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Brief communication

Ultrasonic Imaging of Reproductive Events in Muskoxen

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Introduction

Patterns of ovarian follicular development have been well described in horses (Ginther, 1992), cattle (Ginther *et al.*, 1989; Knopf *et al.*, 1989), sheep (Ravindra *et al.*, 1994), goats (Ginther & Kot, 1994), llamas, alpacas (Adams *et al.*, 1990) and, more superficially, in several non-domestic species (Adams *et al.*, 1991) using transrectal ultrasonography. The technique allows the identification of follicles >2 mm. in diameter though analysis is usually restricted to follicles >4 mm. In all the species mentioned above, antral follicles appear in the ovaries in small groups or cohorts, and continue to grow in parallel for the next few days, before the majority degenerate. Such a group is known as a follicular wave. Each wave is initiated by a surge of follicle stimulating hormone from the pituitary which precedes wave emergence (Adams & Pierson, 1995). Follicular waves occur during the breeding and non-breeding seasons as well as during pregnancy. It is not known whether follicular waves are affected by nutrition.

Transrectal ultrasonography has recently been used in muskoxen to characterize the corpus luteum of pregnancy (Rowell *et al.*, 1993) and to examine the accessory genital glands of males (Tedesco, 1996) but the behaviour of ovarian follicles has not been studied. Follicular and luteal dynamics are of particular interest in muskoxen because their reproduction is very sensitive to nutritional (Adamczewski *et al.*, 1997) and photoperiodic (Gray, 1987; Tedesco, 1996; Tener, 1965) effects.

The objectives of this study were to determine the feasibility of daily transrectal ultrasonography of the female reproductive tract, and to characterize ovarian follicular and luteal dynamics during the breeding and non-breeding seasons. Here we report on the technique and preliminary results obtained during the breeding season. A full account will appear elsewhere.

Materials and methods

Female muskoxen at the University of Saskatchewan – Goodale Research Station were examined weekly or daily, by transrectal ultrasonography during selected periods in 1992 and 1995. In 1992 the animals (n=4) ranged in age from 6–10 years and weighed 222±9 kg; in 1995 all the animals (n=6) were two years old and weighed 171±6

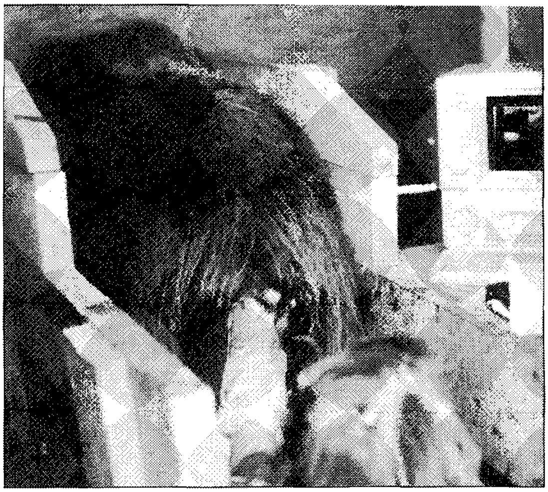

Fig. 1. Ultrasound examination of a muskox showing the animal, the instrument, the examining stall and the operator (who is crouching to give a clear view).

kg. The longest period of continuous daily observation was 35 days.

The muskox cows were normally maintained on pasture with at least one male. With the onset of the rut, the cows were separated from the bulls by a wire fence until one oestrous cycle had been completed. The muskoxen were accustomed to entering a central feeding pen daily, and were halter-tied in individual stalls while feeding. The study animals were led into a raised tie-stall that was enclosed and darkened so the ultrasound screen could be seen (Fig. 1). The side of the stall was adjustable to minimize lateral movements. The animals were fed during the procedure and could be easily restrained without tranquilization or anesthesia. After manual removal of the feces, the reproductive tract was examined as in horses (Ginther, 1986), using a B-mode scanner with a 7.5 MHz linear-array transducer (Aloka SSD 500, Overseas Monitor Corp. Ltd., Richmond, B.C.). On each occasion, the ovaries were sketched to record the size and location of the follicles and the corpus luteum.

