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RESEARCH ARTICLE

Describing Ovarian Cycles, Pregnancy Characteristics, and the Use of Contraception in Female White-Faced Marmosets, *Callithrix geoffroyi*

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Endocrine data and characteristics of nonconceptive ovarian cycling and pregnancy are limited within the genus *Callithrix* to the common marmoset (*C. jacchus*) and Wied's black tufted-ear marmoset (*C. kuhlii*). This article presents patterns of urinary pregnanediol-3-glucuronide (PdG) excretion, as determined by enzyme immunoassay, throughout the course of ovarian cycling and pregnancy in white-faced marmosets (*C. geoffroyi*). Furthermore, characteristics of reproductive parameters including litter size, duration of gestation, maternal age, and information about ovarian cycling following administration of contraceptives are also described. A steep increase in PdG, an indication of ovulation, characterizes normative ovarian cycles, with peak-to-peak intervals between cycles being 27.82 ± 1.49 days in length. PdG excretion (μg/mg Cr) across pregnancy peaked during the 1st and 2nd trimesters (1st = 20.71 ± 2.98, 2nd = 21.16 ± 2.60) and declined gradually to near preconception levels over the 3rd trimester until parturition (3rd = 5.74 ± 1.60). Gestation lasted 148.55 ± 1.89 days. Most pregnancies (82.8%) resulted in an immediate postpartum ovulation (PPO) of 17.45 ± 2.22 days with 58.3% of PPOs resulting in conception. No differences in PdG excretion during the 1st trimester between full pregnancies and miscarriages were found, and pregnancy characteristics such as litter size, duration of gestation, and maternal age were not associated with PdG concentrations. Administration of cloprostenol resulted in shorter peak-to-peak cycle durations, but ovulation was detectable with similar concentrations of peak PdG to a normal nonconceptive cycle. Conversely, medroxyprogesterone acetate (DMPA) injections resulted in little to no PdG excretion across the ovarian cycle. Both methods of contraception providing effective prevention of conception. Overall, these results show that strong similarities in reproductive parameters persist within the genus *Callithrix* and to a lesser extent across the Callitrichidae family. Am. J. Primatol. 00:1–10, 2012.

Key words: reproduction; pregnancy; pregnanediol-3-glucuronide (PdG); contraception; primates; white-faced marmoset

INTRODUCTION

New World primates of the family Callitrichidae, including marmosets and tamarins, are a focal model for elucidating physiological and social mechanisms of reproductive biology and behavior. More specifically, Callitrichids are an important model of primate reproductive biology for a variety of reasons. One example within the *Callithrix* (*C.*) genus is the regular occurrence of twinning in common marmosets, *C. jacchus*—though singletons and triplets can also occur—including multiple ovulations and implantation [Hearn, 1983]. Common marmosets and Wied's black tufted-ear marmosets, *C. kuhlii*, also exhibit varying levels of endocrine and social mechanisms of reproductive inhibition of young female marmosets [Abbott et al., 1981, 1998; Smith et al., 1997; Ziegler & Sousa, 2002]. Young male common marmosets undergo reproductive impairment, but this appears to be regulated by incest avoidance and not rank-related father suppression [Baker et al., 1999], and cotton-top tamarins, *Saguinus oedipus*, another Callitrichid, show similar patterns of reproductive suppression [Ginther et al., 2002].

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Conversely, young white-faced marmosets, *C. geoffroyi*, show little sensitivity or suppression of gonadal activity, that is, excretion of urinary androgens, as a response to social cues [Birnie et al., 2010]. Wied's marmosets exhibit genetic chimerism in a variety of tissues through the transfer of cells by siblings in utero, which make marmosets possess interesting relatedness characteristics [Ross et al., 2007]. Marmoset reproduction is also sensitive to changes in nutritional availability making differences in ecological environments, such as changes in food quality and availability, a potentially important reproductive parameter [Tardif & Jaquish, 1994].

All marmosets are diurnal and arboreal New World primates who spend the majority of their time roaming and monitoring home ranges. They are socially monogamous breeders, and both the mother and father participate in the rearing of offspring. Marmosets also display alloparental behavior, where siblings still residing in the family unit assist in many parental behaviors, such as carrying, food sharing, and playing, which are all critical for young developing marmoset offspring [French, 1997]. In spite of the many similarities displayed within the Callitrichidae family, there is still considerable variation worth noting, including differences in body size and ecological characteristics such as diet, home range size, and location [Rylands, 1993]. Because such variation exists across the Callitrichidae family, it is important to explore species-level differences in all biological parameters, including female reproductive function.

