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Effect of age and castration on serum anti-Müllerian hormone concentration in male alpacas

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ABSTRACT

The synthesis of anti-Müllerian Hormone (AMH) by the Sertoli cells in males is crucial for sexual differentiation. There are no studies on AMH in Camelids. The objectives of this research were to 1) compare AMH serum concentrations in prepubertal and adult male alpacas and 2) determine the effect of castration on these concentrations in adult males to provide a validation of a commercial AMH test in alpacas. Serum samples were obtained from 15 prepubertal animals (5 for each age groups of 6, 7 and 8 months) and from 5 adult males (age 5–9 years), pre- and 24 h post-castration. AMH was determined with a quantitative ELISA according to the manufacture's instructions. There was not significant difference ($P < 0.05$) in AMH level (pg/ml) between pre-pubertal (549.9 ± 120.8, 789.4 ± 172.3, 597.5 ± 177.3 for ages 4, 7, and 8 months, respectively) and adult alpacas (938.7 ± 175.9). In adult males, AMH concentration decreased significantly following castration ($P < 0.05$) (938.7 ± 383.5 pg/ml) pre-castration, and 222.1 ± 116.5 pg/ml) after castration). There was a positive correlation between testosterone levels and AMH. In conclusion, the quantitative assay used is a reliable test to determine AMH in alpacas. The AMH level in prepubertal and adult alpacas appear to not differ, contrarily from other mammals, this requires further investigation. The decrease in serum AMH concentrations after castration suggests that measurement of this hormone can be used to diagnose bilateral cryptorchid or hemicastrated unilateral cryptorchid animals in this species.

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1. Introduction

Anti-Müllerian hormone (AMH) is a homodimeric glycoprotein belonging to the transforming growth factor-β (TGF-β) family. In the male, it is secreted by the Sertoli cells from the time of fetal sexual differentiation through puberty [1]. AMH plays an important role in sexual differentiation and is responsible for regression of the Müllerian (paramesonephric) ducts in the male fetus [2]. The signaling cascade responsible for Müllerian duct regression is activated by binding of AMH to the specific AMH receptor (AMHR2) in the mesenchyme while allowing development of the Wolffian (mesonephric) ducts and formation of the internal genital tract in the normal male [3]. Disruption of the AMH gene by homologous recombination and point mutation results in persistence of the Müllerian ducts (i.e. persistent Müllerian duct syndrome) [3–5].

AMH serum concentration changes through puberty and adulthood have been described in several species. In human males, serum AMH concentrations are low immediately after birth, increase rapidly to peak around 2–3 years of aged remaining high in the prepubertal period, then decrease to low values after puberty [6]. In horses, serum AMH concentrations are significantly higher in neonates and prepubertal colts than postpubertal stallions. In this species, AMH concentration is affected by season in intact mature stallions [7]. The decrease in AMH concentrations after puberty has also been described in cattle [1,8] and sheep [9]. Serum AMH concentrations were found to be inversely correlated with testosterone concentrations in humans, horses and cattle [8] but not in sheep [9] or Asian/African elephants [10]. In Asian and African elephants it was also demonstrated that serum AMH concentrations were not statistically different between prepubertal (<7 years) and postpubertal animals (11–35 years), but were significantly lower in aged animals (>36 years) [10].

Abnormalities in expression of AMH or its receptors in males have been associated with several pathological conditions

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including abnormal sexual differentiation and testicular neoplasia. In dogs, expression of AMH was found to be a useful marker for immature Sertoli cells, neoplastic Sertoli cells [11–13], and testicular atrophy [14]. AMH concentrations are useful for the diagnosis of persistent Müllerian duct syndrome in dogs [15]. In cattle it has been demonstrated that AMH was expressed in the ovaries of fetal freemartins and young freemartins less than 5–6 months of age. By 11 months of age AMH expression was absent [16].

AMH expression in Sertoli cells persists beyond puberty in cryptorchid testes [7,17]. This results in high serum concentrations of AMH in cryptorchid animals and may be useful for the diagnosis of the condition in calves [18] and horses [7,19,20]. It is important to note that AMH concentrations were similar in intact calves and calves with inguinal testes [21].

Camelidae are economically important in several areas throughout the world. However, reproductive patterns in this species and particularly in males remain poorly studied. Reproductive patterns in camelids present several differences when compared to that of other large animal domestic species. Age at onset of puberty in alpacas varies from 12 to 24 months [22]. The incidence of pathological conditions in breeding alpacas was 18.2% (testicular hypoplasia 10%, cryptorchidism 5.7%, and ectopic testes 2.5%) [22]. Characterization of AMH secretion in this species would be fundamental in further understanding of testicular development and function, but also for clinical and pathological evaluation in cases of testicular abnormalities and infertility. The objectives of the present study were to 1) compare AMH serum concentrations in prepubertal and adult male alpacas and 2) determine the effect of castration on these concentrations in normal adult males to provide a biological validation of a commercial test for AMH in alpacas.

