

Reproductive exocrine and endocrine profile of female langur monkeys, *Presbytis entellus*

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Summary. In 5 caged langurs menstrual cycle length (for 125 cycles) varied from 18 to 45 days with a mean \pm s.d. of 26.3 ± 3.6 days. Menstruation lasted for 2 days. A distinct cycle of vaginal smear cells was observed with a maximum cornification on Day 10 coinciding with the serum oestradiol peak. A monophasic pattern of vaginal temperature with a significant nadir on Day 11, 1 day after the oestradiol peak, was observed. Serum sialic acid concentrations directly reflected the oestrogenic activity and showed a significant peak on Day 10, coinciding with the oestradiol peak and was followed by a progesterone rise. Langurs menstruated throughout the year without showing any sign of summer amenorrhoea. We suggest that maximum cornification, vaginal temperature nadir and serum sialic acid peak could be used as markers for prediction and detection of the time of ovulation in langur monkeys.

Keywords: langur; vaginal temperature; serum oestradiol; vaginal smear patterns; serum sialic acid; ovulation; menstrual cycle

Introduction

Four species of langurs (*Presbytis*) are commonly available in India (Ramaswami, 1977); these are *P. entellus*, *P. geei*, *P. johnii* and *P. pileatus*. Certain aspects of the behavioural studies of these animals have been described by Jay (1965), Sugiyama *et al.* (1965) and Poirier (1969). The gross anatomy and histology of the female reproductive tract of the hanuman langur (*Presbytis entellus*) (David & Ramaswami, 1971) have been reported. However, there is very little information on the reproductive endocrine and exocrine profile of this non-human primate.

This study was undertaken to acquire basic information on the changes in the gonadal hormones during the normal menstrual cycle of langurs. Changes in serum sialic acid levels, vaginal smear cytology and basal body temperature were also studied during the menstrual cycle to determine whether the changes in these characteristics could help to predict the time of ovulation.

Materials and Methods

Animals. Five adult female langur monkeys, weighing between 10 and 14 kg, were caught from places around Jaipur, India. Animals were housed individually in steel cages (measuring $0.75 \times 0.75 \times 1.05$ m) under natural temperature and daylight conditions. Animals were fed with green leaves, roasted or soaked grams, bread, vegetables and fruits. Water was provided *ad libitum*.

Animals were quarantined for a period of 2 months before the study was started. During this period, they were acclimatized to laboratory conditions and were also trained for the taking of vaginal smears and blood samples.

Collection of vaginal smears. Daily vaginal smears were obtained by using a sterile glass dropper after restraint of the animals for about 5 min in the cage. Slides stained with Papanicolaou stain (Riley *et al.*, 1955) were examined for various cell types (Boris, 1940).

Recording of vaginal temperature. Vaginal temperature was recorded with the aid of a veterinary thermometer throughout the menstrual cycle.

Blood samples. Blood samples were collected daily throughout the menstrual cycle from the saphenous vein. Blood sampling was repeated over five alternate cycles of each animal. Serum was separated and stored at -20°C until processed for hormone estimation.

To avoid stress to the animal due to repeated handling, the recording of vaginal temperature, and collection of vaginal smear and of blood samples were done simultaneously around 08:00 h.

Estimation of sialic acid. Serum concentrations of sialic acid were estimated by using the method described by Warren (1959).

Estimation of gonadal hormones. Serum concentrations of oestradiol-17 β and progesterone were assayed in triplicate by the specific radioimmunoassay (RIA) methods described in the W.H.O. Method Manual (WHO, 1981). In brief, oestradiol-17 β was measured by using an antiserum raised against oestradiol-6(O-carboxymethyl)-oxime-bovine serum albumin and [1,2,6,7- ^3H]oestradiol (sp. act. 92 Ci/mmol) as tracer. Antiserum raised against progesterone-11-succinyl-BSA and [2,4,6,7- ^3H]progesterone (sp. act. 94 Ci/mmol) as tracer were used for the estimation of progesterone concentrations. The separation of free steroid from the bound was achieved by using dextran-coated charcoal suspension. The inter- and intra-assay coefficients of variation were, respectively, 10.0% and 3.6% for the oestradiol assay and 11.5% and 4.7% for the progesterone assay. The sensitivities of the assay methods were 15 pmol oestradiol and 30 pmol progesterone.

