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The effects of short-term medroxyprogesterone acetate on rut related behaviors, semen characteristics and fertility in farmed reindeer bulls

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ABSTRACT

The effects of medroxyprogesterone acetate (MPA) on velvet antler cleaning, hypophagia, aggressive behavior and fertility were evaluated in farmed reindeer bulls during a 2-year study. Eight reindeer bulls aged 1–4 years were divided into 2 groups balanced for age. During each year, one group (MPA, n = 4) was treated with MPA 2 wk prior to the expected onset of rut while the other group (CTL, n = 4) served as untreated controls. Feed consumption, behavior and antler cleaning were recorded daily or 3 x weekly for 3 mo. Each year a dominant CTL and MPA bull were put into separate harems of estrous synchronized females for 1 wk in mid-September. Following harem breakup, semen was collected from all bulls via electroejaculation and evaluated. In Year 2 the bulls were switched such that year 1 MPA bulls received the CTL treatment and year 1 CTL bulls received MPA treatment. In Year 2 all eight bulls received MPA treatment/booster following semen collection. In both years, MPA treatment reduced rut associated body weight loss ($p \leq 0.05$), rut associated hypophagia ($p \leq 0.001$), interfered with velvet antler cleaning, and abolished aggressive rut related behavior. All of these changes suggest suppression of testosterone mediated effects. Alternatively, semen parameters differed little between treatment groups with the exception of reduced sperm concentration and total sperm production in MPA bulls ($p \leq 0.05$). All CTL bulls in Year 2 exhibited full rut behavior with the dominant bull successfully breeding 100% of females available for breeding, suggesting no carryover effect of MPA treatment from the previous year. The MPA bull successfully bred 4 of 6 females (Year 1) but the MPA bull in Year 2 failed to sire any offspring. A single 400 mg treatment of MPA just prior to rut was sufficient to suppress rut associated aggression and hypophagia on a short-term (3 mo) basis. It did not however, completely suppress fertility.

1. Introduction

Progestins have been used for decades to reduce male aggression in a wide range of animal species including humans [1], fringe-eared oryx [2], monkeys [3], gerenuk [4], horses [5], and dogs [6]. In humans efficacy with synthetic progestins (Depo-Provera) is achieved at 2.5 mg/kg body weight, hence a dose range of 2.5–5 mg/kg body weight has been recommended for mammals in general [7].

Progestins are most commonly used in male ungulates in zoos and captive wildlife settings to reduce aggression as opposed to contraception. In many of these cases data on associated reproductive parameters were not collected. However, accounts of progestin treated males (Nilgai antelope, Muntjac and Mule deer) siring offspring suggest that the doses used were not sufficient to impair fertility. The effect on antler growth varied considerably [8].

Rutting reindeer bulls are extremely aggressive which frequently results in destruction of fencing and infrastructure. They also stop eating during rut (~2–3 mo), even as subordinate bulls [9], and hypophagia-induced emaciation can be difficult to reverse [10]. There is abundant anecdotal information on the use of progestins in reindeer bulls, especially medroxyprogesterone acetate (MPA). This is frequently administered in two low doses early and late in the breeding season to reduce aggression in rutting males (Deerforums, 2013; The R.O.B.A. Review, 2015). To date, even the most reliable estimates of dosage for reindeer are equivocal and range from 200 to 400 mg per mature bull [10]. Anecdotal information indicates that treatment with MPA produces very manageable males, does

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not alter the normal antler cycle and treated males breed successfully in subsequent seasons. However, there have been no specific studies addressing the use of MPA on rut behavior and fertility in male reindeer. Further, studies in other species have documented that higher doses tend to remain in the system longer and, in female primates and ungulates, this has been associated with uterine fluid accumulation and endometrial and uterine infections, usually after two or more years of continuous use [8]. There is very little information on side effects of high progestin doses in male ungulates. Generally, in reindeer production settings, MPA is used on a short-term basis to reduce aggression in non-breeding reindeer males or is given to males following breeding to reduce rutting behavior and reverse rut-related hypophagia.

The demand for reindeer-specific information is just emerging and knowledge in this area will not only improve our ability to farm reindeer, but will add substantially to the general understanding of this cervid, whether farmed or free-ranging. Additionally, this research also provides critical background information for developing more advanced reproductive technologies i.e. semen collection, freezing and artificial insemination in reindeer.

This study specifically compares changes in velvet antler cleaning, body weight, feed consumption, rut associated behavior and fertility between MPA-treated and control reindeer bulls in a 2-year study.

2. Materials and methods

2.1. Animals

The study was conducted over two rutting seasons at the Robert G. White Large Animal Research Station (LARS), University of Alaska Fairbanks (UAF). Fairbanks is located in the interior of Alaska (64.83778° N; −47.71639° W) and characterized by a dry climate that can reach extremes of both summer heat and winter cold. Mean temperatures over the course of the study (Aug to the end of Oct) were 6.1 °C (range: 12.8 °C–0 °C) in year 1 and 7.2 °C (range: 16.1 °C to −2.8 °C) in year 2. The daily maximum (26.7 °C in Aug) and minimum (−18.9 °C in Oct) were similar in both years. https://w2.weather.gov/climate/xmacis.php?wfo=pafg. The LARS is a closed, 52.6 ha facility divided into several small holding pens (0.1–0.4 ha) and 9 larger paddocks with mixed pasture and tree cover (2–8.1 ha). Reindeer are maintained year round in same sex herds and all have access to mixed pasture grasses and browse. They are fed, *ad libitum,* a custom designed, complete pelleted ration (D-ration, Alaska Pet and Garden, Anchorage, AK): 15% CP, 70% TDN, 18% ADF, and 6% ash (dry matter). Pellet intake is approximately 3–6 kg per day depending upon animal size and time of year. One moveable, covered feeding station (2.5 m long and accessible from both sides) is used for every 8 to 10 reindeer. A loose mineral mix supplement (Buffalo Mineral, Alaska Pet and Garden, Anchorage, AK) is provided at the feeding stations. Water or snow is available *ad libitum* to all animals. Production metrics under this current feeding protocol have been excellent.

