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Effects of GnRH agonist (leuprolide) on reproduction and behaviour in female wapiti (*Cervus elaphus nelsoni*)

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Fertility control offers a potential alternative to traditional methods for regulating the growth of overabundant wild ungulate populations. However, current technology is limited due to practical treatment application, undesirable side-effects and economic considerations. A promising non-steroidal, non-immunological approach to contraception involves the use of a potent GnRH agonist. Two experiments were conducted to evaluate the effectiveness of a GnRH agonist (leuprolide) for controlling fertility in captive female wapiti and to assess physiological and behavioural side-effects of the treatment. In Expt 1, the optimum dose of agonist treatment was determined by measuring serum LH response of eight female wapiti to four formulations of leuprolide (0, 45, 90 and 180 mg) administered as a subcutaneous (s.c.) bioimplant. In Expt 2, the effects of leuprolide on wapiti pregnancy rates, duration of suppression of serum LH and progesterone secretion, and short-term behavioural and physiological side-effects were evaluated. All concentrations of leuprolide in Expt 1 were equally effective in reducing serum LH to non-detectable values throughout the 130 day trial. In Expt 2, leuprolide administered before the breeding season was 100% effective at preventing pregnancy in treated females. Serum LH and progesterone were reduced to baseline values by day 92 and remained at this concentration for 195–251 days after treat-ment, and returned to pretreatment concentrations in the following breeding season. Reproductive behaviour rates were similar for treated and untreated wapiti for all behaviour categories for both the breeding and post-breeding seasons. Haematology and blood chemistry parameters of treated and un-treated females were similar, and seasonal intake and body weight dynamics appeared normal. In conclusion, leuprolide is a safe, effective contraceptive agent and can potentially suppress fertility in female wapiti for one breeding season.

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Introduction

Fertility control offers a potential alternative for controlling the growth of overabundant ungulate populations when traditional methods are not feasible or are unacceptable (Kirkpatrick and Turner, 1985; Bomford, 1990; Garrot, 1995). However, current technology does not provide a means for controlling populations that is practical, economical and without undesirable side-effects (for a review, see Fagerstone *et al.*, 2001).

A promising non-steroidal, non-immunological approach to contraception involves potent GnRH agonists. Chronic treatment with continuous, high doses of GnRH agonists results in temporary suppression of pituitary responsiveness and gonadotrophin secretion. The resulting decreases in plasma concentrations of LH and FSH in females lead to suppression of ovulation, oestrous cyclicity and gonadal steroidogenesis (Belchetz *et al.*, 1978; Macmillan *et al.*, 1985; Evans and Rawlings, 1994). Once GnRH agonist treatments are terminated, normal pituitary function is gradually restored (Gorospe and Conn, 1988; Bergfeld *et al.*, 1996).

GnRH agonists have been shown to inhibit ovulation in several domestic ungulate species including sheep (McNeilly and Fraser, 1987), cattle (D'Occhio *et al.*, 1996; D'Occhio and Aspden, 1999) and horses (Montovan *et al.*, 1990). However, studies on wild ungulates are limited (Lincoln, 1987; Brown *et al.*, 1993; Becker and Katz, 1995) and to our knowledge, none have demonstrated the effectiveness of GnRH agonists as contraceptive agents. GnRH agonists provide a potential biotechnology for achieving a controlled, reversible suppression of fertility in both captive and free-ranging female wild ungulates. However, the practicality of using these agonists as a contraceptive agent is dependent on effective inhibition of reproduction without negative behavioural or physiological side-effects. In the present study, experiments were conducted with sustained release formulations of GnRH agonist in captive female wapiti to evaluate these factors. Specifically, the objectives of the present study were to determine: (i) the effectiveness of GnRH agonist in preventing pregnancy; (ii) the duration of GnRH agonist suppression of LH and progesterone secretion; and (iii) the behavioural and physiological side-effects (if any) of GnRH agonist treatments.

Materials and Methods

Animals

Female wapiti (*Cervus elaphus nelsoni*) are polyoestrous and have seasonal reproductive cycles that are influenced by photoperiod. In temperate North America, the onset of the breeding season occurs in mid-September, during decreasing daylength. Calving takes place in the summer, after a gestation period of about 255 days. Non-pregnant females have five to eight recurrent oestrous cycles each of 18–21 days. Reproductive cycles cease in late March and females remain anoestrus until early autumn (Morrison *et al.*, 1959; Guiness *et al.*, 1971; Haigh and Hudson, 1993).

