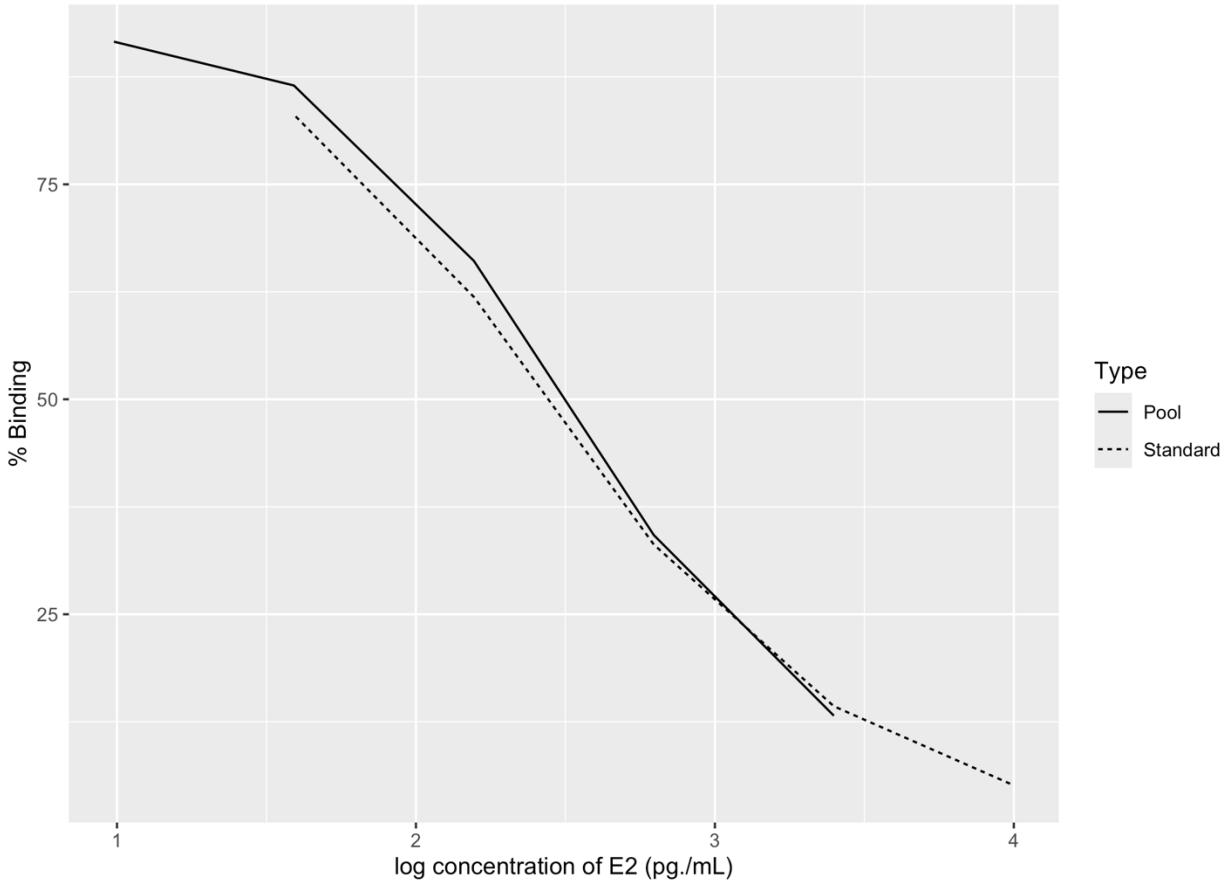
Serial dilution for 1:8 to 1:512 dilutions of 17β-estradiol (E2): observed vs. expected concentrations (pg./mL) [simple linear regression: y = 1.091 x - 17.481 (r^2=0.991)].



Parallelism. The estradiol assays showed good linearity in the detection range around 50% binding (r^2=0.95, $p < 0.001$; figure 2).

Figure 2.

Results of parallelism between known standard concentrations and serially diluted pool samples ($r^2 = 0.95$, $p < 0.001$).



Accuracy and Precision. Using a threshold value of <110% recovery (AOAC, 2012) as ideal, good accuracy was detected for the estradiol assay (Figure 3) with the mean percent recovery at 108.34%. The estradiol assays were found to be precise and the intra-assay variations (CV) are reported in Table 2.

Figure 3.

Accuracy of 17β-estradiol (E2): observed vs. expected concentrations (pg./mL) [simple linear regression: y=0.9387x - 18.1 (r^2=0.9993)].

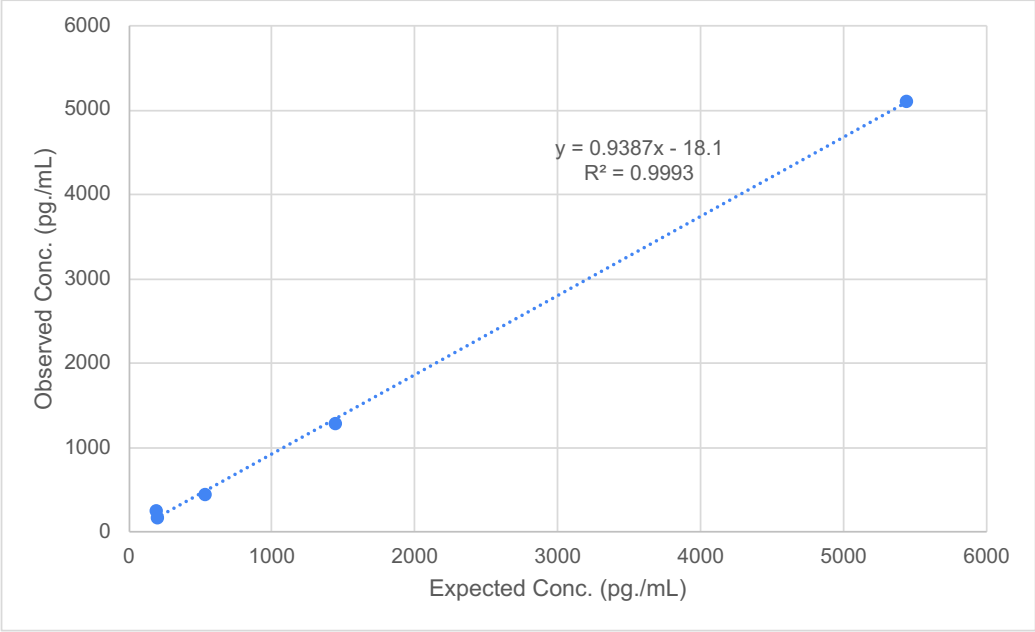


Table 2.

Results for precision validation: intra-assay CVs and average concentration of fecal estradiol (E2) for low, medium, and high pool samples.