Information concerning reproductive endocrinology is already available for many genera of callitrichid primates. These include *Saguinus* [Brand, 1981; French et al., 1983; Heistermann et al., 1993; Heistermann & Hodges, 1995; Lottker et al., 2004; Ziegler et al., 1987], *Cebuella* [Ziegler et al., 1990], *Leontopithecus* [French & Stribley, 1985, 1987; French et al., 1992], and *Callithrix* [Eastman et al., 1984; French et al., 1996; Harding et al., 1982; Harlow et al., 1983; Hearn & Chambers, 1980; Heger & Neubert, 1987; Hodges & Eastman, 1984; Gilchrist et al., 2001; Tardif et al., 2003]. However, this information is limited, as these data are available for only a small number of species. For the genus *Callithrix*, reproductive endocrine information is available for only two species, the common marmoset, *C. jacchus* and Wied's black tufted-ear marmoset, *C. kuhlii* [French et al., 1996].

Endocrine data are critical to assess reproductive and ovarian status. Measuring plasma, urinary, and fecal hormone levels are performed in short time windows with minimal levels of stress and invasiveness. Measuring endocrine substrates noninvasively is important because it helps prevent unintentional activation of nonaccounted for adrenal-cortical functioning, which may alter gonadal function and hence endocrine output [Jurke et al., 1995]. The development of hormone analyses using radioimmunoassay and enzyme immunoassay (EIA) for samples collected noninvasively (e.g., urine or feces) has made the study of reproductive function in small primates, such as marmosets and tamarins, particularly accessible in both a natural and a laboratory setting. There are no external signs of reproductive state (e.g., ovulation) present in female marmosets and tamarins, and behavioral changes across the reproductive cycle in females are subtle [Stribley et al., 1987; Ziegler et al., 1993]. Urinary progesterone metabolites that are excreted can, however, represent a measure of reproductive status in marmosets.

In an effort to improve the overall understanding of endocrine regulation of reproduction in marmosets, the temporal and endocrine changes of ovarian functioning in the white-faced marmoset, *C. geoffroyi* are reported. Progesterone and urinary progesterone metabolites such as pregnanediol-3-glucuronide (PdG) increase following ovulation in the luteal phase of the marmoset ovarian cycle [French et al., 1996; Hearn, 1983], so normative levels of PdG are used to illustrate typical nonconceptive and conceptive cycles. PdG concentrations can change following administration of contraceptives. For instance, Cloprostenol injections are able to terminate *corpus luteum* function during both an ovarian cycle and pregnancy [Hodges et al., 1987; Summers et al., 1985]. Alternatively, medroxyprogesterone acetate (DMPA) inhibits secretion of gonadotropins, which, consequently, prevents follicular development and ovulation [Rivera et al., 1999]. Information about postpartum conception, interbirth intervals (IBI), and the regulatory functioning of contraceptives are described. Finally, the associations of length of gestation, variation in litter size, and maternal age with PdG concentrations are assessed.

METHODS

Animals

The study included eight adult female white-faced marmosets (age range: 2.22–9.13 years) housed in large breeding enclosures with unrelated adult males. These breeding enclosures were located in colony rooms at the University of Nebraska at Omaha's Callitrichid Research Center (CRC). Colony rooms were maintained at 19.7–22.1°C and on a 12:12 light–dark cycle. Wire-mesh enclosures varied in size depending on the number of offspring in the breeding group, with a minimum of 0.8 m^3 per animal. Enclosures included branches, rope vines, nest boxes, and various enrichment items. All animals were fed twice each day, first at 0800 h and then later in the afternoon. All animal use procedures were approved by the Institutional Animal Care and Use Committee at the University of

Nebraska at Omaha (IACUC 07–033-05-FC), and followed all ethical guidelines and principles endorsed by the American Society for Primatologists. Further details of animal housing and husbandry have been previously reported [Schaffner et al., 1995].

Urine Sample Collection

First-void urine samples from breeding females were collected using noninvasive collection techniques. One to three times a week, a researcher collected samples by holding aluminum pans under a stream of urine in exchange for a food reward between 0730 and 0800 hr. Researchers acted to ensure only one individual's urine was present in each pan. Collected urine samples were transferred into individual microcentrifuge tubes, and the samples were centrifuged at 5,000 rpm for approximately 2 min to separate the urine from any sediment contamination. The urine was transferred into a test tube and stored at –20°C until assayed. Urine sampling reflects total circulating hormones levels since the last time the bladder was emptied, and first-void urine samples are an accurate and readily available representation for the composite daily average of PdG [French et al., 1996].

Pregnanediol-3-Glucuronide EIA

Reproductive parameters were evaluated by monitoring excreted levels of the progesterone metabolite, PdG. Urinary PdG levels were monitored by EIA following the protocol established by Munro et al. [1991], and adapted for marmosets [French et al., 1996]. High and low concentration quality control pools were assayed on each plate. Intra- and interassay coefficients of variation were 22.0% and 6.1% (high, $N = 53$) and 28.0 and 6.8% (low, $N = 53$), respectively. Variation in fluid intake and output was indexed by measuring concentrations of urinary creatinine (Cr). The creatinine assay utilized a modified Jaffé end-point assay, previously described and validated for use in marmosets [French et al., 1996].

