Urinary endocrine monitoring of the ovarian cycle and pregnancy in Goeldi's monkey (*Callimico goeldii*)*

J. B. Carroll†, D. H. Abbott‡, L. M. George‡, J. E. Hindle‡ and
R. D. Martin§

†Jersey Wildlife Preservation Trust, Les Augres Manor, Trinity, Jersey, Channel Islands, UK;

‡MRC/AFRC Comparative Physiology Research Group, Institute of Zoology, Regent's Park,
London NW1 4RY, UK; and §Anthropologisches Institut und Museum, Universität Zürich-Irchel,
Winterthurerstrasse 190, CH-8057, Zürich, Switzerland

Summary. A non-invasive study of urinary hormones in 6 captive female Goeldi's monkeys provided accurate information on reproductive function. Conjugated oestrone accounted for 80–85% of the urinary oestrone and oestradiol measured. Radioimmunoassay measurements of conjugated oestrone provided a reliable indicator of cyclic ovarian function (mean cycle length: 24.1 ± 0.9 days; $n = 9$) and pregnancy (gestation: 145, 155 days; $n = 2$). Measurements of urinary progesterone and pregnanediol glucuronide were only reliable as indicators of ovarian cyclicality. Elevations in urinary conjugated oestrone coincided with luteal-phase elevations of urinary progesterone and pregnanediol glucuronide. Urinary LH concentrations provided no indication of pituitary activity. However, the frequencies of female sexual solicitations of males were maximal when oestrone conjugate concentrations rose, suggesting a peri-ovulatory period. Ovulation was suppressed in 1 of 3 subordinate females housed in male–female–female trios.

Keywords: monkey; oestrogen; progesterone; pregnanediol glucuronide, LH; ovarian cycle; pregnancy; urinary hormones

Introduction

Goeldi's monkey, *Callimico goeldii*, is a rare and little-studied South American primate. It is sparsely distributed within a wide range in the upper Amazon basin (Hershkovitz, 1977) and has only been kept in captivity in any numbers since the mid-1970s (Beck *et al.*, 1982; Carroll, 1982). This species is of considerable scientific interest because it is in a monospecific genus which is regarded taxonomically as intermediate between the two New World primate families of the Callitrichidae (marmosets and tamarins) and the Cebidae (the 'true' monkeys) (Hill, 1959; Rosenberger, 1981; Dutrillaux *et al.*, 1988).

Captive breeding programmes have been recently established for Goeldi's monkey (Andrews, 1986; Rettberg, 1986) to improve the management and conservation of this unusual primate species in captivity. Breeding success will benefit from accurate information on the reproductive biology of this primate and from the availability of reliable and practical methods for monitoring reproductive function. Goeldi's monkeys, however, do not demonstrate any overt signs of their reproductive status, such as menstruation or a change in sex skin swelling or coloration. Females have a behavioural oestrous cycle of about 22–24 days, a post-partum oestrus, a gestation length of about 150–160 days, show no breeding seasonality and produce a single offspring at each birth (Lorenz, 1972; Beck *et al.*, 1982; Carroll, 1982). Monitoring of hormonal changes therefore represents the

*Reprint requests to Dr D. H. Abbott.

only reliable way to detect ovulation and pregnancy. Hodges *et al.* (1979, 1981) have shown the usefulness of urinary hormone analysis in delineating the reproductive endocrinology of diverse Old and New World primates. Measurement of urinary oestrogens has proved particularly informative as regards ovarian cyclicity and pregnancy in New World primates (Hodges *et al.*, 1981; Eastman *et al.*, 1984; French *et al.*, 1984; Pryce *et al.*, 1988). Goeldi's monkeys have proved no exception and the first preliminary studies showed that measurements of urinary oestrone conjugates (Carroll *et al.*, 1989; Ziegler *et al.*, 1989) and total urinary oestradiol (Christen *et al.*, 1989) provided useful initial indicators of ovarian cyclicity and pregnancy.

The aim of this study was to (i) identify the major oestrone and oestradiol-17 β metabolites excreted in the urine of Goeldi's monkeys during the ovarian cycle and pregnancy from a representative number of females; (ii) demonstrate that the measurement of the principal urinary oestrone or oestradiol-17 β metabolite provided a reliable marker of ovarian cyclicity and pregnancy; (iii) compare the usefulness of measurements of urinary bioactive luteinizing hormone (LH), progesterone and pregnanediol glucuronide as indicators of ovarian cyclicity or pregnancy; and (iv) determine whether ovarian cycles are suppressed in socially subordinate female Goeldi's monkeys. A concurrent behavioural study examined sexual behaviour changes across the ovarian cycle to ascertain whether a peak in sexual activity occurred at approximately the time the endocrine parameters indicated ovulation.

