Urinary oestrogen and progesterone excretion before and during pregnancy in a pied bare-face tamarin (Saguinus bicolor bicolor)

M. Heistermann, E. Pröve, H.-J. Wolters and G. Mika

Lehrstuhl für Verhaltensphysiologie, Fakultät für Biologie der Universität, Postfach 8640, D-4800 Bielefeld 1, West Germany

Summary. Oestrone, oestradiol-17β and progesterone were measured by radioimmuno-assay in daily urine samples after pairing and during subsequent pregnancy in a pied bare-face tamarin. On the basis of excretion profiles an ovarian cycle length of about 3 weeks and a gestation length of about 160 days are suggested. Oestrone was the predominant urinary oestrogen excreted by the non-pregnant and pregnant pied bare-face tamarin, the oestrone/oestradiol ratio being greater than 100:1. The results suggest that steroid monitoring can provide useful information about reproductive physiology in this species of tamarin.

Introduction

Many species of marmosets and tamarins (Family Callitrichidae) are threatened with extinction in the wild (Mittermeier & Coimbra-Filho, 1982; Ayres et al., 1982). Improved management and conservation of these species in captive breeding colonies is therefore becoming increasingly important. Breeding success greatly depends upon knowledge of basic data on the reproductive biology and the availability of reliable and informative methods for monitoring reproductive function. Marmosets and tamarins, however, do not show any external obvious signs (e.g. menstruation, changes in vaginal cytology, behavioural oestrus) to indicate their reproductive status accurately (Hearn & Lunn, 1975; Brand, 1981; Epple & Katz, 1982). Monitoring hormonal changes, therefore, is the only consistent way to evaluate the reproductive events, including detection of ovulation and conception as well as pregnancy monitoring. Many studies have demonstrated the practical advantages of urinary hormone analyses in this regard (e.g. Lasley et al., 1980; Hodges et al., 1981; Czekala et al., 1981).

Investigations on urinary hormone excretion in callitrichids have mainly dealt with the common marmoset (Callithrix jacchus: Lunn, 1978; Heger & Neubert, 1983; Hodges et al., 1983; Hodges & Eastman, 1984; Eastman et al., 1984). In addition there are data from the cotton-top tamarin (Saguinus oedipus: Brand, 1981; French et al., 1983; Hodges & Eastman, 1984), the saddle back tamarin (Saguinus fuscicollis: Hodges et al., 1981; Epple & Katz, 1982) and the golden lion tamarin (Leontopithecus rosalia: French & Stribley, 1985). So far nothing is known about hormonal data in the pied bare-face tamarin, one of the most endangered primate species.

The present study is the first to deal with basic endocrinological data of reproduction in this New World primate.

Materials and Methods

The female used in this study was an adult specimen at an age of 5–6 years. On 28 December 1983 it was paired with an unrelated male and 7 months later, on 16 July 1984, the female gave birth to male twins one of which died shortly thereafter. The animals were housed in a cage about 1.0 x 2.0 x 2.4 m in size. Branches, a nest box and some toys
were provided. The animals were fed with a special marmoset diet supplemented daily with a mixture of fruits and vegetables. Water was always available. The colony room was maintained at a temperature of 22–24°C and a relative humidity of 60–70% and received natural daylight with additional illumination between 06:00 and 18:00 h.

**Urine collection**

For sample collection which occurred between 06:00 and 07:30 h from 7 January until 1 July 1984 on an average of 5-6 days per week, the female was removed from her home cage into a metabolism cage. Urine samples were usually voided within 30 min and then frozen at −20°C until assayed.

