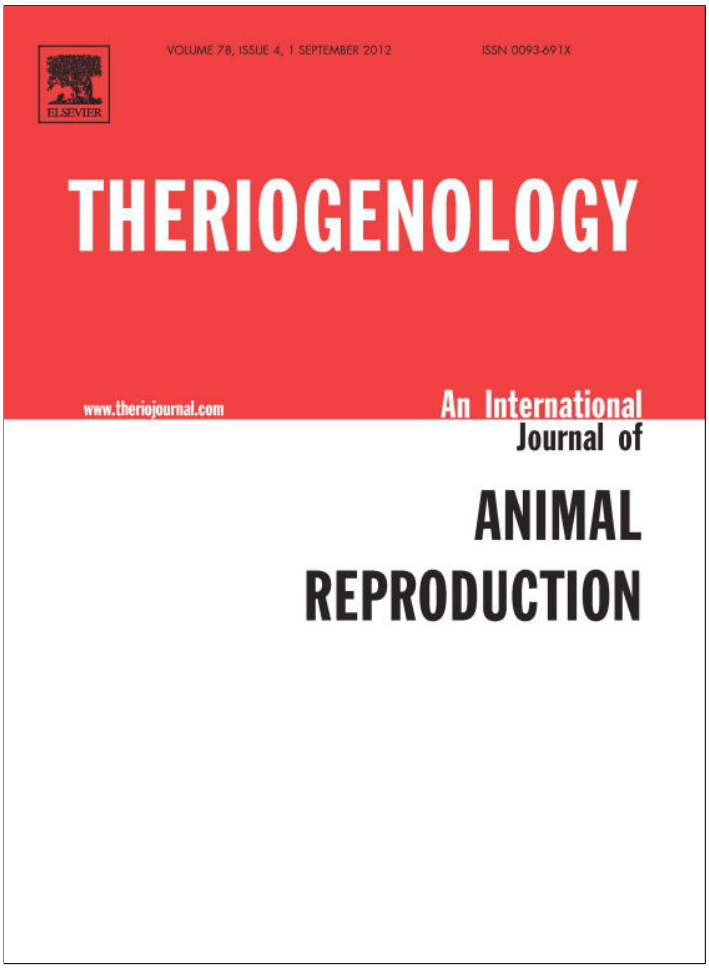

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Passive transfer of maternal GnRH antibodies does not affect reproductive development in elk (*Cervus elaphus nelsoni*) calves

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Abstract

Gonadotropin-releasing hormone is intermittently released from the hypothalamus in consistent patterns from before birth to final maturation of the hypothalamic-pituitary-gonadal axis at puberty. Disruption of this signaling via GnRH vaccination during the neonatal period can alter reproduction at maturity. The objective of this study was to investigate the long-term effects of GnRH-antibody exposure on reproductive maturation and function in elk calves passively exposed to high concentrations of GnRH antibodies immediately after birth. Fifteen elk calves (eight males and seven females) born to females treated with GnRH vaccine or sham vaccine during midgestation were divided into two groups based on the concentration of serum GnRH antibodies measured during the neonatal period. Those with robust (>15 pmol ^{125}I-GnRH bound per mL of serum) titers (N = 10; four females and six males) were designated as the exposed group, whereas those with undetectable titers (N = 5; three females and two males) were the unexposed group. Onset of puberty, reproductive development, and endocrine function in antibody-exposed and unexposed male and female elk calves were compared. Neonatal exposure to high concentrations of GnRH antibodies had no effect on body weight ($P = 0.968$), endocrine profiles ($P > 0.05$), or gametogenesis in either sex. Likewise, there were no differences between groups in gross or histologic structure of the hypothalamus, pituitary, testes, or ovaries. Pituitary stimulation with a GnRH analog before the second potential reproductive season induced substantial LH secretion in all experimental elk. All females became pregnant during their second reproductive season and all males exhibited similar mature secondary sexual characteristics. There were no differences between exposure groups in hypothalamic GnRH content ($P = 0.979$), pituitary gonadotropin content ($P > 0.05$) or gonadal structure. We concluded that suppressing GnRH signaling through immunoneutralization during the neonatal period likely does not alter long-term reproductive function in this species.
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Keywords: Elk; Fertility control; GnRH vaccine; Gonadotropin; Hypothalamic-pituitary-gonadal axis; Wildlife

1. Introduction

Episodic secretion of GnRH from the hypothalamus and signaling at gonadotropic cells of the anterior pituitary gland is required for normal steroidogenesis and gametogenesis in reproductively mature mammals [1]. Increases in pulse frequency mark the shift from reproductive quiescence to activity during puberty and tran-

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sition from anestrus to breeding condition in seasonal breeders [2]. The hypothalamic-pituitary-gonadal (HPG) axis is functional and active during the neonatal period in domestic ruminants [3,4], but is not fully mature until the prepubertal period [5–8]. There is a biphasic increase in gonadotropins during prepubertal maturation in female sheep and cattle [9]. Similar to females, prepubertal males have a critical period of HPG axis maturation during the neonatal period [10,11]. Attainment of reproductive maturity can be hastened by administration of GnRH during early postnatal life [12]. Alternatively, puberty can be delayed by inhibiting GnRH secretion during this early period of development [13,14]. Gonadotropin-releasing hormone signaling during the postnatal/prepubertal time period is important for final maturation of the HPG axis in domestic ruminants.

Vaccination against GnRH is one method of functionally removing the hormone and its effects in vivo. The effects of GnRH exclusion on reproductive system function have been investigated using active and passive GnRH vaccination in mature [15,16] and juvenile [17,18] ruminants. In adults, reproductive function generally returns as antibody titers wane [19,20]. In contrast, immunization of neonatal or prepubertal animals can have a prolonged effect on reproductive function, despite progressive decline in GnRH-antibodies [21–25]. In fact, Brown and colleagues [22,23] reported that active vaccination against GnRH during the neonatal period caused permanent suppression of reproductive function in a subset of mature domestic sheep of both sexes, despite a lack of measurable GnRH antibodies after the prepubertal period. Few studies have investigated the long-term (>1 yr) reproductive effects of GnRH vaccination during the neonatal or prepubertal period.

Gonadotropin-releasing hormone vaccination has been proposed as a method of wildlife contraception [26]. In a previous study, we actively vaccinated midgestation female elk to evaluate the safety, efficacy, and duration of a recently registered GnRH vaccine (GonaCon, National Wildlife Research Center, United States Department of Agriculture, Fort Collins, CO, USA) intended for use in wild ungulates as a contraceptive [27]. Calves born to these females experienced neonatal passive transfer of GnRH antibodies. Before management application of this vaccine, it is imperative to understand the first-generational effects of vaccination in pregnant females to prevent unintended population-level consequences [28].

The purpose of this study was to investigate the long-term reproductive effects of maternal passive transfer of GnRH antibodies to male and female elk calves after birth. Passive vaccination during the neonatal period may delay the onset of puberty and could permanently suppress function of the HPG axis, leading to decreased fertility during adulthood.

