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Natural and Induced Ovarian Synchrony in Golden Lion Tamarins (*Leontopithecus rosalia*)[1]

S.L. Monfort,[2] M. Bush, and D.E. Wildt

Conservation and Research Center, National Zoological Park, Smithsonian Institution, Front Royal, Virginia 22630

ABSTRACT

Ovarian cycle synchrony was assessed in spontaneously cycling female golden lion tamarins by monitoring longitudinal (16 mo) urinary steroid metabolite (estrone conjugates; pregnanediol-3α-glucuronide, PdG) excretion in four pairs (n = 8) of females isolated from males. The overall mean ovarian cycle duration was 18.5 ± 0.3 days (n = 136 cycles; mean range, 15.7–21.0 days), and there was no evidence of reproductive seasonality. Laparoscopic ovarian examinations confirmed that cyclic fluctuations in urinary steroid metabolite excretion were temporally associated with the formation and demise of corpora lutea. Evaluation of ovarian synchronization tested the null hypothesis that urinary hormone cycles were expressed randomly relative to those of cagemates or other females housed in separate cages but within close proximity. Natural ovarian synchrony (expressed as the mean difference in ovarian cycle onset) between cagemates (4.1 ± 0.4 days) and among noncagemates (4.2 ± 0.2 days) did not differ ($p > 0.05$) from a random ovarian cycle distribution. Two trials also were conducted to evaluate the efficacy of the prostaglandin (PG) $F_{2\alpha}$ analogue, cloprostenol, for artificially synchronizing ovarian cycles. Induced ovarian synchrony was not achieved with a single 0.8-μg i.m. injection of cloprostenol. However, doubling the cloprostenol dose (1.6 μg) caused a rapid decrease in mean urinary PdG ($p < 0.05$) within 2 days, and synchronous ovulation was demonstrated by an increase ($p < 0.01$) in mean urinary PdG 10 days after cloprostenol administration. In summary, females housed in pairs, in the absence of males, exhibit spontaneous, year-round ovarian cycles with no evidence of among-female ovarian synchrony. Results also suggest that this New World primate has a reduced sensitivity to cloprostenol (compared to common marmosets) but that a single, midcycle cloprostenol injection of 1.6 μg effectively induces luteolysis and synchronous ovulation.

INTRODUCTION

Golden lion tamarins (*Leontopithecus rosalia*) are an endangered species in the family Callitrichidae that historically inhabited lowland forest of southeastern Brazil [1]. This cooperatively breeding species generally is monogamous with birth peaks between January and August in temperate latitudes and from September to March in native tropical habitats [1–3]. In captivity, golden lion tamarins are the only New World primate in which reproductive synchrony is reportedly high among cagemates as well as among individuals living in separate nearby cages [4]. The original conclusion, however, was based upon examinations of among-female peak discrepancies in urinary estrone. It now is generally accepted that such peaks do not represent preovulatory estrogen surges but instead reflect luteal estrogen production/excretion. Because luteal phase estrogen production in golden lion tamarins occurs over several days, and peak values do not correspond to a discrete physiological event such as ovulation, this is likely to be an inappropriate index for estimating among-female ovarian cycle synchrony. Thus, the existence or absence of ovarian cycle synchrony in the golden lion tamarin warrants further investigation.

Of related interest is regulation of luteal activity. Despite a report that induced luteolysis in the common marmoset (*Callithrix jacchus*) occurs consistently after administration of the prostaglandin (PG) $F_{2\alpha}$ analogue, cloprostenol [5], similar treatments do not consistently induce luteolysis in humans [6, 7] or baboons (*Papio cynocephalus anubis*) [8]. The corpus luteum (CL) of the common marmoset is cloprostenol-sensitive and becomes increasingly responsive to this analogue in vitro at 6–14 days after ovulation [9], with a single i.m. injection (0.5 μg) after Day 8 of the luteal phase being routinely luteolytic [5]. Because callitrichids generally exhibit no obvious signs of impending ovulation (e.g., menses or behavioral receptivity), cloprostenol-induced ovarian cycle synchronization could facilitate timed matings, fixed-time artificial inseminations, and embryo recovery efforts designed to enhance captive propagation of endangered New World primates.

The ovarian cycle of New World primates is distinguished from that of Old World primates by substantial estrogen production/excretion during the luteal phase of the ovarian cycle [10]. Thus, estrone excretion generally parallels progestagen excretion during the luteal phase, and, with few exceptions, estrogen and progestagen excretion profiles are equally effective for noninvasively assessing ovarian cycles in New World primates, including the common marmoset [11–13], cotton-top tamarin (*Saguinus oedipus*) [10, 14–16], saddle-back tamarin (*Saguinus fuscicollis*) [17–20], Goeldi's monkey (*Callimico goeldii*) [21–24], muriqui monkey (*Brachyteles arachnoides*) [25], and white-faced saki (*Pithecia pithecia*) [26, 27]. Urinary estrogen, but not progestagen, metabolites have been described for the golden lion tamarin (*Leontopithecus rosalia*) [28, 29].

