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Physiological and behavioral effects of hormonal contraceptive treatment in captive, pair-bonded primates (*Plecturocebus cupreus*)

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Hormonal contraception is an effective, reversible tool for managing birth rates in humans and nonhuman animals alike. However, manipulating reproductive hormones has behavioral consequences that can impact social and sexual behavior between conspecifics. First, we studied 18 pairs of nonreproductive titi monkeys (*Plecturocebus cupreus*) to test the efficacy of a novel method of hormonal contraception (deslorelin acetate implants) on reproductive hormone cycling in females and found significant reductions in urinary estrogens and progestagens among treated females compared to untreated controls. We then studied 35 nonreproductive pairs of coppery titi monkeys (*Plecturocebus cupreus*) to ascertain whether treating females with one of 2 different forms of hormonal contraception (deslorelin acetate implants (*n* = 17) or medroxyprogesterone acetate injections (*n* = 9)) would influence the relationship between pair mates compared to the relationship between untreated females and their vasectomized male mates (*n* = 9). Over a 5-month period, we found no differences in affiliative behaviors between pairs containing untreated females compared to pairs in which the female was treated with either deslorelin acetate or medroxyprogesterone acetate. Similarly, we found no differences in affiliation between pairs in the 2 treatment groups. This study is the first to examine behavioral consequences of hormonal contraception in a pair-bonding species. The results are encouraging for captive, managed breeding colonies of such social animals, especially those used in behavioral research.

Abbreviations and Acronyms: CNPRC, California National Primate Research Center; Cr, creatinine; E1C, estrone conjugate; FSH, follicle stimulating hormone; GnRH, gonadotropin-releasing hormone; HPG, hypothalamus-pituitary-gonadal; IM, intramuscular; LH, luteinizing hormone; PdG, pregnanediol-3alpha-glucuronide

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Introduction

Reproduction is critical for the continuation of a species. In animal populations, contraception is used to manage the size, ecological impact, and genetic make-up of populations. In the wild, implementing contraception is an ethical way to manage populations that reduces the need for methods like culling to control overpopulation and mitigate food scarcity, and/or to decrease human/wildlife conflict.[12] In captive populations, controlling reproduction is important to allow facilities to financially and physically care for all offspring and manage population-level genetic diversity.[31] Hormonal contraceptives are an effective method for controlling reproduction because they are both long acting and reversible, while physiologic alterations to reproductive anatomy (for example, spaying, neutering, vasectomy, tubal ligation, or hysterectomy) are highly effective, but not usually reversible.[31] However, the use of hormonal contraceptives may alter behavior. In social species, particularly those that form and maintain attachment relationships to their mating partners, the behavioral side effects of hormonal contraceptive use is an important consideration.

Deslorelin is a synthetic GnRH analog that is available as a subcutaneous implant. It functions as a GnRH agonist to inhibit conception in females but also reduces testosterone levels in males, leading to its use in managing male aggression in captive primates (for example, *Papio ursinus*[34]). Aggression was also reduced in female Guinea baboons (*Papio papio*) that received these implants, as was overall affiliation between social group members.[29] However, in a study of Barbary macaques (*Macaca sylvanus*), use of a progestin-based contraceptive implant was associated with increased aggression and increased receipt of grooming, but less grooming of conspecifics.[21] Finally, in stump-tailed macaques (*Macaca arctoides*), females treated with Depo-Provera, a systemic progestin-based contraceptive, engaged in more subordinate behaviors (for example, fear grimaces) and antagonistic behaviors (for example, stare threats) toward males than did untreated females.[19]

Hormone levels in females may also influence a male's choice of social and sexual partners. Male stump-tailed macaques did not approach or inspect the genitals of treated females as often as they did for untreated females and they copulated less often with treated females.[19] For some species, the mechanism for distinguishing treated and untreated females may be olfactory. Male ring-tailed lemurs (*Lemur catta*) preferred the scent of untreated females over the scent of females that were treated with Depo-Provera.[8] In other species, visual cues may help males distinguish treated and untreated females. Among hamadryas baboons (*Papio hamadryas*), a species that displays overt visual signs of ovulation, males were less likely to mate with Depo-Provera treated females compared with untreated females.[13] Taken together, these results suggest that hormonal

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contraceptive treatment can affect both attraction and social behavior of male and female nonhuman primates, possibly through hormonal pathways and sensory cues.