In these experiments female wapiti were trained to repeated handling, blood sampling techniques and holding pens. When not used in the experiments, female wapiti were maintained in fenced paddocks (2 ha) and fed a diet consisting of alfalfa cubes, grass hay and supplement. This study was conducted with the approval from the Colorado Division of Wildlife's Animal Care and Use Committee and in compliance with United States Federal Animal Welfare Regulations.

Experimental protocol

Experiment 1: dose–response. The optimum dose of leuprolide (desGly^{10}-D-Leu^{6}-LH-RH ethylamide acetate) required for suppression of serum LH secretion was determined in eight

female wapiti (6–12 years of age; weighing 240–300 kg). Female wapiti were monitored for the occurrence of oestrous cycles by measuring serum progesterone concentrations at weekly intervals from 1 November 1998 and were considered reproductively active when progesterone concentrations were > 1 ng ml^{-1} for two consecutive sampling periods (Adam *et al.*, 1985). Females were selected randomly to receive one of four doses (0, 45, 90 or 180 mg leuprolide acetate) of 90 day sustained release leuprolide formulation using the ATRIGEL® drug delivery system (Atrix Laboratories, Inc., Ft Collins, CO; Dunn *et al.*, 1994). At lower doses, these formulations have demonstrated a sustained release and activity in rats and dogs for a period of at least 90–120 days (Ravivarapu *et al.*, 2000).

On the day before treatment application, animals were removed from paddocks, weighed (± 0.5 kg), moved to individual isolation pens (5 m × 10 m), sedated with xylazine hydrochloride (Rompun; Bayer AG, Leverkusen; 25–200 mg animal $^{-1}$ i.m.) and fitted non-surgically with indwelling jugular catheters. Sedation was reversed with yohimbine (30 mg) (Antagonil®; Wildlife Laboratories, Fort Collins, CO). The sampling period began the next day (20 November 1998) at 09:00 h. A patch of hair (3 cm in diameter) was shaved in the shoulder region of each female (controls did not receive a placebo formulation) and leuprolide formulations were injected under the skin using an 18 gauge needle and 3 ml syringe. Blood samples (5 ml) were collected at 0, 60, 120, 180, 240, 300, 360 and 480 min and then at 12, 24, 36, 48, 84 and 240 h after injection. Catheters were flushed each day with sterile saline solution. After the last blood collection, catheters were removed and animals returned to 5 ha paddocks.

The effective durations of leuprolide treatments were compared by measuring pituitary responsiveness to an exogenous dose of GnRH analogue (D-Ala^6-GnRH-Pro^9-ethylamide; Sigma Chemical Co., St Louis, MO) administered at days 35, 70, 110 and 130 after treatment. Animal handling and blood sampling protocols were similar to those described previously. A previously determined dose (Baker *et al.*, 1995) of GnRH analogue (1 μg (50 kg body weight)$^{-1}$) was administered through the jugular cannula and blood samples were collected at 0, 30, 60, 90, 120, 180, 240, 300, 360, 420 and 480 min after injection. After collection, blood was stored at 4°C for 24 h until serum was obtained by centrifugation at 1500 ***g*** for 15 min. Serum was then stored at –20°C until analysed for LH.

Experiment 2: anti-fertility effects

The effects of the optimum dose of leuprolide formulation established in Expt 1 on pregnancy rates, duration of suppression of LH and progesterone secretion, blood chemistry and reproductive behaviour of wapiti from 2 November 1999 to 15 May 2000 were evaluated. Fourteen adult female (7–13 years of age; weighing 240–320 kg) and three adult male wapiti (4–13 years of age; weighing 375–400 kg) were used in this experiment. Females were assigned to one of three experimental groups on the basis of their tractability for handling and blood sampling. Four wapiti cows (group A) were treated with 32.5 mg leuprolide and five cows (group B) served as untreated controls and were used to compare pregnancy rates, blood chemistry and reproductive behaviour with those of treated females. These two groups of females were maintained together with three adult male wapiti in adjoining paddocks (2 ha each). The remaining four females (group C) served as untreated, non-pregnant controls and were placed in a separate pasture (1 ha) without direct contact with male wapiti. LH and progesterone secretion of these females was compared with that of leuprolide-treated females (group A).