2. Materials and methods

2.1. Animals and experimental approach

This study was reviewed and approved by Colorado State University (#07–146A–01) and the Colorado Division of Wildlife (#09–2007) Institutional Animal Care and Use Committees. All animals were housed at the Colorado Division of Wildlife, Foothills Wildlife Research Facility in Fort Collins, CO, USA (40°352046“ N, 105°92029”W). This work builds upon a previous study investigating the effects of active vaccination against GnRH in mature female elk during midgestation [27]. The current study used calves born during the previous work. Fifteen elk calves (N = 8 male; N = 7 female) born to females immunized with GnRH vaccine or a sham vaccine (carrier and adjuvant but no GnRH) during midgestation (approximately 100 days of 255 ± 7 days gestational period [29]) were divided into two groups, based on the presence of serum GnRH antibodies measured during the neonatal period (1–30 days). Dam vaccination status was not necessarily indicative of calf GnRH antibody exposure status, due to nursing from multiple females early in the neonatal period. Calves with robust (>15 pmol ^{125}I-GnRH bound per mL of serum) neonatal titers (N = 10; six males and four females) were designated as the exposed group, whereas those with undetectable titers (N = 5; two males and three females) the unexposed group. Calves were dam-raised in mixed exposure groups until 3 to 4 mo of age when they were weaned (September 1, 2006). One male calf exposed to GnRH antibodies was <30 days at weaning and was euthanized due to welfare concerns. At weaning, calves were separated into same sex groups, maintained in fenced paddocks (approximately 5 hectares), and fed a diet of ad libitum alfalfa-grass hay mix, limited supplement, trace mineral blocks, and water. Before experiments, calves were trained to repeated handling, blood sampling, isolation pens, alleyways, and a handling chute. All biological samples, with the exception of semen collection which required complete immobilization, were collected while elk were standing and lightly sedated using xylazine hydrochloride (30–150 mg per animal im; TranquiVed; Vedco, Inc., St. Joseph, MO, USA) in a nonsqueeze handling chute. Tranquilizer effects were

reversed after each sampling session with either yohimbine hydrochloride (30 mg per animal iv; Wildlife Pharmaceuticals, Fort Collins, CO, USA) or tolazoline hydrochloride (600 mg/animal im; Tolazine; Akorn, Inc., Decatur, IL, USA). During the experiment (May 2007), one male calf in the GnRH antibody-exposed group died because of a venomous snake bite. The study was conducted from birth (May to August 2006) to approximately 3 yr of age (March and April 2009).

2.2. Antibodies and growth rates

To measure maternal antibody transfer to calves, blood was opportunistically collected from calves before first nursing (N = 2; one from each exposure group) and from every calf 24 h after birth, then at approximately 2-wk intervals for the first 2 mo and monthly until 6 mo. Calves were weighed (kg) at the same time points. Thereafter, similar measurements were made sporadically, but not less than once every 6 mo. Final antibody concentration measurements were made before the 2008 reproductive season (mid September to mid November). Blood samples (10 to 30 mL) were collected via jugular venipuncture using a 20-gauge blood collection needle, tube holder, and 10-mL blood tubes without anticoagulant (BD Vacutainer SST; Becton, Dickinson, and Co., Franklin Lakes, NJ, USA). Blood was allowed to clot at room temperature, centrifuged for 10 min at 1500 × *g*, and serum was decanted to polypropylene tubes and stored at −80 °C until assays were performed. Peripheral GnRH antibody concentrations (pmol of ^{125}I-GnRH bound per mL serum at 1:1000 dilution) were measured using a modified radioligand binding assay, as described previously [27].

2.3. Puberty and fertility

2.3.1. Males

We measured and compared mean monthly serum concentrations of testosterone (ng/mL) between exposure groups from 14 to 21 mo [30]. Blood was collected as described above at single time points at the beginning of each month between August 2007 and March 2008. In addition, we measured secondary sexual characteristics, including antler length (cm), scrotal circumference (cm), and neck girth (cm) at monthly intervals from 9 to 21 mo (March 2007 to March 2008) as well as before the 2008 reproductive season (August 2008). Finally, we measured antler complexity (number of branch points), and weight (g) before the first and second potential breeding seasons (August 2007, 2008) when hardened antlers were removed for herd management purposes. Antlers were consistently removed (using a Giglis wire saw) 1 cm above the ridge of bone where the antler and pedicle meet.

To estimate fertility and confirm pubertal maturation, we collected semen samples at monthly intervals from August 2007 to March 2008 (14 to 21 mo). Semen samples were collected via electroejaculation [31] using a 60-mm diameter rectal probe (Pulsator III, Lane Manufacturing, Inc., Denver, CO, USA) while elk were chemically immobilized (18 to 23 mg butorphanol tartrate, 15 to 19 mg azaparone tartrate, and 6 to 8 mg medetomidine hydrochloride im; Wildlife Pharmaceuticals). An endotracheal tube (20 mm internal diameter) was placed during the procedure to prevent laryngeal collapse and hypoxemia. No supplemental oxygen was administered. Sedative effects were reversed when procedures were completed (10 mg atipamazole hydrochloride im; Pfizer Animal Health, Exton, PA, USA; and 500 mg tolazoline hydrochloride im). Total and progressive sperm motility (%), as well as components of velocity (straight line [VSL], curvilinear [VCL], and average path [VAP]; μm/sec), were evaluated immediately using computer assisted sperm analysis (IVOS, Hamilton, Thorne Biosciences, Beverly, MA, USA) [32,33]. If necessary, samples were diluted with semen extender (E-Z Mixin "BF", Animal Reproduction Systems, Chino, CA, USA) before motility analysis. Undiluted semen was smeared, stained with eosin-nigrosin (Hancock Stain; Animal Reproduction Systems), and slides were stored at room temperature for future evaluation. Sperm morphology (% normal) was evaluated by a single technician according to standards used for bovine semen [34].

2.3.2. Females

Onset of puberty was estimated in females by measuring peripheral serum progesterone concentrations (ng/mL) every 10 days (estrous cycle length = 21 to 22 days, luteal phase = 13 to 17 days [35–38]) from August 2007 to April 2008. Serum concentrations of progesterone ≥1.0 ng/mL were considered indicative of a functional CL and signified females were postpubertal [35,39]. Female fertility was evaluated by assessing pregnancy after exposure to proven herd sire males for 63 days during their second potential breeding season (September to November 2008) at 2.5 yr. Pregnancy status was determined using pregnancy specific protein B assay [40], transrectal palpation [41], and transrectal ultrasound [42] 45 days after bulls were removed from paddocks. For management purposes, when pregnancy was confirmed, abortion was induced using two doses of prostaglandin $F_{2\alpha}$ 6 h apart (25 mg

im; lutalyse, Pharmacia & Upjohn, Kalamazoo, MI, USA) [43,44].

2.4. Hypothalamic-pituitary-gonadal axis

Pituitary responsiveness to GnRH was measured before the second breeding season (August 2008). All elk were fitted with nonsurgical indwelling jugular catheters (14-gauge 14 cm Abbocath; Abbott Laboratories, Abbott Park, IL, USA) and administered the GnRH analog d-Ala^6-GnRH-Pro^9-ethylamide (1 μg/50 kg body weight iv; Sigma, Chemical Co., St. Louis, MO, USA). Blood samples were collected before treatment then at hourly intervals for 8 h [36,45]. Blood was handled as described above and serum was stored at −20 °C for subsequent analysis of LH [46]. All blood samples collected from males were also analyzed for testosterone [30]. Progesterone was measured in pretreatment serum samples collected from females [47]. Gonadotrope response to GnRH analog challenge was assessed in two ways: (1) mean maximum concentration of LH over all time points (ng/mL); and (2) total amount of LH secreted (ng/mL per min), estimated by calculating the area under the curve for LH response [36]. To avoid confounding female pregnancy estimates, stimulation with GnRH analog was repeated in males but not females during the breeding season (early November 2008).

