Progesterone, oestradiol-17 β and LH during the oestrous cycle of muskoxen (*Ovibos moschatus*)

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Summary. Progesterone, oestradiol-17 β and LH were measured in plasma from 6 non-pregnant, captive, female muskoxen during the 1984 and 1985 breeding seasons. Jugular blood samples were taken on an alternating 3/4-day schedule in 1984 and daily or at 4-h intervals over oestrus, via indwelling jugular cannulae, for 6 weeks in 1985. Oestrous cycle length was 19.6 ± 0.96 (s.d.) days ($n = 19$) and did not vary between the first and subsequent cycles of the season. Progesterone was lowest at oestrus (≤ 0.1 ng/ml), began to rise on Days 4–5, peaked on Days 10–12 (mean = 2.6 ng/ml) and returned to baseline 2–5 days before the next oestrus. A small rise in progesterone before the first cycle of the breeding season was observed on 7 of 12 occasions. Oestradiol-17 β was significantly higher ($P < 0.001$) 1–4 days before, or coincident with, oestrus. The average duration of the LH peak was 24.6 h ($n = 7$) and coincided with observations of behavioural oestrus. In one animal behavioural oestrus and an LH peak preceded a small progesterone rise at the beginning of the breeding season. The temporal relationship of these three hormones during the muskox oestrous cycle is very similar to that seen in domestic ruminants.

Keywords: muskox; oestrous cycle; progesterone; oestradiol-17 β ; LH

Introduction

Although superficially resembling bison, muskoxen have many characteristics in common with sheep and goats (Moody, 1958; Tener, 1965; Rowell *et al.*, 1987) and are in the same subfamily (Caprinae) (Simpson, 1945). However, their lack of close relatives, chromosomal differences ($2N = 48$) and adaptation to an arctic environment make this species of particular comparative interest.

The morphology of the muskox reproductive tract is very similar to that of sheep and goats but little is known of their reproductive physiology. Most information has come from behavioural observations (Gray, 1987) and post-mortem examination (Tessaro *et al.*, 1984; Rowell *et al.*, 1987). Muskoxen are seasonally polyoestrous, generally mating in August (Tener, 1965; Tessaro *et al.*, 1984; Latour, 1987) and September (Smith, 1976; Gray, 1987) in their natural habitat. The oestrous cycle of the muskox is estimated to be 21 days in length (J. Bourque, cited by Gray, 1973; Wilkinson, 1973) but nothing is known of its endocrinology. Accordingly, serial blood samples were taken from 6 captive muskoxen over 2 breeding seasons to measure changes in progesterone, oestradiol-17 β and luteinizing hormone (LH) during the oestrous cycle.

Materials and Methods

Animals. Six adult, female, muskoxen from the Research Herd at the Western College of Veterinary Medicine (Flood *et al.*, 1984) were sampled over 2 breeding seasons (1984–1985). They were 2.5 years old at the beginning of the study and 5 of the 6 females were parous. The females were kept together in outdoor pens with intact and castrated males. They were fed a supplement of oat pellets and had ad-libitum access to brome-alfalfa hay and water. The muskoxen had been bottle raised and were fully accustomed to handling and restraint.

Sample collection. In 1984, blood samples were collected on an alternating 3/4-day schedule and the cows were allowed to mate freely with the dominant bull.

In 1985, the cows were fitted with jugular cannulae about 1–2 weeks before the first expected oestrus. Following immobilization with carfentanil (Janssen Pharmaceuticals, Mississauga, Ontario, Canada) and xylazine (Haver-Lockhart, Rexdale, Ontario, Canada), one jugular vein was cannulated. Immobilization was reversed with naloxone (Wildlife Labs Ltd, Fort Collins, CO, U.S.A.) after 20–40 min. The cannulae were flushed and refilled with heparinized saline (10 U heparin/ml physiological saline) twice daily and following each blood collection. Prophylactic antibiotics were administered at the time of cannulation and again 3 weeks later.