Pregnancy rates, hormonal measurements and blood parameters. The effects of leuprolide on pregnancy rates of treated and untreated wapiti were determined by measuring pregnancy-

specific protein B (PSPB; BioTracking, Moscow, ID) in serum at about 70, 160 and 215 days of gestation (Huang *et al.*, 2000). The effects of leuprolide on the extent and duration of LH and progesterone suppression were compared in treated and untreated, non-pregnant wapiti from 2 November 1999 to 11 November 2000. GnRH challenge trials were conducted before application of leuprolide treatments and at days 30, 90, 145, 180, 225, 250 and 373 after treatment. The final GnRH challenge trial was conducted to assess reversibility of treatment. The protocol for GnRH challenge trials followed the procedures described in Expt 1.

The physiological side-effects of leuprolide were assessed by comparing serum chemistry, haematology and body weight dynamics of treated (group A) and untreated, non-pregnant wapiti (group C). Blood sample collections and body weight measurement were made in conjunction with GnRH challenge trials. Blood samples for haematology and serum chemistry analysis were collected at day 90 after treatment and submitted for analysis to Colorado State University (Veterinary Teaching Hospital, Clinical Pathology Laboratory, Fort Collins, CO).

Serum chemistry profiles were obtained using a Hatachi 917 autoanalyser (Roche/Boehringer Mannheim, Indianapolis, IN) for the following parameters: glucose, creatinine, phosphorus, calcium, magnesium, total protein, albumin, globulin, albumin:globulin ratio, bilirubin, creatinine kinase, aspartate aminotransferase, gamma-glutamyltransferase, sorbitol dehydrogenase, sodium, potassium, chloride and bicarbonate.

Values for the following haematological parameters were obtained using an ADVIA 12O autoanalyser (Bayer Corporation, Tarrytown, NY): nucleated cells, neutrophils, lymphocytes, monocytes, eosinophils, plasma protein, erythrocytes, haemoglobin, packed cell volume, mean corpuscular volume, mean corpuscular haemoglobin concentration, platelets and fibrinogen.

Reproductive behaviour. The effectiveness of the leuprolide formulation as a contraceptive agent is dependent upon suppression of ovulation and steroidogenesis for the duration of the breeding season. Thus, we tested two hypotheses relative to the effects of leuprolide on reproductive behaviour of wapiti: (i) because leuprolide was expected to suppress gonadotrophin secretion and ovulation, we predicted that sexual interactions during the breeding season would be reduced in leuprolide-treated females (group A) compared with untreated controls (group B); and (ii) as depletion of the leuprolide implant (90 days) was expected before anoestrus (late March), we predicted that behavioural oestrus would resume in treated females and the rate of sexual interactions would be higher than that for untreated females.

These two hypotheses were tested by examining the effects of leuprolide on reproductive interactions of male and female wapiti during two periods: the breeding season (defined as the period between 10 November and 23 December 1999) and the post-breeding season (defined as the period between 7 February and 27 March 2000). On 2 November 1999, female wapiti in group A were treated with leuprolide and released with untreated controls (group B) into adjoining paddocks (2 ha each). After 7 days (10 November), three adult male wapiti were placed with these groups and behavioural observations were initiated. All females were individually identified with colour/numeric-coded neck collars. Observers did not know which animals were selected as treatments and controls. Behavioural measurements were made from a distance of 50–250 m from an elevated tower (10 m) situated between adjacent pastures using binoculars and a spotting scope during the day, and a spotlight and night vision scope at night. Selected behaviours were recorded using a lap-top computer with a behavioural software program.

Focal animal sampling procedures were used to sample reproductive behaviours of all experimental animals over 24 h (Lehner, 1996). Preliminary observations indicated that wapiti were most active in the morning (05:00–08:00 h), late day (14:00–17:00 h) and night

Table 1. Description of wapiti (*Cervus elaphus nelsoni*) reproductive behaviour and associated behaviour categories

Behaviour category	Reproductive behaviour
General breeding	Male directed behaviour related to establishing, maintaining and defending a group or harem of female wapiti (for example, herding, guarding, tending)
Male precopulatory	Male courtship behaviour directed toward an individual female to induce or detect oestrus or ovulation (for example, urine testing, flehmen, tongue flick, lick, smell or rub female's body, chivy)
Female precopulatory	Female courtship behaviour directed toward dominant male to arouse copulatory behaviour (for example, lick and rub male, mount, lordosis, twitch hocks)
Copulatory	Male behaviour directed toward a receptive female in oestrus (for example, precopulatory mounts, intromission, pelvic thrust)

