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Changes in plasma concentrations of LH, progesterone and oestradiol in relation to the occurrence of luteolysis, oestrus and time of ovulation in the Shiba goat (*Capra hircus*)

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Summary. Luteolysis in Shiba goats was spontaneous (N = 5) or induced by prostaglandin F-2α (N = 5). Blood sampling and the test for oestrous behaviour were carried out at 2-h intervals, and the time of follicular rupture (ovulation) was determined by culdoscopic observations performed every 2 h around the periovulatory period. In both groups plasma LH concentrations showed a temporary but significant increase during the abrupt fall in plasma progesterone concentrations at luteolysis. This LH rise may be responsible for the preovulatory development of antral follicles and the increase in oestradiol secretion from them. The total number of antral follicles (spontaneous luteolysis 3·6 ± 0·6, induced luteolysis 4·2 ± 0·7; mean ± s.e.m.) and the number of ovulations (1·8 ± 0·4, 2·4 ± 0·2) did not differ significantly between the two groups. There was also no significant difference in the timing of oestrus, LH surge and ovulation following the two modes of luteolysis. The interval from luteolysis to the peak of the LH surge averaged 65 h (range 56–72 h). The period of oestrous behaviour coincided with the acrophase of the LH surge and lasted for 22 h (12–28 h). Ovulations occurred 21 (16–24) h after the LH peak and 7 (2–12) h after the end of oestrus.

Introduction

Endocrine events in the peripheral blood during the follicular phase have been studied in detail in ewes and cows during the past decade (Baird & Scaramuzzi, 1976; Dobson, 1978; Karsch, Foster, Legan, Ryan & Peter, 1979; Baird, Swanston & McNeilly, 1981); it has also been established that the luteal regression induced by prostaglandin (PG) F-2α results in a prompt return to oestrus (Rowson, Tervit & Brand, 1972; Roche, 1974). In contrast to this detailed information for sheep and cattle, almost nothing is known of the sequence of endocrine events during follicular development in the goat.

The Shiba goat is a Japanese native miniature goat, adult females weighing 15–22 kg (Kano, Sawasaki & Oyama, 1977). The reproduction of this goat is characterized by its high fecundity (usually twins or triplets) and complete lack of seasonality (Kano *et al.*, 1977; Kano & Mori, 1982; Mori, Takahashi, Sawasaki & Kano, 1983b). The present investigation was undertaken to define the sequence of endocrine events in the follicular phase of the cycle in relation to the timing of oestrus and ovulation after spontaneous or induced luteolysis in Shiba goats.

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Materials and Methods

Animals

The 10 Shiba goats used were 2–4 years old, weighed 16–22 kg and had been showing regular 20-day oestrous cycles before the experiment. Throughout the experiment they were tied to a stanchion and fed a pelleted diet for ruminants (Matsui *et al.*, 1981) twice a day. Hay and water were supplied *ad libitum*. The experiment was conducted under natural lighting conditions during February 1980 (daylength 10·3–11·0 h, 36·3°N).

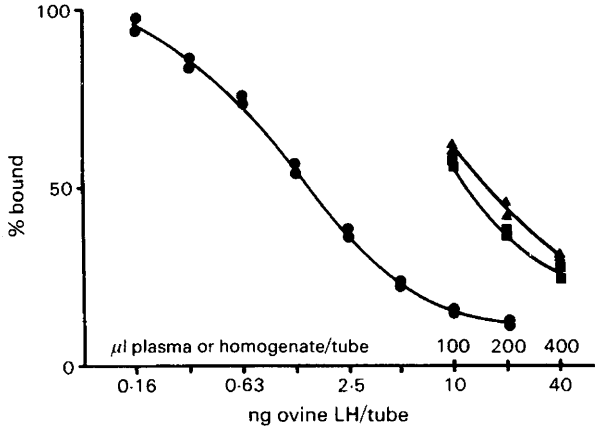
In 5 goats (Group 1), luteolysis was induced by a single i.m. injection of 2 mg PGF-2α given between Days 6 and 10 of the oestrous cycle (onset of oestrus = Day 0), and blood sampling was started 6–8 h before the PG injection. In the remaining 5 goats (Group 2), blood sampling began on Day 17 or 18 of the oestrous cycle, 2–3 days before the predicted onset of oestrus (average length of cycle 20·4 ± 0·2 days; Kano & Mori, 1982).