Because of variations in cycle length, the results were analysed and centred according to the day of the oestradiol peak which served as the reference point and was designated as Day 0.

Analysis of results. The values for samples were determined from the best-fit straight line of the standard curve, linearized on logit-log co-ordinates by using a Hewlett-Packard Mini Computer (Model 9831A). The results on different days of the menstrual cycles were compared by using Student's *t* test.

Results

Menstrual cycle record

Over a 2-year period the 5 langurs had 125 menstrual cycles. The cycle length varied between 18 and 45 days with a mean (\pm s.d.) of 26.3 ± 3.6 days. The median length was 22 days (Fig. 1). The duration of menses ranged from 1 to 3 days with a mean of 2.0 ± 0.1 days. The animals menstruated throughout the year and showed no sign of amenorrhoea.

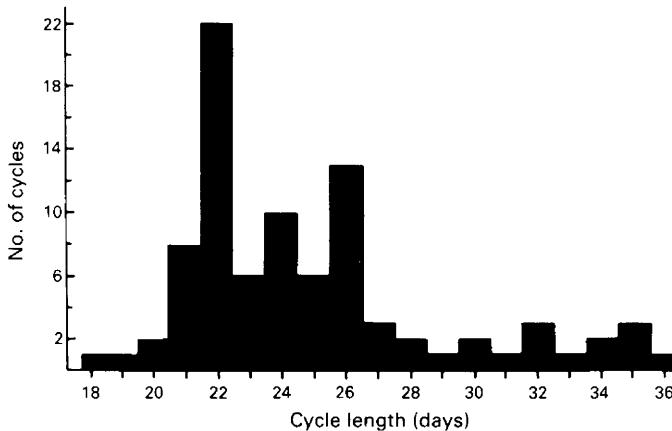


Fig. 1. Frequency distribution of menstrual cycles in langurs.