While some publications[8,13,19] address the impact of contraceptive use on social behavior of nonhuman primates, the species studied to date do not employ monogamous mating strategies and display overt visual signs of ovulation (for example, size and/or color changes of genital region during ovulation). The effects of contraceptives should also be studied in species with other mating strategies in order to better understand how different social systems may be affected by hormonal contraceptive treatment. One social system of particular interest in this regard is social monogamy. Coppery titi monkeys (*Plecturocebus cupreus*) are small South American primates that form deep and lasting attachments to their pair mates.[22] They are socially monogamous[11] and show a consistent preference for their mate, especially in the presence of a stranger.[5] Pair mates groom one another, stay near each other, and twine their tails together—all of which are regarded as signs of affiliation used to maintain the pair-bond and indicate a close, preferential relationship.[10,22,24] While new pairs exhibit low affiliation immediately upon pairing,[15] levels of affiliation rise during the first week and remain consistently high.[28] Based on our estimates, approximately 250 coppery titi monkeys are now being maintained in captivity in 35 facilities (zoos, laboratories, wildlife parks) in the United States and Europe. While some species of titi monkeys are classified as Critically Endangered (for example, *P. caquetensis*, *P. oenanthe*) by the International Union for Conservation of Nature,[35] coppery titi monkeys are currently categorized as of Least Concern. Due to their close social relationships and monogamous mating, titi monkeys are ideal for the study of how hormonal contraception affects social behavior between bonded reproductive partners.

The titi monkey reproductive cycle is like that of humans: estrogen and progesterone fluctuate based on the day and phase. During the follicular phase, estrogen increases and peaks at ovulation, while progesterone peaks in the luteal phase.[30] The average titi ovulatory cycle is approximately 17 d and can be identified from urinary assays of estrone conjugate (E1C) and pregnanediol-3α-glucuronide (PdG).[30] As in humans, the female reproductive cycle of the titi monkey is regulated through the hypothalamic-pituitary-gonadal (HPG) axis (Figure 1A). The hypothalamus produces gonadotropin-releasing hormone (GnRH), which stimulates the anterior pituitary gland to release gonadotropins (follicle stimulating hormone (FSH) and luteinizing hormone (LH)). FSH and LH then stimulate the ovaries to produce estrogens and progestagens. Together, the hormones and gonadotropins drive the phases of the reproductive cycle.

Captive titi monkey reproduction can be managed using hormonal contraception, but no studies to date have examined the physiologic and behavioral consequences of its use. Current knowledge is limited to the endocrinology of the titi reproductive cycle.[7,30] Understanding how titi monkey behavior is affected by hormonal contraceptive use is important both for managing their care in captive populations and comparing our findings to human behavior in the context of monogamous relationships.

In the current study, we investigated the physiologic and affiliative behavioral consequences of hormonal contraceptive treatment on female titi monkeys and their pair relationships. Our colony's reversible contraceptive method was historically medroxyprogesterone acetate injections, but in 2019 we transitioned to deslorelin acetate implants. To validate this new method in coppery titi monkeys, we analyzed urine from implant-treated and untreated females to confirm that the implant altered the female reproductive cycle. Given the aim and efficacy of contraceptive implants in other species, we hypothesized that treated females would have consistently lower levels of urinary estrogens and progestagens compared with untreated females. We also examined how treatment with hormonal contraception would affect the relationship between pair mates and whether the 2 methods used in our colony differed in their effects. To do this, we examined rates of affiliation between pairs in which the female received a contraceptive implant, a contraceptive injection, or no treatment. Given evidence from other species whose social behavior