As in cattle, individual follicles could be identified and monitored (Knopf *et al.*, 1989). The diameter of the largest follicle was recorded from the day at which it was first identified (4 to 5 mm.) to the day on which its identity was lost (regression to ≥4 mm.). The daily diameter profile was then related to the total number of follicles >4 mm detected each day. Follicular development was accepted as wave-like if the number of new follicles detected each day peaked at regular intervals. The largest follicle of a follicular wave was defined as a dominant follicle, and the next largest follicles were defined as subordinate if they appeared within one day of the dominant follicle and increased in size for at least one day after detection (Ginther *et al.*, 1989). Waves in which the size of the dominant and subordinate follicles diverged markedly were defined as major waves. If the largest follicle was only a little larger than its companion follicles, the wave was referred to as a minor wave (Bergfelt & Ginther, 1993). Successive follicular waves in an oestrous cycle (Day 0 = day of ovulation) were designated as Wave 1, Wave 2, Wave 3 and Wave 4 regardless of whether they were major or minor. The day of wave emergence was determined in retrospect as the day on which the dominant follicle was 4 to 5 mm in diameter.

The significance ($P \geq 0.05$) or tendency ($P \geq 0.1$) of fluctuations in the number of follicles detected per day was tested by analysis of variance for repeated measures and confirmed by split-plot analysis of variance. An indication of a day effect in the number of follicles detected per day was followed by a normalization procedure in which the data (follicle number and diameter, corpus luteum diameter) were adjusted to the mean day of wave emergence for each successive wave, and to the mean interovulatory interval.

Results and discussion

Within a week of commencing daily examinations, all of the muskoxen had adjusted to the procedure and could be handled by one person with minimal coaxing (Fig. 1). The animals remained standing when examined and rarely kicked. There was some resistance (restless movement) during the initial period of habituation but very little effort was made to avoid being handled. No signs of learned aversion were observed. On the contrary, the muskoxen became more eager to enter the stall as the study progressed.

The rectum of all the muskoxen ($n = 10$) was large enough to permit the insertion of a gloved hand and an ultrasound probe. The part of the procedure that was most resented was initial insertion of the examiner's hand into the anus. The muskoxen particularly resented the guard hairs around the perineum being pulled and the short tail was clamped tightly over the anus at the start of the examination. Clipping the hair covering the tail and perineum greatly facilitated access and minimized discomfort. The anus was more restrictive than the rectum and operators with small hands (glove size, <7) were most successful. Once the anus was passed, both the tail and the anal sphincter relaxed. Standard methyl-cellulose gel was satisfactory as both a lubricant and contact medium. Insertion of the hand into the rectum was an improvement over an earlier technique in which the transducer was fitted with a stiffened handle manipulated from the exterior, because it allowed controlled pressure on the rectal wall and greater subtlety of movement. Typical images are shown in Fig. 2.

Follicular waves were apparent during the oestrous cycles of all 4 muskoxen examined during the ovulatory season ($P<0.1$); one animal had 3 waves per cycle and the remaining 3 animals had 4 waves. Only the dominant follicle of the last wave of the cycle ovulated; the dominant follicle of other waves regressed slowly over a period of a few days. Intervals and follicle growth characteristics for 3- and 4-wave animals are summarized in Table 1 and Fig. 3. Dominance was clearly manifest in the first and last follicular waves in each of the 4 animals (major waves), whereas the other waves in all but one instance appeared to be minor waves. In cattle and llamas, the largest follicle of a wave consistently suppresses its subordinates, growing in diameter while its subordinates regress (Adams *et al.*, 1990; Adams *et al.*, 1992; Ginther & Kot, 1994; Ravindra, 1994). In sheep (Ravindra *et al.*, 1994), goats (Ginther & Kot, 1994) and horses (Bergfelt & Ginther, 1993), follicle dominance is not seen in some waves and apparently muskoxen show the same phenomenon.

