Wild ungulate vontraception: Use of GnRH agonist or GnRH vaccine to control reproduction in captive and free-ranging female elk (*Cervus elaphus nelsoni*)

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Summary

Limiting the abundance of free-ranging ungulate populations is a significant issue for natural resource managers in many areas of North America. Hunting and culling have traditionally been used to regulate wild animal numbers but there are a growing number of circumstances where these methods pose significant legal, ethical, and ecological challenges, and resource managers often seek alternative approaches to population control. Decreasing fertility offers a potential non-lethal method for limiting population growth; however, current technologies suffer from a variety of technical, physiological, and regulatory problems.

Two fertility control methods, which have been tested in both captive and free-ranging elk to limit individual animal fertility, and show promise as a practical tool at the population level, are gonadotropin releasing hormone agonists and vaccines. While each uses a different method of action, the end result is the same - blocking the endocrine cascade at the level of the pituitary and suppressing gametogenesis. We tested the GnRH agonist, leuprolide acetate, in a slow-release gel formulation in female elk and found it prevented pregnancy in all treated animals for one breeding season with return to fertility the following season. Using similar methods, we tested a single injection GnRH vaccine and found that while it did not have the same level of efficacy; its effects were more persistent, lasting up to three years post-vaccination in some individuals. While our studies demonstrate that it is possible to decrease fertility of individual animals, substantial effort by managers would be needed before population level effects could be realized.

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Introduction

Rocky Mountain elk (*Cervus elaphus nelsoni*) are resilient, adaptable members of the cervid family. In North America, they thrive in a variety of habitats and climes from high altitude mountainous regions to coastal plains including remote pristine habitats, as well as the human dominated urban interface (Skovlin 1982). When elk become locally overabundant, particularly in peri-urban areas, where hunting is not permitted, this plasticity can lead to a variety of human-wildlife conflicts. Unregulated populations can have devastating effects on preferred forage species through herbivory (Zeigenfuss *et al.* 2002), affect biodiversity if they become the dominating force in the ecosystem (White *et al.* 1998), increase density dependent disease transmission (Smith 2013), pose a safety threat for motor vehicles and pedestrians, and damage private property (Walter *et al.* 2010). Traditional management techniques include, public hunting, professional culling, and in limited circumstances, translocation to address many of these issues. As human tolerance for hunting has waned, particularly near urban environments, and protectionism sentiment has grown, the need for alternate wildlife management methods has emerged. Fertility control is a non-lethal method, among a suite of management tools, which may assist in mitigating these conflicts (Bradford & Hobbs 2008).

The idea and science of wildlife fertility control is not new. For more than four decades practitioners have investigated the efficacy and practicality of manipulating wildlife reproduction using a variety of methods in a wide range of species (Fagerstone *et al.* 2010). However, for many physiological, ecological, and socio-political reasons, wildlife contraception has not been widely adopted as a means for population management (Ransom *et al.* 2013). Despite these barriers, resource managers, researchers and wildlife advocates continue to advance and promote the development of wildlife fertility control as a potential management tool. It has been suggested that the ideal fertility control agent would be: 1) highly effective, 2) free from toxicity and harmful side-effects for the target animal, 3) reversible to preserve the reproductive capacity of the individual and genetic integrity of the population, 4) inexpensive, 5) have little if any impact on social interactions and behavior, 6) be effective with a single administration preferably through remote delivery, and 7) be incapable of passing through the food-chain to predators, scavengers, or humans (Kirkpatrick & Turner 1991). To date there is no product which meets all of these criteria. Not surprisingly, wildlife contraception involves a number of diverse challenges not seen in human or domestic species (Turner & Rutberg 2013).

Recently, the extended duration gonadotropin releasing hormone (GnRH) agonist, leuprolide acetate, and the GnRH immunocontraceptive vaccine, GonaCon, have overcome some of the obstacles to successful application of a wildlife fertility control agent. Through a series of trials in both captive and free-ranging female elk, we investigated the safety, efficacy, and behavioral effects of these two methods of contraception. Here, we summarize and report the results of these findings and suggest areas for future research efforts.

Elk reproductive ecology

Elk have a polygamous harem oriented breeding structure (Bubenik 1982). While mature males dominate breeding activity, subordinate males also contribute to reproduction (Noyes *et al.* 1996). It is generally accepted that decreasing female rather than male fecundity is necessary to reduce the rate of population growth or initiate population decline (Garrott 1995).