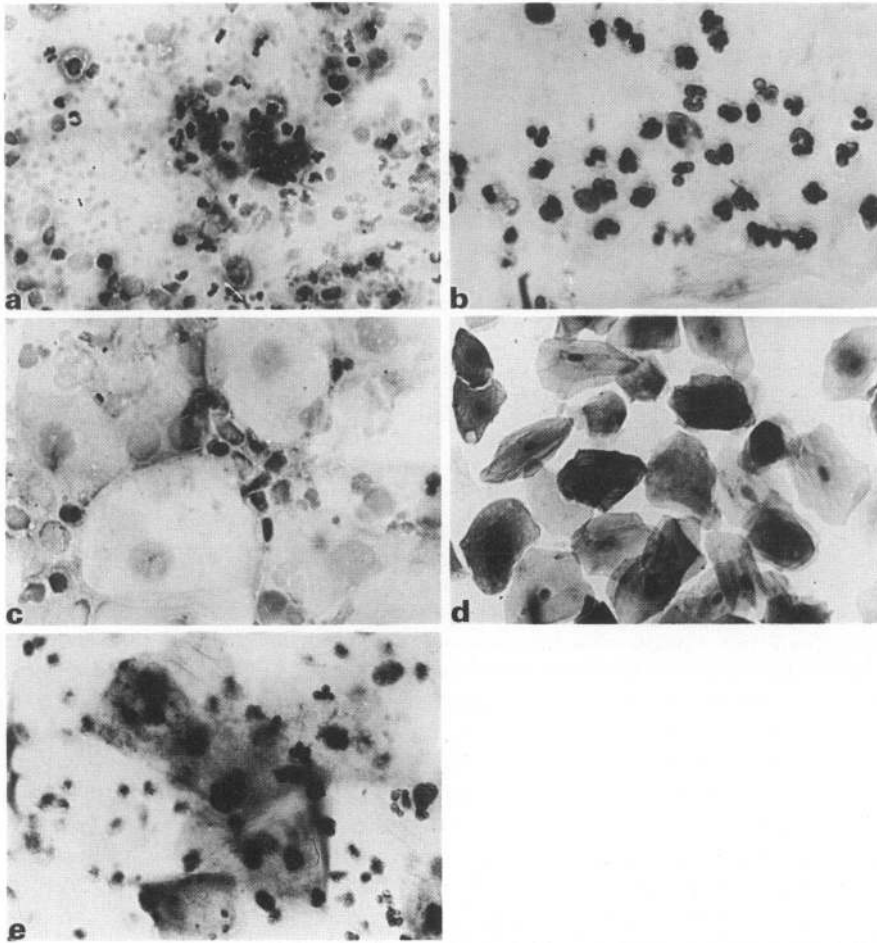


Fig. 2. Vaginal smear cytology during different phases of the menstrual cycle in langurs: (a) menstrual phase: $\times 170$; (b) post-menstrual phase: $\times 170$; (c) follicular phase: $\times 170$; (d) ovulatory phase: $\times 170$; (e) luteal phase: $\times 170$.

Vaginal smear cytology

On the basis of vaginal smear cell morphology different phases of the menstrual cycle were distinguished and their characteristics (Fig. 2) are as follows.

Menstrual phase. A 'thin' vaginal smear with considerable blood and mucus was observed. Large numbers of leucocytes with few epithelial and cornified cells were also noticed (Fig. 2a). Menses lasted for 1–3 days.

Post-menstrual phase. Proliferation of the vaginal mucosa was just beginning and the leucocytes were predominant (Fig. 2b). This phase lasted for 2–3 days.

Follicular phase. The follicular-phase smear was characterized by large epithelial cells with a large oval shaped nucleus and few leucocytes (Fig. 2c). This phase ranged from 3 to 4 days.

Preovulatory phase. During this phase, lasting 2 days, epithelial cells were progressively more cornified.

Ovulatory phase. There was marked desquamation of cornified cells, which had a degenerated nucleus or were enucleated. Leucocytes were almost absent. The cornified cells tended to remain separate from each other during this phase (Fig. 2d) which ranged from 2 to 3 days.

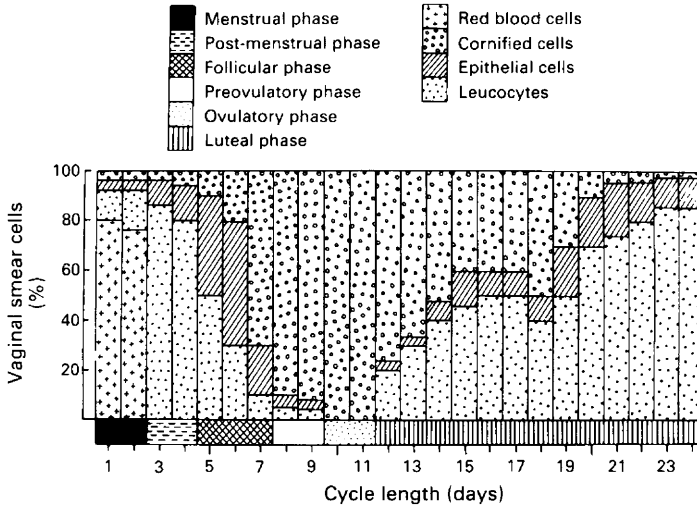


Fig. 3. Percentage distribution of vaginal cell types in the vaginal smear.