Daily blood samples were collected until oestrus was detected, then samples were taken at 4-h intervals for 36–48 h to detect the LH peak. The cannulae remained patent for 2–6 weeks. Those that blocked before the end of the trial were removed and blood was taken by jugular venepuncture every second day. After 6 weeks the two remaining cannulae were removed and sampling in all animals was continued on the alternating 3/4-day schedule until the next observed oestrus when the females were allowed to mate with a fertile male.

Daily samples (~10 ml) were collected between 09:00 and 12:00 h in heparinized vacutainers, refrigerated immediately and centrifuged at 2500 *g* for 10 min within 1 h of collection to optimize progesterone preservation (Rowell & Flood, 1987). Plasma was stored at –22°C until assayed. All samples were assayed for progesterone and the 1985 samples were also assayed for LH and oestradiol-17 β .

Detection of oestrus. In 1984 white adhesive tape was fixed to the rump hair of females and coloured petroleum jelly applied to the briskets of the bulls. Continuous observations for evidence of oestrus were made between 16:00 and 17:00 h each day and the cows were checked each morning for colour marking.

In 1985, an experienced bull was vasectomized, his brisket was painted and the cows were taped as before. The cows were separated from the rest of the herd during the day (09:00–16:00 h) after which they were placed with the vasectomized bull and a 1-h check for oestrus was conducted. The cows were left with the bull overnight and checked for colour marks the following morning. The first day of oestrus, indicated by behaviour and colour marking, was designated Day 0.

Hormone assays. Progesterone, oestradiol-17 β and LH were measured by radioimmunoassays (RIA) following the methods of Rawlings *et al.* (1984).

Progesterone was measured in duplicate samples using a double-antibody method and ¹²⁵I-labelled progesterone. The antiserum (raised against 4-pregnen-11 α -ol-3,20-dione hemisuccinate–BSA) (Steraloids Q3253) had the following cross-reactions: 5 β -pregnan-3,20-dione, 20%; 5 α -pregnan-3,20-dione, 14%; corticosterone, 0.8%. The reaction was stopped using a sheep anti-rabbit γ -globulin solution. Extraction efficiency was 75 $\pm$ 4% (mean $\pm$ s.d., *n* = 19). To confirm extraction efficiency from muskox plasma, 0.5 and 2.5 ng/ml progesterone were added to plasma of a castrated muskox. The samples (*n* = 4) were assayed as above and found to contain 0.64 ng/ml and 2.75 ng/ml (CV = 11.3% and 3.0% respectively). The labelled ligand was significantly displaced from the antiserum by 5 pg progesterone. Coefficients of variation were calculated from a high (~2.5 ng/ml) and a low (~1.3 ng/ml) reference pool of sheep plasma. The mean ($\pm$ s.d.) intra-assay coefficients of variation were 7.4 $\pm$ 4.1% (*n* = 18) and 9.8 $\pm$ 5.4% (*n* = 15) for high and low references respectively. The inter-assay coefficients of variation were 11.7% (*n* = 37; high reference) and 14.6% (*n* = 24; low reference).

Oestradiol-17 β was measured in 1-ml samples of muskox plasma. The antiserum (raised in rabbits against 1,3,5,10-oestratriene-3,17 β -diol-6-one-6-O-carboxy-methyloxime–bovine serum albumin) had the following cross-reactions: oestrone, 2.7%; testosterone, 0.02%; progesterone, <0.02%; cortisol, <0.01%. Extraction efficiency was (85 $\pm$ 1%, *n* = 10). The labelled ligand was significantly displaced from the antiserum by 1 pg oestradiol-17 β . The mean intra-assay coefficients of variation for a high and low concentration reference ewe plasma were 6.7 $\pm$ 3.2% (*n* = 10) and 12.1 $\pm$ 1.7% (*n* = 10) respectively. Inter-assay variation was 10.1% (*n* = 30; high reference) and 14.1% (*n* = 37; low reference).

LH concentrations were measured in duplicate samples using a double-antibody RIA (Rawlings *et al.*, 1984). The first antibody was prepared in rabbits and had the following cross-reactions: ovine thyroid-stimulating hormone, 5%; ovine FSH, 1.1%; ovine prolactin, 0.1%; growth hormone, 3.1%. The range of the standard curve was 50 pg–12.8 ng (NIH-LH-S18) and 100 pg LH significantly displaced the labelled ligand from the antiserum. The intra- and inter-assay coefficients of variation were 4.5% (*n* = 17) and 16% (*n* = 47) respectively.