The peak of breeding season for elk in North America occurs between mid-September and mid-October (Haigh & Hudson 1993). Endocrine and behavior patterns in both sexes are highly seasonal and driven by decreasing photoperiod (Bubenik 1982). Testosterone is at its nadir in

males at the time of antler drop in March/April; then increases to a peak during the breeding season (Haigh *et al.* 1984). Similarly, females are seasonally polyestrus with waves of follicular development occurring during the ovulatory (McCorkell *et al.* 2006), anovulatory (McCorkell *et al.* 2004), and transitional (McCorkell *et al.* 2007) seasons. Ovulation occurs at approximately 21 day intervals and the ovulatory season can persist as late as April although generally ends in mid-February (McCorkell *et al.* 2006, McCorkell *et al.* 2007).

Pregnancy rates for mature females (2-12 yr) can approach 1 calf/female/year in populations which are not forage limited (Sargeant & Oehler 2007). Nearly half of yearling females become pregnant at 16-18 months of age if an optimum body mass is achieved (~ 220kg, 10% body fat) (Sargeant & Oehler 2007, Cook *et al.* 2004). Reproductive senescence is not well described but fertility may decline by 13 to 17 years of age (Sargeant & Oehler 2007); however, effects of body condition are likely more important to fertility than age (Cook *et al.* 2001, Cook *et al.* 2004). Elk are monotocous and twinning is exceptionally rare. Calving occurs from late May to early July after a gestation of approximately 255 days and coincides with spring nutrient flush in North America (Bubenik 1982, Haigh & Hudson 1993).

Elk tend to concentrate in large, primarily single sex herds during the winter months, whereas during the spring and summer, they are more dispersed and in smaller groups. Only during the breeding season do they consistently group in mixed-sex herds (Geist 1982).

Gonadotropin releasing hormone agonist

Gonadotropin releasing hormone is a small protein hormone naturally secreted in a variable pulsatile fashion from the hypothalamus (Clarke & Cummings 1982). It controls the reproductive hormone cascade that eventually results in signaling at the gonad and ovulation in females (Hazum & Conn 1988). Although GnRH receptors on the anterior pituitary and their reproductive consequences have been recognized the longest, GnRH may have other important physiological targets (Skinner *et al.* 2009).

The gonadotrophs of the anterior pituitary are exquisitely sensitive to small changes in GnRH pulse amplitude and frequency (Belchetz *et al.* 1978). Non-basal luteinizing hormone (LH) release is directly correlated to GnRH pulses (Clarke & Cummins 1982), whereas follicular stimulating hormone (FSH) is less tightly regulated by GnRH and has a significant component which is constitutively derived (McNeilly *et al.* 2003). Gonadotropin releasing hormone signaling can be temporarily suppressed using chronic treatment with continuous, high doses of GnRH agonists. Initially, there is a large LH release followed by a return to baseline levels which persists until removal of agonist treatment. Gonadotropin synthesis and release from the pituitary is highly dependent on the episodic nature of endogenous GnRH signaling (Counis *et al.* 2005). The exact mechanisms of second messenger signaling associated with down-regulation of pituitary gonadotrophs are complex and not completely understood (McArdle *et al.* 2002). Regardless, GnRH analogs, particularly GnRH agonists, have become powerful non-steroidal, non-immunological agents for manipulating reproduction and reproductive pathologies in a variety of species (Padula 2005). Effects of GnRH analogs external to the hypothalamic-pituitary-gonadal (HPG) axis have only recently garnered attention but may have important consequences for normal physiology (Skinner *et al.* 2009).

Initially developed for human therapeutic use in treating reproductive hormone activated tumors, precocious puberty, and more recently infertility, GnRH agonists have also been used to prevent ovulation and reproduction in many ruminant species including sheep (McNeilly & Fraser 1987) and cattle (D'Occhio *et al.* 1996). To succeed as a wildlife reproductive inhibitor, GnRH agonists need to suppress gonadotropin secretion for a full reproductive season (approximately 210 days in elk) thereby preventing pregnancy for at least one year.