Materials and Methods

Animals

Six adult female Goeldi's monkeys were used in this study (Table 1: June 1984–April 1986). The animals were housed in the marmoset buildings or the Behavioural Research Unit at the Jersey Wildlife Preservation Trust (Mallinson, 1977; Oliver, 1983) and their husbandry has been previously described (Carroll, 1982). The females were kept in male–female pairs ($n = 2$) or in trios of 1 male and 2 females ($n = 4$). Three of the females were also studied before the introduction of a male (in one pair and one trio: Table 1).

Table 1. Details of Goeldi's monkey females in the study groups and times of male introduction, urine sampling and behavioural observations

Female	Group	Period of urine sampling	Period of behavioural observation
M440	Single	12 June–16 August 1985	—
M440	Paired with male on 17 August 1985	17 August–26 November 1985	17 August–16 September 1985
M820	Paired with male on 3 July 1984	27 June–7 August 1984	3 July–2 August 1984
M947	Single	12 June–20 July 1985	—
M947 } M922 }	Trio (No. 3): male introduced on 3 November 1985	24 October–27 February 1986	November–December 1985
M866 } M872 }	Trio (No. 2): male introduced on 16 June 1985	17 February–21 April 1986	—
M786 } M817 }	Trio (No. 1): male introduced on 5 September 1984	—	—

Urine sample collection

Urine rather than blood samples were collected to avoid the stress induced in blood sampling non-habituated monkeys. Samples were collected by using a syringe to remove urine from the cage floor of an isolated female, or by

using a cup to catch the first morning urine as it was voided. In the former situation, samples were collected between 07:30 and 11:00 h and, in the latter, shortly after the room lights were switched on at 07:30 h. Samples were normally collected at intervals of 1–3 days for periods of 1.5–5.5 months (see Table 1 for details). Directly after collection, urine samples were stored in polypropylene tubes at -30°C until assayed.

Creatinine determination

The creatinine content of each urine sample was determined to control for variations in the volume and concentration of the voided urine (Hodges & Eastman, 1984). Urinary hormone concentrations were expressed as mass/mg creatinine (mg Cr).

Immunoreactive oestrogens

Hydrolysis of oestrogen conjugates. Primates generally, and callitrichid monkeys in particular, excrete high proportions of oestrogen in a conjugated form, such as glucuronides, sulphates and other unidentified conjugates (Hodges *et al.*, 1979, 1983; Hodges & Eastman, 1984). To determine the most abundant urinary oestrone or oestradiol-17 β metabolite during the follicular and luteal phases of the ovarian cycle and during early pregnancy, sequential enzyme hydrolysis and solvolysis was carried out on the urine samples from the females. Enzyme hydrolysis with β -D-glucuronide glucuronosylhydrolase (Sigma No. G3510; Sigma Chemical Co., Poole, Dorset, UK (no sulphatase activity); activity 600 000 Fishman units/g; 300 Fishman units added per tube to 50 μl portions of Goeldi's monkey urine diluted 1:20), then with sulphatase (Sigma Chemical Co.; 20 000 Fishman units/g; 20 Fishman units added per tube), followed by solvolysis, was performed sequentially (Eastman *et al.*, 1984) on urine samples from 3 females in the follicular phase of the ovarian cycle, 3 females in the luteal phase and one female during the early stages of pregnancy. Solvolysis (Hawkins & Oakey, 1974) is a stringent procedure known to liberate most oestrogen conjugates (Eastman *et al.*, 1984; Hodges & Eastman, 1984). The oestrone and oestradiol-17 β released by each procedure were measured by specific radioimmunoassay (Eastman *et al.*, 1984) and are expressed as $\mu\text{g}/\text{mg Cr}$ in Table 2. Oestrone accounted for 90–97% of the urinary oestrone and oestradiol-17 β measured. Between 85 and 96% of the urinary oestrone measured was released by β -glucuronidase enzyme hydrolysis (glucuronide fraction in Table 2), indicating oestrone glucuronide as the principal urinary oestrogen metabolite, of the two oestrogens measured, at all stages of the reproductive cycle.