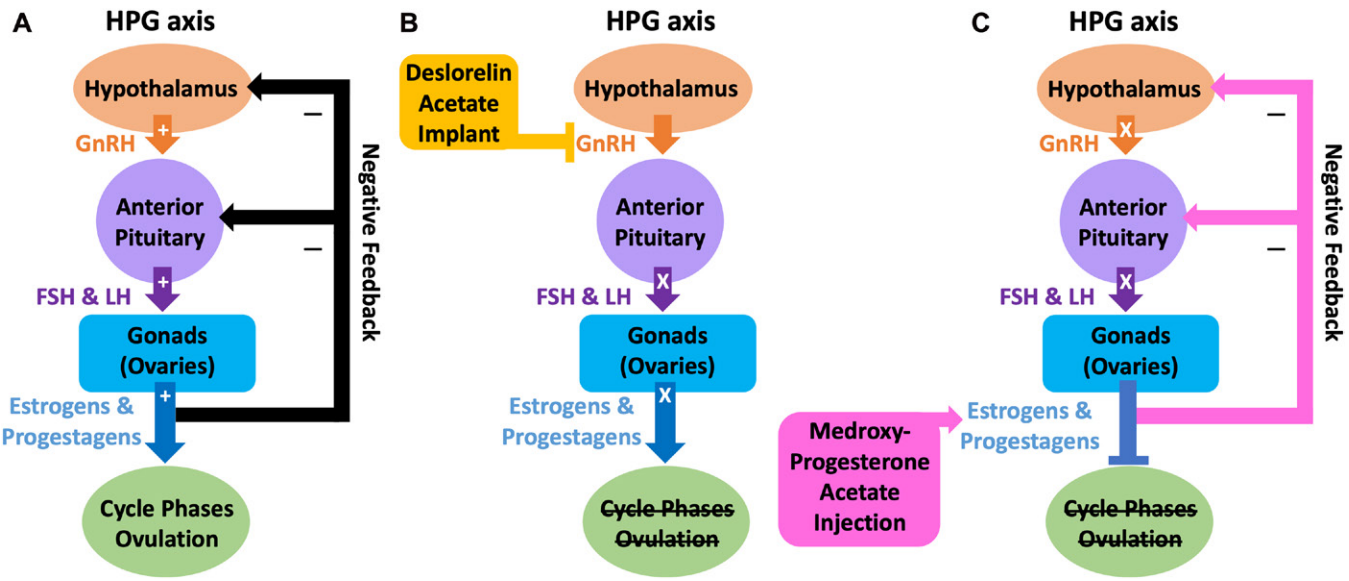

Figure 1. Physiologic mechanisms that underlie the ovulatory cycle and hormonal contraception. (A) The HPG axis as it typically functions. (B) The HPG axis as it functions with the addition of a deslorelin acetate implant, which results in the cessation of cycling through GnRH agonism and the subsequent inhibition of HPG function. (C) The HPG axis as it functions with the addition of a medroxyprogesterone acetate injection, which results in the cessation of cycling through the addition of exogenous progesterone, which in turn triggers the negative feedback loop and inhibits HPG function.

was examined after treatment with hormonal contraceptives, we hypothesized that pairs with treated females would have lower average rates of affiliation compared with pairs with an untreated female.

Methods

Subjects. All subjects were coppery titi monkeys born and housed at the California National Primate Research Center (CNPRC). The physiological effects of deslorelin were assessed in 18 nonreproducing female titi monkeys. Half of the females (n = 9) received hormonal contraceptive implants, while the other half were not treated, but were paired with vasectomized males.

Another set of nonreproductive pairs (n = 35) were used to compare the effects of deslorelin and medroxyprogesterone acetate treatment on affiliation between pair mates. This study had 3 groups: 1) control pairs (n = 9) in which the female was untreated, and the male was vasectomized; 2) deslorelin pairs (n = 17) in which the female received a deslorelin acetate implant and the male was intact; and 3) medroxyprogesterone acetate pairs (n = 9) in which the female received medroxyprogesterone acetate injections and the male was intact. The subjects in this study represent a convenience sample, as contraceptive decisions for these pairs were determined based on colony management priorities for a research colony. Contraception is used in the titi monkey colony at the CNPRC to manage the size and genetic diversity of a small colony of about 100 animals, meet specific project needs in ongoing experiments (for example, subjects must be nonreproducing pairs), and maintain the health of animals with a history of uterine abnormalities or gestation/birth-related trauma.[16]

All monkeys in this study were housed in enclosures measuring at least 1.2 × 1.2 × 0.7 m. The environment was maintained at 21 °C on a 12-h light cycle with lights on from 0600 to 1800. Titi monkeys were fed monkey chow (Lab Diet, New World Primate Diet #5040), carrots, bananas, apples, and rice cereal twice daily. Water was available ad libitum and additional edible foraging enrichment was provided twice daily. Subjects were housed in male-female pairs and did not conceive offspring during the project. This housing situation is the same as described in previous studies.[23,28] This study was approved by the Institutional Animal Care and Use Committee at the University of California, Davis, and complied with legal requirements of the United States and National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Hormonal contraception and reproduction. Females treated with deslorelin received one 4.7 mg Suprelorin F implant (Virbac AH, Fort Worth, TX) that was inserted subcutaneously with a single-use syringe using a 14-gauge needle; the implant was inserted at the midback, off-center of the midline, by a trained animal health technician. Suturing was not needed to close the skin puncture after insertion. Device placement was confirmed with manual palpation. Monkeys received 5 mg/kg of meloxicam via an intramuscular injection to manage discomfort related to device insertion. Implanted monkeys were monitored for complications and pregnancy via ultrasound every 3 mo and received new implants every 6 mo. Deslorelin acetate is a GnRH agonist (Figure 1B) that initially increases gonadotropin and steroid hormone production but then causes downregulation of GnRH release due to saturation of pituitary GnRH receptors.[20] This ultimately leads to suppression of the gonadotropins FSH and LH and reduction of ovarian-produced estrogens and progestagens necessary for reproductive cycling.[6]

Females treated with medroxyprogesterone acetate received monthly IM injections of 15 mg (Depo-Provera, Amerisource-Bergen, Conshohocken, PA). This contraceptive provides exogenous progesterone, which triggers a negative feedback loop that shuts down production of GnRH, FSH, and LH, thereby decreasing the body's production of estrogens and progestagens necessary for reproductive cycling (Figure 1C). The efficacy of these injections is reported in a variety of species.[2] This treatment suppresses reproductive hormones in a manner similar to what occurs in nonovulatory cycles.[25]

The groups of paired monkeys were not significantly different with regard to their prior numbers of live births. Pairs in which the female was untreated, and the male received a vasectomy had successfully reared the same number of offspring with their pair mate (2.3 ±,1.3) as had females treated with medroxyprogesterone acetate (1.1 ± 0.1) and females treated with deslorelin acetate (1.6 ± 0.4) ($F_{(2, 32)} = 0.70$, $P = 0.50$).

