EVALUATION OF REMOTELY DELIVERED LEUPROLIDE ACETATE AS A CONTRACEPTIVE AGENT IN FEMALE ELK (CERVUS ELAPHUS NELSONI)

Authors: Baker, Dan L., Wild, Margaret A., Hussain, Muhammad D., Dunn, Richard L., and Nett, Terry M.

Source: Journal of Wildlife Diseases, 41(4) : 758-767

Published By: Wildlife Disease Association

URL: https://doi.org/10.7589/0090-3558-41.4.758

Journal of Wildlife Diseases, 41(4), 2005, pp. 758–767

EVALUATION OF REMOTELY DELIVERED LEUPROLIDE ACETATE AS A CONTRACEPTIVE AGENT IN FEMALE ELK (*CERVUS ELAPHUS NELSONI*)

Dan L. Baker,[1,6] Margaret A. Wild,[2] Muhammad D. Hussain,[3] Richard L. Dunn,[4] and Terry M. Nett[5]

[1] Colorado Division of Wildlife, Research Center, 317 West Prospect, Fort Collins, Colorado 80526, USA
[2] National Park Service, Biological Resource Management Division, 1201 Oak Ridge Dr., Suite 200, Fort Collins, Colorado 80525, USA
[3] School of Pharmacy, Department 3375, 1000 East University Avenue, University of Wyoming, Laramie, Wyoming 82071, USA
[4] Atrix Laboratories, Incorporated, 2579 Midpoint Dr., Fort Collins, Colorado 80525, USA
[5] Animal Reproduction and Biotechnology Laboratory, Department of Physiology, Colorado State University, Fort Collins, Colorado 80523, USA
[6] Corresponding author (email: dan.baker@state.co.us)

ABSTRACT: Practical application of fertility control technology in free-ranging wild ungulates often requires remote delivery of the contraceptive agent. The objective of this investigation was to evaluate the potential of remote delivery of leuprolide acetate for suppressing fertility in female elk (*Cervus elaphus nelsoni*). Fifteen captive adult female elk were randomly allocated to one of three experimental groups. Six elk were injected intramuscularly with a dart containing leuprolide, and the remaining nine elk received the same formulation without leuprolide. We determined pregnancy rates, suppression of luteinizing hormone (LH) and progesterone concentrations, and reversibility of treatments during 1 August 2002 to 3 September 2003. Leuprolide formulation caused a decrease in concentrations of LH and progesterone, temporary suppression of ovulation and steroidogenesis, and effective contraception (100%) for one breeding season. These results extend the practical application of this contraceptive agent to include dart delivery, where in the absence of such technology, wild elk must first be captured and restrained before treatment.

Key words: *Cervus elaphus nelsoni*, contraception, elk, GnRH agonist, leuprolide, luteinizing hormone, remote drug delivery, reproduction.

INTRODUCTION

There is an increasing need in protected environments such as urban parks and conservation areas for nonlethal methods of managing overabundant wild ungulates. Unregulated populations, if left unchecked, can have adverse effects on natural and human-dominated systems (Jewell and Holt, 1981; Diamond, 1992; Garrott et al., 1993). Hunting and culling have traditionally been used to regulate animal numbers but there are a growing number of circumstances where these methods pose a significant liability (Decker and Connelly, 1989; McCullough et al., 1997), and as a result, resource managers are seeking novel approaches to population control (Kirkpatrick and Turner, 1985; Bomford, 1990; Brush and Ehrenfeld, 1991). One alternative is controlling the fertility of female animals. Fertility control offers a potential nonlethal method for controlling the growth of overabundant ungulate populations and considerable research has been directed toward the development of different contraceptive technologies (Fagerstone et al., 2002). Additionally, development of this technology may provide benefits beyond its value as a management tool for balancing wild ungulates and their food resources. Fertility control has the potential to reduce the rate of disease transmission by regulating local host densities and pathogen shedding (Rhyan and Drew, 2002; Miller et al., 2004). Simulation modeling indicates that, in some situations, fertility control can be as effective as culling in reducing endemic disease or the density of susceptible hosts (Hone, 1992; Barlow, 1996).

Fundamental to practical application of contraceptives to wildlife is a safe and effective antifertility agent that can be remotely delivered to the target species. To

attain this goal, research efforts have focused on the development and testing of ballistic systems and controlled drug release formulations that can remotely administer contraceptive agents to wild ungulates (Kreeger, 1997). Contraceptive agents have been delivered via projectile dart or biodegradable implant to a variety of wild ungulate species including deer (*Odocoileus* spp.) (Turner et al., 1992; Jacobsen et al., 1995; DeNicola et al., 1997), elk (*Cervus elaphus nannodes*) (Shideler et al., 2002), wild horses (*Equus caballus*) (Kirkpatrick et al.,1990), burros (*Equus asinus*) (Turner et al., 1996), and elephants (*Loxodonta africana*) (Delsink et al., 2002).