Pool	Average Concentration (pg./mL)	Standard Deviation	CV (%)
Low Pool	55.39	14.78	26.68
Medium Pool	307.20	29.70	9.67
High Pool	1843.5	152.44	8.27

Estradiol and Old-Age Individuals

In looking at the effect of aging on estradiol concentration, I determined that the model which contained age was a significantly better fit than the null model (age model AIC = 881.99, null model AIC = 900.30, $p < 0.001$). Further examining of age as a predictor of a menopausal shift, I found that estradiol significantly decreased with age ($\beta = -0.04$, SE = 0.01, $p < 0.001$; table 3 and figure 4) such that older adults (>30 years of age) had lower estradiol values than adults (mean old age = 434.76 ng/g, mean adult = 1336.41 ng/g; figure 5).

Table 3.

Effects of Age on Estradiol Concentration

Predictors	B	SE	*t*-value	*p*-value
(Intercept)	7.44	0.16	40.13	$< 2e^{-16}$ ***
Age	-0.040	0.007	-5.57	$3.39e^{-05}$ ***

Fecal estradiol concentrations were compared between the adult and old-age individual groups. Results show a decrease in fecal estradiol with age.
Full versus null model $\chi 2$ (df = 1) = 20.31, $p < 0.001$.
Significance at *0.05, **0.01, ***0.001.

Figure 4.

Decline of fecal estradiol (E2) concentration with age from adult to old-age individuals. Age was measured at time of fecal sample collection.

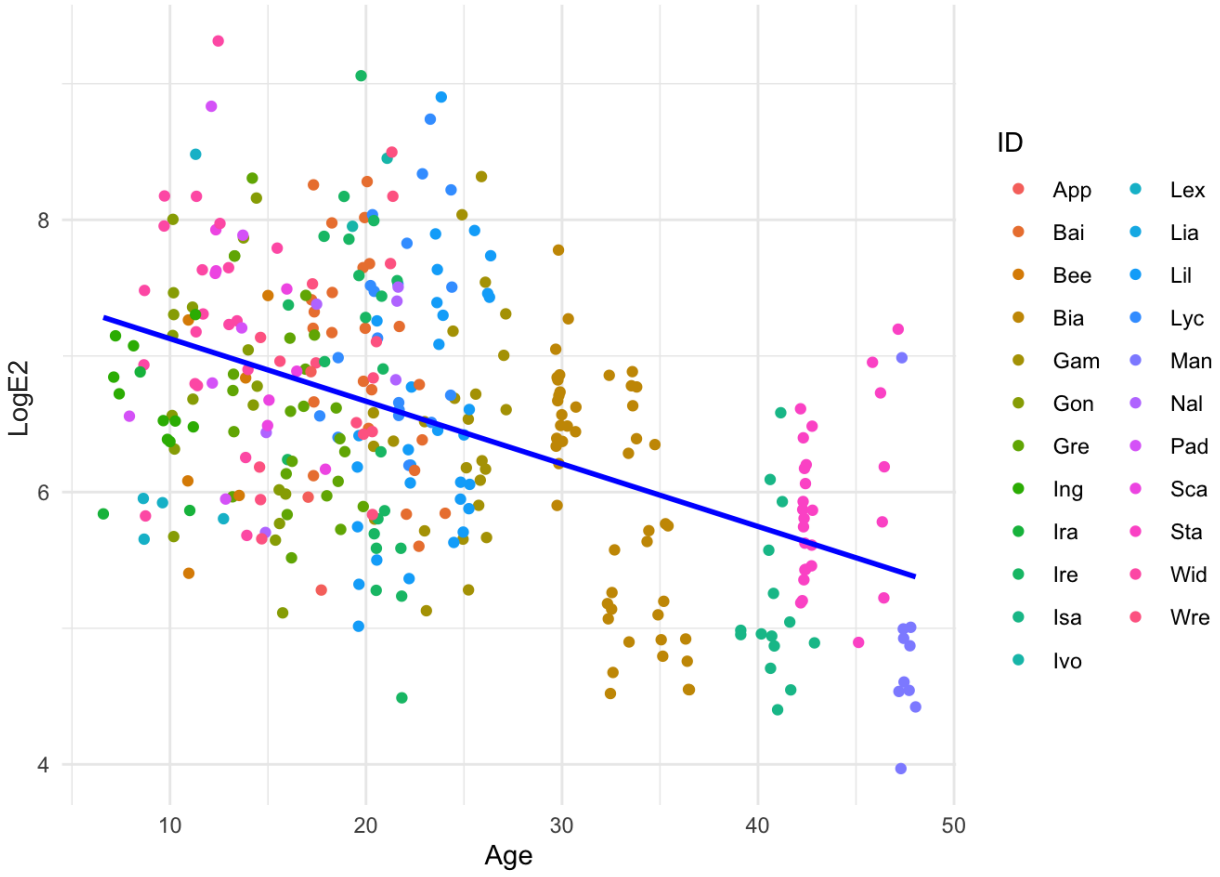
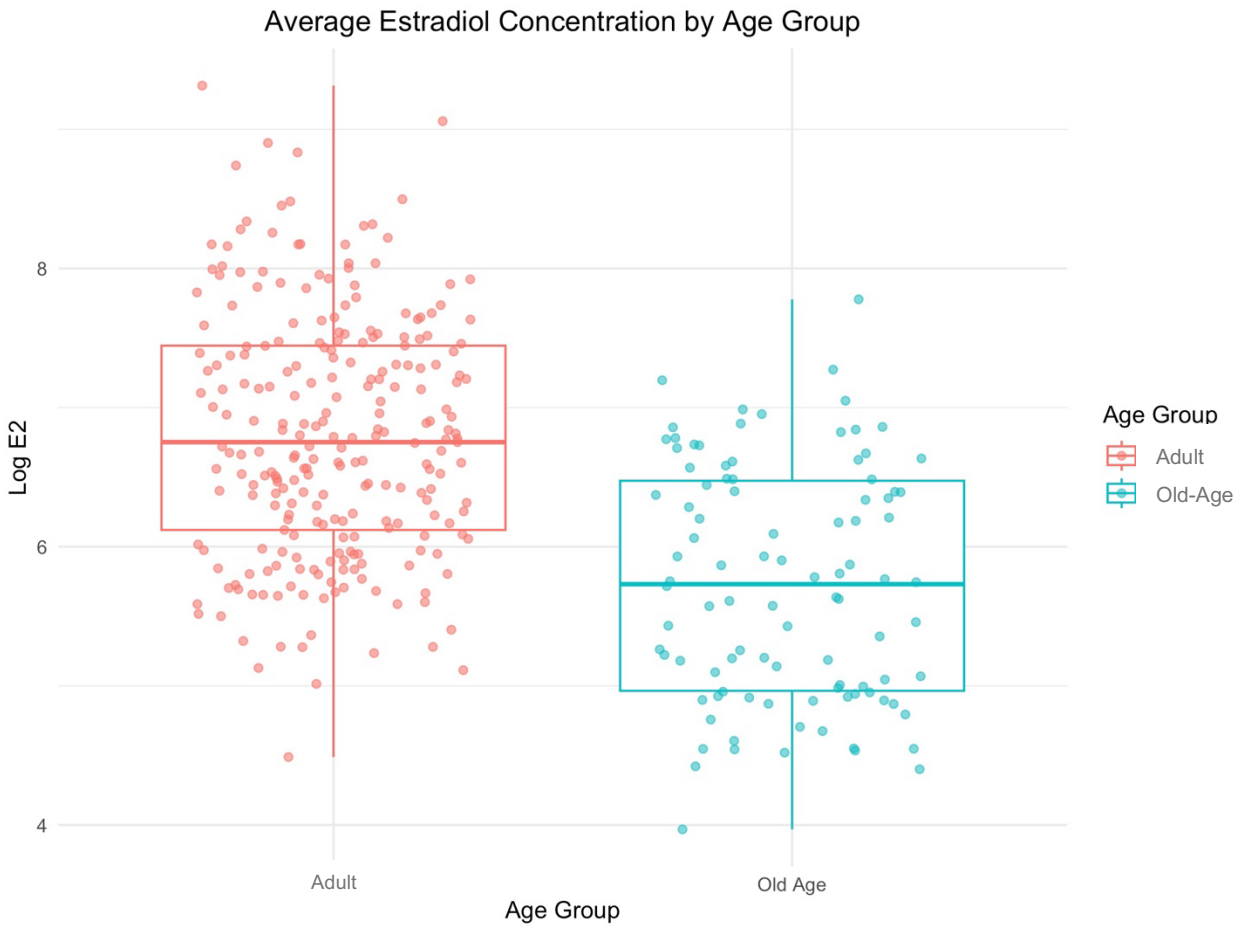