Urine collection and analysis. To confirm that the deslorelin-treated females were not cycling, we obtained 12 urine samples from each female. Samples were collected over 24 to 25 d on Mondays, Wednesdays, and Fridays during the collection period. Samples were analyzed for estrogen (E1C) and pregnanediol (PdG) concentrations. Luteal phases were defined as PdG concentrations that exceeded 100 ng/mg of creatinine in 2 consecutive samples that together exceeded 400 ng/mg of creatinine. Ovulation was assumed to have occurred if these conditions were met.[7]

Urine collection methods were similar to those described in past studies (for example, see ref. 32). Experimenters entered the animal's home cages prior to lights-on (approximately 0600) and waited for the monkey to urinate spontaneously. Urine was caught free-fall in a collection cup and taken to the laboratory, where it was aliquoted and frozen at -80 °C until assay. Subjects were habituated to urine collection procedures, which have been performed multiple times a week in the colony since 2005. Each sample represented a single urination, rather than a 24-h summed urine collection.

Assays for E1C and PdG were conducted as previously described[7,30] in the Clinical Endocrinology Laboratory at the University of California, Davis. Briefly, urine was diluted in water (E1C 1:200, PdG 1:4) so that concentrations would be within the range of the standard curves for each analyte. Samples were then added to the solid phase of plates to allow binding. Horseradish peroxidase conjugated to E1C (1: 240,000 dilution) or PdG (1:150,000 dilution) was added. Plates were then thoroughly mixed and incubated at 4 °C overnight. The next morning, plates were washed 4 times in wash solution and 100 µL of freshly prepared substrate solution (0.05 M citrate, pH 4.0, 0.4 mM ABTS, 1.6 mM H_2O_2) was added. Wells were read individually when the average optical density of the total binding wells in the plate reached an absorbance of 1.0. Intra- and interassay coefficients of variation were <17% for both E1C and PdG assays.

Behavioral data. Behavior was assessed up to 6 times daily in 2-h intervals during daytime hours (0630 to 1630) 5 d a week by using a scan sampling technique.[1] For each observation, a trained observer recorded affiliative behaviors between pair mates from an existing ethogram.[17] Our laboratory has been assessing these behaviors since 2008 to obtain a measure of intrapair affiliation.[17] Every 2 h, the observer recorded whether pairs were in physical proximity, social contact, tail twining, or none of the above. The frequency of these behaviors was recorded across 5 consecutive months (20 wk) during contraceptive treatment. For our analyses, social affiliation was assessed

as the proportion of time a pair spent in proximity, contact, or tail-twining as compared with the time that none of these behaviors occurred for all of the observations collected on that pair over a particular period. This method has been previously used to summarize longitudinal affiliation in titi monkeys using similar data.[17,33] Pairs were observed an average of 399 ± 25 times (range = 98 to 536), with variation in the number of observations per pair attributable to the research hiatus caused by the COVID-19 pandemic in 2020.

Data analysis. All statistical analyses were performed in R[27] with a significance level set at $\alpha = 0.05$. Descriptive values are presented as (mean ± SE) or as median and interquartile range.

To determine whether females treated with deslorelin acetate implants were cycling, we calculated mean hormone (E1C and PdG) values for each female, and then compared hormone levels between treatment groups. As E1C ($W = 0.71$, $P < 0.001$) and PdG ($W = 0.54$, $P < 0.001$) were not normally distributed, Kruskal-Wallis tests were used to make comparisons.

To investigate the relationship between hormonal contraceptive treatment and affiliation within pairs of titi monkeys, we compared affiliation over a 5-mo period between nonreproducing pairs in which either the female was treated with 1 of 2 hormonal contraceptives or the female was untreated, and the male was vasectomized. To calculate rates of affiliation within a pair, we condensed daily behavioral observations into weekly means of affiliation (sum of all tail twining, contact, and proximity observations/total number of observations).

To examine changes in the rate of pair mate affiliation between groups, we employed mixed-effects models using the lmer() function from the lme4 package.[3] Degrees of freedom and *P* values were estimated using the lmerTest() package,[18] which estimates significance using Satterthwaite approximations. Our model included the fixed effect of contraceptive method (levels: deslorelin acetate, medroxyprogesterone acetate, control) and a random effect of pair identity. In our study population, pair identity encompasses all variance attributable to the pair (for example, pair tenure, duration of contraceptive use, previous pairing experience, previous parenting experience, and subject age). Models that accounted for these factors individually fit the data less well and were confounded by the inclusion of the random effect. Therefore, the random factor was included in the final models, but not individual factors of pairing tenure, duration of contraceptive use, previous pairing or parenting experience, and subject age. Effect sizes were calculated using the effect-size package.[4]