(20:00–24:00 h). Thus, time-of-day sampling periods were assigned randomly each week using a randomized block design. Each sampling period consisted of at least 2 h of continuous observations. On the basis of previously reported breeding behaviour of wapiti (Morrison *et al.*, 1959; Geist, 1982; Rapley, 1985), 19 sexual interactions were identified and recorded. As sample sizes were small, individual behaviours were grouped into four general categories: male copulatory, male precopulatory, female precopulatory and general breeding (Table 1). Copulatory, male precopulatory and general breeding were interactions of a male directed toward a specific female, whereas female precopulatory behaviours were actions of a specific female toward a male. Thus, our experimental unit for analyses was the individual female in each breeding group. Behavioural interactions were generally short (< 30 s) relative to sampling interval and, therefore, we recorded the number of occurrences of each event rather than the length of time, and calculated rates of sexual interaction as acts per animal per hour, then multiplied hourly behavioural rates by 24 for a daily rate.

Hormone radioimmunoassay. Serum concentrations of LH were quantified by means of an ovine (o) LH radioimmunoassay (Niswender *et al.*, 1969). Wapiti serum was demonstrated to inhibit binding of ^{125}I-labelled oLH to LH antiserum in a parallel manner. Similarly, when different quantities of oLH standard (NIH-OLH-S24) were added to wapiti serum and samples were subjected to radioimmunoassay, the values obtained were increased by the quantity of oLH added ($r^2 = 0.99$, slope = 0.92, $SE_b = 0.22$, $P = 0.002$). These data indicate that the radioimmunoassay provided a quantitative assessment of LH in wapiti serum. The limit of sensitivity of the LH assay was 0.4 ng ml^{-1}. Serum concentrations of progesterone were determined by radioimmunoassay (Niswender, 1973). Sensitivity of the progesterone assay was 0.12 ng ml^{-1}. Intra- and interassay coefficients of variation for each of these assays were < 10%.

Statistical analysis. Hormone concentrations are reported as untransformed arithmetic means ± SE. Responsiveness of the pituitary gland to GnRH analogue challenge was assessed in two ways: (i) maximum response (highest concentration of LH (ng ml^{-1}) achieved after injection minus baseline); and (ii) total amount of LH secreted (ng ml^{-1} min^{-1}) estimated by calculating the area under the LH response curve (Abramowitz and Stegun, 1968).

The differences among hormone concentrations were analysed using least squares ANOVA for general linear models (SAS Institute, 1993). Responses to treatments were analysed with

one-way ANOVA for a randomized complete block design with repeated measures. Levels of leuprolide formulations were treatments; individual animals were blocks. Factors in the analysis were dose and time. Treatment effects were tested using the animal-within-treatment variance as the error term. Time was treated as a within-subject effect using a multivariate approach to repeated measures (Morrison, 1976). A 'protected' least significant difference test (Milliken and Johnson, 1984) was used to separate means when the overall F test indicated significant treatment effects ($P < 0.05$).

We tested specific reproductive behaviour hypotheses that mean behaviour rate was not different between treatment and control groups for both the breeding and post-breeding seasons using an ANOVA model with a repeated measures structure. In a similar manner to the hormonal analysis, time was treated as a within subject effect using a multivariate approach to repeated measures (Morrison, 1976). The time-of-day, date effects and their interactions were accounted for to test for treatment effects. PROC GENMOD (SAS Institute, 1993) was used to estimate and test for differences in mean behaviour rate by treatment, time-of-day and date. Means and standard errors were estimated using least squares, and hypothesis tests were based on type III generalized estimating equations that accounted for correlation in repeated measurements.

Results

Experiment 1: dose–response

Administration of sustained release formulations of leuprolide to female wapiti resulted in an acute, transient increase in serum LH concentrations irrespective of dose. Maximum LH concentrations (15.6 ± 0.93 ng ml^{-1}) occurred approximately 3 h after treatment and were similar across all treatments (Fig. 1a). After the peak response, there was a rapid decline in LH to basal concentrations during the next 24 h. Total LH secretion (ng ml^{-1} min^{-1}) did not differ among treatments and all treatments resulted in higher LH secretion than in controls ($P \leqslant 0.002$). Leuprolide reduced serum LH secretion to non-detectable amounts in treated females for 130 days after treatment (Fig. 1b). Differences in mean maximum serum LH were significantly lower ($P \leqslant 0.031$) in leuprolide-treated wapiti compared with untreated controls at all sampling periods. For untreated females, mean maximum LH fluctuated from 19.3 ± 4.2 to 3.5 ± 0.06 ng ml^{-1}. This variation was probably related to the phase of the oestrous cycle when control females were challenged with GnRH and the influence of fluctuating concentrations of oestradiol and progesterone on LH secretion (Goodman and Karsch, 1980).