Collection of blood samples

Blood (5 ml) was collected at 2-h intervals through an indwelling vinyl chloride jugular catheter (o.d. 2 mm, i.d. 1·32 mm, length 70 cm). Plasma was immediately separated by centrifugation and stored at −20°C until assayed for LH, progesterone and oestradiol concentrations.

Hormone assays

LH was assayed by the double-antibody radioimmunoassay for ovine LH (Niswender, Reichert, Midgley & Nalbandov, 1969), using rabbit anti-ovine LH antiserum (GDN No. 15), highly purified ovine LH for iodination (LER 1374A) and NIH-LH-S19 as standard. Caprine anti-rabbit gamma globulin antiserum, developed in our laboratory, was used as second antibody at a final dilution of 1:240. The specificity of the assay was validated by comparing displacement curves for serial dilutions of plasma from oestrous goats and a goat pituitary homogenate with the displacement curve of the reference preparation of ovine LH (Text-fig. 1); the 3 curves did not depart from parallelism (2 × 3 points design, $P > 0{\cdot}05$). Samples of 200–400 μl goat plasma were assayed in duplicate and the LH concentration was expressed in terms of NIH-LH-S19. The limit of detection (95% confidence limits of buffer control) averaged 0·8 ng/ml for 200 μl plasma. The intra- and inter-assay coefficients of variation based on 8 replicates were 8·6% and 11·4%, respectively.



Text-fig. 1. Displacement curves for serial dilutions of oestrous goat plasma (■) and a goat pituitary homogenate (▲) demonstrating parallelism with the displacement curve of a reference preparation of ovine LH (NIH-LH-S19: ●). Radioactivity in all cases is expressed as the percentage of ^{125}I-labelled LH found in the buffer control tubes.

Plasma concentrations of progesterone and oestradiol were analysed in duplicate by radioimmunoassays. The specificity of the anti-progesterone antiserum, prepared in the Laboratory of Veterinary Physiology, University of Tokyo, has previously been described (Murakami, Takahashi & Suzuki, 1979). The anti-oestradiol antiserum (DA-27) was obtained from Teikoku Zoki Pharmaceutical Co., Kawasaki, Japan, and the cross-reactivity with other steroids was as determined by Makino (1973). Plasma (50 μl) was extracted with 2 ml diethyl ether and the ether extract was assayed for progesterone without chromatography. For oestradiol, 2 ml plasma were extracted with 10 ml diethyl ether and the ether extract was redissolved in benzene and methanol (85 :15, v/v) and purified by LH-20 microcolumn chromatography (Wu & Lundy, 1971). Mean recovery after extraction of [^{3}H]progesterone added to the pooled goat plasma was 92% and mean recovery after extraction and purification of [^{3}H]oestradiol was 83%. Free and bound ligands were separated by dextran-coated charcoal after an overnight incubation at 4°C with labelled steroid and the antiserum. The limits of detection were 0·2 ng progesterone/ml and 3 pg oestradiol/ml. The intra- and inter-assay coefficients of variation based on 8 replicates were 9·6 and 11·2% for progesterone and 10·2 and 12·4% for oestradiol.

Test for oestrous behaviour

Each goat was tested for oestrous behaviour with a vasectomized male once every 2 h until the female refused to stand for the male on two consecutive occasions after showing oestrus. Behavioural oestrus was defined as the period during which the female showed reflex deviation of the tail and allowed the male to mount. Copulation was not permitted.

Detection of ovulation

Direct observation of the ovary by culdoscopy (Mori, Kano & Sawasaki, 1983a) was performed 7–10 times in each animal; once just before the start of blood sampling, once or twice before the onset of oestrus and then several times at about 2-h intervals after the end of oestrus until ovulation. Follicular sizes were estimated from the photographs taken at each culdoscopy. Additional culdoscopies were performed on one or two occasions to investigate the development of newly formed corpora lutea. The goat was anaesthetized by an i.v. injection of a mixture of ketamine–HCl (Ketalar: Parke-Davis), 10 mg/kg body weight, and xylazine–HCl (Celactal: Bayer), 0·02 mg/kg, and placed prone in a head-down position on the observation stand. Then the culdoscope with a 90° visual angle (Olympus, Tokyo) was inserted into the pelvic cavity via the vaginal fornix and the whole surface of both ovaries was observed. The entire procedure took place within 15 min on each occasion. In a preliminary study it was established that a goat subjected to culdoscopy fully recovered within 30 min of administration of the anaesthetic (Mori *et al.*, 1983a).