Ovarian Cycles and Postpartum Characteristics

Ovulation was confirmed by at least three consecutive samples of PdG concentrations > 10 μg/mg Cr, and pregnancy was defined as PdG concentrations > 10 μg/mg Cr for a minimum of 30 days. The highest and lowest observed PdG concentrations are defined as peak and nadir values, respectively. Day of conception was identified as a nadir PdG concentration immediately preceding a steep increase in PdG concentration that exceeds 10 μg/mg Cr. The interval in days between parturition and the first ovulation is the postpartum ovulation (PPO) interval. Only PPOs following parturition without the interruption of contraceptives were evaluated. The interval in days between parturition and the next estimated day of conception is the birth to conception interval (BCI). Estimated day of conception was the first sample proceeding the beginning of consecutive samples of PdG concentrations > 10 μg/mg Cr. BCIs that were interrupted by the use of contraceptives were not included. The interval in days between the two most recent parturitions is the IBI. IBIs that were interrupted by cycles not carried through parturition or use of contraceptives were not included.

Administration of Contraceptives

Female white-faced marmosets ($N = 8$) were treated with contraceptives to aid in the control of reproduction. The Species Survival Program (SSP) regulates breeding of white-faced marmosets, and contraceptive use coincided with the current breeding priority of a given female–male pair. White-faced marmosets were treated with either cloprostenol ($N = 6$) (Estrumate: Butler Schein Animal Health, Des Moines, IA) or medoxyprogesterone acetate ($N = 8$) (DMPA) injectible suspension (Depo-Provera®: Pfizer, New York, NY) and two females were treated using both methods, though not at the same time. Cloprostenol was used from 2004 to 2008, and DMPA was used thereafter. All treated white-faced marmosets received an intramuscular injection of 0.75 μg cloprostenol (in 1:50 sterile saline day of use; diluted from concentration 250 μg/ml) every 20–30 days in the thigh. Animals were treated approximately every 20–30 days to coincide with ovulation. For DMPA, all treated white-faced marmosets received an intramuscular injection of 20 mg/kg DMPA (from concentration of 150 mg/ml) every 30 days in the thigh. Administration of contraceptives began approximately 30 days following parturition, when risk to the health of the mother and offspring was minimized. Association of Zoos and Aquariums Wildlife Conservation Center determined scheduling of contraceptive injection intervals [Porton & DeMatteo, 2005].

Data Analysis

Means ± SE were reported to describe the temporal and endocrine characteristics of nonconceptive and conceptive ovarian cycles. Ranges and medians were reported when appropriate. Differences in PdG concentrations across trimesters were evaluated by a repeated measures ANOVA of calculated individual means of all pregnancies for each female ($N = 8$), and differences between individual trimesters were confirmed by post-hoc testing. An independent samples t-test was used to access differences in 1st trimester PdG concentrations in a single

female comparing pregnancies carried to parturition ($N = 6$) and pregnancies not carried to parturition ($N = 6$). All the pregnancies that were not carried to parturition were carried at least through the 1st trimester. The comparison of cloprostenol and normal nonconceptive cycling was evaluated by an independent samples t-test comparing peak PdG and full cycle duration means of a single female who both received successive cloprostenol-treated cycles and successive noninterrupted nonconceptive cycles (i.e., consecutive cycles that were not interrupted by administration of cloprostenol or pregnancy) ($N = 1$: Dar). One other female met these criteria but was removed from this analysis because she did not have a breeding partner present and the number of cycles while on cloprostenol was only two (Bes). The comparison of DMPA and normal nonconceptive cycling was evaluated by an independent samples t-test comparing nadir PdG means of a single female presented with multiple DMPA-treated cycles and successive noninterrupted nonconceptive cycles ($N = 1$: Dar). Overall, only a few females in the study had successive nonconceptive cycles ($N = 3$: Uri, Bes, Dar) because they were either actively breeding (and most cycles were conceptive) or on contraception. The association between maternal age and PdG concentrations was evaluated by correlations within three individual females (Lor, Pop, and Dar) who had six or more pregnancies. To assess the effect of litter size on PdG concentrations across pregnancy, differences in small litter size (1 or 2 offspring) and large litter size (3 or 4) were compared using a repeated measures ANOVA (litter size $\times$ trimester). Only females in the study who each had small and large litters ($N = 4$) were used in the analysis. All statistical tests used $P < 0.05$ criterion for statistical significance.