Luteal phase. Clumping and folding of cornified cells and an abrupt appearance of large numbers of leucocytes were the characteristic features of the early luteal phase. After 2–3 days, reversal of the process of cornification was noticed. The nuclei of the cells became progressively large and more vesicular whereas the cytoplasm was progressively less keratinized. The smear continued to contain abundant cornified cells, epithelial cells and large numbers of leucocytes (Fig. 2e). This phase ranged from 8 to 14 days.

A composite picture of the percentage distribution of various cell types in the vaginal smear during different phases of the menstrual cycle is shown in Fig. 3.

Vaginal temperature

Vaginal temperature ranged from 37.0 to 39.5°C on different days of the cycle. No significant difference in mean vaginal temperature during the follicular phase ($38.79 \pm 0.46^\circ\text{C}$) and the luteal phase ($38.74 \pm 0.43^\circ\text{C}$) was observed. However, a significant drop (0.50°C) in the vaginal temperature was observed during the ovulatory phase in 84% of the cycles. The drop in the temperature was observed between Days 9 and 13 of the cycle. In 40% of the cycles, the temperature drop was on Day 11 (Fig. 4).

Sialic acid concentrations

Serum concentrations of sialic acid did not show any marked variation on different days of the follicular phase. The mean values were 22.1 ± 0.3 mg/100 ml (range 21.2–22.5 mg/100 ml). However, between Days 8 and 11 of the cycle, an increase in the values was observed which lasted for 1 day. The mean peak levels were 23.6 ± 0.3 mg/100 ml (range 23.0–24.0 mg/100 ml). Thereafter, a sharp decline in the concentrations occurred. In 67% of the menstrual cycles the peak was observed on Day 10. After the mid-cycle peak, the concentrations of sialic acid during the luteal phase were lower than those observed during the follicular phase (Fig. 4).

Oestradiol concentrations

A gradual increase in the serum concentrations of oestradiol was observed during the follicular phase before the peak levels were attained between Days 8 and 11 of the cycle. In 49% of the cycles,

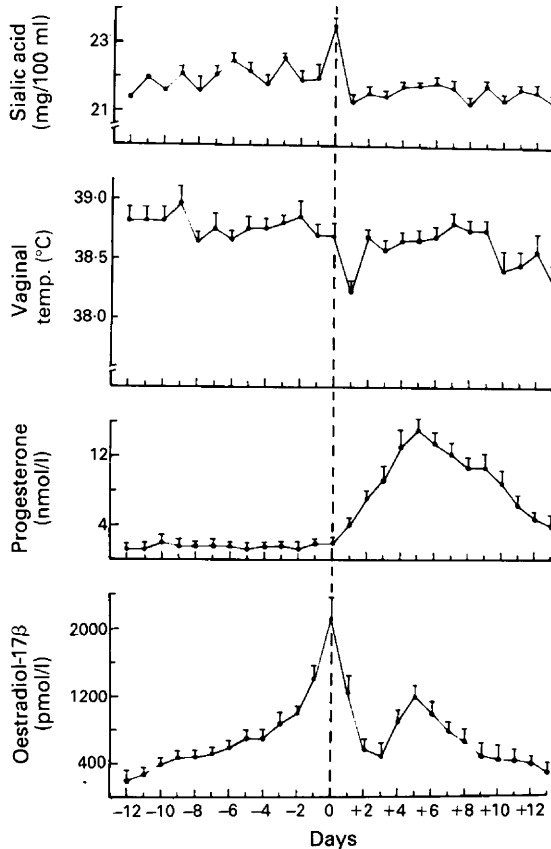


Fig. 4. Serum concentrations (mean \pm s.e.m.) of oestradiol, progesterone and sialic acid and vaginal temperature during the menstrual cycle. Day 0 = day of oestradiol peak.

the major oestradiol peak was on Day 10 of the cycle. During Days 1–6 of the cycle, concentrations were < 600 pmol/l. The mean peak values were 2116 ± 217 pmol/l (range 1792–2620 pmol/l). After the mid-cycle peak, a sharp decline in the oestradiol concentrations occurred. Between Days 12 and 14 of the cycle the levels had dropped to 410 pmol/l, but a second rise (1200 ± 120 pmol/l) occurred 4–6 days after the main oestradiol peak (Fig. 4).