Eight reindeer bulls with an age range of 1–4 years were used in this study. Bull weights varied with age. Mean body weight (±SD) was 143.5 ± 15.6 kg (range: 123–157 kg) for yearlings; 189.3 ± 31.6 (range: 173–224 kg) for 2 year old bulls; 230.5 ± 6.6 (range: 226–240 kg) for 3 year old bulls; and 229.5 ± 28.8 (range: 197–265 kg) for 4 year old bulls. To reduce fighting injuries and facilitate handling, it was necessary to remove the large main antler beam from the dominant males. Altering antler size can affect a bull's dominance and subsequent behavior; therefore it was necessary to similarly remove antlers from all the bulls in both treatment groups. In Year 1 (July 8 to 9) the older males aged 3 years (n = 2) and 4 years (n = 2) were anesthetized (details in section 2.2.4. and supplementary materials) and had the main beam of their velvet antlers surgically removed approximately 13 cm above the bifurcation of the brow tine. Antlers from the 1–2 year bulls were removed within 2 d of velvet shedding using a sawzall with the animal restrained in a hydraulic deer chute. In Year 2, all eight bulls had their antlers similarly removed prior to the onset of the study (July 12 to 13). Antler removal did not change the dominance hierarchy in either group.

Each year healthy females (judged by general condition and body weight) were used to form harems as a test of bull libido and fertility. Pubertal weight for female reindeer is considered 60–80 kg, although females below 100 kg are generally not put in harems. In Year 2, five yearlings over 100 kg were placed in harem, three in the CTL group and two in the MPA group.

Animal protocols were approved by UAF Institutional Animal Care and Use Committee, Protocol #760181-5.

2.2. Study design - year 1

On July 24, when all reindeer bulls were still in velvet antler, animals were divided into 2 groups (n = 4) balanced for age and allocated to one of two separate pens. One group (MPA) received medroxyprogesterone acetate (400 mg im; Rood and Riddle Veterinary Pharmacy, Lexington, KY). The other group served as untreated controls (CTL). The groups remained separate until mid-winter (January). Although it was impossible to block all sensory contact between pens (sight, sound, smell), the two groups were never close enough to have fence line contact.

2.2.1. Body weight and feed consumption

Body weights were recorded on July 27 (initial treatment) and again on October 19 to 20. Semen was collected following the second weighing, after peak rut and breeding. Feed intake was measured on a per group basis and was monitored daily starting with the initiation of MPA treatment and continued until October 16. Each morning the pelleted ration remaining in the single communal pen feeder was removed, weighed and replaced with a measured quantity of feed (consumption plus approximately 10% additional feed). Daily group consumption was recorded for both pens.

2.2.2. Rut related aggressive behavior

Observations of bull behavior were made three times a week at feeding (10 a.m.) from initial treatment to harem and breeding then weekly until semen collection. Rut associated and aggressive behaviors within each pen were scored on a 0 to 5 point scale (Table 1). These observations were intended to identify the presence or absence of specifically defined rut displays. Feeding time was chosen because the presence of handlers often provokes behavioral displays in rutting bulls.

2.2.3. Harems and breeding

On Sept 2, estrus was synchronized in breeding age female reindeer (n = 12) using progesterone-impregnated controlled internal drug release devices (Eazi-Breed™ CIDR® Sheep Insert; Zoetis Animal Health, Troy Hills, NJ). The CIDRs were placed intravaginally for 1 wk and at the time of CIDR removal females were treated with Prostaglandin $F_{2\alpha}$ (Lutalyse, 15 mg im; Zoetis Animal Health, Troy Hills, NJ). Every effort was made to balance the harems for age and weight. In Year 1 the mean body weight (±SD) for the CTL group was 122 ± 7.4 kg with a mean age of 4.0 years; range 2–6 years and for the MPA group 130 ± 9.2 kg with a mean age 5.1 years; range 3–8 years. Harems were randomly assigned to the 4 year old bull from either the MPA or the CTL group (both males were experienced breeders). Harems remained together for

Table 1
Criteria used to rank rut related activity going from least (0) to most aggressive (5).

Rank	Description
0	No Rut - Velvet antlers - eat and sleep as a group – no aggression directed towards pen mates or people at feeding
1	Antler cleaning –occurs abruptly; actively scrapping velvet off; completed in 24–48 h
2	Displacement at feeder becomes obvious – dominance ranking evident - still interested in food - threatening motion with antlers when people approach but does not charge
3	Interest in food declining – guarding feeder, sniffing/licking/yawning at urine from subordinates – sporadic grunting, yearling separates from more dominant bulls – still feeding
4	Dominant bull starts to control pen - grunting continuously/guards feeder/urinating on legs/distinct odor/herds other males around – clear pecking order
5	Full Rut - Males all in separate areas of the pen – dominant bull controls feeder/constant grunting/urinating/controls fence line closest to females – attacks water trough & feeders/displays and charges people/focused on females – does not eat and prevents pen mates from eating.

1 wk (September 10 to 17) after which the bulls were returned to their respective groups and females were placed together in a large pen separate from males for the remainder of the breeding season and winter. Live calf production was recorded for each harem and every calf was tagged and weighed within a day of birth. No twins were born.

Based on past breeding records using this technique of estrus synchronized females with a 1 wk period of bull exposure (harem), the CTL bull was expected to successfully breed >50% of his harem. The MPA bull was not expected to breed any females. A single pregnancy in the MPA harem was considered a positive result.