RESULTS

Nonconceptive Ovarian Cycles

Three breeding females exhibited successive nonconceptive PdG cycles with no interruption with use of contraception. In all nonconceptive cycles, patterns of PdG excretion were characterized by a steep increase in PdG over the course of a few days, a steady plateau near cycle peak in PdG excretion, which is typical of ovulation, followed by a steep decrease in PdG over the remaining few days (Fig. 1). Concentrations of PdG excretion were variable among females, with female mean peak concentrations ranging from 18.30 to 37.13 μg/mg Cr and mean nadir concentrations ranging from 0.77 to 2.80 μg/mg Cr (Table I). The average peak-to-peak interval for nonconceptive cycles sampled from three females was 27.82 ± 1.49 days (Table I). The median cycle length was 29 days with an overall range of 22–34 days (Table I).

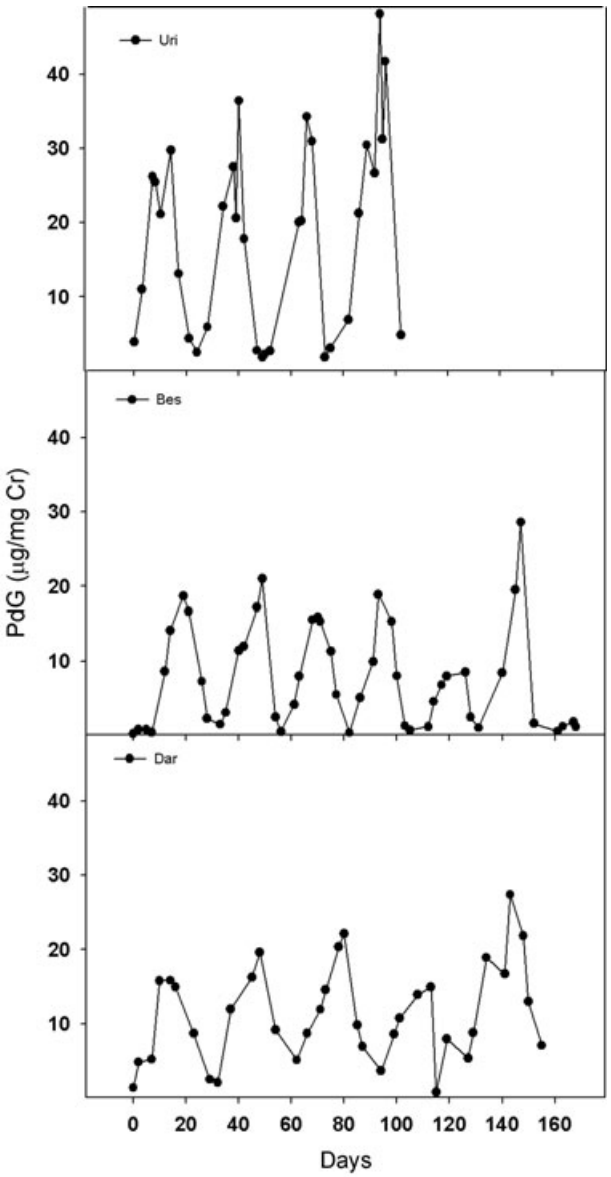

Fig. 1. PdG μg/mg Cr excretion for three individual female white-faced marmosets over consecutive nonconceptive ovarian cycles in days.

Pregnancy

In all conceptive cycles, female PdG excretion was characterized by a sharp increase during the first 10 days from conception, with PdG concentrations remaining elevated through the 2nd trimester, followed by a gradual decrease of PdG concentrations below 10 μg/mg Cr in the 3rd trimester. The average length of gestation was 148.55 ± 1.89 days. The median gestation length was 148 days with an overall range of 145–157 days. Concentrations of PdG (μg/mg Cr) changed significantly over the course of the conception cycle where the 1st and 2nd trimesters PdG concentrations were higher than preconception and 3rd trimester concentrations

TABLE I. Nonconceptive Urinary PdG μg/mg Cr Cycle Details for Breeding White-Faced Marmoset Females

Female	No. of cycles	Peak-to-peak interval (days)	Range (days)	Peak	Nadir
Uri	4	26.67 ± 1.07	26–28	37.13 ± 2.80	2.80 ± 1.19
Bes	6	25.80 ± 1.92	22–31	18.30 ± 2.46	0.77 ± 0.64
Dar	5	31.00 ± 1.47	29–34	19.99 ± 2.25	2.66 ± 1.37
Mean		27.82 ± 1.49	22–34	25.14 ± 2.50	2.07 ± 1.07

All values indicate mean ± SE and total mean reflects mean of individual female means. All nonconceptive cycles excluded periods when female received contraception and conceptive cycles that were not carried out to parturition.