Experiment 2: anti-fertility effects

Pregnancy rates, hormonal measurements and blood parameters. As Expt 1 did not establish a minimum effective dose of leuprolide for suppression of LH, the leuprolide formulation was arbitrarily reduced by approximately 20% below the lowest concentration tested in Expt 1, to 32.5 mg. This dose of leuprolide prevented pregnancy in all treated females (group A), whereas the pregnancy rate of control females (group B) was 100%. Leuprolide-treated females tested negative and controls tested positive for PSPB on all sampling dates. Estimated conception dates for pregnant wapiti ranged from 10–19 November 1999 and parturition occurred between 12 and 26 July 2000.

Leuprolide caused a significant reduction ($P \leqslant 0.035$) in mean maximum serum LH (Fig. 2) and progesterone (Fig. 3) concentrations in leuprolide-treated females (group A) with a return to pretreatment concentrations in the following breeding season (11 November 2000). Serum

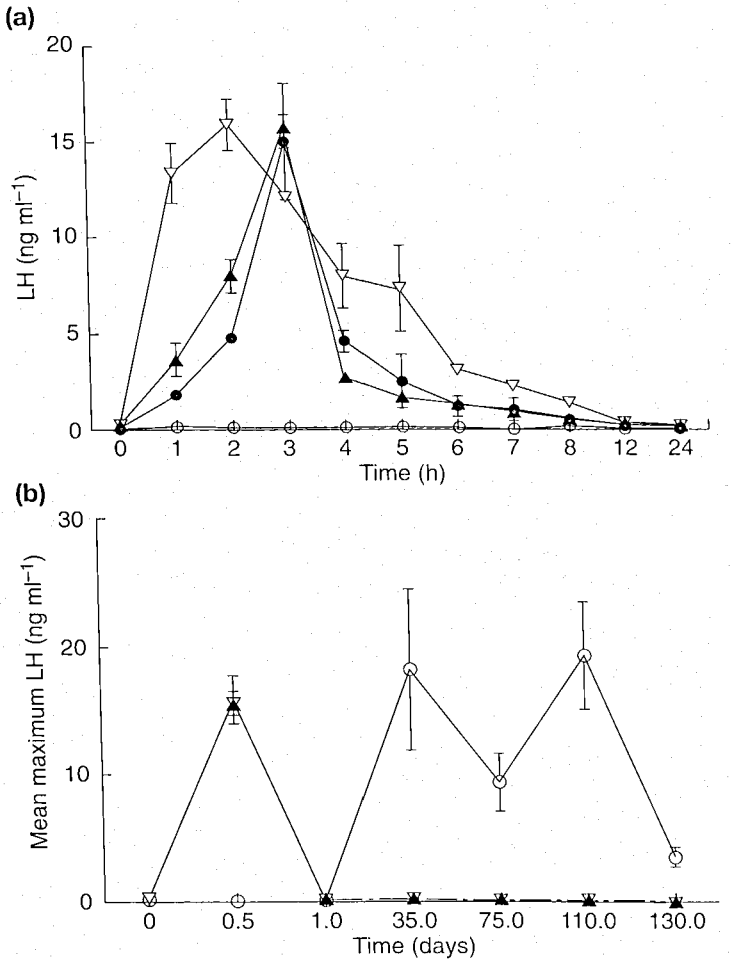

Fig. 1. (a) Twenty-four hour and (b) 130 day profiles of serum LH concentrations in untreated female wapiti (*Cervus elaphus nelsoni*) (○, $n = 2$) and female wapiti treated with sustained release formulations containing 45 (●, $n = 2$), 90 (▲, $n = 2$) and 180 mg (▽, $n = 2$) leuprolide. Results are shown as means ± SE.

LH was reduced to non-detectable concentrations by day 92 after treatment and remained at this concentration until day 225. In one leuprolide-treated female, LH concentrations remained non-detectable for 250 days after treatment. Maximum LH response was lower ($P \leq 0.012$) in leuprolide-treated compared with non-pregnant controls (group C) at days 30, 92, 135, 165 and 193 after treatment. Serum LH of untreated wapiti declined significantly ($P = 0.024$) between April and May with the onset of anoestrus, and then returned to pretreatment concentrations indicative of oestrus in November 2000.

