

Expanded abstract

Seasonal melatonin and luteinizing hormone rhythms in muskoxen at 52° N.

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On northern Ellesmere Island (79° N) muskoxen experience over 4 months of continuous daylight and breed near the end of this period, but in southern regions of their range (which extends to 63° N) breeding occurs in early fall, when nights are lengthening. Because they breed under such different light/dark cycles the influence of photoperiod on their reproductive cycle is not clear. Tener (1965) suggested that the annual rhythm of reproduction in muskoxen may be entrained by photoperiodic cues surrounding the vernal equinox. Photoperiodic information is conveyed to the neuroendocrine axis by melatonin, a hormone produced in the pineal gland at night (Reiter, 1991). Our objective was to characterise daily and annual melatonin and luteinizing hormone (LH) rhythms in muskoxen kept outdoors at 52° N, where the annual photoperiodic cycle approximates that at the southern extremity of their natural range.

Blood was collected outdoors, without sedation, from 14 mature animals (7 females, 3 males, 4 castrated males) near the solstices and equinoxes of 1990 and 1991, under the prevailing photoperiod augmented by dim red light at night. Samples were drawn through jugular ca-

theters at 15 minute intervals over a period of 24 hours and serum was stored at -20° C. Throughout sampling periods muskoxen were fed a standard 10–12 % protein grass hay and a daily pelleted supplement. Ambient temperatures varied from 36° C to -40° C, and night-lengths from approximately 7.25 to 16.5 hours. Preliminary results reported here include seasonal melatonin patterns in 14 animals and LH patterns in 2 castrated males.

Details of the radioimmunoassays used to measure LH (Rowell and Flood, 1988) and melatonin (Fraser *et al.*, 1983) concentrations have been described previously. LH values are from a single assay in which the coefficients of variation (cv) were 8–12 % and assay sensitivity was 0.06 ng/ml for a 200 µ l aliquot. For melatonin, intra-assay cv's were 5–8 %, inter-assay cv's were 11–13 % and assay sensitivity was 10 pg/ml for a 250 µ l aliquot. LH and nighttime melatonin patterns were analysed using PULSAR, a standard peak identification algorithm incorporating assay variability criteria with the most stringent «cut-off» and peak-splitting parameters recommended (Merriam and Wachter, 1982). Mean and basal concentrations, pulse amplitudes and pulse frequencies were calculated