Procedural losses during extraction were estimated in triplicate by the addition of tracer amounts (2000 c.p.m./20 μl) of [^3H]oestrone (sp. act. 91 Ci/mmol) to 50 μl of a diluted urine pool and recoveries were $86.0 \pm 1.8\%$ (mean $\pm$ s.e.m., $n = 5$; these values relate to the recovery of unconjugated fractions in Table 2). The efficiency of each hydrolysis step was determined by hydrolysing trace amounts of [^3H]oestrone-3-glucuronide (sp. act. 53 Ci/mmol) and [^3H]oestrone-3-sulphate (sp. act. 60 Ci/mmol) to oestrone in 50 μl diluted urine. The recoveries obtained were as follows: β -glucuronidase, $68.9 \pm 10.4\%$ ($n = 5$) and $3.5 \pm 0.4\%$ ($n = 5$), respectively, which relate to the glucuronide fraction in Table 2; sulphatase, $75.9 \pm 6.3\%$ ($n = 4$) and $69.4 \pm 9.7\%$ ($n = 5$), respectively, which relate to the sulphate fraction in Table 2; solvolysis, $79.2 \pm 2.4\%$ ($n = 5$) and $65.7 \pm 6.5\%$ ($n = 5$), respectively, which relate to the residual fraction in Table 2. These results confirmed the specificity of β -glucuronidase in cleaving glucuronide conjugates (Eastman *et al.*, 1984).

Oestrogen conjugate assay. On the basis of the above data, the oestrogen content of the urine of study females was monitored by a direct, non-extraction radioimmunoassay for oestrone-3-glucuronide (Hodges & Eastman, 1984; Hodges *et al.*, 1984). Duplicate portions (5–20 μl) of urine were taken for assay. The antiserum used in the assay was raised in a rabbit against oestrone-3-glucuronide-BSA and showed the following cross-reactivities: 120% for oestrone, 62% for oestrone-3-sulphate, 2.0% for 17 β -oestradiol-3-monosulphate, <0.1% for 17 β -oestradiol-17-sulphate, 17 β -oestradiol-17-glucuronide and oestradiol-16 α -glucuronide. Serial dilutions of Goeldi's monkey urine (0.1–10 μl) from 2 females in the luteal phase of the ovarian cycle and 1 pregnant female gave displacement curves parallel to that obtained with oestrone glucuronide standards. The mean $\pm$ s.e.m. recovery of unlabelled oestrone- β -D-glucuronide (sodium salt, Sigma No. E1752; values expressed as $\mu\text{g base}/\text{mg Cr}$) added to a Goeldi's monkey urine pool was $95.5 \pm 6.5\%$ ($n = 7$) over the standard curve range of 29.7–1900 pg/tube. The sensitivity limit of the assay at 90% binding was 5.8 pg/tube. Inter-assay precision was 10.0% with a urine pool of $9.5 \pm 0.4 \mu\text{g}/\text{mg creatinine}$ (8 assays). Intra-assay precision was 4.0% with a urine pool of $3.70 \pm 0.03 \mu\text{g}/\text{mg creatinine}$ ($n = 24$).

Progesterone immunoreactivity

Urine samples (100 μl) were extracted with 1 ml petroleum ether before assay. Urinary progesterone in Goeldi's monkey urine was measured using a modification of the heterologous enzyme immunoassay procedure (Sauer *et al.*, 1986) described by Hodges *et al.* (1988). Briefly, the antiserum was raised in a sheep against progesterone-11 α -hemisuccinate conjugated to ovalbumin and showed the following cross-reactivities: 11 α -hydroxyprogesterone 52.9%, 5 β -pregnane-3,20-dione 36.3%, 5 α -pregnane-3,20-dione 4.7%, other C21 steroids <2%, cortisol <0.1% and C19 and C18 steroids <0.1%.

Serial dilutions of Goeldi's monkey urine (1.25–10 μl), from 3 females during the follicular phase and 3 females during the luteal phase gave displacement curves parallel to that obtained with progesterone standards (6.5–20 pg/

Table 2. Urinary oestrogen conjugates during the ovarian cycle and one conception cycle in Goeldi's monkeys