2.2.4. Semen collection

On Oct 19–21, the three older bulls (2–4 years old) from each group were anesthetized. Rutting reindeer are very sensitive to commonly used anesthetic agents [10]. Therefore, anesthetic dose was determined individually after assessing the animal's body condition and intensity of rut behavior. Details of immobilizations are provided in supplementary materials. Briefly, bulls were administered a mixture of ketamine (Ketathesia, 100 mg/mL, Henry Schein Animal Health, Dublin OH) and xylazine (X-Ject E, 100 mg/mL, Henry Schein Animal Health, Dublin OH) while physically restrained in a hydraulic chute following which they were released into an adjacent pen for induction. All reindeer were monitored (temperature, pulse and respiration, as well as oxygen saturation) and administered supplemental oxygen at a rate of 1.5–2 L/min via intranasal tube.

Once immobilized, semen was collected using an electroejaculator: Briefly, the anesthetized animals were maintained in lateral recumbency. Feces were evacuated from the rectum, the prepuce flushed with saline (to reduce sample contamination) and the accessory sex glands massaged briefly with the probe. A Lane Pulsator IV electroejaculator with a ram rectal probe (Lane Manufacturing Inc, Denver, CO), was used. Electrical stimulation was applied in pulses beginning with 3 s on, 3 s off at low voltage (±1 V) gradually building up to 2.5 V over approximately 5 min. This was programmed into the machine and voltage changes occurred automatically. The procedure was repeated 10 min later and following collection of the second sample the anesthetic was reversed with Tolazoline 250–300 mg (1.5 ± 0.3 mg/kg) (Tolazine, 100 mg/mL, Akorn, Inc., Lake Forest, IL) and the animal released.

Semen was collected into a pre-warmed (approximately 37 °C) artificial vagina fitted with a collection cone at one end, designed for semen collection of rams and domestic deer. None of the bulls achieved a full erection making a clean collection difficult.

2.2.5. Semen evaluation

Semen was evaluated immediately following collection using a modified breeding soundness evaluation (BSE) that included measures of semen volume, sperm motility, morphology, and concentration. Briefly, 10 μL of neat semen was evaluated on a pre-warmed (37 °C) microscope slide for gross motility (×100 magnification). Ejaculate contamination was estimated based on the amount of debris in the bottom of the collection cone and whether white blood cells were present in the ejaculate as determined by microscopy (×400 magnification). A 1:40 dilution of semen in Sperm-TL buffer (Item #IVL03, Caisson Labs, Logan, UT) was assessed (×400 magnification) for percentage and strength of progressive motility by a single technician. Sperm concentration was determined using a 1:100 dilution of semen in phosphate buffered saline using an AccuRead Photometer (model #019956, IMV Technologies, Maple Grove, MN). An aliquot of each ejaculate was stained using Morphology Stain (Item 23-4, Lane Manufacturing Inc, Denver, CO) and used to determine semen morphology and viability by counting 100 sperm for each measure (X 1000 magnification). When ejaculate volume was sufficient, 0.5 mL semen was washed in Sperm-TL buffer (1:20 dilution) and fixed in 2.0% formaldehyde. Additional measures of sperm fertility, using specific biomarkers for the assessment of sperm acrosome integrity, cell membrane integrity, DNA integrity, and mitochondrial energy potential were conducted on fixed sperm using the Guava easyCyte HT (IMV Technologies, Maple Grove, MN) flow cytometer and protocols according to manufacturer's directions.

2.3. Study design - year 2

To evaluate breeding competence following MPA treatment, individual bulls from CTL and MPA groups were switched and every effort was made to maintain identical protocols in both years. However, some changes were unavoidable. The 4 year old bull from the original CTL group died (traumatic injury unrelated to the study) prior to the onset of the study in Year 2. To maintain age balance within groups the 4 year counterpart bull was removed from the study and yearling bulls were added to both groups maintaining the 1–4 year old age structure. It is important to note that only bulls aged 2, 3 and 4 years old in Year 2 were actually switched between treated and control. All other aspects of the protocol, metrics, and data collection were the same with the following exceptions: In Year 2 both harems increased to nine females with a mean weight of 119.8 ± 6.4 kg and mean age 3.5 years (range: 1–7 years) (CTL) and 115.5 ± 12.7 kg with a mean age of 3.8 years (range: 1–8 years) (MPA). The MPA bull placed in harem in Year 2 was not an experienced breeder, although he had displayed full aggressive rutting behavior the previous year. The control harem bull was an experienced breeder. In Year 2, semen was collected from anesthetized reindeer bulls that were suspended upright in a harness (Munks Mfg. Inc., Anacortes, WA) on September 26 to 28, 10 d following harem break-up and 30 d earlier than the previous year. The semen collection protocol and evaluation were identical except that recovered semen was extended in Triladyl extender containing egg yolk (1:1:3 Triladyl:egg yolk:dH_20 by volume) at a concentration of 1×10^6 sperm/mL at 20 °C. Extended semen was placed in 0.5 mL straws, cooled to 4 °C for 2–6 h and frozen in liquid nitrogen vapor by suspending straws approximately 2 cm above liquid nitrogen for at least 10 min before plunging into liquid nitrogen. Flow cytometric analyses of sperm from Year 2 were conducted on frozen/thawed sperm using the same procedures as Year 1. In Year 2, all eight bulls (CTL and MPA) received MPA (400 mg im) following semen collection to improve care of the animals and decrease the hypophagia observed in Year1.

2.4. Analyses and statistics

Mean body weight loss was compared between CTL and MPA groups (*t*-test and paired *t*-test) and analysis of variance, a general linear model was used to analyze group feed consumption with week and treatment as factors and the Holm-Sidak test for post hoc comparisons between MPA-treated and CTL groups (Systat Software – Sigma Plot 11). Because of timing and protocol differences between Year 1 and Year 2, years were treated separately unless otherwise indicated. Semen characteristics were analyzed using the mixed model in SAS (Version 9.4) including year, bull, and treatment as independent variables. In all cases $P \leq 0.05$ was considered significant and $P \leq 0.10$ was considered indicative of a trend.