Results

Physiologic effects of deslorelin. In this study, we investigated the effects of hormonal contraception on a managed captive population of titi monkeys. First, we wanted to document the physiologic effects of deslorelin acetate implants in titi monkeys as a prelude to questions about behavior. To do this, we compared reproductive hormone levels between pairs (18 pairs) in which the female was either treated with deslorelin (9 pairs) or untreated (9 pairs). Overall, the data span 24 d of reproductive cycling, which encompasses at least one full reproductive cycle in this species.[30] As expected, treated females had significantly lower levels of urinary estrogens than did untreated females (median, IQR of 201, 113 and 690, 650, respectively) ($X^2(1) = 124$, $P < 0.001$, $d = 1.35$, 95% CI [-1.65, -1.06]). Similarly, treated females had significantly lower levels of urinary progestagens than did untreated females (median, IQR of 14, 10 and 53, 145, respectively) ($X^2(1) = 81$, $P < 0.001$, $d = 0.93$, 95% CI [-1.21, -0.65]). Although all of our treated females failed to meet criteria to be considered cycling, 4 of our untreated females were also not cycling (Figure 2). Cycling females had higher levels of both E1C ($t_{(2)} = 9.30$, $P < 0.001$, $\beta = 0.62$, $\eta^2 = 0.98$, 95% CI [0.64, 1.00]) and PdG ($t_{(2)} = 10.41$, $P < 0.001$, $\beta = 0.68$, $\eta^2 = 0.98$, 95% CI [0.70, 1.00]) as compared with treated noncycling females (Figure 3). Compared with untreated noncycling females, untreated cycling females had similar levels of E1C ($t_{(2)} = 1.63$, $P = 0.11$, $\beta = 0.11$, $\eta^2 = 0.99$, 95% CI [0.85, 1.00]), but higher levels of PdG ($t_{(2)} = 7.36$, $P < 0.001$, $\beta = 0.48$, $\eta^2 = 0.96$, 95% CI [0.48, 1.00]). Among noncycling females, untreated females had higher levels of E1C ($t_{(2)} = 6.82$, $P < 0.001$, $\beta = 0.49$, $\eta^2 = 0.96$, 95% CI [0.42, 1.00]), but similar levels of PdG ($t_{(2)} = 1.45$, $P = 0.15$, $\beta = 0.10$, $\eta^2 = 0.51$, 95% CI [0.00, 1.00]) compared with treated females. Among untreated females, cycling status was not predicted by either age ($F_{(1, 7)} = 2.64$, $P = 0.15$, $\eta^2 = 0.27$, 95% CI [0.00, 1.00]), time since leaving their natal group for pairing ($F_{(1, 7)} = 2.59$, $P = 0.15$, $\eta^2 = 0.27$, 95% CI [0.00, 1.00]), or time since their current pairing ($F_{(1, 7)} = 1.57$, $P = 0.25$, $\eta^2 = 0.18$, 95% CI [0.00, 1.00]).

