Relationship between sexual skin colour of female rhesus monkeys and midcycle plasma levels of oestradiol and progesterone

J. A. Czaja, J. A. Robinson, S. G. Eisele, G. Scheffler and R. W. Goy

*Wisconsin Regional Primate Research Center, University of Wisconsin,
1223 Capital Court, Madison, Wisconsin 53706, U.S.A.*

Certain Old World primates show marked and systematic changes in colour and area of the perineal or sexual skin which are thought to reflect endocrine condition (Young & Orbison, 1944; Bullock, Paris & Goy, 1972). In the rhesus monkey, definite colour changes occur, but they are subtle and have been difficult to relate to reproductive state (Hartman, 1932; Valerio, Pallota & Courtney, 1969). However, Czaja, Eisele & Goy (1975) have reported a distinct midcycle alteration from the maximum skin coloration found during ovulatory menstrual cycles of rhesus monkeys. The association of ovulation with plasma oestrogen levels (Yamaji *et al.*, 1971; Weick *et al.*, 1973) and the increase in intensity of the colour of the sex skin in oestrogen-treated ovariectomized rhesus females (Allen, 1927; Michael & Saayman, 1968) suggest that midcycle colour changes in the intact female rhesus may provide a simple way of estimating the time of ovulation. Concurrent determinations were therefore made of skin coloration and circulating steroid levels in adult female rhesus monkeys.

The 31 females used all had a history of ovulatory menstrual cycles and were housed in single cages within environmentally controlled animal quarters (12 h L:12 h D, $22 \pm 2^\circ\text{C}$) at the Wisconsin Regional Primate Research Center Breeding Colony. Daily observations were made to record skin coloration and the occurrence of menses. The animals were accustomed to being examined and each female had previously been trained with the aid of food reward to turn her perineum toward an investigator for inspection. This allowed the vaginal orifice to be checked for signs of menstrual discharge and the skin colour evaluated without removing the female from her home cage, thereby eliminating the stress of handling which we find to influence skin coloration in some females. Although colour changes are seen around the perineum and on the thighs, on the abdomen and on the face, the monkeys' thigh region was the area primarily monitored for skin colour since it is here that the greatest colour change occurs throughout the menstrual cycle in most females. Skin coloration was scored by a general system of grading colours from 0 (virtually white) to 4.25 (very deep red) in steps of 0.25 (Czaja *et al.*, 1975). Colour estimations were normally performed between 10.00 and 12.00 h and preceded collection of blood by 2–5 h. The most easily observable midcycle change in colour in rhesus monkeys is a relatively rapid and consistent fading from peak sex skin colour. We define this change (colour breakdown) as a decline in female colour for 2 or more consecutive days. The first day on which such a colour decrease is observed is labelled as the day of colour breakdown, and in the present study this day was determined for each cycle before steroid levels had been measured. Analyses involved a total of 39 cycles in which colour breakdown was observed (1–2 cycles per female). In 21 of these cycles, blood samples (2.5 ml) were obtained for analysis of oestradiol-17 β concentrations by saphenous vein puncture every 3 or 4 days except for the 5–10 day period of daily sampling when skin coloration was intense. In the other 18 cycles studied, blood was withdrawn daily throughout the cycle: oestradiol-17 β levels were measured in every sample and progesterone levels in samples obtained every 2nd day. The steroid levels were determined by radioimmunoassay as previously described (Bielert *et al.*, 1976).