3. Results

3.1. Body weight

In both years of the study, CTL bulls lost more weight than MPA-treated bulls over the period of rut ($P < 0.05$; Fig. 1). The loss was greatest in older bulls while weight loss in yearlings ranged between 10 and 20 kg. Among the MPA-treated bulls weight change was more variable. Yearling and subordinate bulls gained 10–20 kg while the older, MPA-treated males still lost weight (2–26 kg loss). The magnitude of this weight loss was variable and less ($P \leq 0.002$) than weight loss when the same bull was in the CTL group (Fig. 2).

3.2. Feed consumption

Initially feed consumption in the CTL group exceeded ($P < 0.001$) that of the MPA group for the first 3 wk after which feed consumption among CTL bulls declined sharply, approximately 2 wk after the dominant bull cleaned his antlers and was lower ($P \leq 0.001$) than MPA–treated bulls for the remainder of the trial

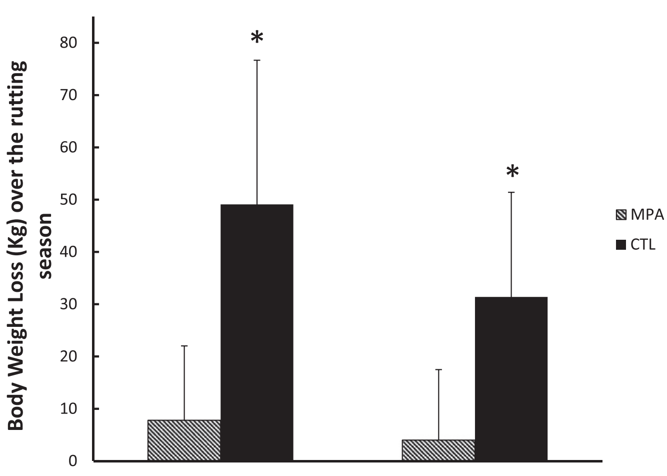

Fig. 1. Mean (±SD) body weight loss between pre-rut (Jul 27) and post-harem (Oct 19, Year 1; Sept 26, Year 2) in reindeer bulls receiving no treatment (CTL) or medroxyprogesterone acetate (MPA, 400 mg im) in a two year study. The ∗ indicates differences ($p \leq 0.05$).

with the exception of harem exposure for one week (Fig. 3). The pattern was repeated in Year 2 with feed consumption among CTL bulls lower ($P \leq 0.001$) than MPA-treated bulls by week 4 where it remained until week 9 (10 d post-harem) when the CTL bulls were treated with MPA. Control bulls began eating within days of this treatment and were consuming an amount of feed equal to MPA bulls within 2 wk (Fig. 3). Among MPA bulls, a seasonal decline in feed consumption was evident; however, this was a gradual decline and always significantly exceeded feed consumption relative to CTL bulls. The MPA group in both years continued to graze and eat pellets throughout the study.

3.3. Rut related behavior

Rut behavior intensified quickly in the CTL group of bulls following antler cleaning (Fig. 4). Alternatively, the MPA bulls remained together sharing the feeder and bedding down in close proximity without any evidence of rut related aggression. Prior to harem formation, they were never observed challenging people, grunting, urinating on their hind legs or displaying overt dominance behaviors among each other. Dominance was inferred on the basis of weight and age in the selection of a harem bull. After harem formation in Year 1 there were subtle signs of dominance and aggression (mostly chasing and grunting) (Fig. 4).

3.4. Antler cleaning and casting

Cleaning of velvet antlers among CTL bulls occurred in early August (Fig. 4). In both years all the CTL bulls actively cleaned their antlers over 24–48 h, usually beginning with the dominant, older males. Antler cleaning spanned August 10 to 21 (Year 1) with the yearling bull cleaning 1 wk after the end of antler cleaning by older bulls and August 1 to 13 (Year 2) with the 2 year old bull the last to clean. The MPA bulls were never observed actively scrapping or cleaning their antlers. By the end of August (both years) the velvet appeared very dead and boney antler was visible among older MPA bulls (Fig. 5). The first evidence of any antler cleaning among MPA bulls in Year 1 was observed among the 3 older bulls following harem break up (September 10 to 17). In Year 2, a similar pattern of antler cleaning was observed. However, this was a very wet summer and the dead velvet on one MPA bull became gangrenous and had to be manually stripped from a large part of the antler. Among MPA bulls, antler cleaning was a passive, protracted event spanning months making it difficult to pinpoint a cleaning date.

Antler casting in CTL and MPA bulls (Year 1) started in early January (older dominant bulls) and continued through March (young/subordinate bulls) which is the normal casting time frame for this herd. In Year 2, MPA bulls cast in late January ($n = 1$) and March ($n = 3$) while the CTL bulls ($n = 3$) started casting antlers, approximately 3 wk post-MPA treatment at the end of October. The youngest CTL bull retained antlers until early February.