In a series of experimental trials in captive, mature female elk, we investigated the appropriate dose of the GnRH agonist leuprolide acetate in a 90 day slow-release gel matrix (ATRIGEL®) to suppress gonadotropin release and ultimately prevent pregnancy (Baker *et al.* 2002). We also studied the effect of this treatment on reproductive behaviors both during and after the captive breeding season (October-November). Finally, we tested whether this high viscosity gel could be effectively delivered from a remote dart delivery system (Baker *et al.* 2005). Interestingly, we found elk to be quite sensitive to GnRH agonist suppression. All doses from 45 to 180 mg of long-acting leuprolide acetate delivered subcutaneously, late in the breeding season (late-November), first initiated an acute LH release ($\sim$ 15 ng/ml maximum) and then maintained LH suppression for greater than 130 days post-treatment (Baker *et al.* 2002). This was determined via GnRH analog (D-Ala^6-GnRH-Pro^9-ethylamide) challenge and measurement of LH response on days 35, 70, 110, and 130 of the experiment. In light of this finding the leuprolide dose was decreased to 32.5 mg in subsequent trials attempting to prevent pregnancy.

During the next breeding season, female elk were treated with this lower dose of long-acting leuprolide, prior to their exposure to males. All treated females failed to become pregnant. Their response to GnRH analog challenge was also suppressed well into the anestrous period (April-May). In fact, luteal function was suppressed for approximately 200 days, notwithstanding the 90 day formulation, resulting in a full year of infertility. Remarkably, rather than having diminished reproductive behaviors as predicted, leuprolide treated females continued to display and receive precopulatory behaviors throughout the breeding season. In fact, they showed similar copulation rates as control females which all became pregnant (Figure 1) (Baker *et al.* 2002). We surmise that this is likely due to progesterone priming prior to treatment and sufficient, albeit basal, estradiol release due to continued follicular development in response to FSH despite lack of an LH surge which would induce ovulation (Baker *et al.* 2002, Powers *et al.* 2011).

In a successive studies, we found that leuprolide delivered intramuscularly in the same gel matrix through remote dart delivery (1 ml, 13 mm diameter, 32 mm needle, gel collar darts, Type P with fin stabilizers; Pneu-Dart Inc., Williamsport, Pennsylvania, USA) was equally efficacious as syringe hand-injection at preventing pregnancy and suppressing LH release (Baker *et al.* 2005). Regardless of method of administration, either by hand injection or dart delivery, all treated females returned to fertility one year post-treatment (i.e., the following breeding season).

In accordance with our findings in captive elk, free-ranging female elk treated in Rocky Mountain National Park (Colorado, USA) failed to become pregnant for one year when similarly hand-injected with long-acting luprolide gel, 30-60 days prior to the peak of breeding season (mid-October). In a natural setting it was possible to more accurately compare daily activity patterns, display of reproductive behaviors, and changes in body condition in leuprolide treated and sham-polymer administered control females. We used similar methods to those used in the captive study to observe and record reproductive behaviors associated with general breeding (i.e., herding, tending), male courtship (i.e., flemen response, urine testing, chivy), female courtship (i.e., circle and rub male, lordosis), and copulation (i.e., male mounting female with pelvic thrust, intromission) (Baker *et al.* 2002, Conner *et al.* 2007). In addition, we observed non-reproductive behaviors (i.e., feeding, moving, idling) during the breeding and post-breeding seasons at various times during the day. Finally, we estimated body condition and measured rump fat thickness at the time of initial capture (fall), at the time of pregnancy diagnosis (following spring), and finally during the subsequent breeding season (fall).