In addition to challenge with GnRH, we evaluated potential changes to the HPG axis by collecting hypothalamus along with the median eminence, pituitary, and gonads on March 25 (males) and April 14, 2009 (females). We assessed morphologic and morphometric differences in gonads between exposure groups. Gonad mass (g) was determined for each animal. Transverse sections of testes were cut, weighed, and frozen until sperm per gram of testes was measured [48]. Remaining testes tissue and all ovarian tissue was preserved in Bouin's fixative (Polysciences, Inc., Warrington, PA, USA). Pituitary and hypothalamus were hemisectioned through the midsagittal plane. One half of each brain was preserved in 4% paraformaldehyde and the other half wrapped in aluminum foil and frozen at −80 °C. Frozen sections of hypothalamus were assayed for total GnRH content (ng) [49]. Frozen pituitary sections were processed as described by Hart and colleagues [49], and analyzed for LH and FSH content (mg/g tissue) [46,50]. Fixed tissues were trimmed, placed in cassettes, maintained in 10% neutral buffered formalin, and sectioned using standard histology techniques at the Colorado State University Veterinary Diagnostic Laboratory. Hematoxylin and eosin stains were used for histologic examination. A veterinary pathologist (Spraker) qualitatively examined sections of gonad, pituitary, and hypothalamus (magnification × 2 to × 40) for pathologic changes and evidence of differences in morphology.

2.5. Hormone assays

Concentrations of progesterone [47], testosterone [30], LH [46], FSH [50], and GnRH [49] were measured using RIA. Samples were assayed in duplicate in single batches for each hormone at each time point. Intra-assay coefficients of variation for the upper and lower reference standards (20% and 80% ligand-labeled hormone-bound, respectively) were 4% to 15% for progesterone, 3% to 17% for testosterone, 3% to 11% for LH, 3% and 6% for FSH, and 5% and 10% for GnRH. Interassay coefficients of variation were 2% for progesterone, and <20% for testosterone and LH. Both FSH and GnRH were measured in single assays. Mean limits of detection for each hormone (1 SD of the assay) are as follows: progesterone, 5 pg; testosterone, 2.5 pg; LH 30 pg; FSH 0.2 ng; and GnRH 0.5 pg.

2.6. Statistical analysis

Dependent response variables, including body weight, concentrations of progesterone, testosterone, and LH measured at multiple time points, sperm parameters measured after electroejaculation, and secondary sexual characteristics were analyzed using one-way ANOVA models for a nonrandomized design with a repeated measures structure (SAS 9.2, Proc MIXED; SAS Institute, Cary, NC, USA). The independent variables exposure status, sire, date, and time, were included as fixed effects. Sex was also treated as a fixed effect if a response variable was measured in both sexes. Individual animal was included as a random effect. We first modeled the variance-covariance structure for each dependent variable using the restricted maximum-likelihood method, with the most global model of fixed effects (response variable = exposure status + sire + date or time as categorical variables ± sex). We modeled the following variance-covariance structures appropriate for unequal time intervals; variance components (VC), compound symmetric (CS), and spatial power [SP(POW)]. We selected the most appropriate variance-covariance structure using Akaike's Information Criterion with correction for small sample sizes (AICc) and then used the top-ranked structure for subsequent modeling of fixed effects. The best structure for all models had constant variance within an exposure group; some models allowed heterogeneous variance between exposure groups, whereas others were homogeneous for all study

animals. The best covariance structure for all variables except body weight was variance components, which implies no covariance between repeated measurements. The best covariance structure for body weight was spatial power. Because date and time were the only significant fixed effects in the model describing total LH released after GnRH analog stimulation, a Student *t* test was used to compare differences between sexes and between months for males.

Single time point concentrations of LH, FSH, and GnRH at necropsy, as well as gonad mass, and sperm per gram of testis were analyzed using generalized linear ANOVA models (Proc GLM), with exposure status, sire, and sex (if appropriate) as classification variables. Arcsine transformation was performed for all data expressed as percent. Means and standard errors were estimated using least squares analysis and tests for differences between GnRH antibody-exposed and unexposed groups were based on type III generalized estimating equations. Descriptive statistics were used to explain concentrations of GnRH antibody.

3. Results

3.1. Antibody concentrations and growth

Antibodies to GnRH were measured in exposed calf serum between 1 and 200 days after birth (Fig. 1). Antibodies were undetectable before nursing in blood samples taken from two calves, one with a GnRH vaccinated dam and the other with a sham-vaccinated dam. Maximum antibody concentrations (± SEM) in exposed calves were often higher (37.7 ± 4.1 pmol/mL; range, 15.2 to 54.1 pmol/mL) than those previously measured in the GnRH vaccinated dam cohort at similar time points (May 2006) (28.8 ± 3.8 pmol/mL; range, 12.2 to 42.3 pmol/mL [data not shown]). Antibodies declined exponentially over time in exposed calves with a half-life of approximately 7 days (Fig. 1). Exponential antibody decay was represented by the following equation: $[A] = 67.1e^{-0.1(d)}$ where A = serum GnRH-antibody concentration and d = calf age in days ($r^2 = 0.64$).

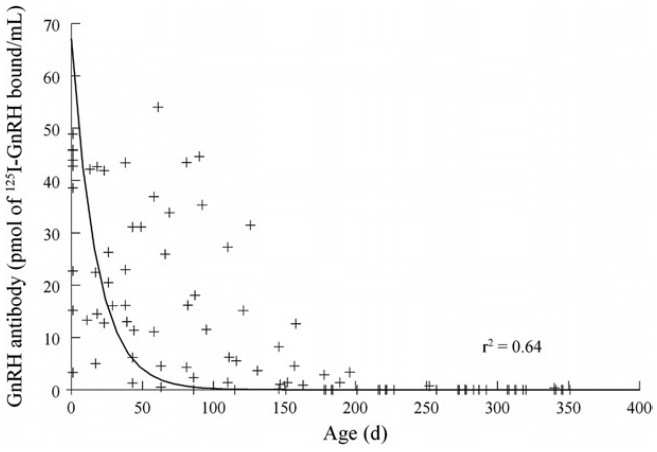

Fig. 1. Persistence of maternal antibody from birth to 1 yr in elk calves (N = six to eight males, two died August 2006 and May 2007; N = 4 females) exposed to colostral transfer of GnRH antibodies during the first 24 h of life. Calves unexposed to GnRH antibodies had undetectable antibody titers (data not shown).

There was no effect of neonatal exposure to GnRH antibodies on body weight (P = 0.968) between birth and 3 yr. Although males were heavier than females at birth (P = 0.024) and at 3 yr (P < 0.001), neither sex (P = 0.905) nor sire (P = 0.913) were significant variables in the model. Age was the most important variable describing body weight (P < 0.001) and there was an age by sex interaction (P = 0.001) with males gaining weight faster than females. Males gained approximately 0.83 kg per day during the first 100 days and then gained more slowly at 0.2 kg per day between 200 and 800 days. Females gained an average of 0.75 kg per day during the first 100 days but decreased their growth rate to 0.1 kg per day between 200 and 800 days.

3.2. Puberty and fertility

3.2.1. Males

There was no effect of exposure on mean monthly concentrations of testosterone (P = 0.659) between 15 and 21 mo. Likewise, sire did not affect concentrations of testosterone (P = 0.275); however, concentration did vary by month (P = 0.001; Fig. 2). Highest concentrations occurred in October and November. With the exception of one calf exposed to GnRH-antibodies, which had a maximum concentration of 1.7 ng/mL during November, all males had at least one testosterone measurement ≥2 ng/mL.