	Oestrone ($\mu\text{g}/\text{mg Cr}$)				Oestradiol ($\mu\text{g}/\text{mg Cr}$)			
	Unconjugated fraction	Glucuronide fraction	Sulphate fraction	Residual fraction§	Unconjugated fraction	Glucuronide fraction	Sulphate fraction	Residual fraction§
Ovarian cycle								
Follicular phase*	Mean conc. ($\pm \text{s.e.m.}$)	1.00 $\pm$ 0.34	0.07 $\pm$ 0.02	0.02 $\pm$ 0.01	—	0.07 $\pm$ 0.02	0.02 $\pm$ 0.01	0.01 $\pm$ 0.01
	Mean % of $E_1 + E_2$	81.8	5.6	2.5	—	6.1	1.6	1.0
Luteal phase†	Mean conc. ($\pm \text{s.e.m.}$)	8.73 $\pm$ 0.93	0.74 $\pm$ 0.11	0.11 $\pm$ 0.02	0.04 $\pm$ 0.01	0.88 $\pm$ 0.08	0.22 $\pm$ 0.03	0.01 $\pm$ 0.01
	Mean % of $E_1 + E_2$	79.5	6.7	1.0	0.4	8.0	2.0	0.1
Conception cycle								
1–14 days after conception‡	Mean conc. ($\pm \text{s.e.m.}$)	10.30 $\pm$ 2.42	1.50 $\pm$ 0.36	0.08 $\pm$ 0.01	—	0.20 $\pm$ 0.02	0.07 $\pm$ 0.03	0.01 $\pm$ 0.01
	Mean % of E_1	82.8	12.0	0.6	—	2.1	0.9	0.1

*14 samples from 3 females.

†19 samples from 3 females.

‡15 samples from 1 female.

§Oestrogen liberated by solvolysis after enzyme hydrolysis.

¶Mean percentage of all oestrogen measured ($E_1 + E_2$): E_1 = oestrone; E_2 = oestradiol.

well). The recovery of unlabelled progesterone added to a Goeldi's monkey urine pool was $100.4 \pm 7.2\%$ ($n = 14$) over the standard curve range. The sensitivity limit of the assay at 90% binding was 6.5 pg/well: inter-assay precision was 8.1% (12 assays) and intra-assay precision was 1.8% ($n = 22$).

High-pressure liquid chromatography (h.p.l.c.)

As progesterone was not identified as an extracted urinary progestagen from gas chromatography/mass spectrometry (GC/MS) analysis of Goeldi's monkey urine (Christen *et al.*, 1989), h.p.l.c. analysis was performed to provide additional circumstantial evidence for the presence of progesterone. A chromatographic system, consisting of two single piston pumps (501 and 510; Waters, Milford, MA, USA) and an automated gradient controller, was fitted to a Lichrosorb straight-phase silica 60 column (250 $\times$ 4 mm, 10 μ m particle size; Merck 50387). A linear solvent gradient strength of 3.3%/min (0–2% methanol in *n*-hexane:chloroform (70:30 v/v) within 30 min at a flow rate of 2 ml/min) was used to separate the urinary progestagens (E. Möstl, personal communication).

Two portions of 1 ml of urine from one female Goeldi's monkey in the luteal phase of her ovarian cycle were prepared for chromatography by extracting with 10 ml freshly distilled diethyl ether, after the addition of approximately 10,000 c.p.m. [^{3} H]progesterone. The ether was dried down and the aliquots reconstituted in 150 μ l *n*-hexane:chloroform (70:30 v/v). Then 100 μ l of each solvent aliquant was injected onto the column and fractions were collected by an automatic fraction collector (Frac-100; Pharmacia, Uppsala, Sweden) over a 30 min period. The fractions were dried down and reconstituted in assay buffer. Part (100 μ l) of each fraction was taken to measure recovery of the added [^{3} H]progesterone and a further 30 μ l were taken for assay. [^{3} H]Progesterone and immunoreactive progesterone were both found in fractions 23 and 24, supporting the validity of urinary progesterone measurement in female Goeldi's monkeys. This was the only immunoreactive peak found. The elution pattern of this peak was separate and distinct from the ^{3} H-labelled and immunoreactive elution patterns of other related progestagenic compounds run separately through the column, such as 5 α -pregnan-3,20-dione (fraction 12), 5 β -pregnan-3,20-dione (fraction 16), pregnanolone (fraction 31), 4-pregnen-20 α -ol-3-one (20 α -dihydroprogesterone: fraction 36) and 5 β -pregnanediol (fractions 45 and 46).