Serum progesterone concentrations of leuprolide-treated females followed a similar pattern to that observed for serum LH (Fig. 3). Progesterone concentrations were similar in leuprolide-treated and control wapiti until day 30; thereafter, serum progesterone concentrations remained at basal concentrations in leuprolide-treated females until day 225 of the trial, indicating that additional ovulations did not occur. Control females maintained increased

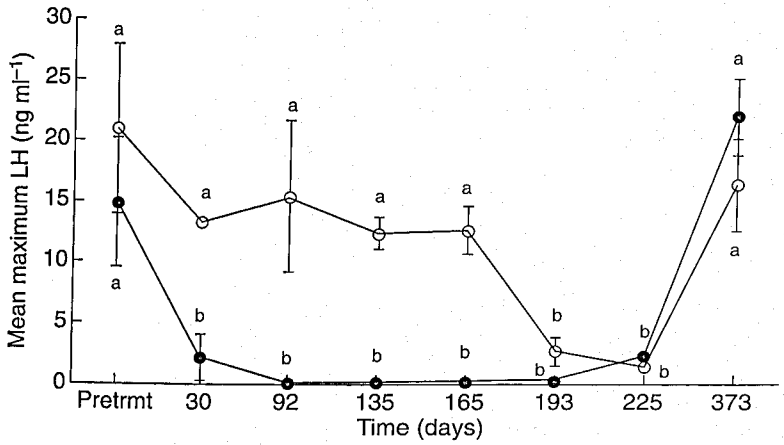

Fig. 2. Profiles of mean maximum serum LH concentrations for untreated female wapiti (*Cervus elaphus nelsoni*) (○, $n = 4$) and female wapiti treated with a sustained release formulation containing 32.5 mg leuprolide (●, $n = 4$). Results are shown as means ± SE. Pretrmt: pretreatment. Different lower case letters indicate significant differences between means ($P \leq 0.05$).

serum progesterone concentrations, reflecting continued regular oestrous cycles within this group until day 165 (18 April) when the effects of anoestrus reduced progesterone to basal values. In a similar manner to serum LH, progesterone concentrations then increased during November 2000 to pretreatment concentrations in both leuprolide-treated and untreated wapiti (Fig. 3).

We evaluated 13 haematology and 19 serum chemistry parameters in treated and untreated female wapiti. With the exception of creatinine kinase, a muscle-derived enzyme, all individuals were clinically similar. Females that were treated with leuprolide showed moderately increased creatinine kinase concentrations (400–702 iu l^{-1}). Creatinine kinase concentrations can increase in unconditioned animals after vigorous exercise and remain increased for 4–6 h (Lefebvre *et al.*, 1994). Handling procedures for blood sampling in leuprolide-treated females were often more physically rigorous than those for controls due to the need to separate females from males. Thus, the increased creatine kinase concentrations in leuprolide-treated wapiti compared with controls probably reflect a bias due to a difference in animal handling before blood sample collections, rather than a treatment-induced response.

Reproductive behaviour. Male to male dominance interactions were observed immediately after release of wapiti into the pastures with treated and untreated females. Within 2.5 weeks, one male established dominance over the other two males. Thereafter, subdominant males retreated to remote locations in the pastures and rarely interacted with females or the dominant male for the remainder of the experiment.

During the breeding season, reproductive interactions of males and females were observed for 34 days from 10 November to 23 December 1999. We analysed 63 sampling periods (134.5 h): 20 periods at dawn (45.7 h), six at midday (13.5 h), 20 at dusk (42.8 h) and 17 at night (32.6 h). The average duration of the observation period was 2.1 (SE = 0.10) h. Observations after breeding occurred for 14 days from 7 February to 27 March. Data were analysed from 16 sampling periods (54.7 h): six periods at dawn (22.5 h), two at midday (7.5 h), seven at dusk (22.2 h) and one at night (2.5 h). Observation periods averaged 3.4 (SE = 0.24) h.