The use of gonadotropin-releasing hormone (GnRH) agonist implants to suppress short-term ovarian follicular growth and ovulation are well documented for a number of species including cattle (McLeod et al., 1991; D'Occhio et al., 1996), sheep (McNeilly and Fraser, 1987), monkeys (Fraser et al., 1987), and humans (Broekmans et al., 1996). However, few studies have established the efficacy of these agents for long-term suppression of ovarian activity and contraception (Trigg et al., 2001; Baker et al., 2002, 2004; D'Occhio et al., 2002) and, to our knowledge, none have previously demonstrated effective contraception by dart delivery of the implant.

In previous research, we administered GnRH agonist leuprolide acetate by hand injection to captive female elk (*Cervus elaphus nelsoni*) (Baker et al., 2002) and mule deer (*Odocoileus hemionus hemionus*) (Baker et al. 2004) as a sustained release injectable implant and achieved 100% infertility for one breeding season. The implant formulation consisted of 45% w/w 75/25 poly(DL-lactide-co-glycolide) (PLG) polymer having an intrinsic viscosity of 0.20 dl/g dissolved in *N*-methyl-2-pyrrolidone (NMP) and containing 6% w/w leuprolide in the polymer solution. This formulation was designed to release the drug for a period of 3–4 mo after subcutaneous injection (Ravivarapu et al., 2000).

In these previous studies, leuprolide formulation was demonstrated to be highly effective when delivered subcutaneously; however, it's not known if similar effectiveness can be achieved when administered as an intramuscular (IM) injection via dart. Differences in drug pharmacokinetics and metabolism between muscle and subcutaneous tissues could affect release dynamics of the implant and possibly decrease the antifertility properties of leuprolide.

Therefore, the objectives of this experiment were to determine in captive female elk (1) the effectiveness of remotely delivered intramuscular leuprolide implant in preventing pregnancy, (2) the effective duration of suppression of luteinizing hormone (LH) and progesterone secretion, and (3) the reversibility of infertility (if achieved).

MATERIALS AND METHODS

Experimental animals

During 1 August 2002 to 3 September 2003, we evaluated the effects of remotely delivered leuprolide formulation on pregnancy rates, LH, and progesterone secretion in captive female elk. Controlled experiments were conducted with 15 adult females (2–14 yr of age; 220–275 kg body weight [BW]), two intact adult male elk (3 yr of age; 350–400 kg BW), and one epididymectomized adult male elk (3 yr of age; 340–375 kg BW) at the Colorado Division of Wildlife's Foothills Wildlife Research Facility in Fort Collins, Colorado, USA. Captive elk used in this experiment were permanently maintained at this facility and were trained to repeated handling, weighing, blood-sampling techniques, and isolation pens. When not involved in the periodic intensive sampling procedures required by this study, elk were maintained in fenced pastures (5 ha) containing native vegetation and fed a diet consisting of ad libitum quantities of grass-alfalfa hay, grain supplement, trace mineral block, and water.

In an effort to induce normal cyclic ovulatory responses and synchronize estrus, we released an epididymectomized male elk with 15 seasonally anovulatory female elk on 20 July 2002 (McComb, 1987). Four weeks later (21 August) and before assigning elk to experimental treatments, we assessed the reproductive status of each female by: (1) manual rectal palpation of

the reproductive tract to diagnose ovarian status and identify any abnormalities, and (2) measuring the responsiveness of pituitary gonadotropes to an exogenous dose of GnRH analog. Females showing evidence of reproductive tract abnormalities or suppressed gonadotrope function were excluded from the experiment.

Experimental design

Fifteen female elk were randomly assigned to one of three experimental groups. Six elk (group A) were injected with a dart containing the polymeric matrix formulation of leuprolide acetate (D-Leu^6-GnRH-Pro^9-ethylamide). Four elk (group B) were designated as pregnant controls. They received the polymer solution without leuprolide to compare the effects of leuprolide formulation on pregnancy rates between treated and untreated elk. These two groups of elk were maintained together in the same pastures with two intact adult male elk from 13 September 2002 to 10 April 2003. The five remaining elk (group C) served as nonpregnant controls and were placed in a separate pasture (2 ha) without direct contact with male elk. We compared concentrations of LH and progesterone of these females to those treated with leuprolide formulation (group A). Nonpregnant control females (group C) provided a more representative comparison with treated elk for evaluating treatment-induced hormonal responses than potentially pregnant elk, thus the need for two separate control groups.

Treatments

Leuprolide implant formulation: The polymer, 85/15 poly (DL-lactide-co-glycolide) (PLG) with intrinsic viscosity 0.31 dl/g (Absorbable Polymer Technologies, Pelham, Alabama, USA) and *N*-methyl-2-pyrrolidone (NMP, International Speciality Products, Wayne, New Jersey, USA) were mixed in a ratio of 50:50 in a vial until the polymer was completely dissolved. The polymer solution was sterilized by γ-irradiation at a dose of approximately 25 Gy (Isomedix, Morton Grove, Illinois, USA) and an appropriate amount of the sterilized polymer solution was filled into 1.2 luer-lock female syringes. For the leuprolide part of the system, the calculated volume of filtered aqueous solution of leuprolide acetate (Mallinkrodt, St. Louis, Missouri, USA) was filled in 1-ml male syringe barrels (Becton-Dickenson, Franklin Lakes, New Jersey, USA) and lyophilized. This formulation was designed to deliver a 32.5 mg dose of leuprolide at a controlled rate over a 180-day therapeutic period. A similar formulation was previously shown to suppress ovulation and pregnancy for one breeding season in captive elk when delivered subcutaneously by hand injection (Baker et al., 2002).