Our general aim was to utilize noninvasive urinary steroid hormone monitoring to assess reproductive synchrony and the regulation of luteal function in the rare golden lion tamarin. The primary objectives were to 1) investigate the existence of reproductive synchrony among females housed in pairs, as well as among females housed in separate cages in close proximity, and 2) assess the efficacy of the $PGF_{2\alpha}$ analogue, cloprostenol, for artificially synchronizing ovarian cycles. Because urinary progestagen excretion had not been previously evaluated in golden lion tamarins, a secondary objective was to compare urinary pregnanediol-3α-glucuronide (PdG) and estrone conjugates (EC) for monitoring longitudinal ovarian cycles and to relate endocrine evaluations to direct ovarian observations via laparoscopy.

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[2]Correspondence: Dr. S.L. Monfort, Conservation and Research Center, National Zoological Park, 1500 Remount Road, Front Royal, VA 22630. FAX: (540) 635-6571.

MATERIALS AND METHODS

Animals

Eight female golden lion tamarins (1–5 yr of age; 0.6–0.7 kg BW) were maintained at the National Zoological Park's Conservation and Research Center, Front Royal, VA (38°N). Animals were housed in pairs within individual cages (3.0 × 4.9 m) connected to outdoor enclosures (3.0 × 8.2 m) and exposed to ambient fluctuations in photoperiod. Two pairs of siblings (#205–206 and #643–644) were 49 and 21 mo of age, respectively, at study onset. For the entire study interval, female #512 (45 mo) was paired with #884 (37 mo) and female #694 (65 mo) was paired with #917 (14 mo). Solid concrete walls separated adjacent cages, whereas cage fronts were covered with wire mesh. Paired females were permitted auditory and olfactory, but not visual, communication with other female pairs housed within the same building; four cages were aligned in a side-by-side linear array, and all females were completely isolated from males. Diet consisted of Zu/Preem Marmoset Diet (Premium Nutritional Products, Inc., Topeka, KS) and mixed fruit; animals had ad libitum access to water. Animal keepers observed females for 15–30 min at least twice daily (early morning, late afternoon), and detected bouts of aggression were recorded in the daily log.

Urine Sample Collection

Although permitted free access to the indoor-outdoor enclosures during daylight hours, each female was locked inside a wooden nestbox (0.3 × 0.3 × 0.6 m, w × h × l) overnight (2000–0700 h). A clear plastic partition was used to separate each female into one half of the nestbox, and urine samples (1–5 ml) were collected at 0730 h from separate, clean drain pans left overnight underneath each one half of the nestbox. While confined within the nestbox, female pairs were in continuous olfactory, auditory, and visual contact with each another. In general, urine samples were collected 5–7 days per week during 16 mo. However, daily urine samples were collected during all laparoscopic trials, as well as during attempts to synchronize ovarian cycles with exogenously administered cloprostenol. Urine samples were centrifuged briefly (1500 × *g* for 10 min) to remove particulate matter and stored frozen immediately (−20°C). All samples were indexed by creatinine (Cr) measurement [30–31], and hormone values were expressed as mass units/mg Cr excreted.

Assay for EC

Urinary EC was analyzed as described by Monfort et al. [30]. Initially, each urine sample was diluted (1:50) in PBS (0.1 M, 0.1% gelatin, pH 7.0) 1:10 000, and a 50-μl aliquot was adjusted to a final assay volume of 300 μl in Tris buffer (0.1 M Tris, 0.9% NaCl, 0.1% NaN_3, 0.1% gelatin, pH 8.4). Antiserum that cross-reacts 100% with estrone glucuronide and estrone sulfate (anti-estrone-3-glucuronide serum, 100 μl, 1:1500; D. Collins, Emory University, Atlanta, GA) and ^{3}[H]-estrone sulfate (100 μl, 7000 cpm, specific activity 55 Ci/mmol; Dupont-New England Nuclear, Wilmington, DE) were combined with unknowns and standards (4.9–2500 pg/tube; Sigma Chemical Co., St. Louis, MO) and incubated overnight at 4°C. After the addition of 300 μl charcoal-dextran (0.0625% Norit A charcoal, 0.00625% dextran in 0.1 M PBS, pH 7.0) and a 30-min incubation at 4°C, tubes were centrifuged (10 min, 1500 × *g*), decanted into scintillation vials, combined with 5.0 ml Ready Solv HPb (Beckman Instruments Inc., Fullerton, CA), and counted for 5 min.

Serial dilutions of golden lion tamarin urine yielded displacement curves parallel to that obtained for estrone sulfate standards. The mean ± SEM recovery of estrone sulfate (range, 4.9–2500 pg/tube) added to a pool of golden lion tamarin urine was 90.2 ± 6.2% ($y = 0.94x + 27.7$; $r^2 = 0.96$). Assay sensitivity was 4.9 pg/tube; interassay coefficients of variation for three separate internal controls (n = 23 assays) were 9.9% (11–16% binding), 8.3% (41–53% binding), and 11.2% (74–85% binding); and intraassay variation averaged < 10%.