Figure 5.

Difference in mean estradiol (E2) concentration between adult (<30 years old) and old-age individuals (>30 years old).



Effects of an Ovariectomy on Estradiol

I found that estradiol concentrations in samples collected after Irene's ovariectomy were significantly lower that before her ovariectomy (mean pre-ovariectomy = 1763.36 ng/g, mean post- ovariectomy = 181.28 ng/g). Her pre-ovariectomy samples differed significantly from the post-ovariectomy samples (β = 1.89, SE = 0.63, $p < 0.001$; Figure 6). However, none of the old-age females' mean estradiol concentration values differed from the Irene post-ovariectomy samples (see Table 4).

Table 4.

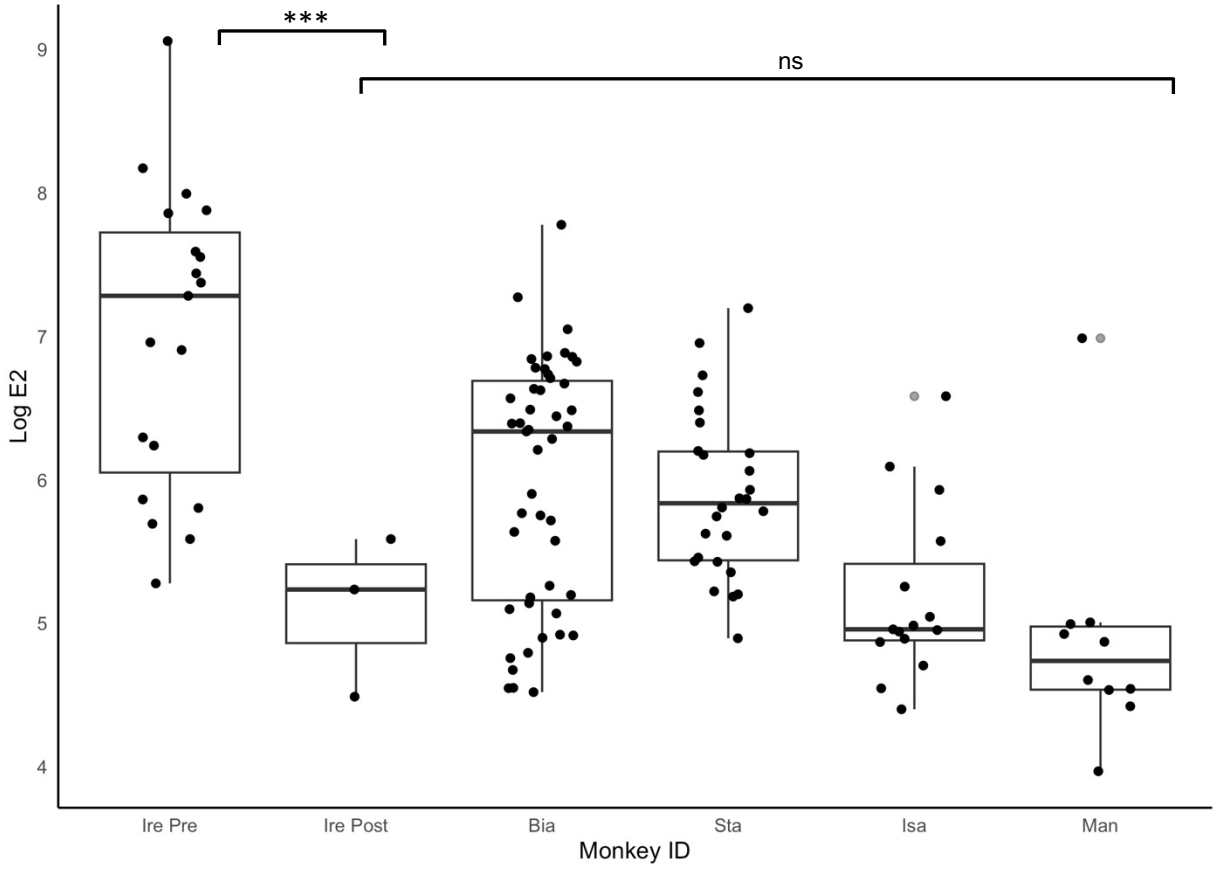
Relationship between Estradiol Concentrations Post-Ovariectomy vs. in Old Age

Predictors	B	SE	CI	*p*-value
(Intercept)	5.10	0.47	4.18 - 6.03	**< 0.001**
Bias	0.89	0.48	-0.07 – 1.84	0.068
Irene (Pre)	1.89	0.50	0.90 – 2.88	**< 0.001**
Isabelle	0.08	0.51	-0.93 – 1.09	0.878
Mango	-0.22	0.53	-1.27 – 0.83	0.682
Star	0.80	0.49	-0.18 – 1.77	0.108

Fecal estradiol concentrations were compared between the Irene's pre- and post-ovariectomy samples and the old-age individuals. Irene's post-ovariectomy samples were used as the reference level and the label "Irene" refers to the pre-ovariectomy samples. Results show the pre-ovariectomy samples have a higher concentration of fecal estradiol compared to post-ovariectomy samples and that there is no significant difference between post-ovariectomy samples and old-age individuals.