Pregnanediol glucuronide

Urine samples (2.5 μ l) were submitted to a direct non-extracted enzyme immunoassay for pregnanediol glucuronide, as previously described (Hodges & Green, 1989). Briefly, the antiserum, raised in a rabbit against pregnanediol-3 α -glucuronide-BSA showed major cross-reaction only with 5 β -pregnanediol (45.1%), 5 β -pregnanedione (4.1%), 20 α -dihydroprogesterone (12.1%), 5 β -pregnanetriol (<0.1%), 17 α -hydroxyprogesterone (0.1%) and oestradiol-17-glucuronide (0.1%). Serial dilutions of Goeldi's monkey urine (0.625–5 μ l), from 3 females in the follicular phase and from 3 females in the luteal phase gave displacement curves parallel to that obtained with pregnanediol glucuronide standards (12.5–6000 pg/well). The recovery of unlabelled 5 β -pregnane-30 α ,20 α -diol glucuronide (in free acid form: Sigma No. P3635) added to a Goeldi's monkey urine pool was $102.8 \pm 9.7\%$ ($n = 16$) over the standard curve range. The sensitivity limit of the assay at 90% binding was 12.5 pg/well. Inter-assay precision was 14.2% (8 assays) and intra-assay precision was 9.7% ($n = 16$).

LH bioactivity

Bioactive LH was measured in Goeldi's monkey urine using a modified mouse Leydig cell bioassay (Hodges *et al.*, 1987; Abbott *et al.*, 1988). Duplicate portions of urine (2.5–5 μ l) at two appropriate doubling dilutions, with the initial dilution ranging from 1:50 to 1:1000, and triplicate aliquants of reference standard (6.25–200 i.u./100 μ l of the 2nd International Reference Preparation of the human pituitary gonadotrophin; code no: 78/549) were incubated with a suspension of Leydig cells (1×10^5 cells/200 μ l) obtained from 5- to 7-week-old mice (Tucks No. 1; an outbred strain now bred at the Institute of Zoology). Testosterone production by the Leydig cells was measured by radioimmunoassay (Hodges *et al.*, 1987). Intra- and inter-assay coefficients of variation were 13.4% (15 assays) and 9.3% ($n = 15$), respectively. The sensitivity limit of the bioassay was 10 μ i.u./tube. Serial dilutions of Goeldi's monkey urine gave displacement curves parallel to that obtained with the human reference preparation.

Estimation of the presumed time of ovulation

Sustained elevations of urinary oestrone conjugates above 5 μ g/mg Cr were designated as forming part of the luteal phase of the ovarian cycle until the time when a rapid and sustained fall in urinary oestrone conjugate values occurred, to below 5 μ g/mg Cr. The follicular phase then continued until urinary oestrone conjugate concentrations rose above 5 μ g/mg Cr again. Ziegler *et al.* (1989) showed that the urinary LH peak occurred at the time of the onset of the rise in concentrations of urinary oestrone conjugates, and so an estimated time of presumed ovulation was taken as the day before urinary oestrone conjugate concentrations reached 5 μ g/mg Cr. This estimate may be inaccurate by 1–2 days, but this is a sufficiently small source of error for the purpose of this study.

The estimated time of presumed ovulation was further supported by the fact that the expected increase in female sexual solicitations of males occurred at this time (Table 4) and that, immediately following this time, prolonged elevations of urinary oestrone conjugate concentrations occurred during all 3 observed conception cycles (e.g. Fig. 3).

Behavioural observations

Four of the 6 females were observed in their home cages for 1 month after the introduction of a male (2 pairs and 1 trio: Table 1). Each pair or trio was observed for 1 h every 2 days, between 11:00 and 15:30 h, as previously described (Carroll, 1985). Quantitative behavioural measurements were recorded on check-sheets and the following behavioural categories were scored (Carroll, 1985). Female proceptive behaviour was sexual solicitation of the male by the female whereby (i) the female approaches the male and stands bipedally, with her sternal area close to the male's face, and arms held at right angles to the body (bipedal stance: Carroll, 1985) and (ii) the female stands quadrupedally in front of the male, with her posterior region close to his head, frequently looking back at the male over her shoulder, sometimes reaching back towards the male with one of her hands (present: Carroll, 1985). Copulation was typical primate copulation with accompanying pelvic thrusts by the male. Statistical analysis of the behavioural data was made using the Kruskal–Wallis one-way analysis of variance and the Mann–Whitney U test (Siegel, 1956). For the purposes of these behavioural analyses, the follicular phase of the cycle was taken as Days –11 to –3 from the estimated day of ovulation, the peri-ovulatory period was taken as Days –2 to 2 and the luteal phase was taken as Days 3–12.