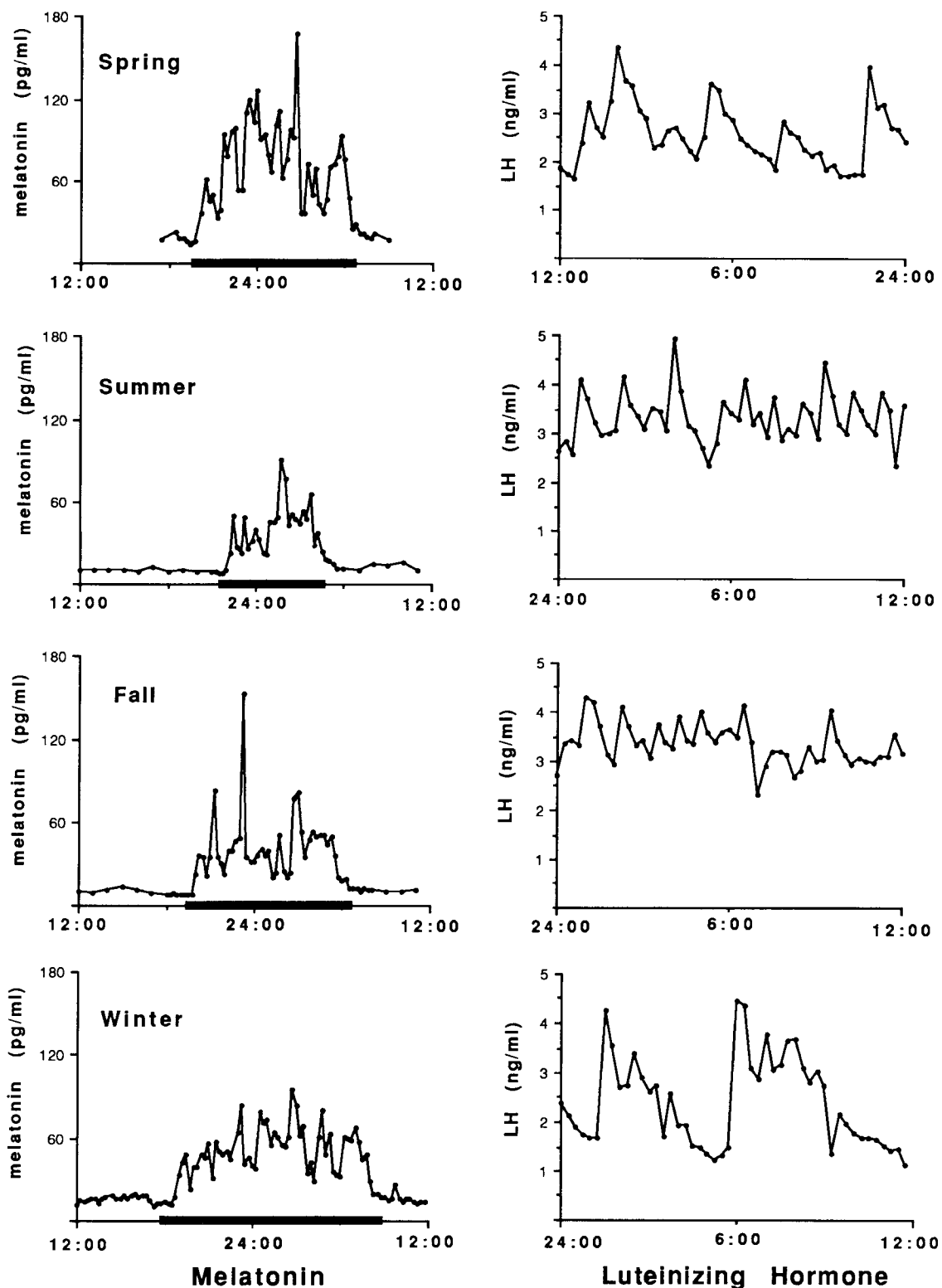


Fig. 1. Day/night patterns of melatonin and luteinizing hormone in a mature, castrated male muskox during different seasons at 52° N. Dark bars indicate the night (sunset to sunrise).

for each profile after which data were compared by season using repeated measures analysis of variance modified by the Greenhouse-Geisser epsilon (GLM procedure, SAS Version 6). Tukey's HSD method was used for multiple comparisons of means.

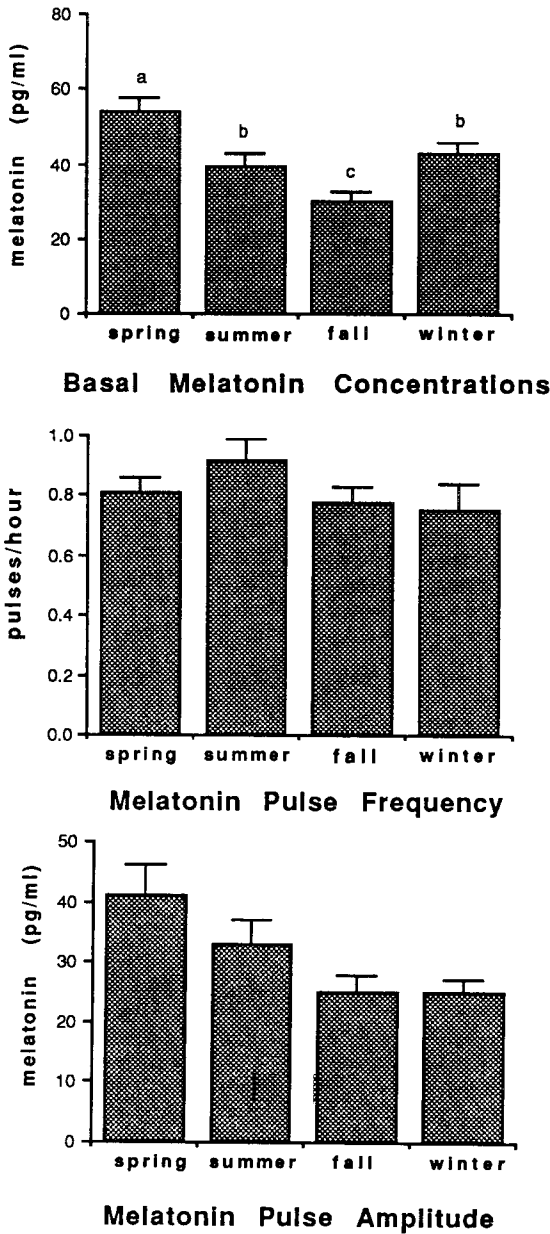


Fig. 2. Results (\pm s.e.) of PULSAR analysis of nighttime serum melatonin rhythms in 14 mature muskoxen at 52° N. Samples were drawn at 15 minute intervals. Values with different letters differed significantly ($p < 0.05$).