Assay for PdG

Urinary PdG immunoreactivity was analyzed using the methods of Monfort et al. [30]. A 200-μl urine aliquot diluted (1:50) in PBS was combined with 100 μl each of PdG antiserum (1:20 000), which crossreacts 100% with PdG and 6.7% with pregnanediol, and ^{3}[H]-PdG (7000 cpm, specific activity 42 Ci/mmol), both supplied by Courtauld Institute of Biochemistry, London, UK. Urine samples and standards (19.5–5000 pg/tube, Sigma) were incubated overnight (4°C), and antibody-bound and free steroid were separated after a 45-min incubation with 300 μl charcoal-dextran suspension and centrifugation for 10 min (1500 × *g*). Supernatants were combined with 5 ml Ready Solv HPb and counted for 5 min.

Serial dilutions of golden lion tamarin urine yielded displacement curves parallel to that obtained with standard preparations. Recovery of known amounts of PdG (range of 19.5–5000 pg/tube), which was added to a pool of diluted urine (100 μl, 1:50), gave a mean of 94.2 ± 6.3% ($y = 0.96x - 7.2$; $r^2 = 0.99$). Assay sensitivity was 19.5 pg/tube; interassay coefficients of variation for two separate internal controls (n = 26 assays) were 10.4% (35–55% binding), 10.7% (58–70% binding), and 23.8% (74–88% binding), and intraassay variation was < 10%.

Laparoscopic Ovarian Examinations

During Year 1, urine samples were collected daily from six females that were subjected to laparoscopy five times per individual at 5-day intervals encompassing one complete ovarian cycle. For laparoscopy, females were induced into a surgical plane of anesthesia using ketamine hydrochloride (10 mg/kg i.m.) and then maintained on 1–2% halothane (administered by face mask). After anesthesia induction (usually within 10 min of injection), each female was surgically prepared after being placed in dorsal recumbency on a surgical table and tilted head-down at 30°. By means of a Verres needle and hand pump, the abdominal cavity was inflated with room air. A trocar-cannula unit was inserted through a 1.5-mm skin incision and through the midventral abdominal wall and peritoneum. A 5-mm-diameter, rigid laparoscope was inserted to permit the viewing of all ovarian aspects and structures (follicles, CL; number, size). Size estimates were made by comparing ovarian structures to the 2-mm Verres needle (which also served as a probe) and the 5-mm-diameter laparoscope. After the cannula was removed, the abdominal insertion site was sutured, and each female was given 0.25 ml long-acting penicillin (G.C. Hanford Manufacturing Co., Syracuse, NY) as a prophylactic measure. Ovarian observations were compared with corresponding urinary hormone metabolites profiles (see above).

Ovarian structures also were observed laparoscopically

in eight females on two separate occasions during Year 2, 10 days after i.m. administration of either 0.8 or 1.6 μg cloprostenol (Miles Laboratories, Inc., Shawnee, KS). Cloprostenol trial doses were directly extrapolated from previously published reports in the common marmoset (0.5 μg/female; Summers et al. [5]). To compare endocrine profiles among treatment groups, all hormone data were aligned to the day of cloprostenol administration.

Statistical Analyses

Although baseline hormone concentrations varied among females (15–150 ng/mg Cr), within-female basal hormone concentrations were consistent across cycles. For each individual animal, intercyclic troughs in PdG excretion were readily identifiable by visual inspection of the data; individual hormone baselines were estimated as the mean of the PdG concentrations within these troughs across repeated ovarian cycles. Day 1 of the ovarian cycle was defined as the first day on which PdG excretion increased above baseline concentrations and then remained elevated for at least 2 successive days. In < 5% of the cycles monitored, one or more data points were missed because of a missed urine collection. In cases in which this occurred within the intercyclic trough, the first PdG increase above baseline after the skipped day was designated Day 1. Missed samples had no effect during laparoscopic and cloprostenol trials because urine samples were collected daily. The luteal phase duration was defined as the interval between the first elevation in urinary PdG above basal (Day 1) until the subsequent decline to basal concentrations. The follicular phase was defined by the duration of the intercyclic troughs in PdG excretion. Ovarian cycle length was estimated by measuring the interval between Day 1 of successive ovarian cycles. Standard descriptive statistics including mean and standard error of the mean (± SEM) were used to describe the general features of the ovarian cycle as well as hormonal metabolite values. Statistics were performed using Statview 512+ (Version 1.1; BrainPower, Inc.; Calabasas, CA) on an Apple Macintosh (Cupertino, CA) computer.

Evaluation of ovarian synchronization tested the null hypothesis that urinary hormone cycles were expressed randomly relative to those of cagemates or other females housed in separate cages but within close proximity. A one group *t*-test and chi-square statistic were used to determine whether the distribution of ovarian cycles among females differed from random [4]. Absolute among-female differences in ovarian cycle duration (in days) were determined (relative to Day 1 of the ovarian cycle), and results were clustered into 2-day intervals (0–1, 2–3, 4–5, 6–7, and 8–9). For the *t*-test, the mean distribution of the ovarian cycle difference was estimated as 4.5 days (based upon an average cycle of 19 days with an assumed distribution of absolute differences ranging from 0 to 9 days and a mean of 4.5 days) [4]. For the chi-square statistic, expected values for each 2-day interval were equal to the total number of among-female comparisons divided by five (the total number of 2-day intervals).