**Hormone analyses**

**Oestrogens.** Urine (20 μl) was mixed with a trace amount (~1000 c.p.m. in 20 μl phosphate buffer, pH 7·0 ± 0·1) of tritiated oestrone-3β-glucuronide (sp. act. 19-6 Ci/mmol; Amersham Buchler, Braunschweig, FRG) and oestradiol-17β-glucuronide (sp. act. 50 Ci/mmol; New England Nuclear (NEN), Dreieich, FRG), equilibrated with 270 μl 0·5 M-acetate buffer (pH 4·7 ± 0·1) for 30 min and hydrolysed with 30 μl β-glucuronidase/sulphatase (from *Helix pomatia*, Type H-2; Sigma Chemie, Deisenhofen, FRG) for 12–18 h at 37°C. Oestrogens were extracted twice with 5·0 and 3·0 ml dichloromethane. The combined extracts were evaporated, reconstituted twice in 0·5 ml 10% ethyl acetate in iso-octane and subjected to celite column chromatography according to the method of Wingfield & Farner (1975). The dried extracts were reconstituted in 2·0 ml ethanol. Aliquots of 100 μl oestradiol-17β and 20 μl oestrone (previously diluted 1:21 in ethanol) were assayed in duplicate. Procedural losses were monitored by counting the radioactivity in 400 μl of the ethanol extract. Recovery values were 87·6 ± 4·2% and 85·3 ± 7·4% (mean ± s.d., n = 170) for oestrone and oestradiol-17β respectively.

Oestrone assay tubes contained the evaporated sample, 0·1 ml [3H]oestrone (~4500 c.p.m. in phosphate buffer; sp. act. 45·8 Ci/mmol; NEN) and 0·1 ml of an oestrone/oestradiol antiserum (antiserum no. E 17–94; Endocrine Sciences, Tarzana, U.S.A.) diluted with phosphate buffer to give approximately 50% binding of labelled oestrone in the absence of unlabelled steroid. Cross-reactivity values for the antiserum provided by the supplier were 130% for oestrone, 100% for oestradiol-17β and ≤3% for all other compounds tested.

Oestradiol assay tubes contained the evaporated sample, 0·1 ml [3H]oestradiol-17β (~4500 c.p.m. in phosphate buffer; sp. act. 47·4 Ci/mmol; NEN) and 0·1 ml of an oestradiol antiserum (antiserum no. 1702; Arnel Products Co., New York, U.S.A.) diluted with phosphate buffer to give approximately 50% binding of labelled oestradiol-17β in the absence of unlabelled steroid. Cross-reactivity with this antiserum was 14% for oestrone, 5% for oestriol and ≤0·001% for all other compounds tested.

For both steroids a duplicate standard curve (dose range 10–1000 pg) was included in sample determination. After incubation on ice for 3 h separation of free from bound steroid was achieved by the addition of 1·0 ml ice-cold dextran-coated charcoal and centrifugation of the mixed samples at 900 g and 4°C for 18 min. Supernatants were decanted into scintillation vials containing 4·5 ml scintillant (Insta Fluor: Packard Instrument, Frankfurt, FRG) and counted the next day for 10 min in a scintillation spectrometer.

Water blanks for oestrone and oestradiol-17β were 8·8 ± 0·7 pg and 33·7 ± 4·4 pg (mean ± s.d., n = 10) respectively, yielding assay sensitivities (mean ± 2 s.d.) of 10·2 pg for oestrone and 42·5 pg for oestradiol. Accuracy was tested by measuring in triplicate known amounts of oestrogens added to phosphate buffer and treated the same way as unknown samples. Linear regressions were found for estimated oestrogens against added hormones (r = 0·89x + 24·04 for oestrone and r = 0·85x + 0·36 for oestradiol) with slopes and intercepts being not significantly different from the expected values of 1 and 0 respectively. Intra-assay variations, studied in 10 replicates of a urine pool and calculated as the coefficient of variation, was 11·2% and 22·9% for oestrone and oestradiol respectively. Inter-assay variability, calculated by studying replicates of 4 different urine pools in successive assays, varied between 10·8% and 25·5% and 10·1% and 23·6% for oestrone (n = 2) and oestradiol-17β (n = 4) respectively.