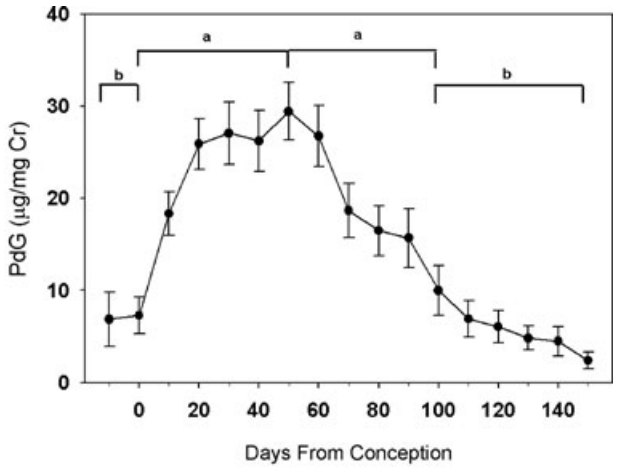

Fig. 2. PdG μg/mg Cr excretion across pregnancies of eight female white-faced marmosets in days postconception. Brackets represent trimesters and the 10 days prior to conception. Different letters represent mean differences in trimester PdG concentrations across pregnancy, where 1st and 2nd trimesters showed higher overall PdG concentrations than the 3rd trimester and 10 days preconception ($P < 0.001$).

(Fig. 2: $F_{3,21} = 21.11$, $P < 0.001$). Overall, peak PdG excretion across the first two trimesters ranged from 20.26 to 53.05 μg/mg Cr, and 3rd trimester PdG concentrations across females ranged from 1.00 to 2.75 μg/mg Cr (Table II). For an individual female, pregnancies carried out to parturition ($N = 6$) did not differ in PdG concentrations during the 1st trimester from pregnancies that resulted in miscarriage ($N = 6$), ($t_{10} = 0.45$, $P = 0.66$).

Postpartum Characteristics

In the pregnancies sampled from the eight females ($N = 29$), 82.8% followed with an immediate PPO. The 17.2% of female PPOs that did not present with an immediate PPO (i.e., an ovulation that did not occur within the normal ovarian cycling range 27.82 ± 1.49) following parturition did eventually present with a normal ovarian cycle. Of the 82.8% of immediate PPOs ($N = 24$), 58.3% resulted in a conceptive cycle. Figure 3 displays an exemplary sample of postpartum ovarian cycling profiles of PdG excretion (Lor: $N = 6$). Overall, the length of 24 immediate PPOs, the interval between parturition and ovulation, was 17.24 days in length (range = 11–31 days, median = 17 days). The median BCI was 10 days with an estimated range of 5–254 days and a mean ± SE of 57.11 ± 8.68 ($N = 17$). The individual BCI of 254 days was considerably larger than the next highest BCI (165) and may be the result of a particularly old male breeding partner (> 15 years in age). The median IBI was 155 days with a range of 149-399 days and a mean ± SE of 202.18 ± 8.67 ($N = 17$). Of the 17 IBIs, 64.7% were within 149–200 days. In the same instance as the BCI, the individual with the highest IBI was an individual female with a particularly old breeding partner older than 15 years in age.

Ovarian Cycling During the Use of Contraceptives

Nonconceptive ovarian cycles in females who received cloprostenol injections showed a peak PdG concentration similar to normal peak PdG concentrations of noninterrupted nonconceptive cycles ($t_{16} = 0.99$, $P = 0.34$), but not cycle duration (i.e., peak-to-peak interval, 19.58 ± 1.12) ($t_{15} = 6.66$, $P < 0.001$) (Fig. 4A). DMPA injections resulted in attenuated levels of PdG concentrations over the course of the ovarian cycle resembling nonovulatory nadir concentrations of PdG ($t_6 = 1.59$, $P = 0.15$). No peaks of PdG concentrations exceeding 10 μg/mg Cr were observed for females receiving DMPA injections over successive nonconceptive cycles (Fig. 4B). These observations illustrate two findings: first, cloprostenol's effect of terminating the *corpus luteum* corresponds to urinary PdG excretion that resembles normal nonconceptive cycling (i.e., similar peak PdG concentration); however, the peak-to-peak interval between cycles is shorter. Second, DMPA's inhibition on follicular development results in a near absence of urinary PdG excretion over the duration of the nonconceptive cycle (i.e., no peaks exceeding the PdG concentration threshold of ovulation) (Fig. 4A and B).

TABLE II. Conceptive Urinary PdG μg/mg Cr Cycle Details for Breeding White-Faced Marmoset Females