Statistical analysis

Results were expressed as mean ± s.e.m. and Student's *t* test was used to determine the significance of differences between means.

Results

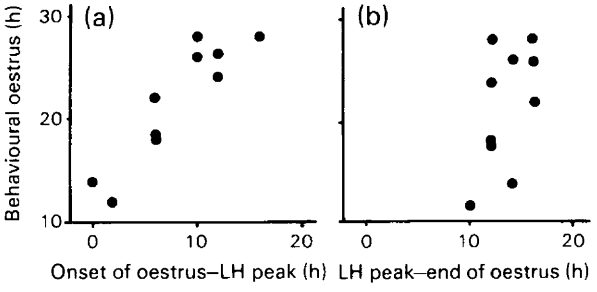
Chronological relationships between luteolysis, oestrous behaviour, the LH surge and ovulation are presented in Table 1. There were no significant differences in any of the characteristics measured in goats in Groups 1 and 2. Oestrous behaviour began abruptly about 55 h after PG injection in Group 1, and 19 days after the previous oestrus in Group 2. In Group 2, the entire sequence of spontaneous luteolysis (including the onset of luteolysis) was observed in only one goat.

Table 1. Chronological relationships between luteolysis, oestrus, the LH surge and ovulation during the follicular phase following PG-induced (Group 1) or spontaneous (Group 2) luteolysis in Shiba goats

	Group 1 (N = 5)	Group 2 (N = 5)	Total (N = 10)
Onset of luteolysis* to oestrus (h)	54·8 ± 2·6 (46–60)	54§	—
Onset of luteolysis* to LH peak† (h)	64·8 ± 2·9 (56–72)	70§	—
Onset of oestrus to LH peak† (h)	10·0 ± 1·1 (6–12)	6·0 ± 2·8 (0–16)	8·0 ± 1·5 (0–16)
Duration of oestrus (h)	24·4 ± 1·7 (18–28)	18·8 ± 2·9 (12–28)	21·6 ± 1·8 (12–28)
Duration of LH surge‡ (h)	7·6 ± 0·8 (6–10)	7·6 ± 0·8 (6–10)	7·6 ± 0·5 (6–10)
LH peak to end of oestrus (h)	14·4 ± 1·2 (12–18)	12·8 ± 1·0 (10–16)	13·6 ± 0·8 (10–18)
LH peak to ovulation (h)	20·0 ± 0·9 (20–24)	21·2 ± 0·5 (16–24)	20·6 ± 0·5 (16–24)
End of oestrus to ovulation (h)	5·6 ± 1·0 (2–8)	8·4 ± 0·7 (6–12)	7·0 ± 0·8 (2–12)

Values are means ± s.e.m. with ranges in parentheses.
* PG injection in Group 1.
† Time when plasma LH concentration was maximum.
‡ Period when plasma LH concentration was >10 ng/ml.
§ Observed only in one goat.

The acrophase of the LH surge (>10 ng/ml plasma) occurred during the period of oestrus. The interval between the onset of oestrus and the LH peak (maximum concentration) was more variable than the time from the LH peak to the end of oestrus. As shown in Text-fig. 2, the time between the onset of oestrus and the LH peak was significantly correlated with the duration of oestrus ($r = 0{\cdot}91$, $P < 0{\cdot}05$, N = 10), while the time from the LH peak to the end of oestrus was not ($r = 0{\cdot}21$), indicating that the variability in the duration of oestrus is mainly due to variability in the time to the LH surge. Ovulations occurred 21 h after the LH peak.



Text-fig. 2. Comparison of the duration of behavioural oestrus with (a) the time from the onset of oestrus to the LH peak and (b) the time from the LH peak to the end of oestrus in Shiba goats.

The number of follicles ovulated or becoming atretic was not significantly different between the two groups (Table 2). On average, 4 Graafian follicles developed to about 3 mm in diameter or larger after luteolysis and half of them ovulated. The gross morphological changes seen in the ovary during ovulation in Shiba goats have been described by Mori *et al.* (1983a).