**Progesterone.** Urinary progesterone was assayed directly after dichloromethane extraction: 20 ml urine were mixed with a trace amount (~1000 c.p.m. in 0·1 ml phosphate buffer) of [3H]progesterone (sp. act. 53·4 Ci/mmol; NEN) and 0·3 ml phosphate buffer, equilibrated for 30 min, extracted with dichloromethane and evaporated and reconstituted in 2·0 ml ethanol as described for the oestrogens. The mean recovery value was approximately 100%. Duplicate aliquants of 750 μl were assayed. Progesterone assay tubes contained the evaporated sample, 0·1 ml [3H]progesterone (~4500 c.p.m. in phosphate buffer; sp. act. 53·4 Ci/mmol) and 0·1 ml of a progesterone antiserum (supplied from NEN) diluted in phosphate buffer to give ~50% binding of labelled progesterone in the absence of unlabelled steroid. Cross-reactivity values for this antiserum were ≤3·3% for all compounds tested. For sample determination a duplicate standard curve (dose range 20–2000 pg) was included in the assay. The mean water blank value was 104·1 ± 26·9 pg (n = 10), yielding an assay sensitivity of 157·9 pg. Accuracy was determined by measuring in triplicate known amounts of progesterone added to phosphate buffer and treated like unknown samples. A linear regression was found for recovered progesterone against added progesterone (r = 0·88x + 116·7), the slope and intercept of which were not significantly different from the expected values of 1 and 0 respectively. Intra- and inter-assay variability, calculated as described for the oestrogens, were 14·7% (n = 10) and 6·4–17·9% (n = 3) respectively.

**Creatinine analysis**

Creatinine values were determined in triplicate using an alkaline picric acid–sodium borate–SDS reagent according to a modification (I. Kiidering, personal communication) of the method of Heinegard & Tiderström

Downloaded from Bioscientifica.com at 12/01/2018 05:07:04AM via free access
Fig. 1. Excretion profiles in urine of (a) oestrone, (b) oestradiol-17β and (c) progesterone before and during pregnancy in a single pied bare-face tamarin. The data are normalized to the day of parturition (= Day 0). The arrows (↓) indicate peak values of excretion before the estimated day of conception (C).
(1973). Urine (20 µl) was diluted in optical cuvettes to 0·5 ml with distilled water. Then 3·0 ml of the picric acid reagent was added, and the absorbance at 500 nm was read 10 min later on a spectrophotometer. The readings were corrected for the presence of non-specific creatinine-like compounds by the addition of 100 µl of a solution of concentrated sulphuric and acetic acid. The absorbance was again read 3 min later and the difference between the two readings yielded the value for creatinine-induced absorbance. Intra-assay variability was calculated as the coefficient of variation based on duplicate sample agreement of a urine pool according to the method of Cekan (1975) and was 2·6% \( (n = 10) \). Inter-assay variation, monitored by the repeated assay of the same urine pool in successive assays, was 2·7% \( (n = 10) \).

Results

The urinary excretion profiles of the three measured steroids, beginning 10 days after pair formation until 15 days before the end of the resulting pregnancy are shown in Fig. 1. The profiles observed 190–156 days before parturition are characterized by the appearance of two distinct hormonal peaks in oestrogen and progesterone excretion. The oestrone and progesterone maxima were easily identifiable but the oestradiol-17β peaks were less pronounced. Peak-to-peak intervals were 21 days for oestrone, 22 days for oestradiol and 23 days for progesterone.

After the second maximum at about 162 days before parturition, the concentrations of the three steroids decreased \( (P < 0·001; \) Mann–Whitney U-test), remaining low for at least 4 weeks before they began to rise. Oestrone concentrations showed a marked, steady rise over the next 12½ weeks, reaching a maximum of 120–140 µg/mg creatinine at about 38 days before parturition after which there was a progressive decline until termination of the study. The increments in oestradiol-17β and progesterone concentrations were less marked and there were considerable fluctuations of daily excretion. After an initial significant rise \( (P < 0·0001; \) Mann–Whitney U-test) oestradiol concentrations fluctuated around 327·0 ng/mg creatinine until about 1½ months before parturition when a second significant increase occurred \( (P < 0·0001; \) Mann–Whitney U-test). The elevated concentrations of 563·2 ± 188·4 ng/mg creatinine (mean ± s.d.) were maintained until the end of the study. Urinary progesterone concentrations showed an initial significant increase \( (109·9 ± 19·8 \) ng/mg creatinine; \( P < 0·0001; \) Mann–Whitney U-test) and then a slight rise until the end of the study.

The chromatographic separation of the oestrogens revealed that oestrone was the predominant urinary oestrogen excreted by the non-pregnant and pregnant pied bare-face tamarin. Of the total immunoreactive oestrogen measured, more than 99·0% was oestrone. The oestrone/oestradiol ratio was therefore greater than 100:1.