Treatment application: On the day before treatment application (6 September 2002), experimental elk were moved from holding paddocks to individual isolation pens (5 × 10 m), weighed (±0.5 kg), sedated with xylazine hydrochloride (Rompun; Bayer AG, Leverkusen; 25–200 mg/animal, IM) and fitted nonsurgically with indwelling jugular catheters. The next day, and just before injection, separate syringes containing the polymer and the leuprolide were connected and the contents mixed with 60 back-and-forth mixing cycles. The resulting homogenous dispersion was drawn into the male syringe, and the formulation was transferred into single-use, 1 ml, 13-mm-diameter, barbless darts equipped with gel-collared 32-mm-long needles (Pneu-dart, Williamsport, Pennsylvania, USA). The final concentration of leuprolide was 12% in the homogenous mixture of polymer solution and leuprolide acetate after mixing and was designed to deliver approximately 32.5 mg of leuprolide acetate to the elk. Control elk received only the polymer solution processed the same way but without leuprolide.

Prior to darting, individual elk were placed in a handling chute and lightly sedated with intravenous (IV) xylazine hydrochloride (15–20 mg/animal). This dose allowed animals to remain standing in the chute and minimized excitation associated with discharge of the dart gun. All elk were remotely injected with a dart fired from a CO_2-powered pistol (DanInject™, Wildlife Pharmaceuticals, Fort Collins, Colorado, USA). To determine accurately the precise dose of leuprolide formulation delivered to each elk, darts were weighed (0.001 g) before and after injection. With the exception of two animals, one dart per animal was fired from approximately 3 m into the area of the biceps femoris muscle of the standing elk. In two animals, the dart failed to discharge or only partially injected the prescribed dose. In these cases, we reweighed and fired additional darts until the complete dose was delivered to each animal. Once all elk had been treated, sedation was reversed with yohimbine (30 mg, IV) (Antagonil®, Wildlife Laboratories, Fort Collins, Colorado, USA) and animals were returned to individual isolation pens.

Measurements

Twenty-four hour LH response to leuprolide treatment: Immediately following application of treatments to groups A and group C, we determined the amount of LH released during

the initial 24 hr of the treatment period. Blood samples (5 ml) were collected via jugular catheters at 0, 120, 180, 240, 300, 360, 480, 600, 960, and 1,440 min after drug injection. Catheters were flushed after each collection with sterile saline solution. After collections, blood was stored at 4 C for 24 hr until serum was obtained by centrifugation (1,500 RCF for 15 min), then stored at −20 C until analyzed for LH and progesterone. After the last blood collection, catheters were removed and animals were returned to holding paddocks. Eight days later, two intact male elk were placed into the same pasture with these females.

Duration of LH and progesterone response to leuprolide treatment: The effect of leuprolide formulation on the duration of suppression of LH and progesterone was determined by periodically conducting pituitary stimulation trials. These trials were performed before treatment application as an aid in the selection of animals for this experiment and periodically during 29 October 2002 to 3 September 2003 to determine pituitary responsiveness to an exogenous dose of GnRH analog (D-Ala^6-GnRH-Pro^9-ethylamide; Sigma Chemical Company, St. Louis, Missouri, USA).

Pituitary stimulation trials were conducted with elk in groups A and C elk at 50, 100, 150, 185, 215, and 361 days posttreatment. The final challenge trial (3 September 2003) provided hormonal evidence of the reversibility of leuprolide treatment. Stimulation trials were conducted according to the following procedures: On the day of testing, elk from groups A and C were moved from 5 ha pastures to individual isolation pens, weighed, sedated (as previously described), and fitted nonsurgically with indwelling jugular catheters. A bolus dose of GnRH analog (1 μg/50 kg BW) was administered through the cannula and blood samples (5 ml) were collected at 0, 60, 120, 180, 240, 300, 360, and 480 min postadministration. Serum for progesterone analysis was obtained from the 0 hr blood sample for each animal on each of the trial days. Blood samples were handled as described previously. Following the last blood collection, catheters were removed, and elk were returned to holding pastures.

Reproductive response to leuprolide treatment: The effect of leuprolide formulation on reproduction in groups A and B was determined in two ways: (1) by measuring pregnancy rates using the presence or absence of pregnancy-specific protein B (PSPB) (BioTracking, Moscow, Idaho, USA) in serum collected at approximately 100 and 215 days of gestation (Huang et al., 2000) and (2) by observing the presence or absence of calves the following summer.