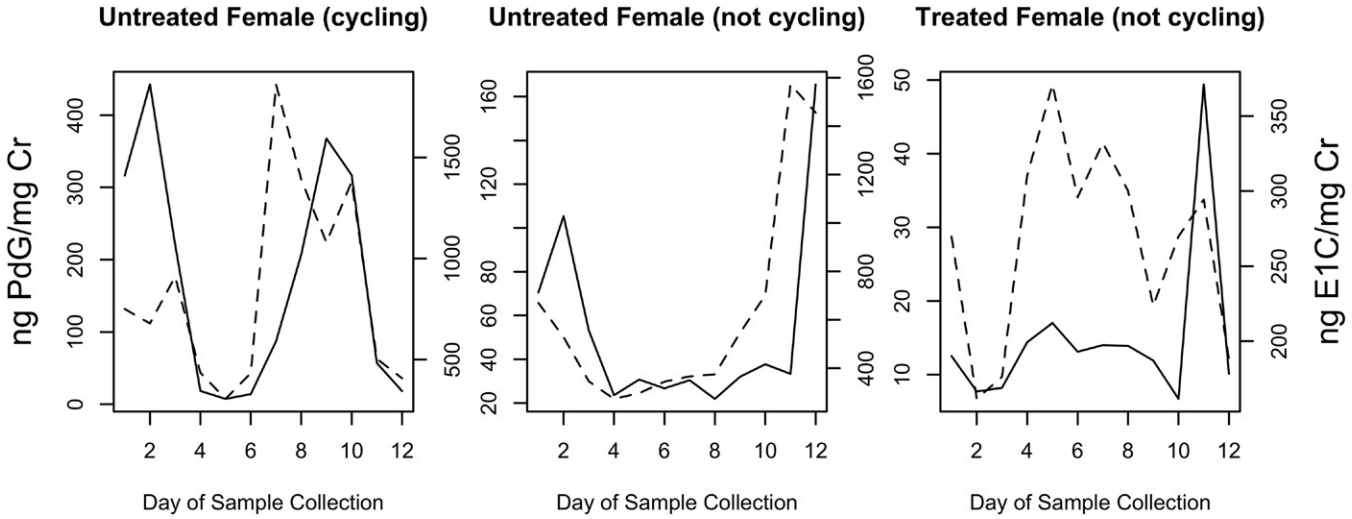

Figure 2. Examples of hormone patterns across treatments. Pregnanediol-3α-glucuronide (PdG) and estrone conjugate (E1C) concentrations in urine as a function of day of sample collection, PdG is graphed as a solid line with values on the left *y*-axis while E1C is graphed as a dashed line with values on the right *y*-axis. Some untreated females were cycling (left panel), while some untreated females were not cycling (center panel). None of the treated females were cycling (right panel).

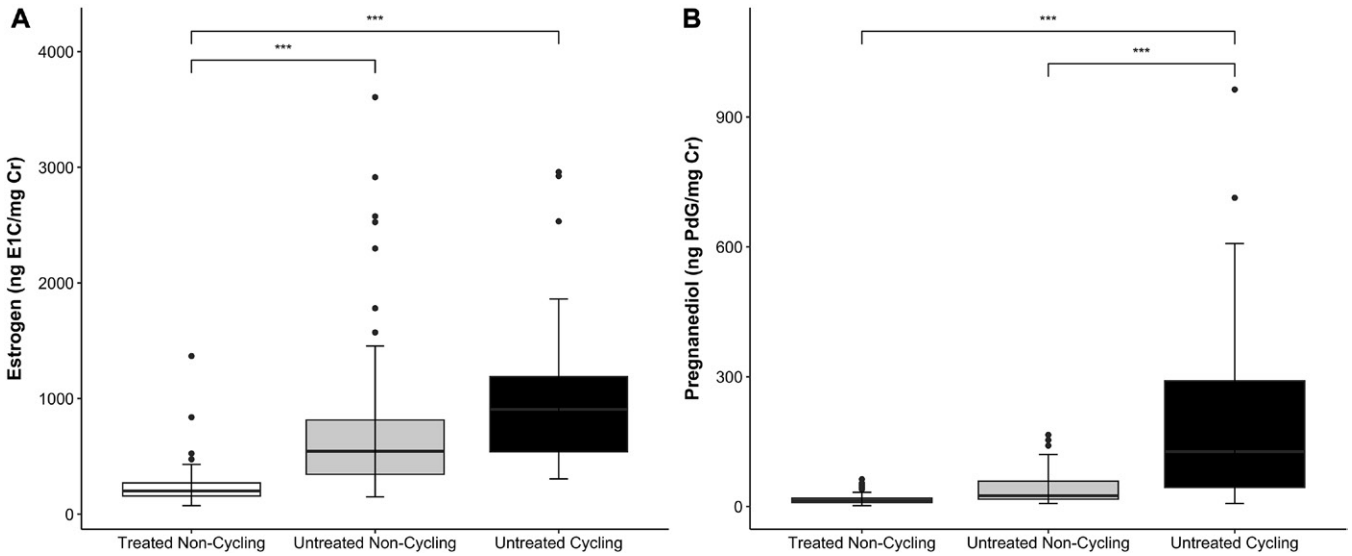

Figure 3. Hormone levels in females by treatment and cycling status. E1C (A) and PdG (B) concentrations in females that were treated with deslorelin acetate implants and not cycling (n = 9), untreated females that were not cycling and were paired with vasectomized males (n = 4), and untreated females that were cycling (n = 5). Estrogen (E1C) concentrations differed significantly between treated and untreated females, such that treated females had lower levels of E1C than did both cycling and noncycling untreated females. Pregnanediol (PdG) concentrations differed significantly between cycling and noncycling females, such that cycling females had higher levels of PdG compared with both treated and untreated noncycling females. The figure shows the median as well as hinges indicating upper and lower quartiles, whiskers indicating the spread of data up to 1.5 IQR from each hinge, and all other outlying data points. Significant comparisons between groups are noted with "***".

Behavioral effects of contraception. The main goal of this study was to determine whether hormonal contraceptive use (deslorelin or medroxyprogesterone acetate) altered affiliative behavior between pair mates in our colony (35 pairs). To do this, we compared affiliation between pairs in which the females were treated with 1 of 2 hormonal contraceptives or were untreated. In total, our data represent a mean of 149 d of data per pair (range = 142 to 150 d). Overall, pairs showed affiliative interactions during an average of 27 ± 1% of observations.