Female	No. of cycles	Tri1	Tri2	Tri3	Full	Peak	Nadir
Swe	5	13.52 ± 2.11	12.66 ± 1.51	3.68 ± 1.25	9.56 ± 2.34	20.26 ± 2.36	1.86 ± 0.86
Pop	6	25.33 ± 2.96	16.85 ± 2.51	4.32 ± 0.96	14.80 ± 3.26	38.18 ± 3.64	2.52 ± 1.33
Bes	2	19.74 ± 3.13	22.20 ± 2.85	5.45 ± 1.63	15.15 ± 3.22	32.60 ± 3.43	2.75 ± 1.70
Dar	6	25.32 ± 3.93	38.40 ± 2.17	10.54 ± 3.03	23.86 ± 3.94	49.53 ± 4.36	2.10 ± 0.95
Lor	7	19.30 ± 2.40	19.89 ± 2.77	6.59 ± 1.53	14.72 ± 2.88	35.13 ± 3.76	2.53 ± 1.21
Uri	2	30.75 ± 3.98	24.51 ± 3.40	4.39 ± 1.19	18.91 ± 3.95	53.05 ± 6.73	1.80 ± 1.06
Ann	2	21.12 ± 2.81	23.49 ± 3.13	8.19 ± 1.86	17.01 ± 3.14	42.40 ± 1.91	1.95 ± 1.53
Zep	1	10.66 ± n/a	11.31 ± n/a	2.77 ± n/a	7.91 ± n/a	24.1 ± n/a	1.00 ± n/a
Mean		20.71 ± 2.98	21.16 ± 2.60	5.74 ± 1.60	15.24 ± 3.15	36.56 ± 4.02	2.06 ± 1.24

All values indicate mean ± SE and total mean reflects mean of individual female means. Only conceptive cycles carried out to parturition were used. Tri refers to trimester and Full refers to the full duration of the conceptive cycle.

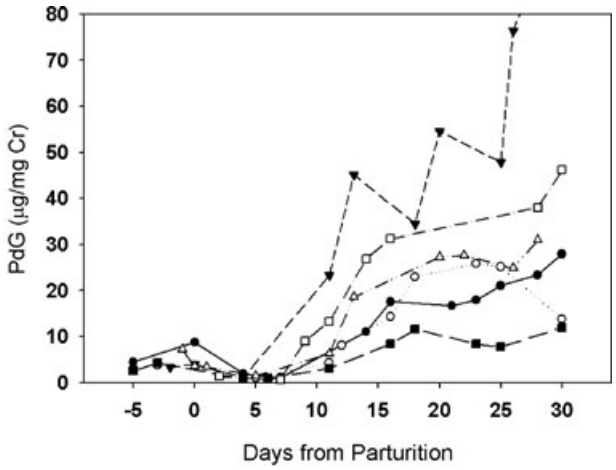

Fig. 3. PdG μg/mg Cr excretion over 30 days from parturition for an exemplary female (Lor). Each line represents one cycle following parturition, with all five cycles representing postpartum ovulations (PPOs).

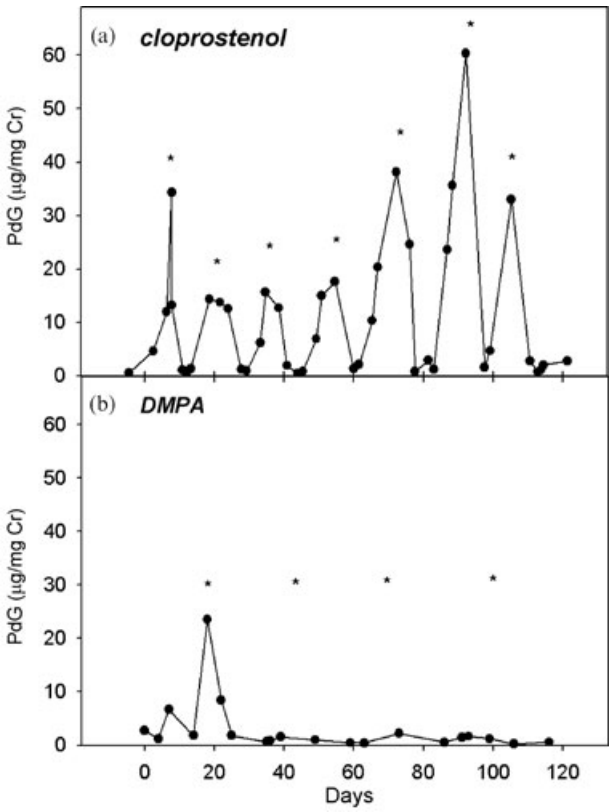

Fig. 4. Exemplary individual female white-faced marmoset consecutive nonconceptive ovarian PdG μg/mg Cr cycles during administration of contraceptives cloprostenol (panel A) (Dar) and DMPA (panel B) (Dar). Asterisks designate day of administration.

PdG Concentrations and Maternal/Gestational Parameters

The relationship between maternal age and PdG concentrations was evaluated for each individual female who had six or more pregnancies ($N = 3$). For each of the three females, there was no relationship between the increase in age with successive pregnancies and PdG concentrations at any trimester (all Ps > 0.20). Mothers who had both large litters and small litters did not differ in overall PdG concentration ($F_{1,3} = 0.21, P = 0.68$) or by litter size across trimesters ($F_{2,6} = 0.92, P = 0.45$). These mothers show a similar relationship of PdG change with the larger full sample where PdG is highest during the 1st and 2nd trimesters and drops significantly during the 3rd ($F_{1,6} = 20.17, P < 0.01$). Overall, PdG concentrations at any trimester were not associated with length of gestation (all Ps > 0.13).