Figure 6.

*Comparison between estradiol (E2) concentrations in Irene pre- and post-ovariectomy samples and old-age individuals (Bias, Star, Isabelle, and Mango). Box plots show median and quartiles, whiskers show the 95% CI, and circles indicate individual samples. Significant differences between concentrations denoted as *p < 0.05, **p < 0.01, ***p < 0.001.*



Effect of Age on Estrus Behaviors

For rates of estrus behaviors, I determined that the model which contained age was a significantly better fit than the null model (age model AIC = 312.64, null model AIC = 324.09, $p < 0.001$). I found that as individuals get older the rate of estrus behaviors declines and age was a significant predictor of this decrease in behavioral estrus ($\beta = -0.059$, SE = 0.02, $p < 0.001$; table 5 and figure 7). When focusing on 2023 – 2024, there is variation in the level of consistent estrus behavior cycles per individual. However, there was no reported estrus behavior for any old females in this timeframe and no behavioral estrus for Irene following her ovariectomy in October 2023 (see figure 8).

Table 3.

Effects of Age on Occurrence of Behavioral Estrus

Predictors	B	SE	*z*-value	*p*-value
(Intercept)	0.89	0.33	2.65	**0.008**
Age	- 0.059	0.018	- 3.34	**0.001**

Rates of the occurrence of behavioral estrus were compared between the adult and old-age individual groups. Results show a decrease in behavioral estrus with age.
Full versus null model $\chi 2$ (df = 1) = 13.45, $p < 0.001$.

Figure 7.

*Rates of estrus behavior cycles per month across all individuals. Box plots show median and quartiles. Significant differences in the occurrence of behavioral estrus per month between the adult age group and the old-age group denoted as *p < 0.05, **p < 0.01, ***p < 0.001.*

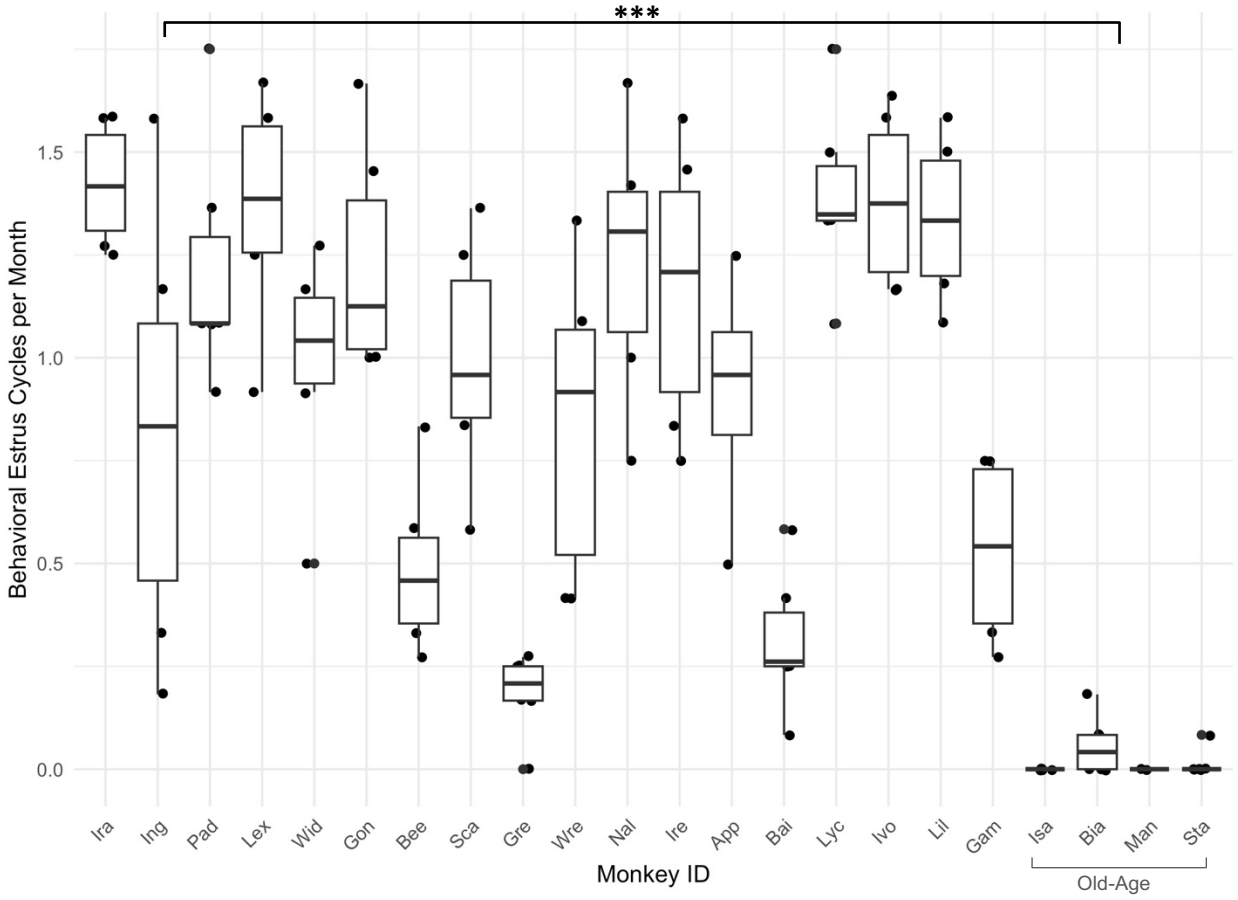
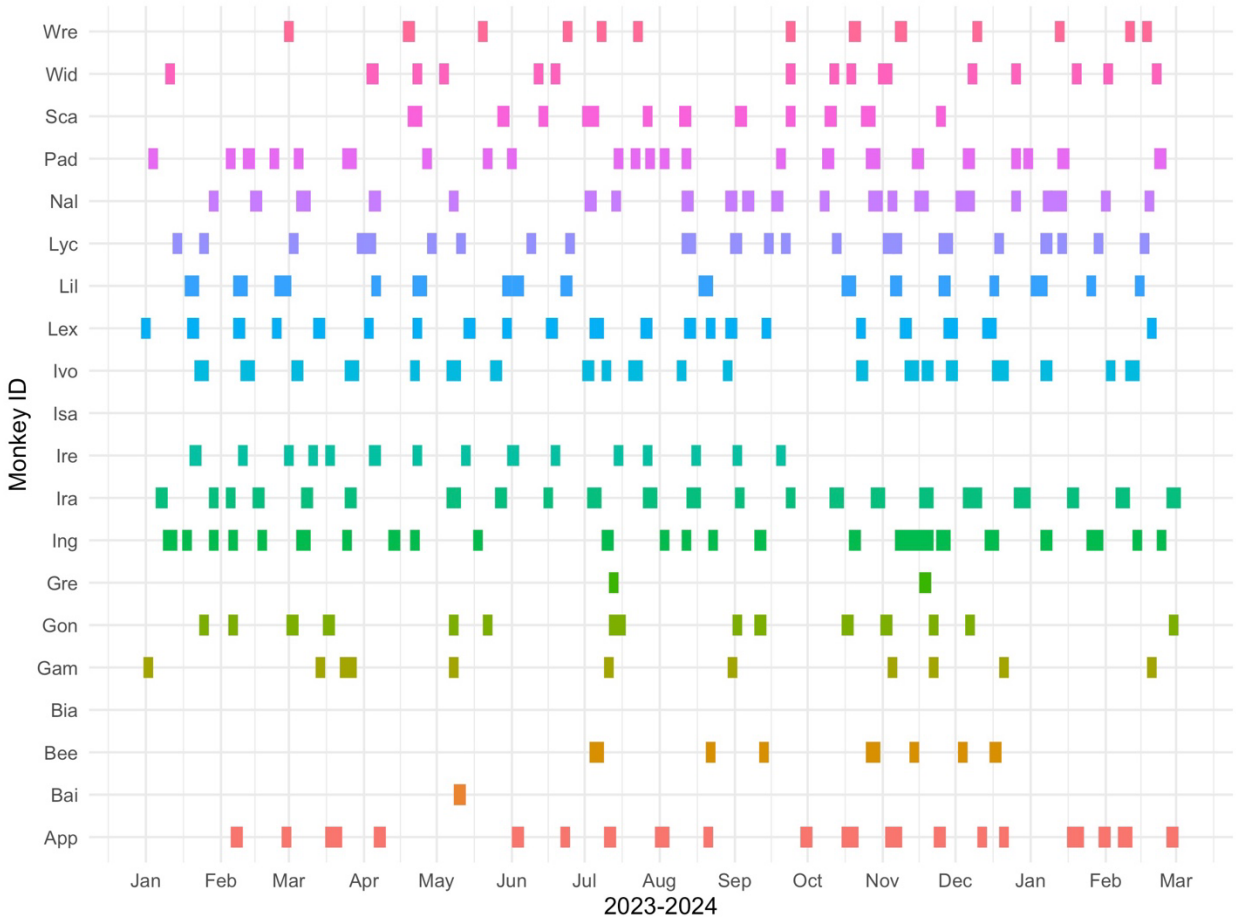