We assessed several demographic features of individuals and pairs to identify potentially influential confounding variables. Neither female age ($F_{(2, 32)}$ = 0.60, P = 0.56, η^2 = 0.04, 95% CI [0.00, 1.00]) nor male age ($F_{(2, 32)}$ = 0.08, P = 0.92, η^2 = 0.004, 95% CI [0.00, 1.00]) differed between groups (Table 1). Similarly, pair tenure (the time between pair introduction and collection of behavioral data) was not different between groups ($F_{(2, 32)}$ = 0.17, P = 0.85, η^2 = 0.01, 95% CI [0.00, 1.00]). Contraceptive tenure (the time between the administration of a hormonal contraceptive or vasectomy procedure and the collection of behavioral data) was different between groups ($F_{(2, 32)}$ = 3.79, P = 0.03, η^2 = 0.19, 95% CI [0.01, 1.00]). Specifically, pairs with vasectomized males had longer contraceptive tenures than did pairs in which the females were treated with deslorelin ($t_{(32)}$ = 2.59, P = 0.01, β = 0.50, η^2 = 0.17, 95% CI [0.02, 1.00]) or with medroxyprogesterone ($t_{(32)}$ = 2.24, P = 0.03, β = 0.43, η^2 = 0.14, 95% CI [0.01, 1.00]) (Table 2).

We found no significant effect of contraceptive method on affiliation between pair mates (Figure 4). We did not detect a difference in affiliation rates between pairs in which the female was untreated (0.31 ± 0.01) and pairs in which the female was treated with either deslorelin acetate (0.27 ± 0.01; $t_{(32)}$ = 0.64, P = 0.53, β = 0.08, η^2 = 0.01, 95% CI [0.00, 1.00]) or medroxyprogesterone acetate (0.26 ± 0.01; $t_{(32)}$ = 0.88, P = 0.38, β = 0.11, η^2 = 0.02, 95% CI [0.00, 1.00]). We also found no difference in affiliation between the 2 groups in which the females received hormonal contraception ($t_{(32)}$ = 0.37, P = 0.72, β = 0.04, η^2 = 0.004, 95% CI [0.00, 1.00]). With regard to the random effect, pair identity accounted for only about 2% of variation in affiliation across groups (σ^2 = 0.02, SD = 0.12). Residual variance indicated that only 3% of within-group variance was due to differences between pairs (σ^2 = 0.03, SD = 0.17).

Table 1. Subject age (days) by sex and contraceptive treatment group

	Age of females (d)		Age of males (d)	
Experimental groups	*N*	Mean ± SE	*N*	Mean ± SE
Untreated control	9	2,799 ± 819		3,421 ± 751
Deslorelin acetate	17	3,217 ± 406		3,122 ± 562
Medroxyprogesterone acetate	9	2,418 ± 385		3,408 ± 566

There were no significant differences in female or male age between groups.

Discussion

The goal of this study was to determine whether and how hormonal contraception affects the affiliative behavior of pair-mated titi monkeys. Before studying behavior, we used 18 pair of monkeys to confirm that our new method of hormonal contraception altered female reproductive hormones. Deslorelin acetate implants have only recently been used for contraception in our colony of titi monkeys and its physiologic effects had not yet been documented. Consistent with data from other primate species,[6] we found that females treated with contraceptive implants had lower overall urinary levels of estrogen and pregnanediol than did untreated females. This finding confirms that the deslorelin-treated females in our sample experienced reproductive suppression during treatment, as was intended.

In addition to cessation of cycling in our treated females, we observed that nearly half of our untreated control females were not cycling. We conducted post hoc analyses to better understand this finding, but did not identify a connection to female age, duration of current pairing, or time since the

Table 2. Pair and contraceptive tenure (days) by contraceptive treatment group

Experimental groups	*N* (pairs)	Total pair tenure (d) (mean ± SE)	Contraceptive tenure (d) (mean ± SE)
Untreated control	9	841 ± 386	74 ± 45
Deslorelin acetate	17	1,028 ± 163	2 ± 2
Medroxyprogesterone acetate	9	961 ± 143	3 ± 3

Contraceptive tenure is calculated as the first day of hormonal contraceptive administration or vasectomy procedure in relation to the first day of affiliation data collection.

female had been removed from her natal group. Little is known about reproductive cycling in titi monkeys, especially in reference to cessation of cycling. While previous work suggests that prepubertal females do not cycle while in their prenatal group,[7] all 18 females had been out of their natal group from a minimum of 2 y at the time of data collection. A period of anovulation has been identified in our colony after the birth of surviving offspring,[30] but none of the females in our sample had reproduced in years, if at all. Age-related cessation of cycling has not been identified in titi monkeys, and while it did not appear to be a factor in our sample, this would be an excellent topic for future research.