Correlation analysis was used to assess the correspondence between EC and PdG in same-day urine samples. Factorial analysis of variance was used to compare within- and among-group differences in ovarian cycle lengths before and after cloprostenol administration. Repeated measures analysis of variance was used for the following: 1) to compare differences in ovarian cycle synchrony before and after cloprostenol administration within treatments; and 2) to determine the day of first significant increase in urinary PdG concentrations above the postcloprostenol induced nadir in PdG excretion; pairwise comparisons were made using Fisher's Protected Least Significant Difference test.

RESULTS

Longitudinal Urinary EC and PdG Excretion

Matched daily urinary EC and PdG profiles from four pairs (#205–206, #644–643, #512–884, #694–917) of golden lion tamarins are presented in Figure 1, A-H. Overall correlation (*r*) between urinary EC and PdG averaged 0.48 ± 0.07 (n = 8; *r* range, 0.25–0.75; *df* range, 100–187; all $p < 0.05$). Mean urinary EC ranged from < 1 to 35 μg/mg Cr, whereas PdG ranged from 10 to 2500 ng/mg Cr. Among-female differences in absolute concentrations of excreted PdG were more pronounced than for EC (note different scales in Fig. 1, A-H). Although urinary EC and PdG excretion were qualitatively similar, there was less within-female, cycle-to-cycle variation in PdG excretion (data not shown). Thus, urinary PdG profiles were chosen to examine ovarian cycle synchrony. Subsequent longitudinal assessments of urinary PdG excretion revealed that the temporal patterns depicted in Figure 1 continued through Year 2, indicating that spontaneous ovarian cyclicity continued year-round. Overall mean ovarian cycle duration was 18.5 ± 0.3 days (n = 136 cycles), whereas individual mean cycle durations ranged from 15.7 to 21.0 days. Mean follicular (5.4 ± 0.2 days; mean range, 4.7–6.3 days) and luteal phase (13.1 ± 0.5 days; mean range 10.3–14.3 days) durations constituted 28.9 ± 1.1% and 71.1 ± 1.1% of the ovarian cycle, respectively.

Laparoscopic Ovarian Observations

Mean urinary EC and PdG profiles during concomitant laparoscopic ovarian examinations are presented in Figure 2. Endocrine data are aligned by the first day of PdG increase above basal, and laparoscopic observations are subdivided into 5-day intervals (1–5, 6–10, 11–15, and 16–20 days). The numbers of small follicles (< 1 mm and 1–1.5 mm diameter) evident on the ovaries (soon after presumed ovulation) were maximal during the first interval (Fig. 2, inset), gradually declining thereafter. Most large follicles (2–3 mm) were observed during the third and fourth intervals (Days 11–20), during the latter part of the luteal phase and into the follicular phase. As expected, most CL were detected between Days 1 and 10 after presumed ovulation, and an average of 1.8 ± 0.1 (range, 1–3) CL per ovarian cycle were observed. Corpora lutea were observed in 3 of 7 individuals only on the left ovary, whereas 4 females had CL present on both ovaries; CL diameter ranged from 1.5 to 4.0 mm. Additionally, CL were observed on three of six occasions when laparoscopy was conducted on Days 1–2 of the ovarian cycle. Ovarian cycle duration during laparoscopy cycle (17.8 ± 0.8 days) was similar ($p > 0.05$) to that of cycles in which laparoscopy was not performed (see above).

Natural Ovarian Synchrony

The mean difference in ovarian cycle onset between cagemates (4.1 ± 0.4 days) and among noncagemates (4.2 ± 0.2 days) did not differ ($p > 0.05$) from a random ovarian cycle distribution with a mean of 4.5 days. Similarly, nonparametric analyses confirmed that the mean differences in ovarian cycle onset between cagemates and among