Analyses

Serum concentrations of LH were quantified by means of an ovine luteinizing hormone (oLH) radioimmunoassay (Niswender et al., 1969). Elk serum was demonstrated to inhibit binding of ^{125}I-labeled oLH to LH antiserum in a manner that paralleled the standard (NIH-oLH-S24). Similarly, when different quantities of oLH standard were added to elk 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, β1=0.22, P=0.002). These data indicated that the radioimmunoassay provided a quantitative assessment of LH in elk serum. The limit of sensitivity of the LH assay was 0.02 ng/ml. Serum concentrations of progesterone were also determined by radioimmunoassay (Niswender, 1973). Sensitivity of the progesterone assay was 0.12 ng/ml. Intra- and-interassay coefficients of variation for each of these assays were <10%.

Hormone concentrations are reported as untransformed arithmetic means (±SE). Responsiveness of the pituitary gland to GnRH analog stimulation was determined by the total amount of LH secreted (ng/ml/min), which was estimated by calculating the area under the LH response curve (Abramowitz and Stegun, 1968). Differences among hormone concentrations were tested using least-squares analysis of variance (ANOVA) for general linear models (SAS Institute, 1997). Responses to treatment were analyzed with one-way ANOVA for a randomized complete block design with a repeated measures structure. Treatment effects were determined by using the total animal-within-treatment variances as the error term. Time was treated as a within-subject effect, using a multivariate approach to repeated measures (Morrison et al., 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).

RESULTS

Intramuscular injection of leuprolide formulation via dart was 100% effective in suppressing ovulation and preventing pregnancy in captive female elk for one breeding season. All leuprolide-treated females (group A) tested negative and untreated controls (group B) tested positive for PSPB at approximately 100 and 215 days of gestation. No calves were born to treated elk, whereas the calving rate of untreated elk was 100%. The amount of leu-

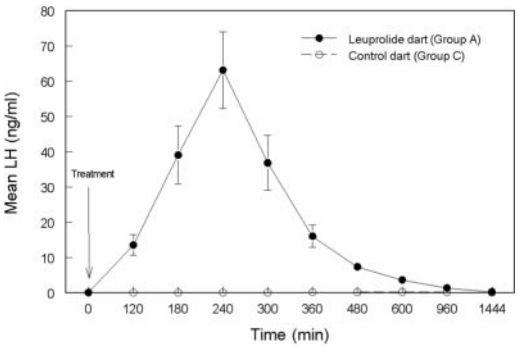

FIGURE 1. Twenty-four hour serum LH concentrations (ng/ml, mean±SE) for untreated female elk (○, n=5) and female elk (●, n=6) treated with a 180-day sustained release implant formulation, containing approximately 32.5 mg of leuprolide acetate, remotely delivered via projectile dart.

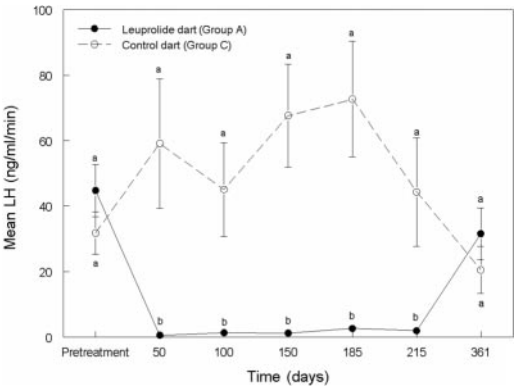

FIGURE 2. Total serum LH concentrations (ng/ml/min, mean±SE) for GnRH analog-induced release of LH for untreated female elk (○, n=5) and female elk (●, n=6) treated with a 180-day sustained release implant formulation, containing approximately 32.5 mg of leuprolide acetate, remotely delivered via projectile dart. Different lower case letters indicate significant differences between means ($P \leq 0.05$).

prolide acetate delivered to each elk ranged from 22. 6 to 38.1 mg ($\bar{x}$=33.1, SE=2.4). The lowest individual dose delivered (22.6 mg) was equally as effective as higher doses in suppressing hormone concentrations and pregnancy, suggesting that the minimum effective dose in elk could be substantially lower than the estimated dose (32.5 mg) used in this experiment.

We did not observe any unusual bleeding, swelling, or trauma at the injection site nor did any of the elk show evidence of impaired mobility, posttreatment tissue necrosis, or abscesses related to dart delivery of the bioimplant. However, additional research and testing is needed to improve the success rates of darts containing the leuprolide formulation. All (9/9) control darts (polymer solution only) discharged the entire prescribed dose, whereas 30% (2/6) of the darts containing the leuprolide formulation (leuprolide + polymer solution) failed and an incomplete dose was delivered. Improved performance of dart delivery technology may require a lower viscosity polymer solution, a lower dose of leuprolide, or both.

Mean serum concentrations of LH increased (P=0.015) in treated elk (group A) within 2 hr of leuprolide injection, peaked at 63.12±10.8 ng/ml (mean±SE) 4.3±0.65 hr (mean±SE) later, then gradually declined to baseline levels by 16 hr posttreatment (Fig. 1). Levels of LH in group A were greater (P=0.032) than those of untreated controls (group C) for 2–10 hr posttreatment, after which values decreased to baseline levels and were similar (P=0.285) for both groups.