In respect to the relationship between affiliation and hormonal contraception, we evaluated 35 different pairs of monkeys and found no effect of contraceptive treatment on affiliation between pair mates. Our results contrast with findings in other species of primates, which found that males engaged less with females treated with medroxyprogesterone acetate[8,13,19] and treated females showed less affiliation toward conspecifics.[21,29] However, several species-specific and design factors are relevant here. First, female titi monkeys tend to be the individuals that regulate affiliative contact with their mate,[9] so male-driven interest may have a lesser role in titis compared with other species. Second, titi monkeys appear to have visually-concealed ovulation, meaning that they do not display visual signals of ovulation—at least as a signal that humans can perceive. A previous study on behavior and contraceptive treatment included nonvisual stimuli (for example, olfactory stimuli[8]), but most stimuli are either visual-specific or nonspecific (that is, the sensory stimuli used in the study are not distinct so the mechanism by which conspecifics differentiate cycling and noncycling females are unknown). While signs of ovulation may not be apparent to human perception, such signs may be apparent to conspecifics, and numerous primate species display overt visual signals of ovulation.[13,19] Such overt ovulation signaling often coincides with mating strategy: females in nonmonogamous species, specifically those with multimale social groups, typically display exaggerated genital swellings.[26] While several hypotheses may explain mating strategies in these species, most agree that paternal certainty—or lack thereof—is an important factor.[26] Monogamous species have greater paternal certainty by virtue of their breeding strategy, which may alter mating strategy, social behavior, and the importance of timed copulation. Finally, our measure of affiliation was conditional, meaning that both members of the pair had to engage in the behavior for it to be scored. Because we did not record approaches and their acceptance or rejection, we do not know whether a nonsignificant finding in our sample reflects unaltered male behavior or female compensation for lowered male interest.

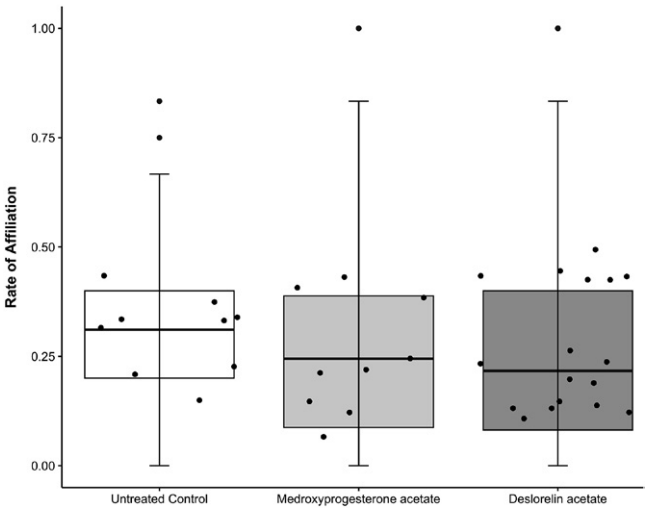

Figure 4. Distribution of affiliative rates between pair mates in each contraceptive treatment group. Untreated females paired with vasectomized males (white), females treated with medroxyprogesterone acetate injections (light gray), and females treated with deslorelin acetate implants (dark gray). Median and IQR are shown with mean rates of affiliation for each pair overlaid within their respective groups. Rates were consistent between all 3 groups.

To examine the impact of individual differences on affiliation between pair mates, our model also included a random effect of pair identity. We found that individual differences between pairs accounted for only 2% of variation in affiliation across all pairs, and only 3% of within-group variance could be explained by differences between pairs. This relatively small inter-pair variation may be explained by the similarities between our subjects in terms of age and pair length, as previous work has identified these as salient factors in affiliation between titi pair mates.[28] Additional sources of variance in affiliation between pairs in previous studies may be attributable to reproductive status, which was controlled for in this study. Among titi monkeys, pair bonds are sensitive to reproductive status. Males that reproduced with their female pair mate ("fathers") showed different neural responses to reunion with their pair mate than did nonfathers.[14] Furthermore, titi pairs show lower rates of affiliation while engaging in active parenting (for example, before and during pregnancy), an effect which is sustained even after the offspring are largely independent.[17] These findings motivated our decision to examine affiliation only in nonreproductive pairs, especially since pair bonds in titi monkeys appear to be resilient to reproductive failure.

This study had a few key limitations. As discussed above, we evaluated affiliation using conditional measures: both the male and female had to be participating in the behavior for it to be scored. This approach does not indicate whether the male or female drove the patterns of affiliation. Although we can hypothesize based on prior research in this area, we cannot truly identify which animal steered affiliative interactions. Future research should focus on more nuanced behavior, such as approach acceptance and rejection, to identify sex-specific changes in behavior. Second, because we used a convenience sample (driven by colony management interests), we had relatively little control over the timing of current or prior contraceptive treatment. Our deslorelin acetate-treated group was almost double the size of the other 2 groups because our colony is currently primarily managed with this type of contraception. Finally, while we only evaluated affiliation between pair mates, other studies have observed increased aggression and submission in hormonally contraceptive-treated females as compared

with untreated females.[19] To support management of a captive, breeding colony, future work should examine aggression between pair mates and within the family unit for pairs that are receiving contraceptives while also rearing offspring.

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