Similar to endocrine results, in male calves neither neonatal antibody exposure nor sire had an effect on semen parameters or most secondary sexual characteristics, including antler mass, neck girth, and scrotal circumference (Table 1). Date was an important variable describing differences in antler length (P = 0.002), antler mass (P = 0.007), neck girth (P = 0.0003), and scrotal circumference (P < 0.0001), but not semen parameters (P > 0.05). Only antler length was affected by exposure status (P = 0.037) with a single male exposed to GnRH antibodies contributing most of the variance. This animal had an unusually short antler, which may have been physically damaged, during his

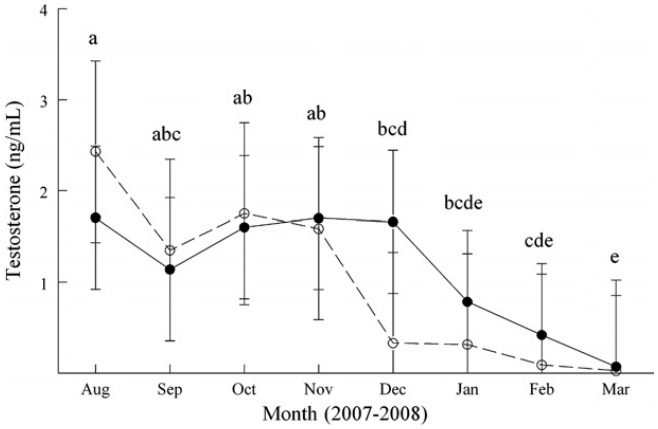

Fig. 2. Mean monthly testosterone concentrations with 95% confidence intervals, between 14 and 21 mo, in male elk exposed (N = 4; ● solid line) and unexposed (N = 2; ○ dashed line) to neonatal GnRH antibodies. Letters denote differences between months ($P < 0.05$); there were no differences between exposure groups within months.

second reproductive season but had a similar number of branch points (five) as other males (five or six per antler). Regardless of exposure group, all males had at least one semen sample which met criteria for adequate progressive motility (≥30%) and morphology (≥70%) of sperm in yearling male domestic cattle [34] and was consistent with acceptable sperm parameters from red deer (*Cervus elaphus*) (>40% motility, >40% normal morphology) [51]. Scrotal circumferences were consistent with those reported for 2-year-old elk [31].

3.2.2. Females

Monthly serum concentrations of progesterone in females were not affected by presence of neonatal antibody titer ($P = 0.727$) or sire ($P = 0.805$); however, concentrations varied by month ($P = 0.002$). All females had evidence of CL formation and regression, based on progesterone profiles, suggesting puberty had been reached by November 2007 (Fig. 3A). All had cyclic changes in serum concentrations of progesterone with multiple samples >1 ng/mL until March 2008 (Fig. 3B). Females were exposed to proven herd sire bulls between September and November 2008 at 28 to 32 mo. All seven females were pregnant when examined in January 2009. Transrectal palpation, ultrasound examination, and pregnancy specific protein B results were concordant.

3.3. Hypothalamic-pituitary-gonadal axis function and structure

In August 2008, before their second potential reproductive season, both males and females responded to GnRH analog stimulation with an acute LH release that peaked between 2 and 5 h and approached baseline levels within 8 h. Although mean maximum concentration of LH was not different between sexes ($P = 0.069$), females (16.1 ± 2.3 ng/mL) had nearly twice the maximum release of LH as males (9.4 ± 2.4 ng/mL; Figs. 4 and 5). Exposure to GnRH-antibodies during the neonatal period did not affect total secretion of LH ± SEM (ng/mL per min) in either males (33.3 ± 7.9 exposed, 35.3 ± 4.1 unexposed; $P = 0.668$) or females (69.7 ± 8.5 exposed, 61.2 ± 8.8 unexposed; $P = 0.333$). Neither mean maximum concentrations of LH nor testosterone were different between months ($P > 0.05$) for males; however, total LH and testosterone released amongst all males in August (42.3 ± 9.2 and

Table 1
Measures of semen quality and secondary sexual characteristics in male elk between 15 and 22 mo of age.

Response variable	Exposed (N = 4)			Unexposed (N = 2)		
	Mean	SEM	Range	Mean	SEM	Range
Normal morphology (%)	59.0	1.6	15–86	67.5	2.4	35–91
Total motility (%)	60.9	1.5	7–98	83.0	1.8	48–98
Progressive motility (%)	39.1	1.3	1–79	64.7	1.5	32–81
Sperm velocities (um per sec)						
VAP	74.5	10.0	31–116	97.6	11.1	76–116
VSL	62.6	10.0	19–100	83.1	11.1	67–104
VCL	119.2	121.2	52–174	153.8	13.5	115–178
Scrotal circumference (cm)	18.7	2.4	13.6–24.7	19.4	2.4	14.8–24.8
Antler length (cm)	148.8	3.1	123–187	166.3	3.8	129–197
Antler mass (g)	2805.5	244.4	1040–4420	2622.3	299.3	1330–4500
Neck girth (cm)	72.7	0.5	62–80	74.8	0.7	68–80

An arcsine transformation was performed on frequency data before analysis. The only end point that differed between groups was antler length ($P < 0.05$).
VAP, average path velocity; VCL, curvilinear velocity; VSL, straight line velocity.

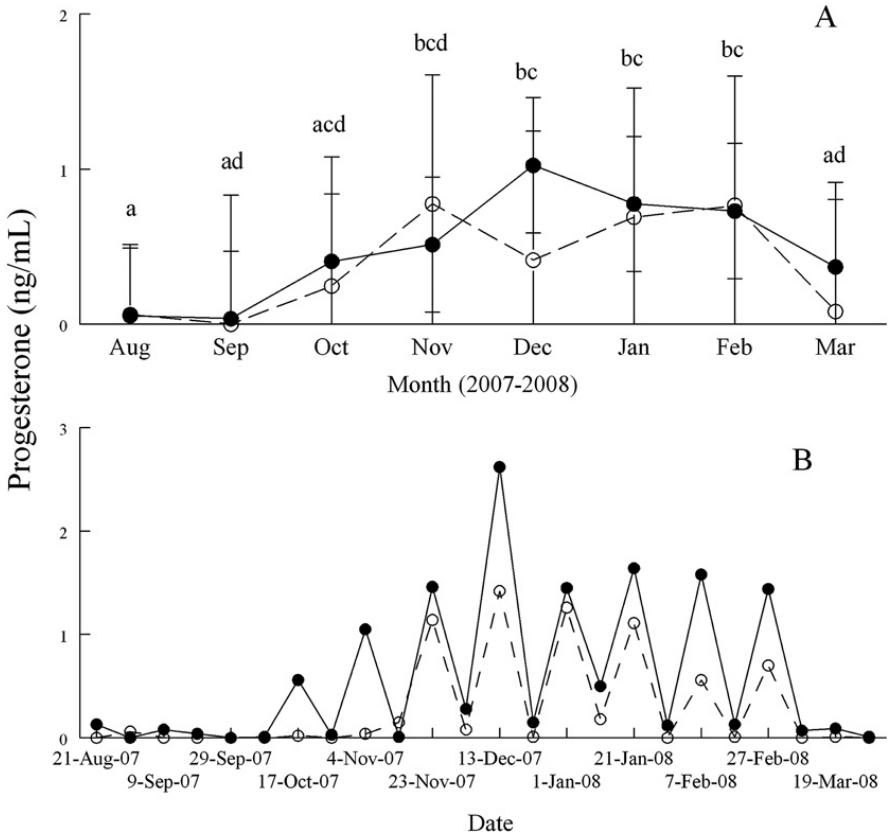

Fig. 3. Top panel (A): Mean monthly progesterone concentrations with 95% confidence intervals, from August 2007 to March 2008, in female elk calves exposed (N = 4; ● solid line) and unexposed (N = 3; ○ dashed line) to GnRH antibodies during the neonatal period. Letters denote differences between months ($P < 0.05$); there were no differences between exposure groups within months. Bottom panel (B): Representative progesterone profiles from female elk calves exposed (● solid line) and unexposed (○ dashed line) to GnRH antibody during the neonatal period. Sampling occurred at approximately 10-day intervals between 15 and 22 mo.