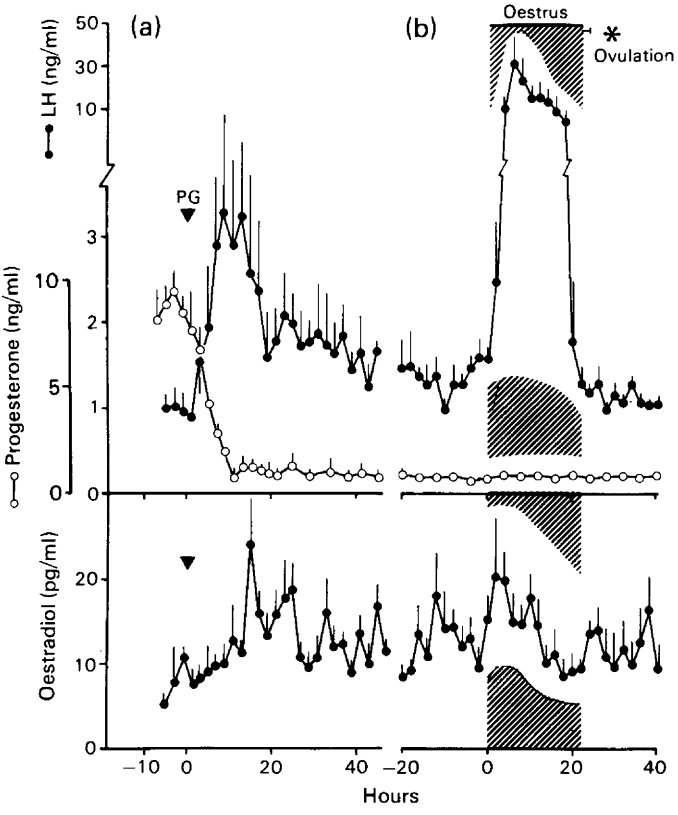
The hormonal profiles during the follicular phase were very similar in the two groups, except for the different timing in onset of luteolysis, and a transitory rise in the basal LH concentration was

Table 2. Number of follicles observed by culdoscopy in the ovaries of Shiba goats during the preovulatory period following PG-induced (Group 1) or spontaneous (Group 2) luteolysis

	No. of follicles		
	Group 1 (N = 5)	Group 2 (N = 5)	Total (N = 10)
Total	4·2 ± 0·7 (2–6)	3·6 ± 0·6 (2–5)	3·9 ± 0·4 (2–6)
Ovulated	1·8 ± 0·4 (1–3)	2·4 ± 0·2 (2–3)	2·1 ± 0·2 (1–3)
Atretic	2·4 ± 0·6 (0–3)	1·2 ± 0·7 (0–3)	1·8 ± 0·5 (0–3)

Values are means ± s.e.m. with ranges in parentheses.

noted in all the goats examined about 50 h before the LH surge. To correlate this LH elevation with luteolysis, the results from Group 1 goats (N = 5) were plotted in relation to the time of PG injection (Text-fig. 3a). The decrease in plasma progesterone concentrations occurred 1–13 h after



Text-fig. 3. Temporal relationships between plasma concentrations of LH, oestradiol and progesterone during the follicular phase in Shiba goats. Around the time of luteolysis the data from the goats in which the luteolysis was induced by prostaglandin (PG) are normalized to the time of PG injection (a), and around oestrus the data from all the goats are normalized to the time of onset of oestrus (b). Each point depicts the mean ± s.e.m. of 5 (a) or 10 (b) observations. The interval between the PG injection and the onset of oestrus was 54·8 ± 2·6 h (N = 5).

the PG injection with an abrupt decline between 5 and 13 h. Thereafter progesterone remained at basal levels (<1·0 ng/ml) throughout the experimental period. Plasma LH showed a marked elevation as the progesterone concentration fell; the rate of increase declined as the plasma oestradiol began to rise. However, the LH concentrations before the preovulatory LH surge were always higher than those before the onset of luteolysis. The plasma oestradiol concentration began to increase after the transitory LH rise at luteolysis, and then fluctuated.