Progesterone concentrations

There was no significant change in the serum progesterone values on different days of the follicular phase. Concentrations until the day of the oestradiol peak were < 1.8 nmol/l (range 1.09–1.74 nmol/l). There was then a gradual rise in the progesterone values and peak levels (mean 13.5 ± 1.7 nmol/l, range 12.6–20.7 nmol/l) were attained between Days 13 and 19 of the cycle. In each ovulatory cycle, concentrations of 10 nmol/l or more were observed for at least 3 consecutive days. Thereafter a gradual decrease in the progesterone values was observed (Fig. 4).

Anovulatory cycles

In 4 menstrual cycles (one in each of 4 animals), blood concentrations of oestradiol and progesterone were indicative of anovulation (Fig. 5). The mean cycle length was 42.0 ± 3 days (range 40–45 days). Oestradiol concentrations during the follicular phase were 2.6 ± 0.6 pmol/l and the

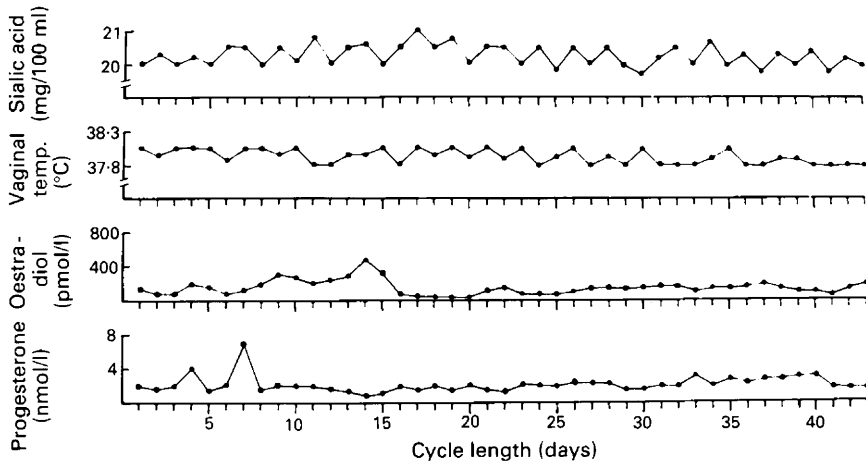


Fig. 5. Pattern of serum concentrations of oestradiol, progesterone and sialic acid and vaginal temperature during 4 anovulatory cycles of langurs.

mid-cycle oestradiol peak was not detected (Fig. 5). Similarly, there was no marked rise in the serum progesterone concentrations at the anticipated time of the luteal phase (Fig. 5).

The mid-cycle rise in serum sialic acid concentrations (Fig. 5) and the drop in vaginal temperature (Fig. 5) were also not evident in these anovulatory cycles. Leucocytes were predominant in the vaginal smear throughout the cycle.

Discussion

This study indicates that the menstrual cycle length, duration of menses and the pattern of endocrine profile of gonadal hormones during the menstrual cycle of langur are very similar to those reported for other commonly used species of non-human primates and women (Boris, 1940; Jenson, 1967; Hutchinson, 1970; Moghissi, 1976, 1980; Graham *et al.*, 1977; Anand Kumar *et al.*, 1980; Murty *et al.*, 1980; Varavudhi & Yodyingyuad, 1980; Bauman, 1981; Aksel, 1981). The changes in vaginal cell cytology, vaginal temperature and in the serum sialic acid levels during the menstrual cycle coincide with the changes in the gonadal hormones.