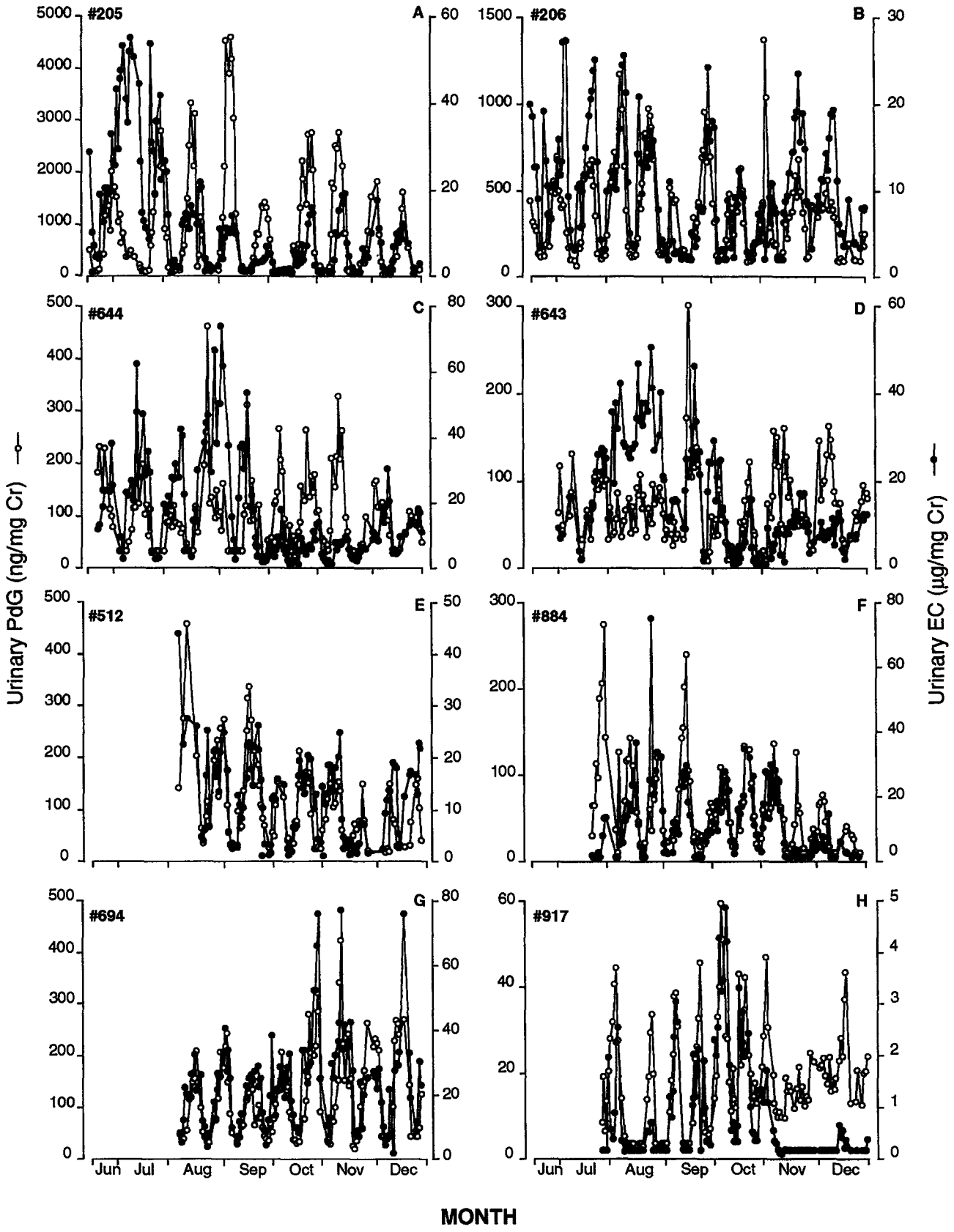

FIG. 1. Longitudinal urinary EC and PdG excretion in four pairs (pairs, #205–206, #644–643, #512–884, #694–917) of golden lion tamarins in which urine was collected five to seven times per week for up to 6 mo. Pairs #205–206 and #644–643 were siblings, whereas other pairs were unrelated. Note different hormone concentration scales.

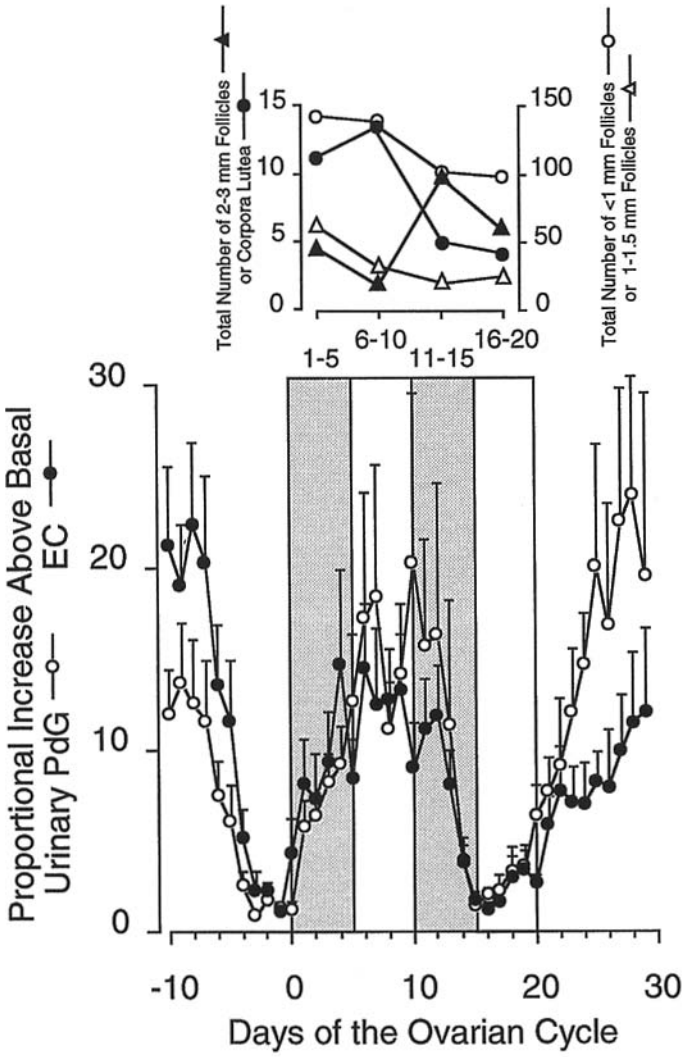

FIG. 2. Mean urinary EC and PdG excretion during concomitant serial laparoscopic ovarian examinations in eight females. Hormonal data are expressed as the proportional increase above basal concentrations and are aligned to the first day of PdG increase (Day 1). Each female was examined by laparoscopy five times at 5-day intervals (open and shaded bars). Observed ovarian structures (inset) were summed and retrospectively plotted relative to the 5-day interval in which observations were made.

noncagemates did not differ ($p > 0.05$) from a random distribution, with only 63.8% (37 of 58) and 65.2% (187 of 288) of the intervals exhibiting a mean difference of 5 days or less, respectively. The lack of ovarian cycle synchrony is apparent when longitudinal urinary PdG excretion profiles are superimposed in a representative pair of females (Fig. 3).