3.5. Semen evaluation and fertility

Semen collection in Year 2 from bulls that were suspended by a harness was much easier than Year 1 when bulls were in the recumbent position. Table 2 presents semen and sperm measurements common to a BSE for bulls in both groups and years. There was no treatment by year effects, so Table 2 does not include interactions. Sperm concentration and total sperm in ejaculate were lower in MPA-treated bulls ($P < 0.05$). The MPA-treated bulls tended ($P = 0.07$) to produce more sperm with knobbed acrosomes than CTL bulls, but otherwise had similar ($P > 0.10$) percentage of normal sperm morphology. Ejaculates from bulls in Year 2 tended to have more ($P = 0.08$) bowed midpiece and more ($P = 0.10$) bent

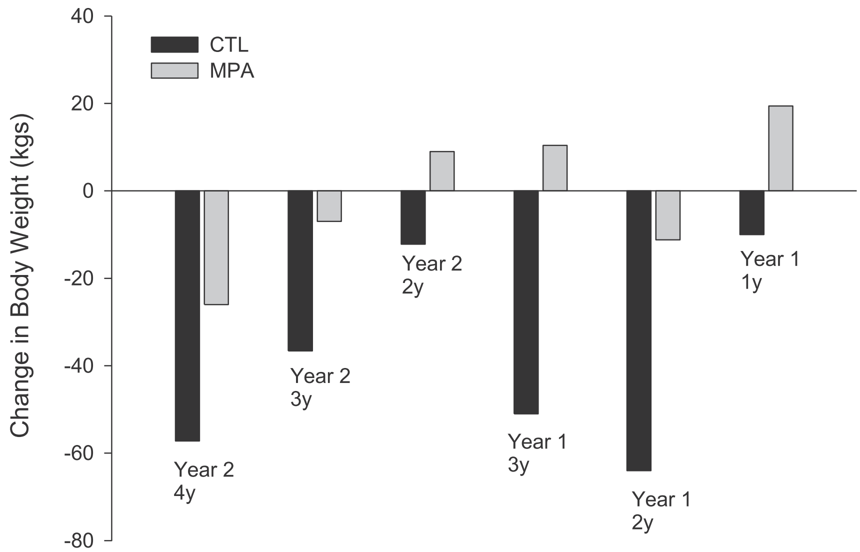

Fig. 2. Body weight change from pre-rut (July 27) to post-harem (Sept/Oct) in six reindeer bulls switched between CTL and MPA treatments in consecutive years. The label over the bar indicates the year and age of the bull when he was in the control group. Weight loss differed ($p = 0.002$) between MPA and CTL treatments for all bulls.

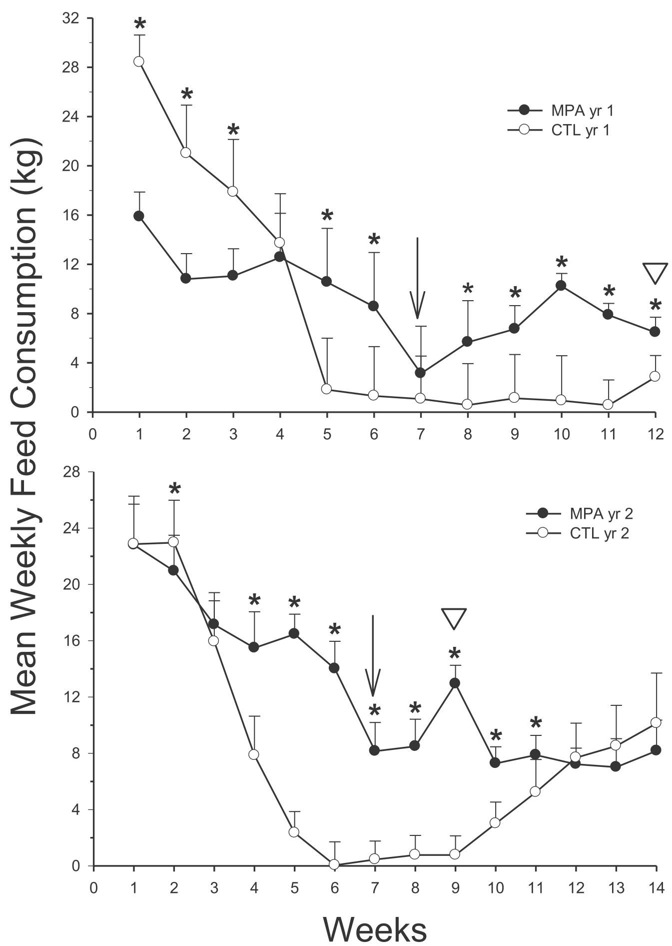

Fig. 3. Mean (±SD) weekly feed consumption in control (CTL) and medroxyprogesterone acetate (MPA 400 mg, im) groups over the period of rut, July 27- Oct 16 (Year 1) and July 27- Oct 31 (Year 2). The long arrow indicates the week the dominant bull from each pen was put in harem. The open arrow head indicates the week of semen collection and, in Year 2 only, the second administration of MPA (400 mg im) to all eight bulls. In Year 2, feed consumption was monitored for an additional 2 wk to capture changes in feed consumption among the CTL animals. The ∗ indicates differences ($p \leq 0.001$) between MPA and CTL pens.

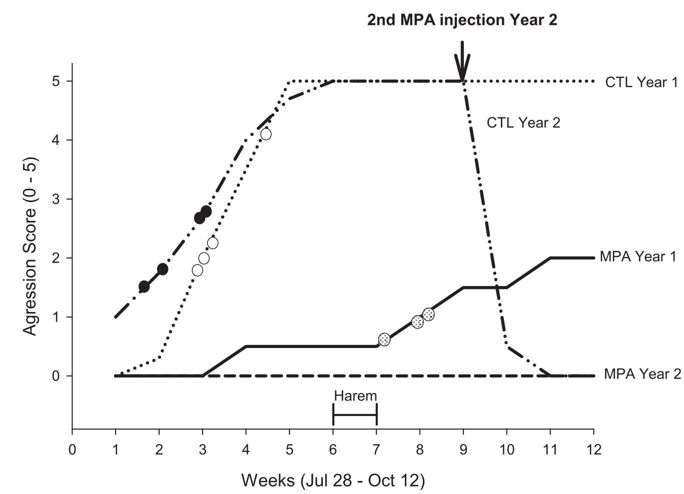

Fig. 4. Mean weekly aggression score (0 = no aggression, 5 = full rut aggression; Table 1) for reindeer bulls that received no treatment (CTL) or medroxyprogesterone acetate (MPA; 400 mg, i. m.) administration on July 27 each year as part of a 2-y study. All bulls received MPA after semen collection on Sept 26–28 of Year 2 (arrow). The circles indicate the time of antler cleaning (rarely apparent in the MPA treated bulls).

principal piece abnormalities than those from Year 1.