In contrast to observations in captive elk, we found that precopulatory courtship behaviors did not persist for a significantly longer time period in non-pregnant treated elk. This effect may have been masked due to dilution effect of relatively few treated females in a large free-
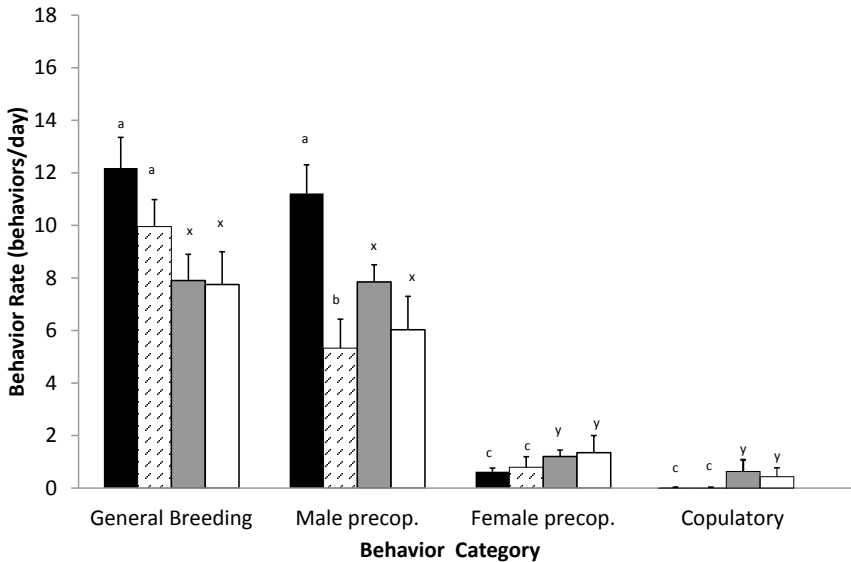
Figure 1. Mean ($\pm$ SE) daily reproductive behavior rates during the breeding season for captive female elk treated with: GnRH vaccine (n = 10, black), saline control (n = 10, hashed), leuprolide acetate (n = 4, grey), and depot control (n = 5, white). During GnRH vaccine/ saline control trial 2 male elk were exposed to two mixed groups of ten vaccinated and control females each. During the leuprolide/ depot control trial two male elk were exposed to all nine experimental females. Therefore, behavior rates between trials cannot be directly compared; however, relative rates between treatment groups are similar between trials. There was a tendency for more persistent behaviors in GnRH vaccinated or leuprolide treated females throughout the breeding season. Columns with different superscripts indicate significant differences between means within a trial; GnRH vaccine (a,b,c) or leuprolide acetate (x, y).

ranging herd with less male/female interaction than occurs in a captive environment. There were no differences in mean reproductive behavior rates between groups. Likewise there were no differences in activity budgets between treated and control females (Conner *et al.* 2007). One particularly interesting finding was that leuprolide treated females showed lower percentage body fat and lower lean body mass in late-winter/ early-spring (March) at the time of pregnancy diagnosis. However, in the fall (September), when leuprolide depots were no longer effective, body mass indicators had returned to parity with control females. We suggest that this may be due to a lack of gonadal steroid hormones, particularly progesterone, along with its effects on food intake, body weight dynamics, and carcass adiposity. This finding is in direct contrast to other fertility control methods, such as porcine zona pellucida vaccination which does not suppress reproductive hormones, and appears to increase body condition in treated animals (Turner & Kirkpatrick 2002).

In summary, 32.5 mg of leuprolide acetate delivered in a slow 90 day release gel formulation and given immediately prior to the breeding season, results in one year of pregnancy suppression in female elk. In the year following treatment fertility returns. Other than persistent precopulatory courtship, it has minimal reproductive behavioral effects in free-ranging elk and no effects on activity budgets. Body condition in non-pregnant leuprolide treated females is decreased during late-winter when compared with pregnant untreated females; however, this effect is no longer observable the following breeding season when fertility returns. The long-term

consequences of this effect on elk fitness are unknown. Long-acting leuprolide acetate can be used as a highly efficacious, reversible, single year fertility control agent in female elk. While GnRH agonists are used routinely in zoo and captive wildlife settings, for a GnRH agonist to be considered a practical wild ungulate contraceptive, longer-acting biodegradable implants that can deliver a sustained release of agonist over a predetermined time period are needed. Given new sustained release polymers for agonist delivery are of interest in human medicine, there may be opportunity for additional research and development in free-ranging ungulates.

Gonadotropin releasing hormone vaccination

Another option for manipulating the HPG axis in ungulates is functional removal of endogenous GnRH using antibodies. A single vaccination against GnRH, using GonaCon Immunocontraceptive Vaccine (GonaCon) (Miller *et al.* 2008), has been found to diminish endocrine and ovarian function within in a variety of ungulate species including white-tailed deer (Miller *et al.* 2008), elk (Powers *et al.* 2011), and horses (Killian *et al.* 2008). Although there are competing theories of action (Molenaar *et al.* 1993), the prevailing hypothesis suggests that antibodies to GnRH induce transient infertility by binding to endogenous GnRH in the hypothalamic-pituitary portal vessels, thus preventing attachment to receptors on gonadotrophs, and suppression of pulsatile LH secretion. As antibodies wane females generally return to fertility (Powers *et al.* 2011).