Induced Ovarian Synchrony

Mean urinary PdG excretion profiles (expressed as a proportional increase above basal) during two cloprostenol treatments (Trial 1, 0.8 and Trial 2, 1.6 μg, respectively) are presented in Figure 4, A and B. Cloprostenol was administered at similar times ($p > 0.05$) during the ovarian cycle for the two trials (Trial 1, 9.6 ± 1.0 days and Trial 2, 10.2 ± 1.4 days, respectively). Although mean PdG concentrations declined ($p < 0.05$) within 2 days of cloprostenol administration in both trials, reaching nadir concentrations by 4 days, PdG subsequently increased above nadir

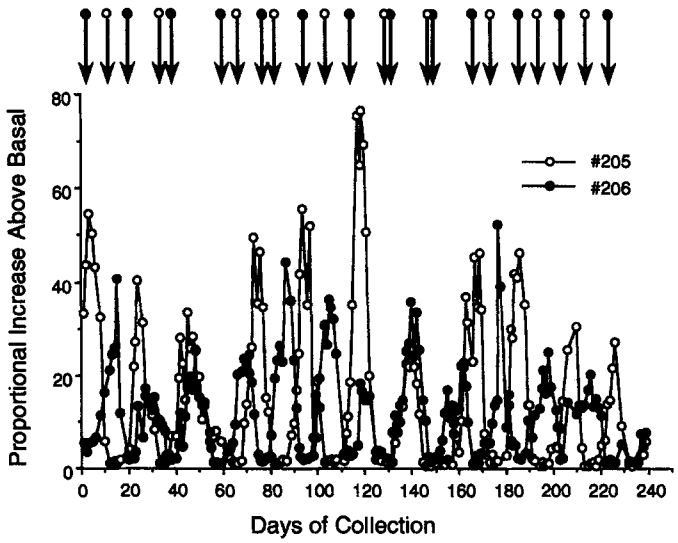

FIG. 3. Superimposed longitudinal urinary PdG excretion profiles (expressed as a proportional increase above basal) in a representative pair of females. All data are aligned by the day of urine sample collection, and the distances between arrows (PdG nadirs) denote the relative differences in ovarian cycle synchrony among cagemates.

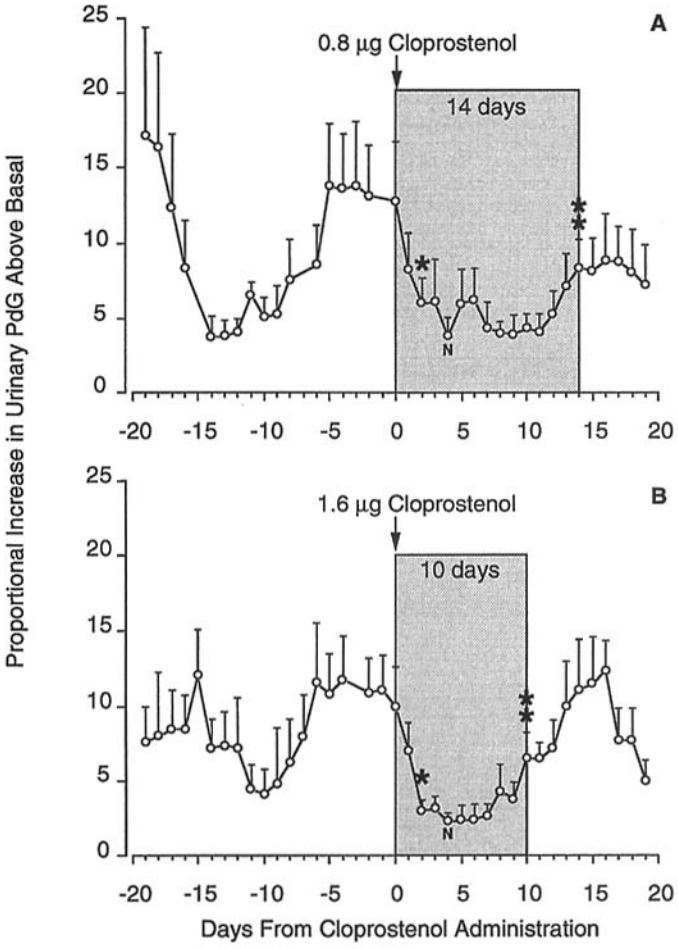

FIG. 4. Mean urinary PdG excretion profiles (expressed as a proportional increase above basal) during two cloprostenol trials (Trial 1, 0.8 and Trial 2, 1.6 μg). All data are aligned to the day of cloprostenol administration (Day 0). Single and double asterisks denote the day at which mean PdG declined and then subsequently increased (both $p < 0.05$), respectively, after cloprostenol administration. The letter N marks the nadir in urinary PdG excretion.