81.8 ± 7.7 ng/mL per min, respectively) was greater than in November (25.6 ± 3.2 and 56.7 ± 6.3 ng/mL per min; $P < 0.001$). A single male with unusually high concentrations of LH (14–22 ng/mL) accounted for most of the variability. Concentrations of progesterone in all females were below the limit of detection at the time of GnRH stimulation in August 2008 indicating lack of luteal tissue.

Endocrine profiles in serum and tissue at the time of necropsy were similar between exposure groups and sexes. There was no effect of exposure to GnRH antibodies on pituitary concentrations of LH ($P = 0.525$) or FSH ($P = 0.349$) in either sex (Table 2). Content of GnRH in hemihypothalmi was not different between exposure groups ($P = 0.979$) or sexes ($P = 0.980$). Serum concentrations of testosterone in males and progesterone in females were nearly undetectable and did not differ between exposure groups ($P > 0.05$). Gonad mass (g) did not vary by exposure status or sire ($P > 0.05$; Table 2).

There were no observed differences in gross or histologic structure of the hypothalamus, pituitary, testes, or ovaries between antibody-exposed and unexposed elk. There was no evidence of overt inflammation or change in structure in the median eminence of any study animal (data not shown). Although gonadotropes were not specifically identified, adenohypophesial structure was similar between exposure groups and was within normal limits for ruminant pituitaries. All ovaries and testes had evidence of gametogenesis. Ovaries had primordial through Graffian follicles, and testes had seminiferous tubules which contained spermatocytes. The only sign of inflammation was infiltration of atretic follicles with eosinophils in four of seven (N = 2 from each exposure group; approximately 60%) females.

4. Discussion

4.1. Antibody effects

Passive transfer of maternal GnRH-antibodies to elk calves shortly after birth did not affect long-term reproductive development in our study elk. There were no significant differences in growth, time of pubertal

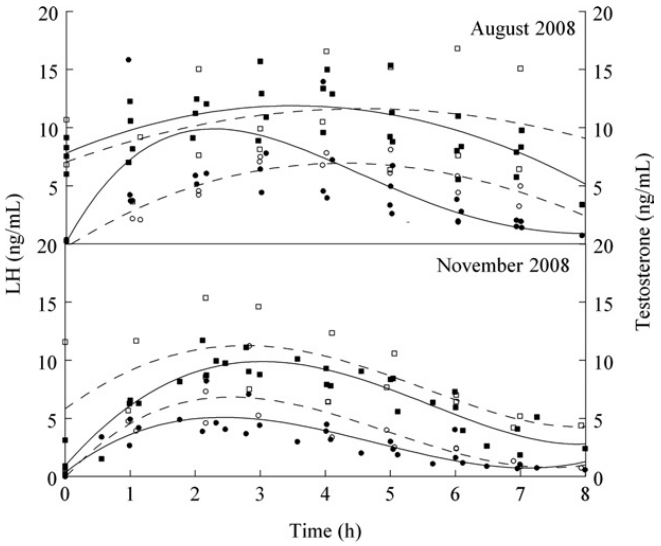

Fig. 4. Serum LH (○ ●) and testosterone (□ ●) concentrations after GnRH agonist treatment at time zero, in male calves (27 to 30 mo) exposed (filled solid line, N = 4) and unexposed (open dashed line, N = 2) to GnRH antibodies during the neonatal period. Total hormone released (ng/mL per min) represented by the area under the curve.

Table 2
Hormone concentrations and gonadal measurements in GnRH antibody-exposed and unexposed male (m) and female (f) elk at the time of necropsy (March/April 2009; approximately 3 yr of age).

Response variable	Exposed (N = 4 m, 4 f)		Unexposed (N = 2 m, 3 f)	
	Mean	SEM	Mean	SEM
FSH (ug/g pituitary)	(m) 1368 (f) 2262	268 268	(m) 1660 (f) 1200	328 568
LH (ug/g pituitary)	(m) 70 (f) 215	67 67	(m) 105 (f) 309	82 142
Hypothalmic GnRH (ng)	(m) 7.1 (f) 5.3	2.2 2.2	(m) 5.3 (f) 6.9	2.7 4.7
Serum testosterone (ng/mL)	0.41	0.73	0.00	0.00
Serum progesterone (ng/mL)	0.18	0.07	0.07	0.08
Testes mass (g)	48.5	5.5	42.6	4.5
Ovary mass (g)	3.1	0.5	2.2	0.2
Number of sperm $\times 10^6$ (per g of testis)	79.9	7.0	77.1	0.6

Gonadotropins and GnRH were measured in both sexes, testosterone in males, and progesterone in females.

onset, or structure and function of the HPG axis between exposure groups in males or females. Therefore, we inferred that the presence of high concentrations of passively transferred GnRH antibodies during the first 60 days of life did not permanently alter the reproductive function of elk. However, because our sample sizes were small, particularly for the unexposed male group, we must be somewhat circumspect about our results. Due to small sample variation, a repeated experiment with relatively large sample sizes (e.g., ≥30) would be more likely to find differences, if they existed. Nevertheless, we consider our results relevant because there are multiple time points per elk for most response variables, and for at least one of those time points, each animal produced a reproductively mature response.

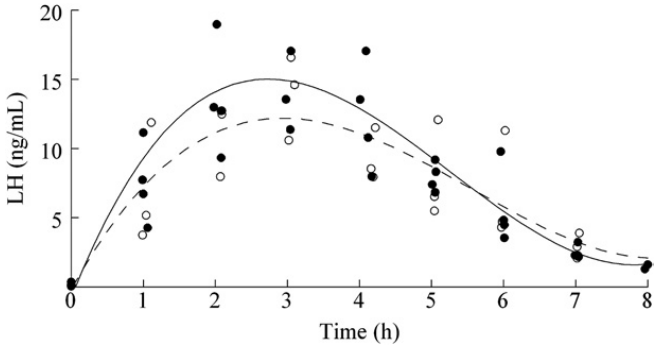

Fig. 5. Serum LH concentrations, after GnRH agonist treatment at time zero, in female elk calves (27 to 28 mo) exposed (● solid line, N = 4) and unexposed (○ dashed line, N = 3) to GnRH antibody during the neonatal period. Total LH released (ng/mL per min) represented by the area under the curve.