DISCUSSION

These data illustrate endocrine and temporal dynamics associated with normative conceptive and nonconceptive ovarian cycles in female white-faced marmosets. Pregnancy was detectable in all conceptive cycles (in all cases of successful parturition, PdG remained over 10 μg/mg into or through the 2nd trimester). PdG concentrations were highest during the first two trimesters of gestation. The 3rd trimester was marked by a gradual decrease in PdG concentrations below 10 μg/mg Cr. Ovulation was detectable in all nonconceptive cycles by a sharp and prolonged increase in PdG excretion with an

eventual decline to near zero. Two contraceptive regimens had different effects on ovarian cycling dynamics. During treatment with cloprostenol, cycle durations were shorter (i.e., shorter peak-to-peak intervals), but ovulation was detectable with similar concentrations of peak PdG to a normal nonconceptive cycle. Conversely, ovulation was undetectable during administration of DMPA, and PdG concentrations remained near zero over the course of DMPA treatment. Length of gestation, maternal age, and litter size were not associated with PdG concentrations across the conceptive cycle.

These data for *C. geoffroyi* reveal similarities in reproductive parameters with *C. kuhlii* and *C. jacchus*. The nonconceptive ovarian cycle length of 27.82 ± 1.49 days in *C. geoffroyi* is similar to the 25-day cycle reported for *C. kuhlii* [French et al., 1996] and 30-day cycle reported for *C. jacchus* [Harding et al., 1982]. Harlow et al. [1983] observed a 27-day cycle that varied by a partner status to a degree of a couple days (single = 28.08, partner = 25.82) in *C. jacchus*. The nonconceptive ovarian cycle lengths reported for marmosets are similar to nonconceptive ovarian cycle lengths reported for other callithrichids [*S. oedipus*, 23.6 days: French et al., 1983; *Cebuella*, 27.0 days: Ziegler et al., 1990; *S. fuscicollis*, 25.7 days: Heistermann & Hodges, 1995]. Likewise, the mean gestation length for *C. geoffroyi* 148.55 ± 1.89 was similar to other *Callithrix* [*C. kuhlii*, 143.1 days: French et al., 1996; *C. jacchus*, 144.0 days: Hearn, 1983]. Unlike nonconceptive cycles, length of gestation varied more with other callitrichids ranging from 125 to 130 days in *Leontopithecus* [French & Stribely, 1985; Kleiman, 1977] to over 180 days in *Saguinus* [Ziegler et al., 1987]. Overall, the PdG and temporal dynamics of ovarian cycling among callitrichids are comparable despite variation in ecological and physical characteristics.

Following parturition, the BCI varied considerably for actively breeding females. Of the 17 cases where conception followed birth without the interruption of contraception administration, 65.7% of those intervals were shorter than 20 days postparturition, with one additional interval shorter than 25 days and the other seven between 38 and 254 days. A total of 58.3% of first conceptions following recent birth occurred around the time of the first PPO, which is considerably lower than the postpartum conception rate of 91.7% reported for *C. kuhlii* [French et al., 1996]. Given the similar reproductive energetics shared between the two marmoset species (e.g., lactation, twinning, and parental behavior), the explanation for this lower fertility during the first PPO warrants further exploration. In some instances, old breeding partners presented with higher BCIs; however, in other cases, long BCIs were also associated with females paired with younger males. This inconsistency suggests that male age does not necessarily predict long BCIs.

During pregnancy, urinary PdG concentrations remained high through the first two trimesters and declined gradually during the 3rd trimester. This pattern is similar to results reported for *C. kuhlii* [French et al., 1996] and *C. jacchus* [Eastman et al., 1984] and typical of most other mammals. Around the onset of the 3rd trimester, the source for the production of progesterone switches from the maternal ovaries to the placenta and progesterone levels therefore decrease [Hearn & Chambers, 1980; Hodges et al., 1983]. These data suggest this transition occurred at 90–100 days postconception, which is roughly similar to reported findings of 70–80 days for *C. kuhlii* and 110–120 days for *C. jacchus*.