Results

Ovarian cycle

Figure 1 illustrates the mean values of urinary hormones during the 24-day ovarian cycle (9 cycles from 4 females; Carroll *et al.* 1989). These excreted urinary products are presumed to be of ovarian origin. Measurement of each of the three urinary steroid products gave a clear indication of ovarian cyclicity, with values clearly elevated during the luteal phase (Fig. 2; Table 3). The approximate time of ovulation was estimated as the day before urinary oestrone conjugate concentrations rose over 5 µg/mg Cr, at about the same time as the urinary LH peak found by Ziegler *et al.* (1989). Unfortunately, in this study there was no measurable elevation in urinary LH concentrations during the cycle and many urinary LH values were below 1.7 m.i.u./mg Cr. Nevertheless,

Fig. 1. Mean $\pm$ s.e.m. urinary concentrations of (a) oestrone conjugates and (b) progesterone and pregnanediol glucuronide (PDG) during the ovarian cycle in female Goeldi's monkeys (9 cycles from 4 females). The estimated time of ovulation is taken as the day before urinary oestrone conjugates rose above 5 µg/mg Cr.

Fig. 2. Urinary concentrations of oestrone conjugates (●—), progesterone (○---) and pregnanediol glucuronide (△...) during individual ovarian cycles in a typical female Goeldi's monkey, M947.

Table 3. Urinary concentrations (grand means $\pm$ s.e.m.) of oestrone conjugates, progesterone and pregnanediol glucuronide in the follicular and luteal phases of the ovarian cycle in 4 female Goeldi's monkeys across 9 ovarian cycles

Urinary hormone measurement	Phase of ovarian cycle	
	Follicular (Days -11 to -1)	Luteal (Days 1 to 12)
Oestrone conjugates ($\mu\text{g}/\text{mg Cr}$)	1.18 ± 0.17	11.79 ± 1.02
Progesterone ($\text{ng}/\text{mg Cr}$)	36 ± 5	116 ± 12
Pregnanediol glucuronide ($\mu\text{g}/\text{mg Cr}$)	0.32 ± 0.05	1.32 ± 0.11

the consistent and repeatable finding of elevated urinary progesterone and pregnanediol glucuronide concentrations concomitantly with elevations in urinary oestrone conjugate concentrations strongly suggested that elevations in the latter reflected the post-ovulatory, luteal phase of the ovarian cycle. Figure 2 shows the very similar hormonal profiles of all 3 urinary steroid products, in a typical female M947, clearly delineating the ovarian cycles.

Fig. 3. Urinary concentrations of (a) oestrone conjugates and (b) progesterone and pregnane-3,20-diol glucuronide (PDG) following parturition and before and after conception in Monkey M866. The arrows indicate the time of parturition (1), death of sucking infant (2), estimated time of conception (3). In (b), ● and ▲ denote values exceeding 300 ng/mg Cr (progesterone) or 3 µg/mg Cr (pregnanediol glucuronide), respectively.

Pregnancy

Urinary oestrone conjugate concentrations were consistently elevated after conception in 3 females (M866 (Fig. 3), M440 and M947). In the 4th pregnant female in the study (M872), urinary oestrone concentrations were 130 ± 7 µg/mg Cr (range: 68.0–213 µg/mg Cr) during the sampling period of –11 to –2 weeks before parturition. At 2–3 weeks after conception, urinary oestrone conjugate concentrations frequently exceeded the maximum value of 58.2 µg/mg Cr found during the luteal phase of non-fertile cycles (compare Fig. 2 and Fig. 3). In Monkey M866 (Fig. 3), the only female to be sampled across the post-partum period, urinary oestrone conjugate concentrations fell from 124 µg/mg Cr on the day after parturition to 1.1 µg/mg Cr 4 days later, when the infant died. Urinary values of oestrone conjugates then remained low until after post-partum conception occurred, about 14 days after parturition. The only 2 pregnancies which went to term (Females M866 and M947), and were monitored for urinary oestrone conjugate concentrations at the time of conception, had gestation lengths of approximately 145 and 155 days, respectively. After the present study, Female M866 was housed singly, except for 3 separate occasions when she was

Fig. 4. Urinary concentrations of oestrone conjugates (—○—), progesterone (---○---) and pregnadiol glucuronide (PDG, ...△.....) in Monkeys M947 and M922. The arrows indicate the time of (a) introduction of a male to the female-female pair (M947-M922), (b) aggression between the females when the subordinate female, M922, was removed and housed singly and (c) the estimated first ovulation in Female M922 after removal.

housed for 1 day with an adult male 294, 160 and 48 days before giving birth (J. B. Carroll, unpublished data). This gave a further gestation estimate of 160 days from the relevant time of mating.