Results of periodic GnRH challenges revealed that leuprolide formulation reduced pituitary content of LH to basal concentrations for at least 215 days posttreatment, which was 35 days longer than the expected 180-day delivery period (Fig. 2) of the implant. Concentrations of GnRH analog-induced LH secretion were lower (P=0.022) in leuprolide-treated elk (group A) than in nonpregnant controls (group C) at 50, 150, 185, and 215 days after treatment. Chronic suppression of LH in treated females was followed by a return to pretreatment levels, indicative of estrus, before the subsequent breeding season (September 2003; Fig. 3). In contrast to leuprolide-treated elk, the pituitary responsiveness of untreated elk (group C) to GnRH analog was elevated and relatively similar (P=0.64) in magnitude during the first 185 days of the experiment, after which, these levels declined

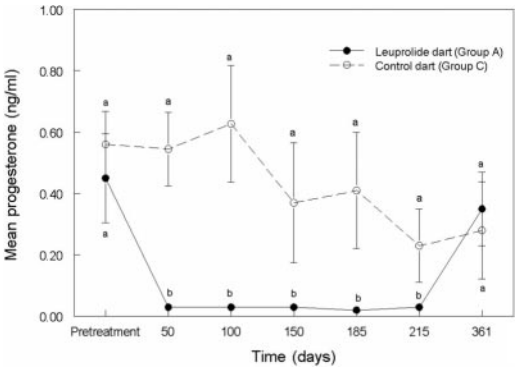

FIGURE 3. Serum profiles of mean progesterone concentrations (ng/ml, mean±SE) for untreated female elk (○, $n=5$) and female elk (●, $n=6$) treated with a 180-day sustained release implant formulation, containing approximately 32.5 mg of leuprolide acetate, remotely delivered via projectile dart. Different lower case letters indicate significant differences between means ($P \leq 0.05$).

($P=0.087$), presumably with the onset of seasonal anestrus (March). Similar ($P=0.582$) to treated elk, pituitary responsiveness in control elk (group C) returned to pretreatment levels in September 2003.

Serum concentrations of progesterone in leuprolide-treated females (group A) followed a parallel pattern to that observed for serum LH (Fig. 3). The suppressive effects of leuprolide on corpus luteum formation and steroidogenesis were readily apparent by the effects on serum progesterone concentrations in treated elk compared with controls (group C). Progesterone levels in treated elk declined ($P=0.017$) to lower limits of detection by 50 days posttreatment and remained at those levels for the duration of the breeding period. For untreated elk (group C), serum progesterone was more variable and consistently higher ($P=0.043$) than that for treated elk at 50, 100, 150, 185, and 215 days posttreatment. As evidence of normal estrous cycles and contraceptive reversibility, progesterone concentrations in both treated and untreated elk (group C) returned to pretreatment levels ($P=0.435$) at the onset of the following breeding season.

DISCUSSION

In the present experiment, we evaluated the effectiveness of projectile dart delivery of leuprolide formulation as an antifertility agent in female elk. Leuprolide treatment resulted in decreased LH and progesterone secretion, presumably suppression of ovulation and steroidogenesis, and effective contraception (100%) for one breeding season, without adverse side effects.

The contraceptive effects of leuprolide followed a two-phase process. The first phase was characterized by an acute, transient rise in serum LH that gradually declined to basal concentrations at about 16 hr posttreatment. The second phase was defined by chronic inhibition of LH and progesterone secretion for the duration of the seasonal breeding period. Subsequently, normal ovarian function and fertility were re-established before the next breeding season. We infer from these patterns of hormone secretion that gonadotrope cells in female elk were down-regulated during treatment with GnRH agonist. The process of down-regulation has been described previously in other species and is initiated by long-term exposure to GnRH agonist. This effect causes a reduction in GnRH receptors on gonadotropes (Clayton, 1989), depletion of pituitary LH and follicle-stimulating hormone content (Aspden et al., 1996), and elimination of the preovulatory LH surge (Gong et al., 1995; D'Occhio et al., 1996). These responses have been shown to result in ovulation failure and infertility, which persists as long as the agonist is present in circulation at therapeutic levels (Melson et al., 1986; D'Occhio et al., 2000). Our findings are consistent with previous observations of acute and chronic responses reported in sheep (Dobson, 1985), cattle (D'Occhio et al., 1989; Gong et al., 1996), horses (Montovan et al., 1990), deer (Becker and Katz, 1995), and elk (Baker et al., 2002) treated with GnRH agonist.

Effective contraception in polyestrous, seasonal breeders depends on suppression

of ovulation from the beginning of the breeding season to the onset of seasonal anestrous, a period of approximately 200 days in elk. Therefore, the timing of treatment application is an important consideration in successful contraception. Because of the acute rise in LH concentrations that occurs following GnRH agonist treatments, ovulation of growing follicles can be induced (Macmillan and Thatcher, 1991; D'Occhio and Aspden, 1999). Therefore, to ensure effective contraception in female elk, leuprolide treatments should be applied before the initiation of seasonal estrus.

In the present study, leuprolide inhibited LH secretion and ovulation for at least 215 days, which is in close agreement with previous research, in which a subcutaneous dose of leuprolide suppressed LH levels for 190–250 days (Baker et al., 2002). In other studies, implants containing GnRH agonist have been shown to suppress ovarian activity for a minimum of 150 days in mule deer (Baker et al., 2004) and almost 400 days in cattle (D'Occhio et al., 2002).