In Text-fig. 3(b) the data from both the groups were plotted from the time of onset of oestrus. Both the magnitude and duration of the LH surge were indistinguishable in the two groups. At the time of onset of oestrous behaviour plasma oestradiol concentrations were elevated in almost all the animals. Oestradiol concentrations declined with the onset of the preovulatory LH surge. Oestrous behaviour ceased when LH and oestradiol values had declined, although plasma concentrations of LH and oestradiol were still higher than they were before the PG injection. In some goats plasma oestradiol showed a second increase after the end of oestrus.

Discussion

Progress in laparoscopic technique has made possible serial observations of the ovary in a variety of experimental animals (Dukelow, 1978). However, there are problems with this technique in the smaller ruminants like sheep and goats, since so much of the space in the abdominal cavity is occupied by the rumen, which makes observations difficult. In this study we used culdoscopy instead of laparoscopy; this gave excellent visualization of the ovaries, and permitted timing of ovulation to within 2 h. To minimize any possible effect of the anaesthetics on the ovulatory process (Robertson & Rakha, 1965; Peet & Lincoln, 1977), most of the culdoscopies were performed after the end of oestrus, and after the LH surge had taken place. The mean number of ovulations observed (2·1) was in close agreement with the mean litter size of this strain of goat (1·8, Kano *et al.*, 1977). The mean time interval between the LH peak and ovulation, 20·6 h, was slightly shorter than the 24 h recorded for ewes (Cumming *et al.*, 1973) and the 26 h for heifers (Mori, Tomizuka, Kariya & Riu, 1982). The interval between the onset of luteolysis and the LH surge, about 65 h, was similar to that found for ewes by Karsch *et al.* (1979) and Wheeler, Baird, Land & Scaramuzzi (1975).

The sequence of endocrine changes occurring after luteolysis in the Shiba goat was very similar to that reported for cows (Christensen, Hopwood & Wiltbank, 1974), and ewes during spontaneous and PG-induced ovulation (Chamley *et al.*, 1972; Baird & Scaramuzzi, 1976; Baird, Land, Scaramuzzi & Wheeler, 1976; Karsch *et al.*, 1979). A rise in the basal concentration of LH after luteolysis has been reported for ewes after the injection of a PG analogue (Baird & Scaramuzzi, 1976). This is probably due to the reduction in circulating progesterone, and removal of its negative feedback effect on LH secretion, as was clearly demonstrated in ewes (Goodman & Karsch, 1980). The decline of LH levels in Shiba goats, coincident with the increasing plasma oestradiol concentrations, suggests that oestradiol was exerting a negative feedback effect on LH secretion. On the other hand, at around the time of onset of oestrus, the elevation in oestradiol was immediately followed by the onset of the preovulatory LH surge, illustrating the positive feedback effect of oestradiol. This initial suppression of LH followed by the surge has been observed in a variety of species in response to exogenously administered or endogenously secreted oestradiol (Scaramuzzi, Tillson, Thorneycroft & Caldwell, 1971; Takahashi, Ford, Yoshinaga & Greep, 1977; Knobil, 1980). Hauger, Karsch & Foster (1977) suggested that there must be a certain threshold level at which oestradiol exerts its positive feedback effect on LH secretion. In this study, there was no obvious difference in the oestradiol concentrations that elicited negative and positive feedback, suggesting that the two effects may be more a reflection of different latent periods than of different thresholds. The precise mechanism by which oestradiol can produce both positive and negative feedback effects remains unknown.

In ewes (Moor, 1974; Baird *et al.*, 1981), cows (Dieleman, Kruip, Fontijne, de Jong & van der Weyden, 1983) and other animals the LH surge is known to terminate oestradiol secretion from Graafian follicles. Dieleman *et al.* (1983) showed that the concentration of oestradiol in the follicular fluid dropped rapidly during the period from 6 to 10 h after the LH peak. This probably explains the relatively constant interval of about 14 h between the LH peak and the end of oestrus that was observed in this study, whilst the interval between the onset of oestrus and the LH peak showed much more variability.

The results presented here show that the chronological sequence of preovulatory events and hormonal profiles of the Shiba goat are in close agreement with those previously described for ewes and cows. The use of the culdoscope has enabled us to relate these endocrine events to morphological changes within the ovaries, and the precise timing of ovulation itself.

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