concentrations ($p < 0.05$) 4 days sooner in Trial 2 (Trial 1, 10 days; Trial 2, 6 days). The total interval from cloprostenol treatment until a significant ($p < 0.05$) PdG increase during the subsequent ovarian cycle for Trial 1 and 2 was 14 and 10 days, respectively. Contrary to expectation, ovarian cycles were less synchronous after cloprostenol treatment in Trial 1 (before, 3.5 ± 0.4 days; after, 5.2 ± 0.6 days, $p < 0.01$). For Trial 1, an average of 1.5 ± 0.4 CL per female (range, 0–3) were observed laparoscopically 10 days after cloprostenol treatment; one female failed to ovulate. Ovarian cycle synchrony improved after cloprostenol treatment in Trial 2 (before, 3.9 ± 0.7 days; after, 2.1 ± 0.4 days, $p < 0.01$), and 1.8 ± 0.3 CL per female were observed by laparoscopy 10 days after cloprostenol. Ovarian cycle durations after cloprostenol treatments did not differ for females in Trials 1 or 2 ($p > 0.05$) when compared to the historical mean cycle durations of each individual (from nonmanipulated cycles). However, the mean ovarian cycle length was shortened ($p < 0.05$) in Trial 2 (16.7 ± 1.0) compared to Trial 1 (18.8 ± 0.4 days).

DISCUSSION

In contrast to the report of French and Stribley [4], we found no evidence of ovarian cycle synchrony between cagemates or among noncagemates housed in close proximity within the same building. However, female golden lion tamarins in the French and Stribley study [4] were housed in mixed-sex family groups or with a male. Under those conditions, females living within the same or different social groups were highly synchronized ($p < 0.001$) with mean intercyclic estrogen peak discrepancies of 1.3 and 2.1 days, respectively. By contrast, we determined that mean differences in ovarian cycle onset between cagemates (4.1 days) and noncagemates (4.2 days) were not different ($p > 0.1$) from a random ovarian cycle distribution among females.

Certainly further experiments are required to elucidate the role of male presence in mediating ovarian cycle synchrony in the golden lion tamarin. Nonetheless, we contend that male absence alone is unlikely to explain the lack of synchrony observed in our study. Ovarian cycle synchrony among group-living females such as rodent (*Rattus norvegicus*) [32], chimpanzee (*Pan troglodytes*) [33], and human [34] females appears to mediated by female-female olfactory signaling [35, 36]. This mechanism is considered important for intrasexual communication in golden lion tamarins, which possess specialized scent glands and display frequent scent marking behaviors [2]. In fact, French and Stribley [4] suggested that intrasexual olfactory signaling might modulate ovarian cycle synchrony among female golden lion tamarins. The importance of female-female interactions in golden lion tamarins is further demonstrated by the fact that polygyny is only expressed within wild family groups in the absence of aggression by the resident dominant female [37]. Yet, despite the importance of female-female signaling and well-developed scent communication, we did not detect ovarian cycle synchrony in pairs of female golden lion tamarins housed together for up to 16 mo. Differences between the present study and that of French and Stribley [4] may be related to our larger sample size (346 vs. 28 cycle comparisons) or to the method by which ovarian cycles were aligned.

Although PG analogues have not proven reliable for inducing functional luteolysis in humans [6, 7] or baboons [8], cloprostenol (0.5 μg, i.m.) consistently induces luteolysis in the common marmoset when administered after Day 10 (~35% of the total ovarian cycle duration) of the ovarian cycle [5]. In those studies, blood progesterone concentrations decreased precipitously within 24 h, and ovulation occurred 10–13 days after cloprostenol administration [5, 38]. Dose extrapolations (based on body weight) ensured that the low cloprostenol dose (0.8 μg) utilized in the present study was similar to that used in the common marmoset [5]. Although this dose was administered to golden lion tamarins midway through the ovarian cycle (mean = 9.6 days, ~50% of the total ovarian cycle duration), 0.8 μg cloprostenol was ineffective for synchronizing ovarian cycles. Mean PdG excretion declined ($p < 0.05$) within 2 days, but among-female differences in ovarian cycle onset paradoxically increased ($p < 0.01$) after treatment, and a significant mean increase in PdG above basal concentrations did not occur until 14 days after cloprostenol administration. Conversely, a doubling of the cloprostenol dose (1.6 μg) not only induced a rapid decline in mean urinary PdG ($p < 0.05$) within 2 days, but posttreatment synchrony improved ($p < 0.01$), and the first significant increase in urinary PdG above baseline occurred 10 days after cloprostenol treatment. Although these data suggested a reduced sensitivity to cloprostenol in the golden lion tamarin (compared to the common marmoset), the time course of endocrine events after cloprostenol administration indicated that a single midcycle cloprostenol injection (1.6 μg administered i.m.) effectively induced luteolysis and synchronous ovulation in this rare species.