Our results were partially in contrast to findings in domestic sheep actively vaccinated against GnRH at 2 wk [21–23] and male rats passively immunized 5 days after birth [24,25]. Clarke et al. found that active vaccination against GnRH during the neonatal period caused permanent HPG axis suppression during adulthood, in a subset of vaccinated males and females, despite a lack of GnRH antibodies. This was caused by decreased GnRH secretion, rather than a decrease in GnRH content of the median eminence [21]. Vogel et al. [24] and Bercu and Jackson [25] found permanent suppression of rat testes development after neonatal passive vaccination, despite normal postpubertal gonadotropin concentrations during adulthood. They suggested that there is a critical window of testes development in rodents at this time that is permanently altered without the timely support of gonadotropins. In our study, we investigated both the potential for passive neonatal vaccination to permanently decrease GnRH secretion and the potential for transient removal of GnRH signaling to alter the structure or function of the pituitary or gonad, even if long-term GnRH secretion was unimpaired during adulthood. We found neither of

these mechanisms altered reproductive function in elk from this study.

Gonadotropin-releasing hormone is secreted from hypothalamic neurons originating within the blood-brain barrier, but terminating near blood vessels of the hypothalamic-pituitary portal system outside of the blood-brain barrier. After active GnRH vaccination in male pigs at 10 and 18 wk and subsequent necropsy at 26 wk, Molenaar and colleagues found lesions, including fibrosis and scar tissue formation within the median eminence which were positively correlated with GnRH-antibody titer and testes atrophy [52]. They suggested that T-cell-mediated autoimmune reactions directed at GnRH neurons or retrograde transport of GnRH antibodies might result in destruction of the neurons or their processes. They demonstrated inflammatory lesions of the median eminence, consistent with the speculation that anti-GnRH immunoglobulin G, in addition to interleukin cytokines, may be responsible for permanent changes to function of GnRH neurons. Their study was different from ours in two important ways. First, the lesions they described were present in animals which had concurrently high concentrations of GnRH antibodies, which may have indicated the inflammatory processes were in progress at the time of death, but the lesions could have been transient. Second, pigs were actively vaccinated with Freund's complete and incomplete adjuvants, potent stimulants of both humoral and cell-mediated components of the immune response [53]. In contrast, elk in our study did not have detectable antibody concentrations by 6 mo and most response variables were measured between 9 and 30 mo. More importantly, elk in the current study were passively immunized with maternal antibodies, rather than stimulating in vivo humoral and cell-mediated immune responses. Although concentrations of GnRH antibodies were certainly higher in our study 24 h after birth, due to reporting inconsistencies, it was not possible to compare concentrations of antibody between the two studies at later time points. Regardless, our elk were not exposed to a full complement of humoral and cell-mediated components of the immune response, whereas pigs exposed to Freund's complete adjuvant along with GnRH conjugated to keyhole limpet hemocyanin (KLH) likely had robust stimulation of the entire immune system. Although we did not examine the histologic structure of the median eminence to the same degree of Molenaar et al. [52], there was no apparent evidence of inflammation or fibrosis in hypothalmi and there was no clinical evidence of HPG axis dysfunction in our elk. Therefore, we inferred that elevated antibody titer alone was not sufficient to induce a permanent decrease in GnRH secretion.

In altricial species, such as the rat, maturation of the HPG axis is incomplete at birth [54]. In precocious species, such as sheep and likely elk, the fetal HPG axis is functional by midgestation [3]; however, hypothalamic signaling is required for development of gonadotrophic cells during the last month of gestation and puberty is reliant on an adequate gonadotrope population [55]. It is unknown exactly when the permanent structure or function of the HPG axis is complete in elk, but we inferred that elimination or at least a decrease in GnRH signaling during the first 60 to 180 days after birth does not delay the process. Because we did not measure gonadotropin secretion during the neonatal period, we do not know if there were adequate concentrations of GnRH antibodies to completely remove GnRH signaling. In our previous study in adult female elk with similar or lower concentrations of GnRH antibodies, there was suppression of fertility, but not complete inhibition of final stages of follicular development, indicating a partially intact HPG axis [27]. Alternatively, ewes with lower concentrations of GnRH antibodies, but with a more aggressive active vaccination schedule, had complete cessation of follicular wave development [56]. It is likely that antibody concentrations were sufficient to influence GnRH signaling in the current study. This finding added strength to our speculation that antibodies alone do not account for long-term suppression of the HPG axis documented in previous studies.

4.2. Comparative reproductive ecology

Our investigation of endocrine changes, direct and indirect measures of fertility, and development of secondary sexual characteristics through puberty in male elk provided results similar to those in untreated and closely related red deer. For male red deer, the highest mean testosterone concentrations occur in late summer and early fall when antlers are hard and velvet has been shed [57–59]. Conversely, in the spring, at the time of antler casting, testosterone is nearly undetectable and testes are at their smallest [58,59]. Male elk in this and other studies [31] appeared to follow a similar seasonal pattern, with testosterone concentrations increasing to a peak in late summer/fall (September to October) then gradually declining through the winter period (December through March). Scrotal circumference mirrored testosterone concentrations, being minimal in late spring and attaining maximum size in late summer. However, as expected, scrotal circumference did not

regress to prepubertal dimensions. Male elk pituitary response to stimulation with GnRH analog before and during the second reproductive season was similar to that reported in red deer stags [57,58]. Maximum concentrations of both LH and testosterone, induced by GnRH stimulation, were higher in August than in November. Interestingly, the single outlier male with an unusually large maximum concentration of LH had a relatively modest corresponding maximum concentration of testosterone. This pattern of large LH release with little secretion of testosterone, was characteristic of the nonreproductive season in red deer [57]. It was our observation that this individual was socially the least dominant male and did not produce a satisfactory semen sample until December 2007.

In elk populations that are not nutrient-limited, males are generally reproductively competent at 14 to 16 mo [31] with similar fertility rates as those seen in older more mature bulls [60]. Although we did not directly measure fertility of males in this experiment, there were no significant differences in semen quality or secondary sexual characteristics associated with fertility between exposure groups. Sperm motility, velocity, and morphology parameters met or exceeded standards for domestic bulls [34] and were similar to findings in red deer [33]. Semen evaluation and sperm analysis have remained elusive as a reliable indicator of fertility in domestic animals [61,62]; however, these assays may be more useful in wild species where strong artificial selection pressures for increased fertility are not in effect [63]. Swimming velocity and sperm morphology account for differences in fertility rates of Iberian red deer (*Cervus elaphus hispanicus*) [33]. Additionally, antler size and complexity are associated with testes size and sperm velocity [64]. Finally, despite seasonal testicular atrophy, all males in our study had maturing spermatocytes within the seminiferous tubules during the early spring at approximately 33 mo. Therefore, we inferred that all males were reproductively mature and likely fertile by the end of their first reproductive season at 15 to 21 mo.

Puberty and ultimately fertility in female elk is a function of body condition. In that regard, 50% of yearling females are fertile at 16 to 18 mo if a minimum body mass (approximately 220 kg, 10% body fat) is achieved [65–67]. The first estrous cycle of the reproductive season often has a short interovulatory interval [68], followed by regular ovulations every 20 to 24 days [38]. In our study, every female had regular increases in serum progesterone concentrations at approximately 20-day intervals. Most females had evidence of functional CL by mid-October and all were cycling by early November. This is later than that reported for mature female elk in North America [68], but is within the range observed in other studies with 2-yr-old females [66]. Three of seven females continued to display elevated concentrations of progesterone into late March when sampling was terminated. This was in accordance with previous research which demonstrated estrous cycling in mature females can occur as late as March but typically ends in late February [68]. Pituitary stimulation with GnRH analog before the breeding season induced an LH release similar to that in nonpregnant mature female elk during the reproductive season [36]. In our confirmatory test of fertility, all females became pregnant. These results provided further evidence for the lack of an effect of GnRH antibody exposure on maturation of the HPG axis or fertility in female elk calves in this study.