Only 2 MPA-treated bulls in year 1 and 1 MPA-treated bull in year 2 compared to 3 CTL bulls each year produced sufficient semen for fixation or cryopreservation to allow flow cytometry analyses. Even the harem bull in Year 2 produced insufficient sperm for freezing or fixing for analysis. There were no differences ($P > 0.15$) in percent live sperm with intact acrosome, live, 3 h post-thaw percent live (Year 2), mitochondrial energy potential, DNA fragmentation, immature sperm, or antioxidant capacity (reactive oxygen species) between treatments or year by treatment (Table 3). Ejaculates obtained in Year 2 had greater ($P = 0.05$) mitochondrial energy potential and percent immature sperm than Year 1 (Table 3).

In Year 1 the MPA bull produced four calves in a harem of six cows compared to five calves from a harem of six cows for the CTL bull. In Year 2, the inexperienced MPA bull produced no calves in a harem of nine cows. The CTL bull in Year 2, an MPA-treated bull

Fig. 5. Appearance of antler velvet in a CTL reindeer bull on Aug 10 (A) and Aug 13 (B) and in a reindeer bull, treated with medroxyprogesterone acetate (MPA 400 mg, im), on Aug 10 (C) and the same bull Aug 31 (D).

Table 2
Breeding soundness evaluation measures on fresh semen for reindeer bulls that received no treatment (CTL) or medroxyprogesterone acetate (MPA; 400 mg, im) administration approximately 3 months (Year 1) or 2 months (Year 2) before semen collection using an electroejaculator. A treatment × year interaction was not detected for any of the variables and therefore has not been included in the table.

Measurement	CTL	MPA	P value
Number of bulls that produced ejaculates	7	7	
Ejaculate volume (mL)	1.0 ± 0.2	0.6 ± 0.2	0.15
Gross motility (Score 0–5; 0 = No swirls, 5 = Rapid swirls)	2.2 ± 0.4	1.2 ± 0.6	0.21
Sperm strength motility rating (Score 0–5)	2.7 ± 0.5	1.7 ± 0.5	0.14
Progressive motility (%)	45.0 ± 11.6	28.5 ± 11.6	0.34
Live (%)	59.3 ± 8.0	62.1 ± 8.0	0.81
Sperm concentration x 10^6	621.4 ± 0.9	220.8 ± 1.0	0.02
Normal morphology (%)	33.6 ± 5.1	35.5 ± 5.1	0.80
Knobbed acrosome	0.8 ± 0.7	2.7 ± 0.7	0.07
Head defects	18.2 ± 2.8	17.1 ± 2.8	0.78
Detached heads	25.7 ± 4.1	19.8 ± 4.1	0.34
Distal midpiece reflex (DMR)	6.0 ± 1.7	6.7 ± 1.7	0.79
DAG defect	5.5 ± 1.4	4.6 ± 1.4	0.69
Bowed midpiece	0.6 ± 0.3	0.4 ± 0.3	0.57
Proximal droplet	5.9 ± 3.6	9.4 ± 3.6	0.51
Distal droplet	1.4 ± 0.7	1.3 ± 0.7	0.94
Coiled principal piece	1.5 ± 0.4	0.7 ± 0.4	0.19
Bent principal piece	1.2 ± 0.7	3.0 ± 0.7	0.11

from the previous year, sired nine calves from nine cows.

4. Discussion

This study evaluated a single application (400 mg) of MPA before onset of the rut and breeding in reindeer bulls. The single dose, which ranged from 1.5 mg/kg in the largest bull to 3.3 mg/kg in the smallest bull, was effective in all individuals regardless of age and weight. It eliminated rut-associated hypophagia, rut-related behaviors and interfered with the final cleaning of velvet antlers. Only when administered to CTL bulls at the height of the rut, did it precipitate antler casting. By the next breeding season, previously treated bulls exhibited full rut expression and normal fertility. Even though treated bulls behaved like castrates, fertility was not abolished by this treatment.

The hypothesis underlying the use of progestins is based on the negative feedback of progestins blocking the release of gonadotropins, specifically LH [5,11]. The subsequent loss of LH stimulation is expected to suppress testosterone production in the testes [11], impacting aggression, libido, sperm production and, ultimately, fertility. We did not measure peripheral testosterone levels in this study. However, previous work on male reindeer at UAF has documented peak testosterone levels in August that remain high through mid-September with the most aggressive, breeding age bulls exhibiting the highest systemic testosterone concentrations [9,12] similar to semi-domestic reindeer in Norway [13]. Rut related aggression and hypophagia are correlated with seasonally increasing levels of circulating testosterone in most deer species [14,15]. The body weight loss in CTL reindeer was dramatic and consistent with the almost total cessation of feeding in the CTL

Table 3
Flow cytometer measures of sperm collected from reindeer bulls approximately 3 mo (Year 1) or 2 mo (Year 2) after no treatment (CTL) or medroxyprogesterone acetate (MPA; 400 mg, i.m.) administration.