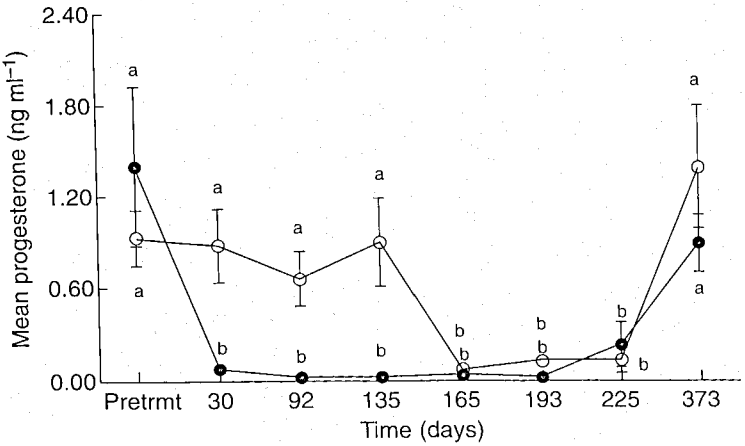

Fig. 3. Profiles of mean progesterone concentrations for untreated female wapiti (*Cervus elaphus nelsoni*) (○, $n = 4$) and female wapiti treated with a 32.5 mg formulation containing leuprolide (●, $n = 4$). Results are shown as means ± SE. Pretrmt: pretreatment. [ab]Different lower case letters indicate significant differences between means ($P \leq 0.05$).

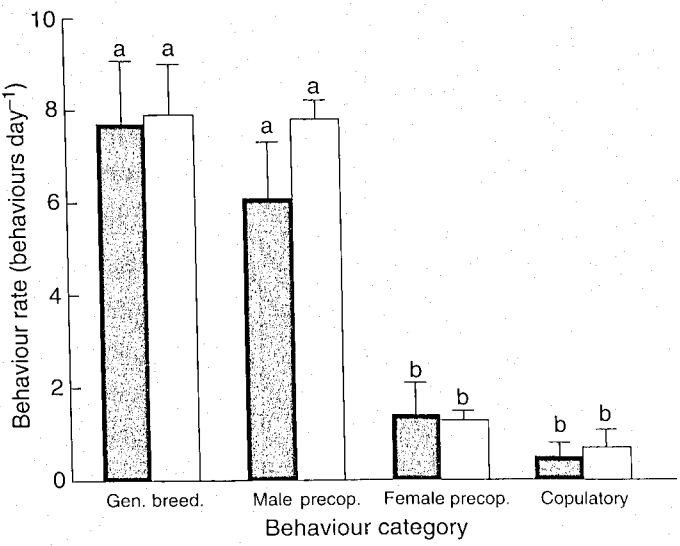

Fig. 4. Mean (± SE) reproductive behaviour rates during the breeding season for untreated ($n = 5$, ■) and leuprolide-treated ($n = 4$, □) female wapiti (*Cervus elaphus nelsoni*) with a sustained release formulation containing 32.5 mg leuprolide. Gen breed: general breeding; male precop: male precopulatory; female precop: female precopulatory. [ab]Columns with different superscripts indicate significant differences between means ($P \leq 0.05$).

Contrary to our first hypothesis, sexual interactions during the breeding season were not diminished in leuprolide-treated females in comparison with controls. Instead, rates of breeding behaviour were similar for leuprolide-treated and untreated females for all behaviour categories (Fig. 4a). Although a significant treatment × time interaction was not

detected, copulatory ($P = 0.064$), male precopulatory ($P = 0.083$) and female precopulatory ($P = 0.072$) behaviours approached significance and are notable because deviations from normal behaviours, even minor ones, are important for contraceptive acceptability. For these three behaviour categories, the daily behaviour rate decreased over time for untreated females, but remained constant for leuprolide-treated wapiti.

We also failed to reject our second hypothesis. Leuprolide-treated females did not resume normal oestrous cycles during the post-breeding season and reproductive behaviour rates did not increase compared with untreated controls. Almost no sexual interactions were observed between the dominant male and leuprolide-treated or untreated females in the post-breeding season. No copulatory or female precopulatory behaviours were recorded, and too few male precopulatory (≤ 0.17 day^{-1}) and general breeding (≤ 0.30 day^{-1}) behaviours were recorded to analyse.

Discussion

Successful application of fertility control technology for wildlife is dependent on development of contraceptive agents that are safe, practical and effective. Current technology is limited due to problems of treatment implementation and concerns for the health of target and non-target species. In the present study, we evaluated a promising non-steroidal, non-immunological contraceptive technology for controlling fertility in female wapiti.

Administration of a sustained release formulation containing leuprolide to captive female wapiti before the breeding season resulted in decreased LH and progesterone secretion, temporary suppression of ovulation and steroidogenesis, and effective contraception without detrimental behavioural or physiological side-effects. The acute increase in serum LH immediately after leuprolide treatment was consistent with studies in cattle (D'Occhio *et al.*, 1996), sheep (Nett *et al.*, 1981), horses (Montovan *et al.*, 1990) and African elephants (*Loxodonta africana*; Brown *et al.*, 1993). There was little variation among wapiti in their serum LH response to different doses of leuprolide, indicating either low variability in the amount and duration of agonist released or doses so high that any variation was masked. The minimum amount of leuprolide needed to suppress oestrus in female wapiti was not determined in this study. All doses of leuprolide were equally successful in reducing LH concentrations for the duration of the 130 day trial. Additional research to establish a minimum effective dose of leuprolide would enhance the economic practicality of this contraceptive agent.