Administering contraceptives affected the normative endocrine patterns and temporal dynamics of ovarian functioning. White-faced marmosets treated with cloprostenol still had detectable ovulation via a peak in PdG concentration. Cloprostenol prevents pregnancy by directly regressing the *corpus luteum*, progesterone synthesis, and release. Consequently, the excretion of progesterone metabolites are rapidly suppressed [Summers et al., 1985]. However, the time scale we used to evaluate PdG excretion, at the order of a PdG sample every few days, was not short enough to evaluate whether a rapid suppression of PdG excretion occurred following cloprostenol administration, which the decline in PdG would presumably occur within or near a day [Summers et al., 1985]. Apart from a potentially rapid decline in PdG excretion, which may lead to the shorter overall cycle durations observed in our data, cloprostenol-terminated ovarian cycles show similar properties to normative nonconceptive ovarian cycles in that peak PdG concentrations were the same. DMPA administration, alternatively, functions by inhibiting follicular development that, in turn, prevents ovulation from occurring [Rivera et al., 1999]. Therefore, marmosets treated with DMPA showed nondetectable ovulation by means of PdG excretion because the DMPA-terminated ovarian cycles show little to no PdG excretion. This pattern is vastly dissimilar to normative nonconceptive ovarian cycles. The two most commonly used contraceptive methods in most New World monkeys, including marmosets, are progestin-based implants or injections [Mohle et al., 1999]. Contraceptive use in primates is usually centered on progestin implants, such as melengestrol acetate, because administration of an effective DMPA dosage and interval frequency in primates can be difficult to determine [Porton & DeMatteo, 2005]. Both cloprostenol and DMPA successfully prevented pregnancy for the white-faced marmosets in this study. There were only two occurrences of a potentially failed DMPA efficacy (none for cloprostenol), and these resulted pregnancies were not included in the pregnancy data analysis.

Reproductive parameters including litter size, maternal age, and length of gestation were not

associated with PdG excretion across the conceptive cycle in white-faced marmosets. Other findings have shown that plasma progesterone concentrations during gestation were higher for larger litter sizes in other species, such as sheep, for instance [Butler et al., 1981]. This relationship likely exists because large litter sizes in many mammals is associated with an increase in placental mass, where the majority of the progesterone is produced during late gestation. However, the positive relationship between litter size and progesterone output appears to be weak in marmosets and there is a limitation of comparing plasma progesterone concentrations and urinary PdG concentrations. Furthermore, the association of litter size, PdG concentrations, and increased placental mass is less predominant in marmosets because their litter sizes are restricted to no more than four (with three or fewer surviving) with two being the normal offspring output in the wild and three being increasingly prevalent in captivity [Tardif et al., 2003]. In marmosets, placental weight is not necessarily higher with a triplet pregnancy compared to a twin pregnancy, and the placenta is actually found to be more efficient (i.e., allocation of placental mass per mass of fetus is much lower) for a triplet pregnancy compared to twins [Rutherford & Tardif, 2008]. Because the mass of the placenta does not augment to accommodate the potentially higher energetic demand of increased litter sizes in marmosets (e.g., increased litter mass), the overall progesterone output from the placenta is not necessarily tied to the number of fetuses. Overall, the magnitude of PdG excretion did not appear to decline with female age for conceptive and nonconceptive ovarian cycles, indicating potentially prolonged fertility among female marmosets under optimal metabolic conditions, where resources are plentiful and obtained with low effort such as conditions that normally exist in captivity. It would be meaningful to further investigate whether the relationship between PdG and reproductive parameters are consistent across natural and captive environments.

These endocrine and reproductive data can provide important insight in the overall conservation of many understudied primate species, and monitoring pregnancies are critical for ensuring healthy and diverse infant development. Previous work has linked decreases in PdG concentrations during the 3rd trimester with infant survival and maternal behavior in titi monkeys, suggesting that decreased progesterone and other sex steroids might play an important role in readying mothers for necessary parental behaviors needed to ensure healthy infant development [Jarcho et al., 2012]. In Wied's black tufted-ear marmosets, infant survival did not differ by prepartum PdG concentrations, but mothers with high prepartum estradiol (E_2) presented with lower infant survival and lower maternal carrying effort, a key behavioral indicator of maternal competency [Fite & French, 2000]. The data presented in this study show that PdG concentrations were not associated with miscarriages, litter size, or maternal age; however, these data do not necessarily rule out that similar PdG or E_2 changes may contribute to overall maternal behavior and infant survival in white-faced marmosets as well. Other hormone variation during the course of pregnancy has been linked to infant development in white-faced marmosets including androgens [Smith et al., 2010] and glucocorticoids [Mustoe et al., 2012]. Overall, neuroendocrine systems act as an important regulator of maternal behavior [review: Saltzman & Maestripieri, 2011], so it is essential to consider the enveloping relationship of maternal hormonal systems, maternal behavior, and infant development when evaluating the contraceptive, captive management, and conservation needs of white-faced marmosets.

Monitoring urinary PdG excretion is a useful and noninvasive method to track reproductive functioning in white-faced marmosets. Nonconceptive ovulation, pregnancy, and contraceptive effectiveness are each observable through patterns of urinary PdG excretion. It appears that white-faced marmosets share similar characteristics of ovarian cycling and reproductive parameters with other marmosets, and these marmosets appear to share, to a lesser extent, similar characteristics of ovarian cycling and reproductive parameters with other members of the callitrichid family. The callitrichid family is a rich and diverse family of behavioral ecology, social systems, and life strategies, yet conservation of the endocrine and temporal dynamics of many reproductive functions persist.

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