Historically, GnRH immunization has required repeat vaccinations to achieve sufficiently high antibody concentrations to alter gonadotroph function. This small peptide hormone is not generally immunogenic but can be made so by conjugation it to a large, highly immunogenic carrier protein (Nett *et al.* 1973). When combined with a potent adjuvant, the vaccine stimulates a persistent immune response resulting in prolonged antibody production against GnRH (Miller *et al.* 2008). The GonaCon vaccine construct we tested is composed of multiple copies of synthetic GnRH peptide linked to hemocyanin protein (Blue Carrier; Biosonda, Santiago, Chile) from a Chilean mollusk (*Concholepas concholepas*; CCH), and combined with the water-in-oil adjuvant, AdjuVac, which contains killed *Mycobacterium avium* ssp. *avium* along with non-biodegradable mineral oil (Miller *et al.* 2008). AdjuVac is based on a commercially available vaccine for protection against *Mycobacterium avium* ssp. *paratuberculosis* (MAP) – Mycopar (Fort Dodge Animal Health, Fort Dodge, Iowa, USA). Vaccination using AdjuVac results in antibodies which often react with commercial tests for MAP and may cause a false positive test for Johne's disease (Powers *et al.* 2011).

In a series of captive elk experiments we investigated the reproductive, behavioral, and first-generational effects of GonaCon vaccination on pregnant female elk. Using a single hand-injection of the vaccine during mid-pregnancy ($\sim$ 100 days gestation) we found that immunization significantly decreased fertility in captive female elk for three years (Powers *et al.* 2011). In the first, second, and third years post-vaccination efficacy was approximately 90%, 75%, and 50% respectively (Table 1). By the fourth year there was little observable treatment effect. Higher serum antibody concentrations were associated with greater contraceptive effects; however, antibody concentrations were not always diagnostic in predicting pregnancy.

Vaccination was associated with persistent precopulatory courtship behaviors throughout the breeding season in non-pregnant treated elk whereas control elk, given a sham vaccine, were no longer receptive to or displaying courtship behaviors once they became pregnant (Figure 1). We suggest that reproductive interactions in elk during the breeding season are driven more by pregnancy status than by the occurrence of ovulation and estrus (Powers *et al.* 2011). Too

Table 1. Mean yearly pregnancy proportions (no. pregnant/ no. exposed to fertile bull) and estimates of treatment effect size (difference in proportions) with 95% confidence intervals for GnRH and sham-vaccinated (captive) or saline administered (free-ranging) female elk.

Years post-treatment		Proportion Pregnant		Treatment Effect
		GnRH-vaccintes [n]	Sham-vaccinates [n]	Difference (95% CI)
0 (pre-treatment)	captive	1.0 [10][a]	1.0 [8][a]	0.0
	Free-ranging	0.85 [60][x]	0.92 [60][x]	0.07 (0.0 – 0.18)
1	captive	0.10 [10][b]	1.0 [7][a]	0.90 (0.71 – 1.0)[*]
	Free-ranging	0.00 [11][y]	0.90 [10][x]	0.90 (0.71 – 1.0)[*]
2	captive	0.25 [8][b]	1.0 [7][a]	0.75 (0.50 – 1.0)[*]
	Free-ranging	0.33 [12][y]	0.75 [13][x]	0.42 (0.08 – 0.79)[*]
3	captive	0.50 [8][b]	1.0 [7][a]	0.50 (0.15 – 0.85)[*]
	Free-ranging	0.65 [14][x]	0.86 [20][x]	0.21 (0.0– 0.50)
4	captive	0.75 [8][a]	0.86 [7][a]	0.12 (0.0 – 0.29)

Proportions with different superscripts are significant ($P \leq 0.05$) letters a and b are differences between treatment groups in captive elk and x and y are differences between treatment groups in free-ranging elk within a given year. Significant treatment effects are indicated with *. Adapted from Powers *et al.* 2011 and Powers *et al.* 2014.

few copulatory behaviors were observed to evaluate whether females were truly receptive to mounting or were only interested in courtship.

In addition to potential behavioral consequences of vaccination, we also investigated possible changes to organ function and overall fitness. Our most important finding was that vaccination is associated with deep, persistent changes to muscle architecture and echogenicity when examined using ultrasound. These changes at the injection site, as observed through ultrasound, occasionally develop into a sterile abscess, rupture, and drain. However, even animals with clinical abscesses did not show any evidence of weight loss or lameness suggesting no change in fitness. Additionally, there were no significant changes in complete blood count or chemistry profiles of treated elk other than a transient increase in inflammatory mediators (Powers *et al.* 2011).