Figure 8.

Instances of behavioral estrus per individual each month in 2023 – 2024. Excluding deceased individuals – Mango and Star.



Relationship between Estradiol and Estrus

I found no relationship between the concentration of estradiol and the onset of estrus behaviors (table 6). A bout of estrus was denoted as day 0; samples were categorized as pre- or post-estrus for each week up to 3 weeks before or after the closest recorded estrus bout. This data was then pooled for individuals which had at least 10 samples near a bout of estrus during the study period. Even with this large range of up to 3 weeks prior and 3 weeks post recorded behavioral estrus dates, I found no correlation with estradiol concentration. Certain individuals seem to show estrus behaviors near a peak of estradiol (see Lily and Lychee, figure 9); however, for most individuals this is not the case.

Table 6.

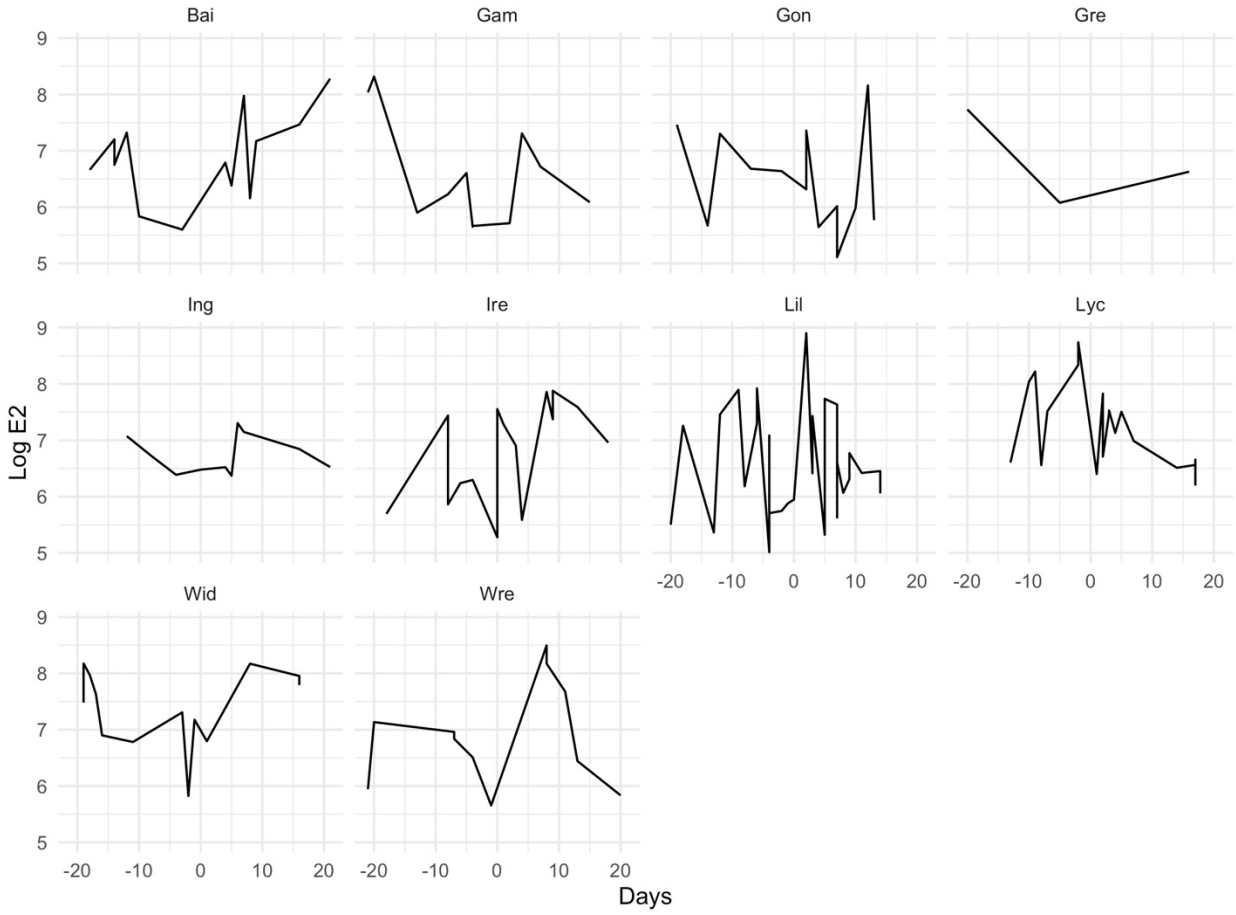
Effect of fecal estradiol (E2) concentration on the onset of behavioral estrus