2. Material and methods

2.1. Sample collection

Healthy prepubertal male alpacas (n = 15; n = 5 per age groups of 6, 7, and 8 months) and healthy, reproductively sound adult male alpacas (n = 5; age 5–9 years) were included in the present study. All animals were housed at the Veterinary Teaching Hospital at Washington State University. A single blood sample was collected from the prepubertal males. Blood samples were collected from the adult males pre-castration and 24 h post-castration. All blood samples were collected by jugular venipuncture. Serum was harvested and stored at −80 °C until it was assayed for AMH and testosterone concentrations.

All adult male alpacas underwent a routine castration. Anesthesia was achieved with an intramuscular (IM) injection of a combination of 3 mg/kg ketamine, 0.3 mg/kg xylazine, and 0.03 mg/kg butorphanol. Males were restrained in lateral recumbency and castrated using a scrotal approach and closed technique. Each testis was removed following transfixing ligation of the spermatic cord with absorbable suture. The castration incision sites were left open for second intention healing.

Experimental protocol was approved by the Institutional Animal Care and Use Committee (IACUC), Washington State University.

2.2. Serum hormone analyses

Serum AMH concentrations were determine in duplicate (20-μl aliquots) according to the manufacturer's instructions. Preliminary investigation used a non-quantitative assay but affirmed that AMH was detectable (SpayCheck®, Preventia LLC, Siasconset, MA 02564). A quantitative assay (AMH ELISA test Kit Cat #KAMH-01; Preventia Diagnostics Inc.) which utilizes the same antisera with calibrators (AMH Calibrator Cat# KAMH-3) was subsequently used to provide to provide quantitative estimates of concentrations. The intra-and inter-assay coefficients of variability were 2.9 and 3.1%, respectively. Serial dilution of sample yielded a straight line with a slope of 1.0 ($r^2 = 0.99$).

Testosterone concentrations were determined by radioimmunoassay (RIA) essentially as published previously [23]. Serum (duplicate 0.5 ml aliquots) was extracted with ethyl ether, the extract was dried and reconstituted. The RIA uses antisera generated in-house and tritiated testosterone tracer (New England Nuclear, Pitscataway, NJ). The sensitivity of the assay is 15 pg/ml and aliquots of extracts are adjusted to fall in the middle of the standard curve. Intra- and inter-assay co-efficient of variation are <10%.

2.3. Statistical analysis

Statistical analysis was performed using a commercial software (Statistix 10®, Analytical Software, Tallahassee, FL.) The effect of age on testosterone and AMH serum concentrations was evaluated using a one-way ANOVA. Scheffe test was used for pairwise comparison between age groups. The pre- and post-castration concentrations of testosterone and AMH were compared using a paired-T test. Pearson correlation was examined between serum testosterone and AMH levels. Significance was set at $P < 0.05$.

3. Results

The mean ± SEM of serum AMH and testosterone for different ages is reported in Table 1. There was no significant difference ($P = 0.34$) in serum AMH concentration between prepubertal and adult intact male alpacas. Serum testosterone concentrations were significantly lower ($p < 0.05$) in prepubertal males compared to adult intact males. There was a weak but significant correlation between testosterone and AMH serum concentration ($r = 0.45$, $P = 0.047$).

The mean ± SEM of serum AMH concentrations in adult males pre- and 24 h post-castration are reported in Fig. 1. In adult male alpacas, there was a 77% decrease in serum AMH concentrations at 24 h post-castration, representing a significant difference ($P < 0.05$) between AMH concentrations in intact adult male alpacas and castrated adult male alpacas.

4. Discussion

To the authors' knowledge this is the first study to report on serum AMH concentrations in male alpacas. Although AMH is a conserved glycoprotein among species, differences still exist in the

Table 1
Mean (±SEM) of serum AMH (pg/ml) and testosterone (pg/ml) concentrations in intact male alpacas of different age groups (n = 5 per group).

Age	Mean AMH (SEM)	Range AMH	Mean Testosterone (SEM)	Range Testosterone
6 months	549.9 ± 120.8[a]	251.2–917.7	84.1 ± 28.8[a]	26.8–182.9
7 months	789.4 ± 172.3[a]	336.4–1333.7	143.1 ± 35.8[a]	62.8–234.1
8 months	597.5 ± 177.3[a]	231.5–1064.6	105.6 ± 50.9[a]	22.75–305.0
>5years	938.7 ± 175.9[a]	571.51–1593.4	7594.0 ± 2011.7[b]	2811.4–14343

[a,b] Significance was set at $p < 0.05$.