After vaccination during mid-gestation, all treated elk gave birth to phenotypically normal calves. Those calves who nursed from treated females all developed high passive antibody concentrations within 24 hours of nursing and maintained titers for approximately 6 months (Powers *et al.* 2012). Changes to early GnRH signaling during the neonatal period can accelerate or impede pubertal development in domestic cattle (Madgwick *et al.* 2005, Chandolia *et al.* 1997, Prendiville *et al.* 1995). We hypothesized that puberty may be delayed or suppressed in elk experiencing high GnRH antibodies during the neonatal period. Therefore, during the following 3 years, calves with and without neonatal exposure to high GnRH antibodies were observed for pre-pubertal and pubertal reproductive development. We measured pituitary responsiveness to GnRH analog at 1.5 years of age and measured primary and secondary indicators of fertility in male and female calves at 2.5 years of age. We found no effect of exposure to high concentrations of maternally transferred GnRH antibodies on the long-term structure or function of the HPG axis of male or female elk. The HPG axis of elk is likely structurally mature at birth and transient disruption in GnRH signaling through antibody neutralization was not sufficient to permanently change function (Powers *et al.* 2012).

Finding relatively good efficacy and low incidence of significant negative side-effects in captive elk, we tested the vaccine in free-ranging female elk at Rocky Mountain National Park (Colorado, USA). In January, mature female elk were captured via remote dart delivered anesthesia, blood was collected, pregnancy was determined, and the same vaccine and dose as

used in the captive trials was given by hand-injection. In conjunction with on-going elk culling management actions at the park, we re-captured treated and control elk in the following three winters, collected samples, and euthanized each animal. Pregnancy was determined at the time of necropsy. Notably, we found that the vaccine was less effective in free-ranging female elk, which remained infertile for approximately two years post-immunization (Powers *et al.* 2014 in press) as compared to three years in captivity. In year one the efficacy was approximately 90% and in year two 42% (Table 1). By the third year there was little measurable treatment effect. We hypothesize that this is due to less robust or persistent immune responses in wild elk due to a combination of stressors not experienced in captivity such as more stressful nutritional, parasitic, and climate encumbrances. This was supported by our GnRH antibody findings which were generally lower in concentration and less persistent over time in free-ranging elk when compared to captive elk (Powers *et al.* 2011, Powers *et al.* 2014 in press).

The most substantial finding other than fertility measures in free-ranging elk was that every animal treated with the GonaCon vaccine had an inflammatory injection site reaction at the time of necropsy up to three years post-vaccination. This is not surprising given the adjuvant contains non-biodegradable mineral oil and highly immunogenic mycobacteria which form a persistent depot and provides on-going antigenic stimulation. Importantly, we found no evidence to suggest that this had a negative influence on fitness even in free-ranging elk. In summary, we found that a particularly potent GnRH vaccine can partially suppress fertility for two to three years with a single vaccination. The vaccine is safe to give during gestation and has no measurable effects on calves born to treated females. Future research needs to address long-term effects of re-vaccination on efficacy, behavior, and side-effects including the possibility of permanent sterility. As with any potent vaccine, the intensity and duration of immune response is likely to increase with additional immunization events. If maintenance of long-term fertility is of paramount importance, using an immunocontraceptive is not the method of choice given individual animal variation in immune response. Additionally, factors affecting lower efficacy in free-ranging animals compared to those in captivity and population level ecological effects deserve considerable attention (reviewed in: Ransom *et al.* 2013).

Conclusions

We conclude from these investigations that it is possible to suppress fertility in elk similarly to domestic ruminants by manipulating the hypothalamic-pituitary-gonadal axis via GnRH agonists or immunization against GnRH. The pharmaceutical effect of leuprolide acetate produces a predictable, highly efficacious, and reversible way to prevent pregnancy but must be reapplied on a yearly basis during a short window of time prior to the breeding season. Vaccination against GnRH produces less predictable, moderately efficacious, and longer-term contraception. Both may be tools for wildlife managers to consider; however, managing free-ranging wildlife is rarely focused on changing the fertility of individual animals. Population level effects are generally more important. The ability to treat sufficient numbers of animals to decrease population numbers poses a significant challenge in most open, free-ranging groups of migratory wildlife such as elk. These products may be useful in small, geographically limited situations but are not likely to be a population management tool in widely dispersed populations of cervids. Additionally, there are significant regulatory, cultural, and ecological hurdles which must be overcome before wildlife fertility control is a legitimate management technique (Ransom *et al.* 2013).

Acknowledgements

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