Predictors	B	SE	*t*-value	*p*-value
(Intercept)	6.79	0.164	41.4	$<2e^{-16}$ ***
1 Week Pre-Estrus	-0.216	0.215	-1.00	0.317
2 Weeks Pre-Estrus	-0.0138	0.231	-0.060	0.953
3 Weeks Pre-Estrus	0.376	0.266	1.41	0.160
1 Week Post-Estrus	-0.0713	0.312	-0.228	0.820
2 Weeks Post-Estrus	0.285	0.235	1.21	0.228
3 Weeks Post-Estrus	0.0287	0.274	0.104	0.917

The occurrence of behavioral estrus was examined in relation to the concentration of estradiol in individuals. The week pre- or post-estrus is presented as a pool of all samples in each pre- or post-estrus category per individual. Results show no relationship between estradiol concentration and onset of behavioral estrus.
Significance at *0.05, **0.01, ***0.001.

Figure 9.

No relationship between peak 17β-estradiol (E2) concentration and the onset of behavioral estrus (measured as day 0) up to 3 weeks pre- and post-recorded estrus bouts. Models below show pooled data from several estrus cycles and the mean fecal estradiol concentration which correspond with the days prior to or following a recorded bout of estrus.



DISCUSSION

I found that old age is associated with a significant decrease in the production of estradiol in tufted capuchin females. In the oldest females, mean estradiol concentrations were significantly decreased from the mean concentration of normally cycling individuals, suggesting a shift into a menopausal state with reduced ovarian function. However, estradiol concentrations in old-age females did not drop to near-zero as in humans (Santoro and Randolph, 2011). This suggests that there may be some interference at the level of the estradiol assay or that tufted female capuchins have higher-than-expected levels of estradiol produced in non-ovarian tissues. It is likely that adipose tissue may be contributing to the production of estradiol in these individuals (Nelson and Bulun, 2001). However, I do not suspect that this is an effect of captivity because the average weight of this population resembles that of wild capuchins. Regardless, there is evidence of a decrease in estradiol production which seems indicative of a distinct age-related transition into a post-reproductive state.

Through biological validation with Irene, I found evidence of surgical menopause in tufted capuchins. Following the removal of Irene's ovaries, her measured concentrations of fecal estradiol declined to that of the old age individuals and did not drop completely to zero. Furthermore, the fact that estradiol values for the older females were not significantly different from Irene's post ovariectomy samples provides strong evidence for the cessation of cycling in the older individuals due to declining of ovarian function.

In terms of behavioral estrus, I found that rates of estrus behavior declined with age, providing further evidence of a shift into a post-reproductive state in the older individuals. This is bolstered by Irene's immediate change from consistent monthly estrus cycles to none

following the removal of her ovaries in October of 2023. In the older individuals and Irene post-ovariectomy, the comparative lack of estradiol may play a role in their reduced behavioral estrus bout. Taken together, the decline in estradiol and lack of reported estrus behaviors in the old-age individuals suggest reproductive cessation is occurring in these individuals.

In addition to a decrease in behavioral estrus with age, I found that the occurrence of behavioral estrus varies considerably between normally cycling individuals. One hypothesis for this is that an individual's rank may be affecting the frequency of their estrus bouts. Several subordinate females (e.g. Bailey and Gretel) showed minimal instances of behavioral estrus between 2023 and 2024 (figure 8). This may suggest that there is some form of suppression of estrus in these females; however, the mechanism of this, whether social or hormonal, is unknown. Additionally, tufted capuchins have a large suite of estrus behaviors and which behaviors are displayed could differ on the individual level. If this was the case, it may be likely that individuals displaying the less exaggerated estrus behaviors (e.g. mutual gaze) may not be recorded as being in estrus. In the case of a rank effect, it may be that subordinate females only display estrus behaviors to males when not in view of dominant females which could lead to an under reporting of estrus bouts from the subordinate individuals.

The lack of consistency I found in the relationship between estradiol and estrus behaviors in normal cycling adults may suggest that estrus behavior is not linked to ovulation in tufted capuchin monkeys. This may provide evidence of potential deceptive estrus behaviors occurring in these individuals. However, with the understanding that there is a delay in detection for fecal estradiol, more intense sample collection is required to explore this. Given that certain individuals display behavioral estrus in concordance with peak estradiol levels and

that the older individuals displayed low-to-no instances of estrus behaviors, it seems clear that estradiol is necessary but not sufficient to induce behavioral estrus in tufted capuchins. Additional research is required to determine the specific function of estradiol in the onset of estrus behaviors and which specific behaviors are correlated with high estradiol levels. Furthermore, determining which behaviors, if any, are most correlated with the periovulatory phase could aid in narrowing down the species-specific adaptive function of behavioral estrus in tufted capuchins and shed light on the presentation of deceptive estrus.

My study is limited by its exclusive use of estradiol as a measure of menopause in tufted capuchins. This provides strong evidence for menopause but does not cover the full range of ovarian hormones which may be at play in individuals undergoing menopause. Additionally, I was unable to collect samples from all individuals daily for estradiol analysis. Although my sample size was robust and I had strong longitudinal data, I had only one individual in my captive population who was between the ages of 30 and 40 which limits the complete interpretation of the menopausal transition in tufted capuchin females. Future research should examine estradiol in tandem with progestins and other ovarian hormones to outline the full hormonal profile of menopause in tufted capuchins.