There was a marked midcycle peak in oestradiol concentration in all 39 cycles, and progesterone values were elevated after this peak in those cycles in which this steroid was measured. Cycles were subsequently aligned by the day of peak oestradiol to assess the midcycle patterns of skin coloration and hormone changes (see Text-fig. 1). The average skin colour index attained its maximum value

1 day after peak oestradiol, but the increase was not a 'surge' like that of oestradiol concentration. The hormone measurement indicated that the variation in cycle length ($24\text{--}50$ days, 29.5 ± 5.0 (S.D.) days) could be accounted for largely by differences in the follicular phase, since the correlation between the lengths of the follicular phase and the cycle were significant ($r = 0.98$, $P < 0.001$). The interval from the beginning of the menstrual cycle to the preovulatory oestradiol peak (12.3 ± 5.2 (S.D.) days, range 6–34 days) was significantly ($P < 0.001$) more variable than that between the oestradiol peak and the end of the cycle (17.2 ± 1.1 (S.D.) days, range 15–19 days). The largest day-to-day change in endogenous oestradiol concentrations during the menstrual cycle is the drop from peak preovulatory levels (Hotchkiss, Atkinson & Knobil, 1971; Weick *et al.*, 1973), and the midcycle shift in skin coloration in the present study appears to reflect this change. Colour breakdown never preceded the day of peak oestradiol values and consistently followed it within 4 days (2.5 ± 0.9 (S.D.) days), giving a highly significant correlation between the days of peak oestradiol and colour breakdown ($r = 0.98$, $P < 0.001$).

Text-fig. 1. The sexual skin colour and steroid levels during the middle of the menstrual cycle of rhesus monkeys. The results are aligned with the day of peak oestradiol = Day 0. (a) Colour index (mean $\pm$ S.E.M.) and the distribution of colour breakdown (hatched columns) in all 39 cycles. (b) Midcycle oestradiol levels (mean $\pm$ S.E.M.) in the 21 cycles with partial hormone data. (c) Hormone levels in the 18 cycles analysed throughout for oestradiol-17 β (—) and progesterone (---). Progesterone is plotted in 2-day blocks; values are mean $\pm$ S.E.M.

The results confirm that changes in the sex skin coloration of rhesus monkeys are closely related to midcycle fluctuations of endogenous oestradiol and, presumably, to ovulation. They also indicate that measurement of skin colour provides a simple technique which can detect shifts in endogenous

oestradiol levels, such as those associated with ovulation, soon after they occur. However, colour breakdown, by itself, does not appear a sufficient clue to positively establish a rhesus menstrual cycle as normal. During more recent observations of abnormal cycles (unpublished), colour breakdown was found to occur in a cycle that had a sharp midcycle drop in oestradiol, but no subsequent increases in progesterone, suggesting that there was a luteal insufficiency or that the cycle was anovulatory. The fact that colour changes were not found in cycles which lacked significant changes in circulating oestradiol levels suggests that oestradiol changes themselves, rather than other variables associated with ovulation, may thus be the significant influence behind the colour fluctuations observed during normal menstrual cycles, although the involvement of other oestrogens or progestagens cannot be discounted.

Consistent with commonly expressed opinion, absolute levels of rhesus sex skin colour were not by themselves reliable for identification of the endocrine conditions during the menstrual cycle. Additionally, intense skin coloration in the rhesus is associated with anovulatory periods, such as during pregnancy (Bielert *et al.*, 1976). However, the present findings show that relative colour changes within the cycle can help to determine whether the cycle is ovulatory and to identify the approximate time of the preovulatory oestradiol surge and ovulation soon after they occur. The existence of an easily observable external sign reflecting conditions of fertility should prove a useful tool in endocrine and behavioural studies, particularly for monitoring and identifying reproductive states of free-ranging rhesus females. The mean interval found between the oestradiol peak and colour breakdown (2.5 days) is not much less than that (2.8 days) between successful insemination and colour breakdown (Czaja *et al.*, 1975). Thus, although ovulation apparently follows the oestradiol surge by approximately 24–48 h (Weick *et al.*, 1973), our data suggest that the optimum time for insemination in the rhesus monkey is coincident with or soon after the oestradiol peak. This hypothesis receives support from the report by Parkin & Hendrickx (1975) of successful inseminations 12.5 h after peak oestradiol levels, and the behavioural findings that the highest probability of intromission and ejaculation in paired rhesus monkeys occurs 2–3 days before colour breakdown (Czaja *et al.*, 1975).

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