As in other mammals, serum melatonin concentrations in muskoxen corresponded to daily light/dark cycles and the amplitude and duration of the nocturnal rise changed with season (Fig. 1). Individual muskoxen differed in the amplitude of the nocturnal melatonin rise as commonly reported in other species (Cozzi *et al.*, 1991) (Fig. 2). Serum melatonin concentrations rose rapidly after sunset to a plateau and fell quickly near sunrise, as in sheep (Arendt *et al.*, 1981). During each night there were short term fluctuations consistent with episodic release of melatonin from the pineal gland. The frequency of the pulses remained relatively constant at approximately one per hour, while pulse amplitude tended to decrease from spring to winter ($p = 0.07$; Fig. 2). Mean and basal concentrations changed throughout the year ($p < 0.006$): nighttime melatonin concentrations were lowest in fall, highest in spring and did not differ significantly ($p = 0.2$) between summer and winter. Because pulsatility did not change with season, episodic release of melatonin may function chiefly to regulate the amplitude of the nocturnal rise, rather than directly creating seasonal effects.

Serum melatonin reached basal nighttime concentrations more quickly ($p < 0.03$) in summer and fall (49 ± 4 and 62 ± 6 minutes after sunset, respectively) than in winter and spring (97 ± 8 and 88 ± 9 minutes). In spring and fall, serum melatonin dropped below basal nighttime concentrations just before sunrise (14 ± 4 and 15 ± 3 minutes) but concentrations were maintained until just after sunrise in summer (17 ± 4 minutes). Melatonin decreased well before sunrise in winter (45 ± 6 minutes; Fig. 3). This probably reflects seasonal differences in the crepuscular light intensity, suggesting that muskoxen respond to a threshold light intensity which initiates or terminates pineal melatonin production. The early decline in melatonin during winter nights indicates that the duration of melatonin release in response to darkness may be limited.

In sheep, photoperiod regulates gonadotropin secretion by altering the pulsatile secretion of GnRH as well as changing the sensitivity of the hypothalamus to negative feedback of gonadal steroids (Bronson, 1988). Changes in LH pulse frequency in the absence of gonadal steroids and under conditions of relatively constant nutrition in sheep, are considered «steroid inde-

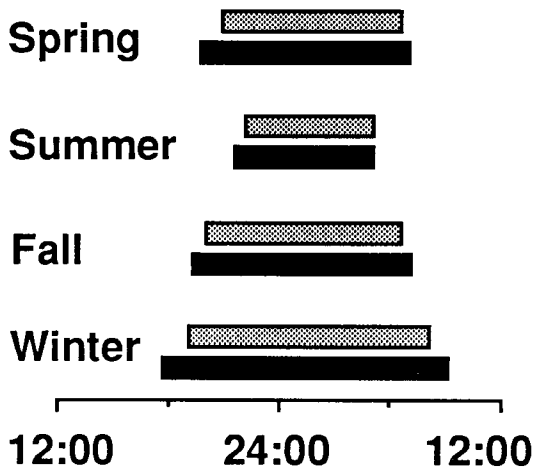


Fig. 3. Average duration of elevated serum melatonin at night in 14 mature muskoxen at 52° N. Dark bars represent the duration of the dark period (sunset to sunrise); gray bars represent mean period during which elevated nighttime levels were maintained. (Elevated levels for each profile were calculated using PULSAR.)

pendent» effects of photoperiod (Goodman, 1988). These include an increase after the summer solstice and a decrease in late fall (Lincoln and Short, 1980). In 2 castrated male muskoxen, basal LH concentrations and LH pulse frequency increased after the vernal equinox and decreased near the autumnal equinox (Fig. 4). Thus, it appears that, in muskoxen at 52° N, LH secretion increases when days are long (L:D > 12:12) and decreases when days are short (L:D < 12:12).