It has been suggested that the differential efficacy of PGs for inducing luteolysis (i.e., effective in New World primates, but ineffective in Old World primates or humans) may be related to fundamental differences in ovarian function [8]. Differences in luteal function are clearly revealed by the fact that callitrichids excrete enormous quantities of estrogen during the luteal phase of the ovarian cycle. There is some in vitro evidence in humans that estrogen stimulates and progesterone inhibits $PGF_{2\alpha}$ production in endometrial tissue [39, 40]. Thus, the relative concentrations of luteal estrogen and progesterone could potentially modulate PG production and/or the ratio of stimulatory to inhibitory PGs, as well as the responsiveness to these agents. Nevertheless, cloprostenol has great potential for safely and reliably synchronizing ovulation in callitrichids. However, even within the Callitrichidae, a broad range of dosages may be required to induce functional luteolysis. Perhaps more importantly, these data reinforce the necessity for baseline studies in endangered species models and illustrate the dangers inherent in broadly applying techniques developed in one species to another, even those that are closely related taxonomically.

In addition to examining ovarian cycle synchrony, we obtained results that extend the existing reproductive database in the endangered golden lion tamarin. Laparoscopic ovarian examinations confirmed that the initial PdG increase above baseline and the subsequent decrease during the late luteal phase were temporally associated with the formation and demise of CL. In general, our results were similar to the findings of Tardif et al. [41], who first described the time course of follicular development in a callitrichid, the common marmoset. Although the interval between successive laparoscopies in the present study was longer (5 days vs. 3 days), peak follicular diameter (2–3 mm) was observed approximately 11–15 days after the previous ovulation in golden lion tamarins; peak follicular surface diameter (2 mm) in marmosets was observed by 8 days

after cloprostenol administration. For both species, only a single wave of follicular growth appeared during each ovarian cycle with up to 3 ovulatory follicles being recruited from a large pool of antral follicles.

Despite reports that progesterone is metabolized and excreted almost exclusively as fecal metabolites in other New World primates (cotton-top tamarin [42]; pied bare-face tamarin [43]; common marmoset [11]), cyclic urinary PdG excretion profiles in the golden lion tamarin were informative with respect to ovarian activity. However, this was the first report describing substantial among-female variation in PdG excretion: 1 of 8 = 35–50 ng/mg Cr, 5 of 8 = 100–250 ng/mg Cr, 1 of 8 = 450–550 ng/mg Cr, and 1 of 8 =1900–2500 ng/mg Cr. These results were in direct contrast to those obtained for the Goeldi's monkey; in that study a single absolute value (200 ng/mg Cr) permitted differentiation between follicular and luteal phases in all members of the population [23]. Unfortunately, among-animal variation in PdG excretion precludes the use of such an "absolute value" in the golden lion tamarin. Yet, despite among-female variation in PdG excretion by as much as 50-fold, follicular and luteal phases of the ovarian cycle were readily distinguished when individual animals were tracked longitudinally.

On the basis of evaluations of cyclic PdG excretion, we observed an ovarian cycle duration of 18.5 ± 0.3 days (n = 136), which is similar to the duration of 19.6 ± 1.4 days (n = 14) duration reported by French and Stribley [28]. Our results also indicated that golden lion tamarins housed in pairs, in the absence of males, exhibited spontaneous year-round ovarian cycles with no evidence of reproductive seasonality. Although both captive [1, 2] and wild [3] golden lion tamarins exhibit distinct seasonal birth peaks, captive individuals maintained at northern temperate latitudes appear to be physiologically capable of conceiving year-round. Laparoscopic detection of approximately 2 CL (range 1–3) per ovarian cycle also is consistent with an observed twinning rate of 56% and 78% in wild and captive golden lion tamarins, respectively [3, 44].

Ovarian cyclicity in subordinate female saddleback tamarins [18], cotton-top tamarins [16, 45], and common marmosets [46, 47], as measured by cyclic patterns in urinary steroid metabolites, is suppressed in the presence of a dominant, reproductively active female. In contrast, golden lion tamarins appear unique among callitrichids because daughters living in natal groups continue to exhibit cyclic urinary estrogen excretion [4]. Despite this presumed ovulatory activity, daughters fail to reproduce and are considered effectively suppressed by an unknown mechanism. Reproductive suppression also appears to occur in free-living groups of golden lion tamarins. Where polygyny is defined as the presence in a social group of more than one reproductive female, fewer than 11% (20 of 211 groups) of social groups are considered polygynous, and polygyny occurs only in the absence of aggression by the dominant female [37]. These observations strongly suggest that reproductive suppression among female golden lion tamarins is socially mediated. Interestingly, among our study animals, all females exhibited ovarian cyclicity, but one individual within each pair tended to excrete more PdG than the other. For example, female #917 (Fig. 1H, 14 mo of age at study onset) excreted the lowest PdG and EC concentrations while paired with a much older, unrelated female #694 (Fig. 1G, 65 mo of age). A similar trend was observed in another pair (Fig. 1, E-F); #512 (45 mo of age) consistently excreted increased PdG (but not EC) compared to #884 (37 mo of age at study onset). Even identically aged siblings (Fig. 1, A-B, C-D) exhibited quantitative differences in PdG excretion. Although among-female differences in steroid metabolite excretion could be related to metabolic/excretory differences (e.g., among-female differences in gastrointestinal clearance), it is tempting to hypothesize that subordinate tamarins may have the capacity to ovulate, yet produce insufficient progesterone to support implantation/pregnancy. However, more work is required to determine the relationship between dominance status and ovarian steroid production and to determine whether such a postovulatory mechanism regulates fertility in golden lion tamarins.

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