The first ovulation of the breeding season was detected in 2 of the 4 muskoxen. The subsequent interovulatory intervals were 6 and 7 days, and included only 1 follicular wave and a short-lived corpus luteum. Growth characteristics of the dominant follicle and corpus luteum of this initial short cycle are given in Table 1.

Corpora lutea were detected soon after ovulation (Day 0.6±0.2). They first appeared as brightly echogenic structures but became less echogenic as the cycle progressed; they remained visible throughout the interovulatory interval and attained a maximum diameter about 5 days after ovulation

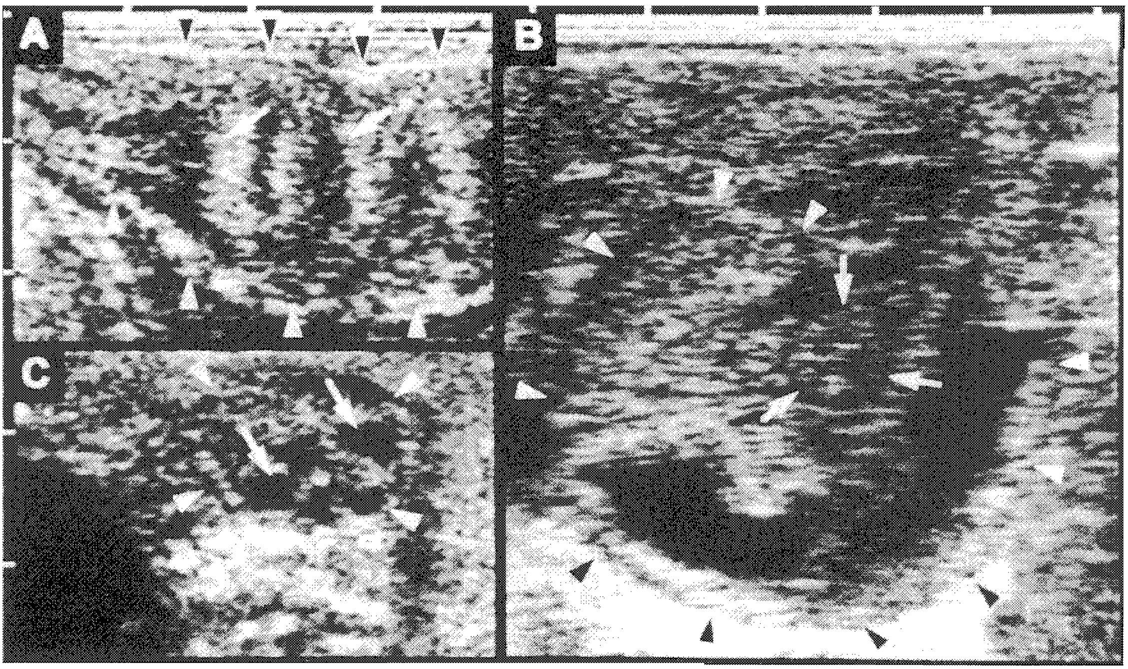

Fig. 2. Ultrasonographic images of the female reproductive tract of the muskox. One cm. scale divisions are shown at the top of each image and cranial is to the right. A) The cervix. The arrows indicate cervical folds and arrow heads the outline of the cervix. B) The female reproductive tract at 25 days gestation. The arrow heads show the greater curvature of the uterus.and the arrows show the outline of the corpus luteum within the ovary. The black area inside the uterus is allantoic fluid. C) An ovary with many follicles. The arrows show follicles of about 4 mm diameter. The arrows heads indicate the outline of the ovary.