This study also uses preliminary behavioral data which does not include more robust measures of instances of behavioral estrus. As a result, monkeys which only displayed estrus behaviors following morning checks may not have had those dates and counts included in this dataset or rate analysis. In future analyses, I plan to utilize the behaviors data from the focal observations to better examine the relationship between estradiol and estrus behaviors in this species. The specific behaviors recorded in the focal observations can provide additional

information about which behaviors may be correlated with estradiol concentrations and open exploration of deceptive estrus research. In looking at the relationship between estradiol concentration and behavioral estrus, I was limited by a lack of samples that were associated with recorded estrus bouts which reduced my sample size for this measure. As a result, I was unable to run a model looking at specific days prior to or following estrus and instead separated samples by weeks leading up to or following estrus bouts. A necessary next step would be to collect daily samples with consistent matched observation to delve deeper into the relationship between estradiol and behavioral estrus.

Given that this study was conducted with captive tufted capuchin females, there may be an influence on the transition into menopause and a post-reproductive lifespan that is a result of captivity factors. One of the hypotheses for why menopause is seen in non-human animals is an increase in survival rate which lengthens the lifespan and leads to a post-reproductive lifespan (Weiss, 1981 as cited in Takahashi, 2017). In this study, I can say there is evidence for menopause in individuals over the age of 30 in a captive population of tufted capuchins, and these individuals may have lengthy post-menopausal lifespans. Captive individuals tend to have longer lifespans than wild individuals due to extensive veterinary care, provisioning, and a lack of natural predators. Therefore, given that my sample was exclusively captive individuals, I am not able to determine whether this transition into menopause is a result of captivity or is identifying an evolutionarily adaptive post-reproductive lifespan in tufted capuchin monkeys.

The discrepancy between the current known captive and wild lifespans of capuchins is notable; however, the longest running capuchin research sites were only established around 30 years ago (e.g. Lomas Barbudal Monkey Project, est. 1990). This means that the current age

data for capuchins in the wild is often an estimation and it may take decades to definitively determine the true maximum lifespan of capuchins. Additionally, the oldest individuals in this study were acquired from other research sites and, as such, their reported birth dates may underestimate their actual age.

As longitudinal research sites continue to collect data on wild capuchins, more evidence for the potential of menopause and a post-reproductive lifespan may emerge in wild tufted capuchins. If seen in the wild, menopause may serve an adaptive function to increase the survival of their social group supporting the Grandmother hypothesis and recent research in killer whales (Williams, 1957; Nattrass et al., 2019). The evolution of menopause in capuchins may also be a result of selection against intergenerational harm from within-group competition due to reproduction (Cant and Johnstone, 2008). Capuchins are highly social and live in large, multimale multifemale groups which would have the multigenerational overlap that is hypothesized to be a factor in the evolution of menopause as a mechanism to separate reproduction between generations. Further research is necessary to investigate the adaptive function of menopause in tufted capuchin monkeys and distinguish the effects of captivity on lengthening lifespans from potential ultimate evolutionary causes of a post-reproductive period in capuchins.

This study is the first, to my knowledge, that utilizes tufted capuchin monkeys as a model for examining the hormonal profile of menopausal individuals and the transition into menopause in New World primates. In addition, I validated and employed a human estradiol assay as an effective measurement of the menopausal transition in tufted capuchin females. My work has an expansive sample size of 22 individuals compared to previous studies using

matched behavioral and hormonal data which had sample populations of fewer than 10 females (e.g. Nagle et al., 1979; Carosi et al., 1999). Unlike previous studies using progesterone, I did not find a strong correlation between high concentrations of fecal estradiol and the onset of estrus suggesting a decoupling of behavioral estrus from ovulation in capuchin monkeys and the potential for deceptive estrus. The results of my research suggest the occurrence of menopause in captive tufted capuchins over the age of 30 and have implications for the capacity to use estradiol to explore menopause in other captively housed, long-lived primates.

References

Altmann, J. (1974). Observational study of behavior: sampling methods. *Behaviour*, *49*(3-4), 227-266.

Association of Official Analytical Chemists (AOAC) (2012). Official Methods of Analysis. Appendix F: Guidelines for Standard Method Performance Requirements. *AOAC International*, 9.

Bates, D., Maechler, M., Bolker, B., & Walker, S. (2015). Fitting linear mixed- effects models using lme4. Journal of Statistical Software, 67(1), 1–48.

Beach, F. A. (1976). Sexual attractivity, proceptivity, and receptivity in female mammals. *Hormones and Behavior*, *7*(1), 105–138. https://doi.org/10.1016/0018-506X(76)90008-8

Cant, M. A. & Johnstone R. A. (2008). Reproductive conflict and the separation of reproductive generations in humans. *Proc. Natl Acad. Sci. USA* **105**, 5332–5336.

Caro, T.M., Sellen, D.W., Parish, A. et al. (1995). Termination of reproduction in nonhuman and human female primates. Int J Primatol 16, 205–220 (1995). https://doi.org/10.1007/BF02735478

Carosi, M., & Visalberghi, E. (2002). Analysis of tufted capuchin (Cebus apella) courtship and sexual behavior repertoire: changes throughout the female cycle and female interindividual differences. American Journal of Physical Anthropology: The Official Publication of the American Association of Physical Anthropologists, 118(1), 11-24.

Carosi, M., Heistermann, M., & Visalberghi, E. (1999). Display of Proceptive Behaviors in Relation to Urinary and Fecal Progestin Levels over the Ovarian Cycle in Female Tufted

Capuchin Monkeys. *Hormones and Behavior*, *36*(3), 252–265. https://doi.org/10.1006/hbeh.1999.1545

Carosi, M., Linn, G. S., & Visalberghi, E. (2005). The Sexual Behavior and Breeding System of Tufted Capuchin Monkeys (Cebus apella). In *Advances in the Study of Behavior* (Vol. 35, pp. 105–149). Elsevier. https://doi.org/10.1016/S0065-3454(05)35003-0

Fragaszy, D. M., & Adams-Curtis, L. E. (1998). Growth and reproduction in captive tufted capuchins (Cebus apella). *American Journal of Primatology*, *44*(3), 197–203. https://doi.org/10.1002/(SICI)1098-2345(1998)44:3<197::AID-AJP2>3.0.CO;2-R

Fragaszy, D. M., Visalberghi, E., & Fedigan, L. M. (2004). *The complete capuchin: the biology of the genus Cebus*. Cambridge University Press.

Hamlett, G. W. D. (1939). Reproduction in American monkeys. I. Estrous cycle, ovulation, and menstruation in cebus. *The Anatomical Record*, *73*(2), 171–187. https://doi.org/10.1002/ar.1090730205

Hakeem A, Sandoval RG, Jones M, Allman J. Brain And Life Span In Primates. In: Birren JE, Schaie KW, editors. *Handbook of the Psychology of Aging.* 4th edition. San Diego: Academic Press; 1996. pp. 78–104.