Discussion

The hormonal profiles observed at 27–23 weeks before parturition indicate a cyclic excretion of urinary oestrogens and progesterone, with oestrone seeming the best indicator of ovarian cyclicity, a finding which was also reported by French et al. (1983) for the cotton-top tamarin. The evaluation of urinary progesterone revealed that both detected cycles might have been ovulatory, and conception occurred at the second of these cycles. Urinary progesterone may therefore provide a useful indicator for ovulation detection in this species.

The intervals between successive oestrogen and progesterone peaks suggested a cycle length of about 3 weeks in this individual tamarin. This is a finding which has to be confirmed but compares well with the available data on reproductive cycle lengths of other New World primates including callitrichids (see reviews by Harlow et al., 1983; French et al., 1983).

The estimate of conception at about the time of the second hormonal peak suggests a gestation length of about 160 days, a period which compares well with the gestation lengths of 140–165 days reported for other callitrichids (see review by Stevenson, 1984). In this single female, there was no rise in concentrations of excreted oestrogens and gestagens around the time of pregnancy initiation, unlike responses in other primates including callitrichids (e.g. Macaca silenus: Shidel et al., 1983; Pan troglodytes; Lasley et al., 1980; Pygathrix nemaeus: Lippold, 1981; Cebus apella: Nagle &
Steroid excretion in a female pied bare-face tamarin 639

Denari, 1982; Callithrix jacchus: Heger & Neubert, 1983; Eastman et al., 1984; Leontopithecus rosalia: French & Stribley, 1985). Later there was an increase in daily excretion of oestrogens and progesterone and the results are consistent with findings from other primates (e.g. Pongo pygmaeus: Czekala et al., 1981; Gorilla gorilla: Seaton, 1978; Pygathrix nemaeus: Lippold, 1981; Papio papio: Townsley, 1974; Callithrix jacchus; Eastman et al., 1984). Nevertheless a decline in oestrogen excretion during the last fourth of gestation (as monitored for oestrone), when in most other primates oestrogen production reaches a maximum, is unusual and it remains to be established whether this is a species-specific phenomenon. Brand (1985) reported a similar finding for a pregnant saddle back tamarin which showed “a decrease in oestrone and oestradiol levels but increase in oestriol levels during late pregnancy, followed by a reversal in each just before parturition”. The close systematic relation of the two species offers the possibility that the same dynamics of late oestrogen excretion obtain, although a decline in oestriol excretion was not observed in the female we studied and oestriol excretion remains to be evaluated. Nevertheless, the profiles, notably that of oestrone excretion, suggest that in the pied bare-face tamarin fetal development is well reflected by increasing urinary oestrogen concentrations throughout gestation. Fetal well-being therefore might be assessed through continuous urine collection and analyses. As in the baboon (Townsley, 1974), Douc langur (Lippold, 1981) and common marmoset (Heger & Neubert, 1983) difficulties in maintaining pregnancy and even abortion might be detected by this application.

Chromatographic separation of the oestrogens revealed that oestrone was the most abundant urinary oestrogen in the non-pregnant and pregnant pied bare-face tamarin. This is in common with findings for cotton-top tamarins (French et al., 1983; Hodges & Eastman, 1984; Brand, 1985), saddle back tamarins (Eppe & Katz, 1982; Brand, 1985), golden lion tamarins (French & Stribley, 1985) and other New World primates (see Hodges et al., 1981) but in contrast to the common marmoset in which oestradiol-17β is reported to be the predominant excreted oestrogen in cyclic and pregnant females (Hodges et al., 1983; Hodges & Eastman, 1984; Eastman et al., 1984). Oestrone is also the principal urinary oestrogen metabolite in a variety of non-hominoid Old World primates (see Lasley et al., 1981).

Although the results reported in this paper are based on one individual only they demonstrate the usefulness of monitoring reproductive function in the pied bare-face tamarin by the measurement of excreted urinary oestrogens and unmetabolized progesterone by a simple method of broad application. The study contributes towards knowledge of the reproductive physiology and may improve the captive management and breeding of this endangered New World primate.

We thank colleagues of our Callithrichid Station for assistance with urine collections; Dr I. Küderling, Deutsches Primatenzentrum Göttingen, for advice on laboratory techniques; E. Geißler for preparing the figures; and Professor K. Immelmann for interest in our research and helpful comments on an earlier draft of this manuscript.

References


Received 19 December 1986