Persistent suppression of ovarian function, beyond the formulated delivery period of the implant, has been reported for several different species. Leuprolide suppressed LH and progesterone levels in elk in this experiment for at least 35 days longer (19%) than the expected 6 mo effective duration and 30–110 days longer in deer and elk in previous studies (Baker et al., 2002, 2004). Similar observations of extended gonadotrope suppression have been reported previously in cattle (Bergfeld et al., 1996; D'Occhio et al., 1996), monkeys (Fraser et al., 1987), men (Hall et al., 1999), and women (Broekmans et al., 1996). The underlying mechanism for this effect is not completely understood, but it is thought to be an associated with prolonged dysfunction of gonadotrope cells rather than direct action on the ovaries (D'Occhio et al., 2000; Aspden et al., 2003). Regardless of the mechanism involved, the extended suppression of ovarian function, as a consequence of leuprolide treatment, is fundamentally essential to effective contraception in deer and elk.

In conclusion, intramuscular delivery of a sustained release formulation of leuprolide via dart resulted in effective suppression of ovarian function and fertility in female elk for one breeding season with a return to normal reproductive function the following year. These results are particularly important for wildlife applications where, in the absence of such technology, animals must first be captured and restrained before treatment.

ACKNOWLEDGMENTS

We sincerely thank E. Jones, J. Ritchie, and A. Mitchell for technical assistance with animal training and handling, sample collection and preparation of samples for laboratory analyses. X. Sha and M. Allen provided valuable laboratory assistance and radioimmunoassay analysis of reproductive hormones. We offer special thanks to J. Powers for reproductive tract examination of experimental elk. We extend our appreciation to D. Bowden for statistical consultation and data analysis. D. Freddy and A. Apa provided particularly constructive reviews of early drafts of this manuscript. This research was supported by a grant from the US Department of the Interior, National Park Service, Rocky Mountain National Park, and the Colorado Division of Wildlife (Federal Aid to Wildlife Restoration, Project 153-R-8). We gratefully acknowledge Atrix Laboratories, Incorporated, Ft. Collins, Colorado, USA, for the generous donation of the leuprolide polymer formulation used in the experiment. This experiment was conducted with the approval of the Colorado Division of Wildlife Animal Care and Use Committee (11-2001) and in compliance with the United States Federal Animal Welfare Act Regulations.

LITERATURE CITED

ABRAMOWITZ, M., AND I. A. STEGUN. 1968. Handbook of mathematical functions. Dover Publishing, Inc., New York, New York.

ASPDEN, W. J., A. RAO, P. T. SCOTT, I. J. CLARK, T. E. TRIGG, J. WALSH, AND M. J. D'OCCHIO. 1996. Direct actions of the luteinizing hormone -releasing hormone agonist, deslorelin, on anterior pituitary contents of luteinizing hormone (LH) and follicle-stimulating hormone (FSH), LH and FSH subunit messenger ribonucleic acid, and plasma concentrations of LH and FSH

in castrated male cattle. Biology of Reproduction 55: 386–392.

———, A. JACKSON, T. E. TRIGG, AND M. J. D'OCCHIO. 2003. Pituitary expression of LHβ- and FSHβ-subunit mRNA, cellular distribution of Lhβsubunit mRNA and LH and FSH synthesis during and after treatment with a gonadotrophin-releasing hormone agonist in heifers. Reproduction, Fertility and Development 15: 149–156.

BAKER, D. L., M. A. WILD, M. M. CONNER, H. B. RAVIVARAPU, R. L. DUNN, AND T. M. NETT. 2002. Effects of GnRH agonist (leuprolide) on reproduction and behavior in female wapiti (*Cervus elaphus nelsoni*). Reproduction 60(Suppl.): 155–167.

———, ———, ———, ———, ———, ———. 2004. Gonadotropin releasing hormone agonist: a new approach to reversible contraception in female deer. Journal of Wildlife Diseases 40: 713–724.

BARLOW, N. D. 1996. The ecology of wildlife disease control: simple models revisited. Journal of Applied Ecology 33: 303–314.

BECKER, S. E., AND L. S. KATZ. 1995. Effects of gonadotropin-releasing hormone agonist on serum LH concentrations in female white-tailed deer. Small Ruminant Research 18: 145–150.

BERGFELD, E. G. M., M. J. D'OCCHIO, AND J. E. KINDER. 1996. Continued desensitization of the pituitary gland in young bulls after treatment with the luteinizing hormone-releasing hormone agonist deslorelin. Biology of Reproduction 54: 769–775.

BOMFORD, M. 1990. A role for fertility control in wildlife management? Department of Primary Industries and Energy, Bureau of Rural Resources Bulletin no. 7, Australian Government Publishing Service, Canberra, Australia.

BROEKMANS, F. J., P. G. HOMPES, C. B. LAMBALK, E. BROEDERS, AND J. SCHOEMAKER. 1996. Short-term desensitization: effects of different doses of gonadotrophin-releasing hormone agonist triptorelin. Human Reproduction 11: 55–60.