Flow Cytometer Measurement		P value	LSM ± SEM			
			Treatment (Trt)		Year[a]	
			CTL	MPA	Year 1	Year 2
Live sperm with intact acrosome, %	Trt	0.69	20.1 ± 3.6	22.9 ± 5.4		
	Year	0.12			15.5 ± 4.0	27.5 ± 5.1
	Year x Trt	0.78				
Live sperm, %[b]	Trt	0.67	25.5 ± 3.7	28.6 ± 5.6		
	Year	0.54			24.9 ± 4.2	29.2 ± 5.3
	Year x Trt	0.87				
Sperm with polarized mitochondria, %	Trt	0.32	15.1 ± 4.1	23.2 ± 6.1		
	Year	0.05			9.4 ± 4.5	28.8 ± 5.7
	Year x Trt	0.46				
Sperm with DNA damage, %	Trt	0.86	10.3 ± 4.1	8.9 ± 6.1		
	Year	0.73			10.9 ± 4.5	8.2 ± 5.7
	Year x Trt	0.63				
Immature sperm, %	Trt	0.35	2.3 ± 0.3	1.7 ± 0.4		
	Year	0.05			1.3 ± 0.3	2.6 ± 0.4
	Year x Trt	0.15				
Sperm with strong antioxidant capacity, %	Trt	0.42	4.1 ± 1.3	6.7 ± 2.2		Year 2 only
Live sperm 3 h post-thaw	Trt	0.85	18.5 ± 3.7	20.2 ± 6.4		Year 2 only

[a] Year 1 samples were fixed and Year 2 samples were frozen/thawed.
[b] Percent live sperm in fixed sample (Year 1) or 0 h post-thaw (Year 2).

group. As aggression in the dominant male increased, he tended to control the feeder, blocking access by subordinate individuals, negatively impacting both feed consumption and body weight among subordinates regardless of their hormonal status. This is consistent with earlier studies demonstrating that reindeer in all age classes lose as much as 36% of body weight during the rut [9].

Suttie et al. [11] administered 150 mg MPA to red deer and abolished the LH and testosterone response to exogenous GnRH within 2 wk. This treatment also resulted in antler casting 3 wk after injection [11]. In a similar manner, treating our CTL reindeer males in Year 2 with MPA 1 wk post-harem had the immediate effect of increasing feed consumption and eliminating rut behaviors. These treated reindeer bulls also cast their antlers approximately 3 wk post-treatment which is consistent with the premise that a sudden loss of testosterone triggers the osteoclast response leading to antler casting [16]. This is temporally similar to antler casting following the experimental application of a testosterone blocker i.e. cyproterone acetate [11,17]. It should be noted that reindeer bulls with newly cleaned antlers did not cast in response to a similar dose of MPA administered in early August (unpublished observations) suggesting that timing of treatment relative to stage of the antler cycle is an important variable contributing to the hormonal control of antlers.

The MPA reindeer behaved generally like castrates. They remained in a bachelor group, continued to eat through the rutting season and failed to display any rut-associated behaviors, at least until after harem formation in Year 1 and not at all in Year 2. The older, larger males in the MPA group still lost weight, again reflected in the slightly decreased feed intake that occurred in late summer/early fall in both years. This represents the seasonal shift in metabolism reported in reindeer of both sexes [18] and is consistent with studies in castrated reindeer that demonstrate a seasonal decline in food intake [19]. The younger bulls in the MPA group gained weight. This was not surprising, considering there was no aggressive impediment blocking access to feed. It also demonstrates that young, growing reindeer can continue to gain weight in the fall and winter with full access to high quality food.

The effect of MPA on antler cleaning and retention was variable. Within this study, the reindeer were treated with MPA while antlers were still in velvet, thus enabling us to use antler cleaning as a metric. Velvet antlers grow when circulating testosterone is low (undetectable). In red deer, the testes are unresponsive to LH stimulation during the period of velvet antler growth [11]. Alternatively, the final ossification and cleaning of antlers is considered a testosterone mediated event [11,20,21]. The MPA-treated reindeer retained velvet antlers, with observable antler growth in some individuals through August, again supporting the inference that testosterone mediated actions were blocked by treatment. The sudden antler cleaning observed in the older 'dominant' MPA bull when he was put in contact with the harem (mid-September) was paralleled by antler cleaning in 2 of the 3 remaining MPA bulls penned directly across a lane from the harem. It is possible that MPA effects were waning by > 30 d post-treatment, although a single administration of MPA (150 mg) to red deer stags suppressed both LH and testosterone response to GnRH challenge >65 d post-treatment [11]. Similarly in the gerenuk study, serum testosterone levels increased gradually over a four month period to reach pre-treatment concentrations [4]. Alternatively, the sudden proximity of estrous females may have precipitated a strong behavioral response, potentially overriding a declining MPA block. In pampas deer, proximity of bulls to females before rut increased testosterone and improved semen characteristics [22]. In general, the protracted, late antler cleaning among MPA animals suggests that the effects of treatment recede slowly. This prolonged pattern of antler cleaning is also consistent with antler cleaning in castrated reindeer. Unlike most deer species, castrated reindeer will clean and, usually, cast their antlers but in a pattern reminiscent of younger, subordinate males [20]. Antler casting among MPA bulls was typical of subordinate and young bulls.

While MPA treatment abolished all overt rut related behaviors, it had little effect on fertility as indicated by similar levels of normal sperm in the collected semen samples from CTL and MPA bulls as well as the breeding success of the MPA bull in Year 1. Although sperm concentration and total sperm in the ejaculate were decreased in MPA compared to CTL bulls, apparently sufficient sperm existed to maintain bull fertility, provided they mated estrous females.