The ovine LH assay was validated for muskoxen using muskox pituitary extract. Pituitaries from 2 muskox calves were homogenized in distilled water, centrifuged and the supernatant diluted 100-, 1000-, 10 000-fold. The binding inhibition curve of the muskox pituitary extract was parallel to the inhibition curve of ovine LH.

Statistical analysis. Analysis of variance and Scheffé's test were used to detect significant differences in the oestradiol-17 β results (Snedecor & Cochran, 1980).

Results

The onset and length of the oestrous cycle

Muskox cows showed their first signs of oestrus from 20 September to 8 October in both 1984 and 1985 (median breeding date, 25 September).

In 1984, 1 cow failed to conceive and blood samples were collected during 4 consecutive cycles. The remaining 5 cows conceived to the first oestrus. In 1985, 2 cycles were followed in each of 4 cows, 3 in another and 4 in a cow that failed to conceive. The 19 cycles for which complete hormonal data were obtained had a mean length of 19.6 ± 0.96 (s.d.) days (range 18–21 days). There was no difference in length between the first cycle of the season and subsequent cycles. No attempt was made to define the end of the breeding season but 1 infertile cow had a total of 7 oestrous cycles that continued until March. Frequent blood samples were not collected during the later cycles; these were inferred from oestrous behaviour and were not included in the hormone profiles.

Progesterone concentrations

The combined data for all 19 oestrous cycles are shown in Fig. 1. Jugular progesterone concentrations were lowest around the time of oestrus (≤ 0.1 ng/ml), began to rise above 0.4 ng/ml on Days 4–5, peaking at 1.4–5.3 ng/ml on Days 10–12 (mean = 2.6 ng/ml). Progesterone began to decline on Days 16–17 reaching basal levels 2–5 days before the next oestrus.

Fig. 1. The combined progesterone concentrations ($\pm$ s.e.m.) for 19 muskox oestrous cycles and oestradiol-17 β concentrations ($\pm$ s.e.m.) for 14 oestrous cycles. All data have been normalized to the day of oestrus (Day 0), indicated by the arrows.

A small rise in progesterone (≥ 0.4 ng/ml) with a mean duration of 4.6 days was observed in 7 of 12 instances at the beginning of the breeding season. One cow had 2 such peaks (both with peak values of 0.7 ng/ml and both lasting 4 days) before the first normal cycle of 1985.

Oestradiol-17 β concentrations

Mean values for oestradiol-17 β from 14 cycles are shown in Fig. 1. The concentrations on the day of oestrus and the preceding 4 days were significantly higher ($P < 0.001$) than those at other times during the cycle. Minimum values were seen in the mid- and late luteal phase.

LH concentrations

The frequent sampling period encompassed the LH peak in 7 cycles from 5 different cows. It occurred 5, 4 and 2 days after progesterone had returned to baseline (0.4 ng/ml) (Fig. 2a & b). The

average duration of 7 LH peaks was 24.6 h (range 20–28 h). The detected amplitude varied from 7.9 to 20.6 ng/ml. Of the 7 LH peaks recorded, 6 coincided with Day 0 of the cycle. In the 7th case the LH peak coincided with behavioural oestrus but was followed by only a slight rise in progesterone (0.7 ng/ml) and the cow returned to oestrus 8 days later. Unfortunately, the cannula in this cow became blocked and LH could not be measured at the second oestrus. Progesterone concentrations indicated that the subsequent cycle was of normal length.

Fig. 2. Representative progesterone, oestradiol-17 β and LH profiles for 2 muskoxen (a, b) during the oestrous cycle. The day of oestrus is Day 0. The days preceding the first oestrus are shown as negative values.