BRUSH, C. C., AND D. W. EHRENFELD. 1991. Control of white-tailed deer in non-hunted reserves and urban fringe areas. *In* Wildlife conservation in metropolitan environments, L. W. Adams and D. L. Leedy (eds.). National Institute for Urban Wildlife, Columbia, Maryland, pp. 59–60.

CLAYTON, R. N. 1989. Gonadotropin-releasing hormone: its actions and receptors. Journal of Endocrinology 120: 11–19.

DECKER, D., AND A. N. CONNELLY. 1989. Deer in suburbia-pleasure or pests? Conservationist 43: 46–49.

DELSINK, A. K., J. J. VAN ALTENA, J. J. KIRKPATRICK, AND R. A. FAYRER-HOSKEN. 2002. Field application of immunocontraception in African elephants (*Loxodonta africana*). Reproduction 60(Suppl.): 117–124.

DENICOLA, A. J., D. J. KESLER, AND R. K. SWIHART. 1997. Remotely delivered prostaglandin F_2 implants terminate pregnancy in white-tailed deer. Wildlife Society Bulletin 25: 527–531.

DIAMOND, J. 1992. Must we shoot deer to save nature? Natural History August: 2–8.

DOBSON, H. 1985. Effects of chronic treatment with a GnRH agonist on oestrous behavior and on the secretion of LH and progesterone in the ewe. Theriogenology 24: 1–11.

D'OCCHIO, M. J., AND W. J. ASPDEN, 1999. Endocrine and reproductive responses of male and female cattle to agonist of gonadotrophin-releasing hormone. Journal of Reproduction and Fertility 54(Suppl.): 101–114.

———, D. R. GIFFORD, C. R. EARL, T. WEATHERLY, AND W. VON RECHENGERG. 1989. Pituitary and ovarian responses of post-partum acyclic beef cows to continuous long-term GnRH and GnRH agonist treatment. Journal of Reproduction and Fertility 85: 495–502.

———, W. J. ASPDEN, AND T. R. WHYTE. 1996. Controlled, reversible suppression of estrous cycles in beef heifers and cows using agonist of gonadotropin-releasing hormone. Journal of Animal Science 74: 218–225.

———, G. FORDYCE, T. R. WHYTE, W. J. ASPDEN, AND T. E. TRIGG. 2000. Reproductive responses of cattle GnRH agonist. Animal Reproduction Science 60–61: 433–442.

———, ———, ———, L. A. FITZPATRICK, N. J. COOPER, W. J. ASPDEN, M. J. BOLAM, AND T. E. TRIGG. 2002. Use of GnRH agonist implants for long-term suppression of fertility in extensively managed heifers and cows. Animal Reproduction Science 74: 151–162.

FAGERSTONE, K. A., M. A. COFFEY, P. D. CURTIS, R. A. DOLBEER, G. J. KILLIAN, L. A. MILLER, AND L. WILMOT. 2002. Wildlife fertility control. Wildlife Society Technical Review 02–02. The Wildlife Society, Bethesda, Maryland.

FRASER, H. M, J. SANDOW, H. R. SEIDEL, W. VON RECHENBERG. 1987. An implant of gonadotropin releasing hormone agonist (buserelin) which suppresses ovarian function in the macaque for 3–5 months. Acta Endocrinology 121: 841–853.

GARROTT, R. A., P. J. WHITE, AND C. A. VANDERBIL WHITE. 1993. Overabundance: an issue for conservation biologist? Conservation Biology 7: 946–949.

GONG, J. G., T. A. BRAMLEY, C. G. GUTIERREZ, A. R. PETERS, AND R. WEBB. 1995. Effects of chronic treatment with gonadotrophin-releasing hormone agonist on peripheral concentrations of FSH and LH, and ovarian function in heifers. Journal of Reproduction and Fertility 105: 263–270.

———, B. K. CAMPBELL, T. A. BRAMLEY, C. G. GU-

TIERREZ, A. R. PETERS, AND R. WEBB. 1996. Suppression in the secretion of follicle-stimulating hormone and luteinizing hormone, and ovarian follicle development in heifers continuously infused with a gonadotropin- releasing hormone agonist. Biology of Reproduction 55: 68–74.

HALL, M. C., R. J. FRITZSCH, A. I. SAGALOWSKY, A. AHRENS, B. PETTY, AND C. G. ROEHRBORN. 1999. Prospective determination of the hormonal response after cessation of luteinizing hormone-releasing hormone agonist treatment in patients with prostate cancer. Urology 53: 898–902.

HONE, J. 1992. Rate of increase and fertility control. Journal of Applied Ecology 29: 695–698.

HUANG, F., D. C. COCKRELL, T. R. STEPHENSON, J. H. NOYES, AND R. G. SASSER. 2000. A serum pregnancy test with a specific radioimmunoassay for moose and elk pregnancy specific protein B. Journal of Wildlife Management 64: 492–499.

JACOBSON, N. K., D. A. JESSUP, AND D. J. KESLER. 1995. Contraception in black-tailed deer by remotely delivered norgestomet ballistic implants. Wildlife Society Bulletin 23: 718–722.