Urinary concentrations of progesterone and pregnadiol glucuronide were not consistently elevated after conception (as illustrated in Fig. 3). Urinary LH concentrations were also mostly below 1.7 m.u./mg Cr during pregnancy. In 2 out of 3 pregnancies monitored immediately after conception, urinary progesterone and pregnadiol glucuronide concentrations rose concomitantly with those of urinary oestrone conjugates (Fig. 3). However, these elevated values were not constantly maintained and fluctuations were frequent. In Female M440, urinary concentrations of progesterone and pregnadiol glucuronide were erratic following conception. Female M440 was the only female not to produce a healthy infant at term. She suffered an apparently spontaneous miscarriage following this study, failed to produce any more infants and had to be hysterectomized approximately 1 year later because of a prolapsed uterus.

Table 4. Median and inter-quartile ranges of frequency of female sexual solicitations of males per hour per day in 3 female Goeldi's monkeys (M440, M947 and M820) across the ovarian cycle

Female	Stage of ovarian cycle		
	Follicular phase (Days -11 to -3)	Peri-ovulatory period (Days -2 to 2)	Luteal phase (Days 3-12)
M947	4.5 (0-7.5)	9.0* (6-23.2)	2.0 (0.5-2.8)
M440	2.0 (0-2.0)	4.0* (1.8-9.2)	1.0 (0-2.0)
M820	0 (0-1.0)	3.0 (0-5.0)	0 (0-1.5)

* $P < 0.05$ compared with luteal phase value (Mann-Whitney U test following Kruskal-Wallis analysis of variance; Siegel, 1956).

Reproductive suppression

In 2 out of the 3 trios, each established with 2 females and 1 male (Table 1), both females became pregnant and gave birth. In Trio 1, both females reared their infants while neither did in Trio 2. In Trio 2, the infant of the dominant female died at 4 days old while the subordinate female's infant died at 3 days old. In both trios, the dominant female gave birth first, the subordinate females giving birth 25 and 80 days later, respectively. Following this, at 325 and 326 days, respectively, after the two trios were established, the subordinate females had to be removed because of aggression received from the dominant female. Both dominant females proved subsequently to be pregnant when the subordinates were removed. Neither subordinate female proved to be pregnant.

In the third trio, no offspring were produced. At the time of the introduction of the male, the dominant female (M947) apparently underwent premature curtailment of the luteal phase of a cycle (Fig. 4), but then proceeded through a normal cycle. Neither cycle was included in the aggregate hormonal profiles shown in Fig. 2, because urinary oestrone conjugate concentrations did not clearly delineate the luteal phases. Urinary progesterone and pregnanediol glucuronide concentrations were used as indicators of ovarian cyclicity in this single instance. The subordinate female, M922, stopped showing ovarian cyclicity after the introduction of the male (Fig. 4). This acyclic state persisted until after the 2 females fought, 46 days after introduction of the male. Subordinate female M922 was partitioned off within the enclosure containing the male and dominant female (M947). The subordinate's ovarian cyclicity recommenced within 2-3 weeks of this separation (Fig. 4).

Sexual behaviour

Of the 4 females observed (Table 1), 3 exhibited proceptive behaviour to males (Table 4). In 2 of the 3 females, these sexual solicitations were demonstrated significantly more frequently during the hormonally defined periovulatory period than during the luteal phase ($P < 0.05$; Mann-Whitney U test). Copulation was rarely observed: only 1 of the 4 observed copulations occurred during the peri-ovulatory period (3 females).

Discussion

Conjugated oestrone was the most abundant form of urinary oestrone or oestradiol-17 β measured, throughout the ovarian cycle and early pregnancy in Goeldi's monkey. Measurement of urinary

oestrone conjugates provided a reliable indicator of ovarian cyclicity and pregnancy in Goeldi's monkey. These results compare well with limited data from other New World primates. Most studies have similarly shown that oestrone is the predominant form of oestrogen in urine during the ovarian cycle or pregnancy (owl monkey, *Aotus trivirgatus*: Bonney *et al.*, 1979; pied bare-face tamarin, *Saguinus b. bicolor*: Heistermann *et al.*, 1987; cotton-top tamarin, *S. oedipus*: French *et al.*, 1984; saddle-back tamarin, *S. fuscicollis*: Epplé & Katz, 1984; golden lion tamarin, *Leontopithecus rosalia*: French & Stribley, 1985; various species: Hodges *et al.*, 1981). Two exceptions to this are the female common marmoset, *Callithrix jacchus* (Eastman *et al.*, 1984) and the pregnant female red-bellied tamarin, *S. labiatus* (Pryce *et al.*, 1988), in which conjugated oestradiol was the most abundant urinary oestrogen.