Hawkes K, O'Connell JF, Jones NG, Alvarez H, Charnov EL. (1998). Grandmothering, menopause, and the evolution of human life histories. Proc Natl Acad Sci U S A. 1998 Feb 3;95(3):1336-9. doi: 10.1073/pnas.95.3.1336. PMID: 9448332; PMCID: PMC18762.

Lahoz, M. M., Nagle, C. A., Porta, M., Farinati, Z., & Manzur, T. D. (2007). Cortisol response and ovarian hormones in juvenile and cycling femaleCebus monkeys: Effect of stress and

dexamethasone. *American Journal of Primatology*, *69*(5), 551–561. https://doi.org/10.1002/ajp.20375

Lima, M. C. M., Scalercio, S. R. R. A., Lopes, C. T. A., Martins, N. D., Oliveira, K. G., Caldas-Bussiere, M. C., Santos, R. R., & Domingues, S. F. S. (2019). Monitoring sexual steroids and cortisol at different stages of the ovarian cycle from two capuchin monkey species: Use of non- or less invasive methods than blood sampling. *Heliyon*, *5*(7), e02166. https://doi.org/10.1016/j.heliyon.2019.e02166

Linn, G. S., Mase, D., Lafrancois, D., O'Keeffe, R. T., & Lifshitz, K. (1995). Social and menstrual cycle phase influences on the behavior of group-housed *Cebus apella*. *American Journal of Primatology*, *35*(1), 41–57. https://doi.org/10.1002/ajp.1350350105

Mayor, P., Pereira, W., Nacher, V., Navarro, M., Monteiro, F. O., El Bizri, H. R., & Carretero, A. (2019). Menstrual cycle in four New World primates: Poeppig's woolly monkey (Lagothrix poeppigii), red uakari (Cacajao calvus), large-headed capuchin (Sapajus macrocephalus) and nocturnal monkey (Aotus nancymaae). *Theriogenology*, *123*, 11-21.

Nadler, R. D., Graham, C. E., Gosselin, R. E., & Collins, D. C. (1985). Serum levels of gonadotropins and gonadal steroids, including testosterone, during the menstrual cycle of the chimpanzee (Pan troglodytes). *American Journal of Primatology*, *9*(4), 273-284.

Nagle, C. A., Denari, J. H., Quiroga, S., Riarte, A., Merlo, A., Germino, N. I., Gómez-argaña, F., & Rosner, J. M. (1979). The Plasma Pattern of Ovarian Steroids during the Menstrual Cycle in Capuchin Monkeys (Cebus apella). *Biology of Reproduction*, *21*(4), 979–983. https://doi.org/10.1095/biolreprod21.4.979

Nagle, C. A., Riarte, A., Quiroga, S., Azorero, R. M., Carril, M., Horacio Denari, J., & Rosner, J. M. (1980). Temporal Relationship Between Follicular Development, Ovulation, and Ovarian Hormonal Profile in the Capuchin Monkey (Cebus apella). *Biology of Reproduction*, *23*(3), 629–635. https://doi.org/10.1095/biolreprod23.3.629

Nattrass, S., Croft, D. P., Ellis, S., Cant, M. A., Weiss, M. N., Wright, B. M., Stredulinsky, E., Doniol-Valcroze, T., Ford, J. K. B., Balcomb, K. C., & Franks, D. W. (2019). Postreproductive killer whale grandmothers improve the survival of their grandoffspring. *Proceedings of the National Academy of Sciences*, *116*(52), 26669–26673. https://doi.org/10.1073/pnas.1903844116

Nelson, L. R., & Bulun, S. E. (2001). Estrogen production and action. *Journal of the American Academy of Dermatology*, *45*(3 Suppl), S116–S124. https://doi.org/10.1067/mjd.2001.117432

Ortiz, R. E., Ortiz, A. C., Gajardo, G., Zepeda, A. J., Parraguez, V. H., Ortiz, M. E., & Croxatto, H. B. (2005). Cytologic, hormonal, and ultrasonographic correlates of the menstrual cycle of the New World monkey Cebus apella. *American Journal of Primatology*, *66*(3), 233–244. https://doi.org/10.1002/ajp.20141

Phillips, K. A., Bernstein, I. S., Dettmer, E. L., Devermann, H., & Powers, M. (1994). Sexual behavior in brown capuchins (Cebus apella). *International Journal of Primatology*, *15*, 907-917.

R Development Core Team. (2015) . R: A Language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing. Retrieved from https://R-project.org.

Rodriguez, M., & Shoupe, D. (2015). Surgical menopause. *Endocrinology and metabolism clinics*, *44*(3), 531-542.

Santoro, N., & Randolph, J. F. (2011). Reproductive Hormones and the Menopause Transition. *Obstetrics and Gynecology Clinics of North America*, *38*(3), 455–466. https://doi.org/10.1016/j.ogc.2011.05.004

Schino, G., & Pinzaglia, M. (2018). Age-related changes in the social behavior of tufted capuchin monkeys. *American Journal of Primatology*, *80*(3), e22746.

Shimizu, K. (2005). Studies on Reproductive Endocrinology in Non-human Primates: Application of Non-invasive Methods. *Journal of Reproduction and Development*, *51*(1), 1–13. https://doi.org/10.1262/jrd.51.1

Talaulikar, V. (2022). Menopause transition: Physiology and symptoms. *Best Practice & Research Clinical Obstetrics & Gynaecology*, *81*, 3–7. https://doi.org/10.1016/j.bpobgyn.2022.03.003

Takahashi, M., Singh, R. S., & Stone, J. (2017). A theory for the origin of human menopause. *Frontiers in Genetics*, *7*, 178732.

Weinbauer, G. F., Niehoff, M., Niehaus, M., Srivastav, S., Fuchs, A., Van Esch, E., & Cline, J. M. (2008). Physiology and Endocrinology of the Ovarian Cycle in Macaques. *Toxicologic Pathology*, *36*(7_suppl), 7S-23S. https://doi.org/10.1177/0192623308327412

Welker, C., Becker, P., Höhmann, H., & Schäfer-Witt, C. (1990). Social Relations in Groups of the Black-Capped Capuchin (Cebus apella) in Captivity Interactions of Group-Born Infants during Their Second Half-Year of Life. Folia Primatologica, 54(1-2), 16-33.

Williams, G. C. (1957). Pleiotropy, Natural Selection, and the Evolution of Senescence: Evolution 11, 398-411 (1957). *Science of Aging Knowledge Environment*, *2001*(1).

Wood, B. M., Negrey, J. D., Brown, J. L., Deschner, T., Thompson, M. E., Gunter, S., Mitani, J. C., Watts, D. P., & Langergraber, K. E. (2023). Demographic and hormonal evidence for menopause in wild chimpanzees. *Science*, *382*(6669), eadd5473. https://doi.org/10.1126/science.add5473