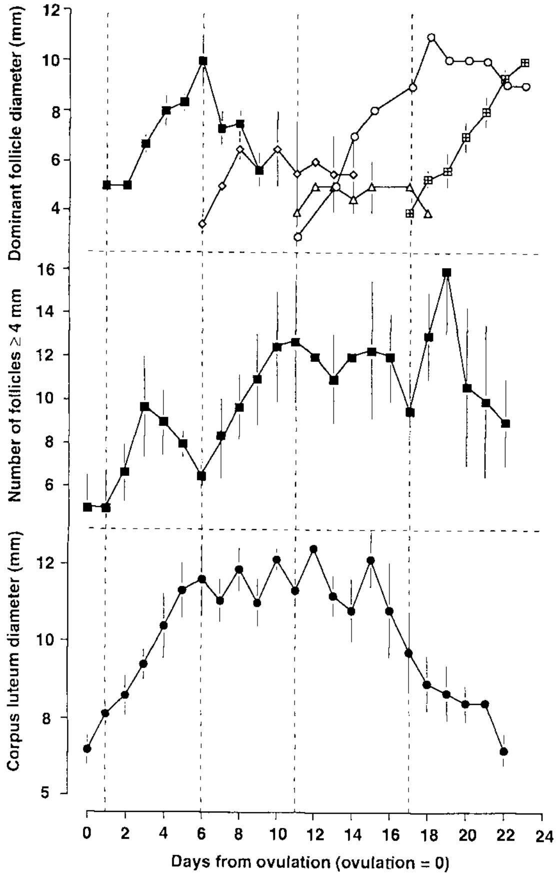

Fig. 3. The diameter of the largest follicle (upper panel), number of follicles >4 mm in diameter (middle panel) and diameter of the corpus luteum (lower panel) throughout the second oestrous cycle (interovulatory interval = 22.8±1.1 days) in 3 muskoxen with a 4-wave cycle (means±SEM; vertical dotted lines show the day of emergence of a follicular wave). Wave 1 (solid squares) and Wave 4 (boxes) were all major waves and Wave 2 (open diamonds) was all minor. In Wave 3, 1 animal (open circles) had a major wave and the other 2 (open triangles) had minor waves; these are shown separately.

(Table 1 and Fig. 3). The corpus luteum of the first cycle of the season was also seen soon after ovulation and was visible throughout the interovulatory interval (Table 1).

Conclusion

These results show the applicability of transrectal ultrasonography to the study of reproduction in muskoxen. The animals were easily examined daily for 5 weeks without apparent stress. The technique could also have been used to assess the reproductive status of immobilized muskoxen in the field. The ovaries of well fed tame muskoxen show regular follicular waves during the breeding season and probably during the non-breeding season, though the latter data are, as yet, incomplete. Each oestrus cycle included 3 or 4 follicular waves at about 6 day intervals. The short cycle at the onset of the breeding season consisted of a single follicular wave.

To our knowledge, this is the first detailed report of ovarian follicular dynamics in a wild species. It seems that the pattern in muskoxen, with major and minor waves and a short first cycle, is similar to that in goats. Insight of this kind is important in the design of appropriate artificial breeding systems and in this respect, the muskox may provide a useful model for the endangered takin (*Budorcas taxicolor*). A detailed knowledge of ovarian events may also be critical to the interpretation of the response of wild populations to environmental stress.

Table 1. Follicular and luteal characteristics of the first and second cycles of the breeding season.

	First cycle	Second cycle
Number of muskoxen	2	3 or 4
Interovulatory		
Interval (days)	6.5±0.5	22.8±1.1
Interwave Interval (days)	–	5.4±0.2
Day of wave emergence*		
Wave 1	0.5±0.5	1.3±0.3
Wave 2		6.0±0.4
Wave 3		11.5±0.3
Wave 4		17.3±0.3
Dominant Follicle		
Number of observations	2	11 or 12
Emerging diameter (mm)	4.5±0.5	4.5±0.4
Growth rate (mm/day)	0.9±0.1	0.9±0.1
Maximum diameter (mm)	10.5±0.7	9.1±0.9
Days to maximum diameter	5.0±1.0	6.0±1.1
Regression rate (mm/day)	–	0.6±0.1
Corpus Luteum		
Number of observations	2	4
Day of first detection	0.5±0.5	0.6±0.2
Diameter at first detection (mm)	6.5±0.5	7.2±0.4
Days to maximum diameter	5.0±0.0	4.7±0.4
Maximum diameter (mm)	14.5±3.5	13.5±1.9
Days retained	> 6	23.5±3.5

*Day of ovulation = Day 0.

Acknowledgements

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