Discussion

The 19.6-day oestrous cycle, based on peripheral progesterone concentrations, agrees well with behavioural observations of a 21-day cycle in captive muskoxen at Kuujuaq, Quebec (J. Bourque, cited in Gray, 1973) and the 19–21-day cycle inferred from the temporal spacing of births of captive

muskoxen in Alaska (Wilkinson, 1973). Although red deer and Père David's deer have an average cycle length of 20 days, considerably longer cycles may occur (Guinness *et al.*, 1971; Curlewis *et al.*, 1988). In fallow deer there is a tendency for the cycle to lengthen as the breeding season progresses (Asher, 1985). Large variations in cycle length were not evident here, although only 4 sequential cycles were intensively studied in a season. The animals were all the same age and their calves had been weaned 1.5–2 months before the breeding season and so the effect of age and lactation could not be assessed.

The changes in progesterone concentration in peripheral plasma were similar to those reported in the ewe (Yuthasastrakosol *et al.*, 1975), cow, buffalo and zebu (see Dobson & Kamonpatana, 1986), red deer (Adams *et al.*, 1985), fallow deer (Asher, 1985) and Père David's deer (Curlewis *et al.*, 1988). A small progesterone rise at the onset of the breeding season is characteristic of many seasonally breeding species (sheep: Legan *et al.*, 1985; fallow deer: Asher, 1985) with the exception of the red deer (Guinness *et al.*, 1971; Adams *et al.*, 1985). The rise has been associated with fluctuating LH levels in ewes (Yuthasastrakosol *et al.*, 1975) and roe deer (Schams *et al.*, 1980) and silent ovulation in other cervids (Asher, 1985). In the ewe, a brief rise in progesterone at the beginning of the breeding season is sufficient to provide the progesterone priming essential for normal luteal function (Legan *et al.*, 1985). Similar mechanisms may be acting in the muskox.

The raised concentrations of oestradiol-17 β in the muskox just before or during oestrus and minimum values in the mid- and late luteal phase are similar to those in sheep (Karsch *et al.*, 1979), cattle (Dobson & Kamonpatana, 1986) and white-tailed deer (Plotka *et al.*, 1980). A rise in oestradiol-17 β was seen in some cows 3–4 days after oestrus. Its occurrence was too irregular to be demonstrable in the pooled data.

The preovulatory LH peak occurred 2–5 days after progesterone had reached baseline. This period is apparently longer and more variable than in the ewe (Baird & Scaramuzzi, 1976; Karsch *et al.*, 1979), goat (Mori & Kano, 1984) and cow (Walters & Schallenberger, 1984). The duration of the LH surge (24 h) was longer than the 6–10 h reported for cattle (Walters & Schallenberger, 1984), 7–8 h for goats (Mori & Kano, 1984) or 14 h for the ewe (Pant *et al.*, 1977). In roe deer the LH peak occurred over a period of days (Schams *et al.*, 1980).

Overall, the pattern and temporal relationships of progesterone, oestradiol-17 β and LH in the muskox are very similar to those reported for domestic ruminants, suggesting that mechanisms for the control of ovulation and luteolysis are similar.

Like sheep, muskoxen are seasonal breeders. In both years of this study the onset of the breeding season was 4–6 weeks later than the estimated time of breeding on Banks Island, Northwest Territories (the island on which these animals were originally captured) (Tessaro *et al.*, 1984; Latour, 1987). Calving dates from other southern locations also indicate delayed onset of breeding (Teal, 1959; Oeming, 1965; Karsten, 1986; Dr G. Glover, Assiniboine Park Zoo, Winnipeg, personal communication). Although this difference could be a consequence of captivity, the effects of temperature and latitude ($\sim 20^\circ$ difference) cannot be excluded. Throughout their natural range, breeding begins at approximately the same time in either decreasing (Thelon Game Sanctuary) or continuous (Northern Ellesmere Island) daylight, suggesting that the stimulus initiating breeding in muskoxen is different from the decreasing photoperiod effective in sheep, goats and deer. Tener (1965) has proposed that the alternating light and dark of the vernal equinox may provide the photostimulatory cue. Given the general similarities between muskoxen and sheep, it would be particularly interesting to investigate this hypothesis and the mechanisms governing the onset of breeding in muskoxen.

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