The cyclic elevations in concentrations of urinary oestrone conjugates apparently reflected the post-ovulatory luteal phase of the ovarian cycle. Cyclic elevations in urinary progesterone and pregnanediol glucuronide concentrations occurred concomitantly with those of urinary oestrone conjugates and thus were suggestive of a luteal origin for the high concentrations of oestrone conjugates in female Goeldi's monkeys, resembling findings in the female common marmoset monkey (Eastman *et al.*, 1984; Hodges & Eastman, 1984). Furthermore, increased sexual solicitations of males by females occurred at the hormonally defined peri-ovulatory period, indicative of a presumed ovulatory event at the time just before the elevation in urinary oestrone conjugates. In the female common marmoset monkey, proceptive displays are almost exclusively limited to around the time of ovulation (Kendrick & Dixon, 1983). Further evidence for the post-ovulatory nature of increased urinary oestrone conjugate excretion in female Goeldi's monkeys has been provided by Ziegler *et al.* (1989, 1990) because the pre-ovulatory urinary LH peak occurred coincident with the onset of sustained elevations of urinary oestrone conjugate concentrations. Similar confirmatory LH data were not found in this study, but this may have been due to the failure to stabilize bioactive LH (Ziegler *et al.*, 1987).

The preponderance of post-ovulatory elevations in urinary oestrone conjugate concentrations in Goeldi's monkeys clearly resembled the pattern of urinary oestrogen excretion found in most marmoset and tamarin monkeys studied (e.g. Eastman *et al.*, 1984) but was distinct from that of a cebid monkey, such as the capuchin *Cebis albifrons* (Hodges *et al.*, 1979, 1981) in which the urinary oestrogen profile clearly identified the preovulatory oestrogen rise preceding or coincident with the urinary LH peak. However, since the cebid owl monkey showed elevations in urinary oestrogen concentrations only in the luteal phase (Bonney *et al.*, 1979), and only preovulatory urinary oestrogen peaks were clearly delineated in the callitrichid saddle-back tamarin (Hodges *et al.*, 1981), these urinary endocrine differences may simply reflect the idiosyncracies of steroid hormone excretion in individual species rather than true differences in hormone metabolism and excretion between the two primate families.

Measurement of urinary progesterone and pregnanediol glucuronide proved particularly useful in this study to delineate the suppression of ovarian cyclicity in a subordinate female (M922). This is the first recorded case of physiological suppression of reproduction in a subordinate female Goeldi's monkey. Normally, in captive monogamous family groups of Goeldi's monkeys, mature daughters also fail to breed (Carroll, 1982). It is not yet known whether ovulation is suppressed in the non-breeding daughters. However, if a strange adult male is introduced to a mother-daughter or sister-sister dyad, both females in the dyads breed (Carroll, 1986; H. Dornbrack, personal communication, respectively). These latter observations might explain why both females (unrelated) bred in Trios 1 and 2 of this study (Table 1) before the subordinate females were attacked and had to be removed. In callitrichid monkeys such as the common marmoset, the suppression of ovulation among subordinate females is widespread (Abbott *et al.*, 1988). In the golden lion tamarin, however, a behavioural block to subordinate reproduction operates (French & Stribley, 1990). Callitrichid monkeys apparently benefit from the presence of non-breeding helpers to aid in caring for the young of the dominant female, which might explain why these primates demonstrate such extreme suppression of subordinate female reproduction. Goeldi's monkeys also

employ non-breeding helpers to help raise the offspring of the dominant female in captive groups. However, unlike callitrichid monkeys, Goeldi's monkey mothers care for their infants exclusively until the infants reach about 3 weeks of age, when typical callitrichid infant-sharing amongst the group commences (Carroll, 1982). Perhaps with less use of non-breeding helpers to raise the offspring of the dominant female, there might also be a lesser need for an extreme form of female reproductive suppression in Goeldi's monkeys.

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