JEWELL, P. A., AND S. HOLT. 1981. Problems in management of locally abundant wild mammals. Academic Press, New York, New York.

KIRKPATRICK, J. F., AND J. W. TURNER, JR. 1985. Chemical fertility control and wildlife management. Bioscience 35: 485–491.

———, I. K. M. LIU, AND J. W. TURNER. 1990. Remotely-delivered immunocontraception in feral horses. Wildlife Society Bulletin 18: 326–330.

KREEGER, T. J. 1997. Overview of delivery systems for the administration of contraceptives to wildlife. *In* Contraception in wildlife management. T. J. Kreeger (ed.). USDA-APHIS Technical Bulletin 1853, Washington, D.C., pp. 29–48.

MACMILLAN, K. L., AND W. W. THATCHER. 1991. Effects of gonadotrophin-releasing hormone on ovarian follicles in cattle. Biology of Reproduction 45: 883–889.

MCCLEOD, B. J., S. E. DODSON, A. R. PETERS, AND G. E. LAMMING. 1991. Effects of a GnRH agonist (Buserelin) on LH secretion in post-partum beef cows. Animal Reproduction Science 24: 1–11.

MCCOMB, K. 1987. Roaring by red deer stags advances the date of oestrus in hinds. Nature 330: 648–649.

MCCULLOUGH, D. R., K. W. JENNINGS, N. B. GATES, B. G. ELLIOT, AND J. E. DIDONATO. 1997. Overabundant deer populations in California. Wildlife Society Bulletin 25: 478–483.

MCNEILLY, A. S., AND H. M. FRASER. 1987. Effect of gonadotrophin-releasing hormone agonist-induced suppression of LH and FSH on follicle growth and corpus luteum function in the ewe. Journal of Endocrinology 115: 273–282.

MELSON, B. E., J. L. BROWN, H. M. SCHOENEMANN, G. K. TARNAVSKY, AND J. J. REEVES. 1986. Elevation of serum testosterone during chronic LHRH agonist treatment in the bull. Journal of Animal Science 62: 199–207.

MILLER, L. A., J. A. RHYAN, AND M. DREW. 2004. Contraception of bison by GnRH vaccine: a possible means of decreasing transmission of brucellosis in bison. Journal of Wildlife Diseases 40: 725–730.

MILLIKEN, G. A., AND D. E. JOHNSON. 1984. Analysis of messy data. Vol. 1. Designed experiments. Lifetime Learning Publications, Belmont, California.

MONTOVAN, S. M., P. P. DAELS, J. RIVIER, J. P. HUGHES, G. H. STABENFELDT, AND B. L. LASLEY. 1990. The effect of potent GnRH agonist on gonadal and sexual activity in the horse. Theriogenology 33: 1305–1321.

MORRISON, J. A., C. E. TRAINER, AND P. L. WRIGHT. 1976. Multivariate statistical methods. McGraw-Hill, New York, New York.

NISWENDER, G. D. 1973. Influence of the site of conjugation on the specificity of antibodies in progesterone. Steroids 22: 413–424.

———, L. E. REICHERT, JR., A. R. MIDGLEY, AND A.V. NALBANDOV. 1969. Radioimmunoassay for bovine and ovine luteinizing hormone. Endocrinology 84: 1166–1173.

RAVIVARAPU, H. B., K. L. MOYER, AND R. DUNN. 2000. Sustained activity and release of leuprolide acetate from an *in situ* forming polymeric implant. American Association of Pharmaceutical Scientist 1: 1–12.

RHYAN, J. C., AND M. D. DREW. 2002. Contraception: a possible means of decreasing transmission of brucellosis in bison. *In* Brucellosis in elk and bison in the Greater Yellowstone Area, T. J. Kreeger (ed.). Greater Yellowstone Interagency Brucellosis Committee, Wyoming Game and Fish Department, Cheyenne, Wyoming, pp. 99–108.

SAS INSTITUTE. 1997. SAS/STAT® User's guide 6.03 edition. SAS Institute Incorporated, Cary, North Carolina.

SHIDELER, S. E., M. A. STOOPS, N. A. GEE, J. A. HOWELL, AND B. L. LASLEY. 2002. Use of porcine zona pellucida (PZP) vaccine as a contraceptive agent in free-ranging tule elk (*Cervus elaphus nannodes*). Reproduction 60(Suppl.): 169–176.

TRIGG, T. E., P. J. WRIGHT, A. F. ARMOUR, P. E. WILLIAMSON, A. JUNAIDI, G. B. MARTIN, A. G. DOYLE, AND J. WALSH. 2001. Use of GnRH analogue implant to produce reversible long-term suppression of reproductive function in male and female domestic dogs. Journal of Reproduction and Fertility 57: 255–261.

TURNER, J. W., JR., I. K. M. LIU, AND J. F. KIRKPATRICK. 1992. Remotely delivered immunocon-

traception in white-tailed deer. Journal of Wildlife Management 56: 154–157.

———, ———, ———. 1996. Remotely delivered immunocontraception in free-roaming feral burros. Journal of Reproduction and Fertility 107: 31–35.

Received for publication 17 September 2004.