4.3. Conclusion

There was no apparent effect of exposure to high concentrations of maternally transferred GnRH-antibodies on the long-term structure or function of the hypothalmo-pituitary-gonadal axis of male or female elk. The elk HPG axis is likely structurally mature at birth and transient disruption in GnRH signaling through antibody neutralization was not sufficient to permanently change function in our study elk. Our findings provide important knowledge to inform wildlife management decisions regarding the application of fertility control in free-ranging populations of elk. Additionally, we have advanced the understanding of the mechanism(s) of how GnRH vaccination may affect long-term fertility in ruminant species. The presence of neonatal antibodies alone did not appear to account for changes in fertility later in life.

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References

[1] Counis R, Laverrière JN, Garrel G, Bleux C, Cohen-Tannoudji J, Lerrant Y, et al. Gonadotropin-releasing hormone and the control of gonadotrope function. Reprod Nutr Dev 2005;45: 243–54.

[2] Smith JT, Clarke IJ. Seasonal breeding as a neuroendocrine model for puberty in sheep. Mol Cell Endocrinol 2010;324: 102–9.

[3] Brooks AN, McNeilly AS, Thomas GB. Role of GnRH in the ontogeny and regulation of the fetal hypothalamo-pituitary-gonadal axis in sheep. J Reprod Fertil Suppl 1995;49:163–75.

[4] Evans A, Adams G, Rawling N. Follicular and hormonal development in prepubertal heifers from 2 to 36 weeks of age. Reproduction 1994;102:463–70.

[5] Polkowska J. Development of the gonadotrophic and somatotrophic axes of sheep. J Reprod Fertil Suppl 1995;49:187–95.

[6] Evans A, Adams G, Rawlings N. Endocrine and ovarian follicular changes leading up to the first ovulation in prepubertal heifers. Reproduction 1994;100:187–94.

[7] Bartlewski PM, Beard AP, Rawlings NC. Ultrasonographic study of antral follicle development during sexual maturation in ewe lambs. Small Rumin Res 2006;63:189–98.

[8] Bartlewski P, Beard A, Cook S, Rawlings N. Ovarian activity during sexual maturation and following introduction of the ram to ewe lambs. Small Rumin Res 2002;43:37–44.

[9] Rawlings NC, Evans AC, Honaramooz A, Bartlewski PM. Antral follicle growth and endocrine changes in prepubertal cattle, sheep and goats. Anim Reprod Sci 2003;78:259–70.

[10] Amann RP, Wise ME, Glass JD, Nett TM. Prepubertal changes in the hypothalamic-pituitary axis of holstein bulls. Biol Reprod 1986;34:71–80.

[11] Collu R, Savoie S, Hamel R, Gibb W, Ducharme J. Maturation of the hypothalamic-pituitary-gonadal axis in the male lamb: a review. Psychoneuroendocrinol 1983;8:213–24.

[12] Madgwick S, Evans AC, Beard AP. Treating heifers with GnRH from 4 to 8 weeks of age advanced growth and the age at puberty. Theriogenology 2005;63:2323–33.

[13] Chandolia RK, Evans AC, Rawlings NC. Effect of inhibition of increased gonadotrophin secretion before 20 weeks of age in bull calves on testicular development. J Reprod Fertil 1997;109: 65–71.

[14] Prendiville DJ, Enright WJ, Crowe MA, Vaughan L, Roche JF. Immunization of prepubertal beef heifers against gonadotropin-releasing hormone: immune, estrus, ovarian, and growth responses. J Anim Sci 1995;73:3030–7.

[15] Clarke IJ, Fraser HM, McNeilly AS. Active immunization of ewes against luteinizing hormone releasing hormone, and its effects on ovulation and gonadotrophin, prolactin and ovarian steroid secretion. J Endocrinol 1978;78:39–47.

[16] Fraser HM, McNeilly AS. Effect of immunoneutralization of luteinizing hormone releasing hormone on the estrogen-induced luteinizing hormone and follicle-stimulating hormone surges in the ewe. Biol Reprod 1982;27:548–55.

[17] Jago JG, Cox NR, Bass JJ, Matthews LR. The effect of prepubertal immunization against gonadotropin-releasing hormone on the development of sexual and social behavior of bulls. J Anim Sci 1997;75:2609–19.

[18] Geary TW, Grings EE, MacNeil MD, de Avila DM, Reeves JJ. Use of recombinant gonadotropin-releasing hormone antigens for immunosterilization of beef heifers. J Anim Sci 2006;84: 343–50.

[19] Delves PJ. How far from a hormone-based contraceptive vaccine? J Reprod Immunol 2004;62:69–78.

[20] Herbert CA, Trigg TE. Applications of GnRH in the control and management of fertility in female animals. Anim Reprod Sci 2005;88:141–53.

[21] Clarke IJ, Brown BW, Tran VV, Scott CJ, Fry R, Millar RP, et al. Neonatal immunization against gonadotropin-releasing hormone (GnRH) results in diminished GnRH secretion in adulthood. Endocrinology 1998;139:2007–14.

[22] Brown BW, Mattner PE, Carroll PA, Hoskinson RM, Rigby RD. Immunization of sheep against GnRH early in life: effects on reproductive function and hormones in ewes. J Reprod Fertil 1995;103:131–5.

[23] Brown BW, Mattner PE, Carroll PA, Holland EJ, Paull DR, Hoskinson RM, et al. Immunization of sheep against GnRH early in life: effects on reproductive function and hormones in rams. J Reprod Fertil 1994;101:15–21.

[24] Vogel DL, Gunsalus GL, Bercu BB, Musto NA, Bardin CW. Sertoli cell maturation is impaired by neonatal passive immunization with antiserum to luteinizing hormone-releasing hormone. Endocrinology 1983;112:1115–21.

[25] Bercu BB, Jackson IM. Response of adult male rats to LH-RH after neonatal immunization with antiserum to LH-RH. J Reprod Fertil 1980;59:501–7.

[26] Fagerstone KA, Miller LA, Killian G, Yoder CA. Review of issues concerning the use of reproductive inhibitors, with particular emphasis on resolving human-wildlife conflicts in North America. Integr Zool 2010;5:15–30.

[27] Powers JG, Baker DL, Davis TL, Conner MM, Lothridge AH, Nett TM. Effects of gonadotropin-releasing hormone immunization on reproductive function and behavior in captive female Rocky Mountain elk (Cervus elaphus nelsoni). Biol Reprod 2011;85:1152–60.

[28] Kirkpatrick JF, Turner JW. Reversible contraception in nondomestic animals. J Zoo Wildl Med 1991;22:392–408.

[29] Haigh JC, Hudson RJ. Farming Wapiti and Red Deer. Mosby, 1993.

[30] Berndtson WE, Pickett BW, Nett TM. Reproductive physiology of the stallion. IV. Seasonal changes in the testosterone concentration of peripheral plasma. J Reprod Fertil 1974;39:115–8.

[31] Haigh JC, Cates WF, Glover GJ, Rawlings NC. Relationships between seasonal changes in serum testosterone concentrations, scrotal circumference and sperm morphology of male wapiti (Cervus elaphus). J Reprod Fertil 1984;70:413–8.

[32] Budworth PR, Amann RP, Chapman PL. Relationships between computerized measurements of motion of frozen-thawed bull spermatozoa and fertility. J Androl 1988;9:41–54.