The cessation of oestrous cycles in females treated with leuprolide and the return to apparently normal ovarian function after depletion of the agonist implant was consistent with findings for females in other species (Fraser *et al.*, 1989; Evans and Rawlings, 1994; D'Occhio *et al.*, 1996). The effectiveness of leuprolide as a contraceptive agent is dependent on suppression of ovulation from the inception of the breeding season to the onset of anoestrus, a period of approximately 200 days for wapiti. Leuprolide inhibited ovulation for > 190 days, two times longer than the formulated 90 day delivery period. The prolonged suppression of gonadotrophin secretion may occur for several reasons. The release of leuprolide from the implant may have continued beyond the predicted 90 day period. Certainly, LH secretion remained suppressed for > 130 days in Expt 1. Similarly, leuprolide treatment may have induced prolonged suppression of gonadotroph function (that is, extending beyond the duration of the implant). In other ruminants, if gonadotroph function is suppressed for an extended period, a recovery period of 30–60 days after the removal of the suppression is necessary before pituitary content of LH and gonadotrophin secretion return to normal values (Nett, 1987). Thus, if the duration of leuprolide release from the implant was 130 days and

recovery of gonadotroph function requires approximately 60 days, this would be sufficient to carry the reduced secretion of LH into the normal anoestrous period when secretion of LH would be photoperiodically suppressed. If this is true, then a single treatment should provide a contraceptive effect for approximately one breeding season.

The overall rates of sexual interactions between leuprolide-treated and control wapiti were not different during the breeding and post-breeding seasons. During the breeding season, the dominant male established a single harem of leuprolide-treated and untreated females. Reproductive behaviours during the breeding season between the dominant male and harem females followed a pattern similar to that described for free-ranging wapiti (Geist, 1982). Treated and untreated females were courted, mated with and defended with equal frequency; however, the pattern of reproductive interactions changed over time. Once untreated females became pregnant, reproductive behaviour rates decreased, whereas copulatory, and male and female precopulatory rates remained constant over time in treated females. These extended sexual interactions were generally intermittent and may have been related to fluctuating concentrations of progesterone and oestradiol. Oestrus can occur with relatively low oestradiol concentrations, if coupled with low progesterone concentration. In domestic sheep, pre-exposure to progesterone stimulates oestrous behaviour at much lower concentrations of oestradiol once there is a decrease in plasma progesterone concentration (Robinson, 1954). Therefore, as these animals had ovulated before leuprolide treatment, they became very sensitive to low amounts of oestradiol, and as ovulation and corpus luteum formation were blocked, they continued to show oestrous behaviour with basal oestradiol concentrations.

Regardless of the mechanism involved, disruption of normal behaviour patterns is not a desirable side-effect of contraceptive treatments. However, without carefully designed large-scale investigations with larger sample sizes, and under more natural conditions, we can only speculate on the significance of these behavioural alterations on the health and social organization of treated populations.

Conclusion

The objective of the present study was to evaluate the contraceptive potential of a GnRH agonist (leuprolide) formulation in female wapiti and provide evidence of physiological and behavioural side-effects of treatment (if any). In conclusion, leuprolide administered as a controlled release formulation before the breeding season offers a new approach to reversible contraception in wild ungulates and overcomes problems associated with existing technology.

Firstly, leuprolide formulation improves practical application of contraception because a single treatment can induce infertility in females without relocating and treating specific individuals each year. Secondly, leuprolide acetate is a neuropeptide and thus the proteinaceous nature of this agent eliminates the possibility of passage through the food chain to non-target species. Thirdly, behavioural side-effects were minimal. Sexual interactions of leuprolide-treated females were extended early in the breeding season but recurrent oestrous cycles and ovulation did not occur. Fourthly, there were no short-term physiological side-effects of treatment. Leuprolide-treated animals appeared healthy and seasonal intake and body weight dynamics were normal. However, before this technology can be considered a practical and efficacious approach for wildlife, additional research is needed to ascertain minimum effective dose, verify effective treatment duration and to develop a remote delivery system for administering leuprolide formulation to unrestrained animals.

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