Semen collection in this study, done under anesthesia, resulted in variable levels of contamination and sample volume. Although natural semen collection (using an artificial vagina) is reported to produce a better quality sample, cervid electroejaculation under anesthesia is a common practice in Red Deer [23], Iberian Red Deer [24], Tufted Deer [25], White-tailed Deer [26,27], Timor Deer [28], Pampas Deer [29], Roe Deer [30]. In the few existing reports of semen collection in reindeer, use of natural service and an artificial vagina have been the preferred collection method [31,32], a technique dependent on the availability of well-trained reindeer bulls [32]. In a more recent report, electroejaculation in physically restrained, standing reindeer was considered preferable to the use of anesthetics [33] although few details were provided. In this study, semen collection was improved the second year by maintaining the anesthetized bulls in sternal recumbency with the hind quarters elevated. Penile extrusion was difficult. Reindeer bulls lack a sigmoid flexure and penile extrusion is normally facilitated by the caudal retraction of the epithelial folds lining the prepuce [31]. Without full erection however, the prepuce, even with flushing and washing, was a major source of contamination.

Ejaculate volume and sperm concentration can vary greatly when electroejaculation is used, however both MPA and CTL bulls were collected in the same manner by the same technicians, so differences in these two measures are most likely a function of treatment. In this study, low semen volume is similar to previous reports [31–34], regardless of collection method, and may well be a characteristic of reindeer. It has been suggested that this represents conservation in reproductive effort [31] supported by the fact that copulation in reindeer is very brief (2–5 s) [35], repeat mounting of the same female uncommon [31,35] and rut-associated hypophagia a constant energy drain [9,10,31].

Sperm concentration of CTL bulls, percent progressive motility and percent normal spermatozoa were within the range of previous reports and were similar to the frozen/thawed semen used in an earlier trial that resulted in 1 of 7 female reindeer conceiving using artificial insemination [36]. The percentage of normal spermatozoa in the current study (30–40%) is lower than the approximate 60–80% normal spermatozoa in a Russian study [33] and may reflect differences in seasonal timing of collection or collection with or without anesthesia. In this study, semen was collected later in the season, as opposed to the height of the rutting/breeding season. Semen collection 1 mo closer to peak rut in Year 2 likely played a role in the increase percentage of sperm with polarized (energized) mitochondria.

In the sperm morphological analysis, detached heads were the most common abnormality (range: 12–22%) occurring in all reindeer regardless of treatment. Dott and Utsi [32] identified over 70% detached heads in some reindeer and suggested elevated temperature exposure of the testes two to three weeks prior to collection as a possible cause. This seems unlikely in the present study where the higher prevalence of this defect was observed in both years and across treatments. The similarities in semen parameters between treatments were surprising given the dramatic effect of MPA on bull behavior in general. Large variation between ejaculate volume, semen and sperm quality traits were likely affected by the small sample size.

The fact that an MPA-treated bull successfully bred four of six females in Year 1, approximately 45 d post-treatment, is consistent with the relatively normal semen profile. It also suggests that the dose and timing of the single application were not sufficient to completely block spermatogenesis. In Year 2 however, the MPA bull showed no interest in estrous females and produced no offspring. Most of the measures related to his BSE were above the mean for the MPA group and should not have impaired fertility, although ejaculate volume was below the mean and insufficient to allow characterization of the traits measured by flow cytometry. A major and likely contributing difference, for the MPA harem bull in Year 2 was the bull's lack of breeding experience, which may have contributed to suppression of libido. It would be interesting to look at the role of breeding experience among MPA treated bulls.

The complete abolition of rut related behavior in reindeer bulls coupled with a relatively normal semen profile and successful mating was unexpected. This presents an apparent contradiction to our understanding of the role of testosterone in antler development, spermatogenesis and rut behavior in Rangifer. As previously stated, hypophagia, antler velvet shedding and a suite of rut related behaviors are normally correlated with increasing testosterone levels in a wide variety of deer species, including reindeer [20] all of which were suppressed in reindeer bulls by a single injection of MPA. The one exception was spermatogenesis. At this point we can only speculate on underlying mechanisms.

Limited histological information on the onset of seasonal spermatogenesis in reindeer indicates testicular reactivation in early spring with clear evidence of complete spermatogenesis by the time of antler cleaning, prior to the onset of rut [37,38]. It is probable that spermatogenesis was well underway at the time of MPA treatment in this study. Low but steadily increasing levels of testosterone, reported in a number of deer species [15,39] including reindeer [12,38], can be detected 6–8 wk before antler cleaning. The ability of androgen-binding protein to concentrate and maintain intratesticular testosterone (or DHT) at levels necessary for continued spermatogenesis [40] is most likely occurring throughout the latter stages of the velvet antler period. Nonetheless, the relatively normal semen profile up to 60 days post-treatment was surprising and raises questions regarding the role of testosterone and rutting behavior in reindeer.

The relationship between systemic testosterone levels and aggression in ungulates is complex. In a study using both MPA and MGA (melengestrol acetate, an orally active synthetic progestin) testosterone levels were significantly suppressed in four gerenuk but aggressive behavior remained unaffected [4]. Conversely, MGA treated fallow bucks, had increased testosterone levels but decreased aggressiveness [41], while in the fringe-eared oryx, both testosterone and aggression were significantly reduced by MGA treatment [2]. The effect of progestins varies with species and dose as well as route and timing of administration [42]. There is also the confounding variable that MPA can have behavioral effects not mediated through the direct suppression of androgens [43–46]. Pharmacological doses of MPA can inhibit the uptake of androgens by target tissues or inhibit the reduction of testosterone to DHT (and other androgen metabolites) or aromatization to estradiol [42]. An enhanced binding affinity of MPA for androgen, estrogen and glucocorticoid receptors has also been reported [44,46].

We did not measure systemic testosterone levels and therefore do not know if MPA is blocking testosterone production or the effects of testosterone on target tissues. Understanding the physiological mechanisms underlying suppression of rut related behavior in reindeer must await further investigation.

Authors' contributions

JR, TG, JB, MS participated in designing the study and collecting the samples. JR, TG and AZ analyzed the data. JR prepared the manuscript and all authors were involved in manuscript revisions.

Conflicts of interest

None.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.theriogenology.2019.08.029.

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