[33] Malo AF, Garde JJ, Soler AJ, García AJ, Gomendio M, Roldan ER. Male fertility in natural populations of red deer is determined by sperm velocity and the proportion of normal spermatozoa. Biol Reprod 2005;72:822–9.

[34] Chenoweth PJ, Hopkins FM, Spitzer JC, Larsen RE. Guidelines for using the bull breeding soundness evaluation form. In: Theriogenology Handbook, Society for Theriogenology: American College of Theriogenologists, 1993, pp. 5

[35] Adam CL, Moir CE, Atkinson T. Plasma concentrations of progesterone in female red deer (Cervus elaphus) during the breeding season, pregnancy and anoestrus. J Reprod Fertil 1985; 74:631–6.

[36] Baker DL, Miller MW, Nett TM. Gonadotropin-releasing hormone analog-induced patterns of luteinizing hormone secretion

in female wapiti (Cervus elaphus nelsoni) during the breeding season, anestrus, and pregnancy. Biol Reprod 1995;52:1193–7.
[37] McCorkell RB, MacDougall L, Adams GP. Ovarian follicle development in wapiti (Cervus elaphus) during the anovulatory season. Theriogenology 2004;61:473–83.
[38] McCorkell R, Woodbury M, Adams GP. Ovarian follicular and luteal dynamics in wapiti during the estrous cycle. Theriogenology 2006;65:540–56.
[39] Kelly RW, McNatty KP, Moore GH, Ross D, Gibb M. Plasma concentrations of LH, prolactin, oestradiol and progesterone in female red deer (Cervus elaphus) during pregnancy. J Reprod Fertil 1982;64:475–83.
[40] Huang F, Cockrell DC, Stephenson TR, Noyes JH, Sasser RG. A serum pregnancy test with a specific radioimmunoassay for moose and elk pregnancy-specific protein B. J Wildl Manage 2000;64:492–9.
[41] Greer KR, Hawkins WW. Determining pregnancy in elk by rectal palpation. J Wildl Manage 1967;31:145–9.
[42] Curran S, Pierson RA, Ginther OJ. Ultrasonographic appearance of the bovine conceptus from days 20 through 60. J Am Vet Med Assoc 1986;189:1295–302.
[43] Asher GW, Fisher MW, Berg DK, Waldrup KA, Pearse AJ. Luteal support of pregnancy in red deer (Cervus elaphus): effect of cloprostenol, ovariectomy and lutectomy on the viability of the post-implantation embryo. Anim Reprod Sci 1996;41:141–51.
[44] Bates GN, Brooks J, Call J. The use of prostaglandin to induce abortion in American elk (Cervus canadensis). J Zoo Anim Med 1982;13:125–6.
[45] Baker DL, Wild MA, Conner MM, Ravivarapu HB, Dunn RL, Nett TM. Effects of GnRH agonist (leuprolide) on reproduction and behaviour in female wapiti (Cervus elaphus nelsoni). Reprod Suppl 2002;60:155–67.
[46] Niswender GD, Reichert LE, Midgley AR, Nalbandov AV. Radioimmunoassay for bovine and ovine luteinizing hormone. Endocrinology 1969;84:1166–73.
[47] Niswender GD. Influence of the site of conjugation on the specificity of antibodies to progesterone. Steroids 1973;22:413–24.
[48] Amann RP, Lambiase JT. The male rabbit. 3. Determination oaily sperm production by means of testicular homogenates. J Anim Sci 1969;28:369–74.
[49] Hart PJ, Squires EL, Imel KJ, Nett TM. Seasonal variation in hypothalamic content of gonadotropin-releasing hormone (GnRH), pituitary receptors for GnRH, and pituitary content of luteinizing hormone and follicle-stimulating hormone in the mare. Biol Reprod 1984;30:1055–62.
[50] Akbar AM, Reichert LE, Dunn TG, Kaltenbach CC, Niswender GD. Serum levels of follicle-stimulating hormone during the bovine estrous cycle. J Anim Sci 1974;39:360–5.
[51] Garde JJ, Ortiz N, García AJ, Gallego L. Postmortem assessment of sperm characteristics of the red deer during the breeding season. Arch. J Androl 1998;41:195–202.
[52] Molenaar GJ, Lugard-Kok C, Meloen RH, Oonk RB, de Koning J, Wensing CJ. Lesions in the hypothalamus after active immunisation against GnRH in the pig. J Neuroimmunol 1993;48:1–11.
[53] Stills HF. Adjuvants and antibody production: dispelling the myths associated with Freund's complete and other adjuvants. Ilar J 2005;46:280–93.
[54] Pang SF, Tang F. Sex differences in the serum concentrations of testosterone in mice and hamsters during their critical periods of neural sexual differentiation. J Endocrinol 1984;100:7–11.
[55] Wańkowska M, Polkowska J, Misztal T, Romanowicz K. Influence of ovarian hormones on endocrine activity of gonadotroph cells in the adenohypophysis of lambs during the postnatal transition to prepuberty. Anim Reprod Sci 2010;121:84–93.
[56] Seekallu SV, Toosi BM, Ziegler A, Reeves JJ, Rawlings NC. Pulsed GnRH secretion and the FSH secretory peaks that initiate ovarian antral follicular wave emergence in anestrous ewes. Anim Reprod Sci 2010;120:56–64.
[57] Suttie JM, Lincoln GA, Kay RN. Endocrine control of antler growth in red deer stags. J Reprod Fertil 1984;71:7–15.
[58] Suttie JM, Fennessy PF, Crosbie SF, Corson ID, Laas FJ, Elgar HJ, et al. Temporal changes in LH and testosterone and their relationship with the first antler in red deer (Cervus elaphus) stags from 3 to 15 months of age. J Endocrinol 1991;131:467–74.
[59] Suttie J, Fennessy P, Corson I, Veenvliet B, Littlejohn R, Lapwood K. Seasonal pattern of luteinizing hormone and testosterone pulsatile secretion in young adult red deer stags (Cervus elaphus) and its association with the antler cycle. Reproduction 1992;95:925–33.
[60] Noyes JH, Johnson BK, Bryant LD, Findholt SL, Thomas JW. Effects of bull age on conception dates and pregnancy rates of cow elk. J Wildl Manage 1996;60:508–17.
[61] Mocé E, Graham JK. In vitro evaluation of sperm quality. Anim Reprod Sci 2008;105:104–18.
[62] Braundmeier AG, Miller DJ. The search is on: finding accurate molecular markers of male fertility. J Dairy Sci 2001;84:1915–25.
[63] Gomendio M, Malo AF, Garde J, Roldan ER. Sperm traits and male fertility in natural populations. Reproduction 2007;134:19–29.
[64] Malo AF, Roldan ERS, Garde J, Soler AJ, Gomendio M. Antlers honestly advertise sperm production and quality. Proc Roy Soc B-Biol Sci 2005;272:149–57.
[65] Sargeant G, Oehler M. Dynamics of newly established elk populations. J Wildl Manage 2007;71:1141–8.
[66] Hudson RJ, Kozak HM, Adamczewski JZ, Olsen CD. Reproductive performance of farmed wapiti (Cervus elaphus nelsoni). Small Rumin Res 1991;4:19–28.
[67] Cook RC, Cook JG, Mech LD. Nutritional condition of northern Yellowstone elk. J Mammal 2004;85:714–22.
[68] McCorkell R, Woodbury MR, Adams GP. Ovarian follicular and luteal dynamics in wapiti during seasonal transitions. Theriogenology 2007;67:1224–32.