Six phases of menstrual cycle described in langur are similar to that reported for women (Boris, 1940), squirrel monkey (Rosenblum *et al.*, 1967; Hutchinson, 1970), cynomolgus monkey (Varavudhi & Yodyingyuad, 1980) and rhesus monkey (David & Mastroianni, 1968; Anand Kumar *et al.*, 1980).

Cyclic changes in the cervical mucus are known to reflect the hormonal changes associated with menstrual cycle (Moghissi *et al.*, 1972). Maximum cornification and increased quantity of clear cervical mucus during the ovulatory phase have been correlated with oestrogen and pregnanediol excretion and serum concentrations of LH, FSH, oestradiol and progesterone (Pauerstein *et al.*, 1978; Marishita *et al.*, 1979). Maximum number of cornified cells during the oestradiol peak was followed by a progesterone rise, and absence of distinct changes in the vaginal cytology in the anovulatory cycles indicates that the vaginal smear cytology can be used as a marker for the occurrence of ovulation in langur monkeys.

The monophasic pattern of basal body temperature observed during the normal ovulatory menstrual cycle of langurs in this study was similar to that reported earlier (David, 1968; Shandilya, 1974). However, the pattern of the basal body temperature in the langur differs from that reported for some other non-human primate species (Hendrickx *et al.*, 1967; Balin & Wan, 1968; Graham *et al.*, 1977). In these animals after a temperature drop, which probably coincides with the oestrogen

peak, a sharp and significant rise in temperature was observed during the luteal phase. This rise is probably due to an increase in the production and secretion of noradrenaline which is a thermogenic neural hormone (Moghissi, 1980; Newill & Mauricekatz, 1982). In women, although the biphasic pattern is more common (Moghissi, 1980; Newill & Mauricekatz, 1982), some ovulatory cycles a monophasic pattern has also been reported (Moghissi, 1976; Lenton *et al.*, 1977; Moghissi, 1980; Bauman, 1981). The reason for the absence of a thermogenic response to ovulatory levels of progesterone in the langur and in women is not known. Nevertheless, the drop in basal body temperature observed in langurs is very similar to that reported for chimpanzees (Graham *et al.*, 1977) and women (Davis & Fugo, 1948; Israel & Schneller, 1950) and it provides an indication of occurrence of ovulation. Absence of a significant drop in temperature in the anovulatory cycles further supports this observation.

A significant rise in the serum sialic acid concentrations coincides with the major oestradiol peak. This pattern of sialic acid in the circulation is similar to the urinary excretion of sialic acid in langurs (Shandilya *et al.*, 1977) and in women (Jenson, 1967). A role for oestrogen in maintaining sialic acid levels of the genital organs has been suggested for ovariectomized langur monkeys (Shandilya *et al.*, 1977). However, progesterone has been reported to suppress the sialic acid contents (Coppola & Ball, 1966). The high progesterone concentrations during the luteal phase explain the low levels of sialic acid and absence of a secondary sialic acid peak. Absence of a sialic acid peak during the anovulatory cycle increases the importance of serum sialic acid measurement for ovulation detection in the langur.

The changes in circulating concentrations of oestradiol and progesterone during the menstrual cycle of langurs are, in general, similar to those described for bonnet monkeys (Stabenfeldt & Hendrickx, 1972; Murty *et al.*, 1980), rhesus monkeys (Hotchkiss *et al.*, 1971; Hodgen *et al.*, 1976; Anand Kumar *et al.*, 1980), baboons (Goncharov *et al.*, 1976; Koyama *et al.*, 1977; Raykling & Westfahl, 1978), chimpanzees (Graham *et al.*, 1972; Reyes *et al.*, 1975; Graham *et al.*, 1977) and women (Landgren *et al.*, 1980; Aksel, 1981). However, the occurrence of a secondary oestradiol peak may bring the langur pattern closer to that of the human than to rhesus and baboon.

We suggest that langur monkeys would be useful for studying aspects of reproduction at basic levels and long-term testing of contraceptives as models for human reproduction.

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