These effects may be regulated by perception of a critical daylength, sometimes in conjunction with an inherently limited reproductive cycle (Bronson, 1988). Alternatively, light/dark cycles may entrain circannual rhythms during an endogenous photosensitive period or «annual window» (Griffiths and Bryden, 1981). Evidence from sheep indicates that an underlying circannual reproductive rhythm may be modified by the development of refractoriness to previously stimulatory or inhibitory photoperiods (Karsch *et al*, 1984). This requires 150–200 days of exposure to a constant photoperiod before LH secretion is altered (Karsch *et al*, 1984). Although this is approximately the time between the vernal equinox and the breeding season of muskoxen in the high arctic, our data do not support the hypothesis that reproductive activity

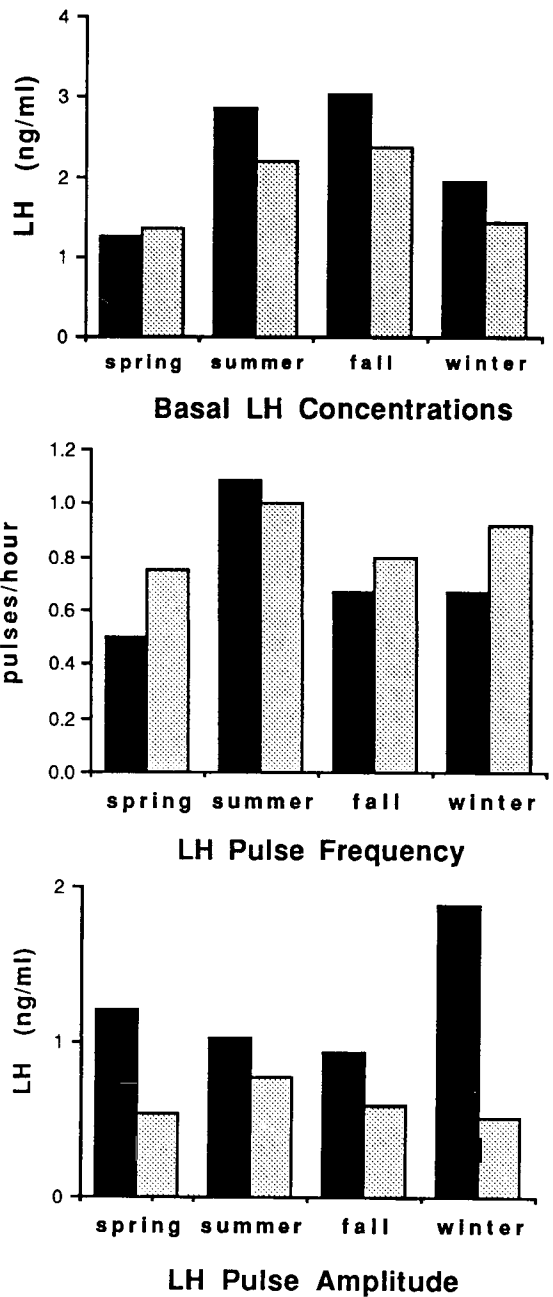


Fig. 4 Results of PULSAR analysis of serum luteinizing hormone patterns in 2 castrated male muskoxen at 52° N, sampled at 15 minute intervals.

□ = «Mervin» ■ = «Dome»

follows the development of refractoriness to continuous daylight. While PULSAR analysis (Fig. 4) indicated an earlier decrease in LH pulse frequency than did visual inspection of the profiles (Fig. 1), elevated LH concentrations

were maintained beyond the autumnal equinox, indicating that muskoxen did not become refractory to the summer photoperiod at 52° N. Long-day photoperiodic cues seem to stimulate reproductive processes in muskoxen, but the factors controlling termination of breeding activity have yet to be defined.

The vernal equinox may represent a time when muskoxen «adjust their annual clocks» and the marked difference between day and night melatonin concentrations around this time may provide a unique and particularly robust photoperiodic cue. Distinct melatonin rhythms are suspended in reindeer (Eloranta *et al.*, 1990) elephants seals (Griffiths *et al.*, 1979) Wedell seals (Barrell and Montgomery, 1988), and Adelie penguins (Cockrem, 1990) during the continuous light of polar summers. This also occurs in sheep exposed to 24-hour light (Rollag and Niswender, 1976), though at temperate latitudes, sheep have higher melatonin concentrations during summer than at any other time of year (Arendt *et al.*, 1981). There is little reason to suspect that muskoxen would differ in their response to continuous daylight, and the relatively low nightly melatonin concentrations we found during summer at 52° N may reflect a decreased significance of, or reliance upon, a daily melatonin rhythm at this time of year. Similarly, the low melatonin concentrations in fall might indicate that musk-oxen do not rely on photoperiodic cues to terminate breeding activity, but rather follow a previously entrained annual reproductive cycle.

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