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Changes in peripheral anti-Müllerian hormone concentration and their relationship with testicular structure in beef bull calves

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ABSTRACT

The aim of this study was to clarify the time-course of changes in anti-Müllerian hormone (AMH) and testosterone (T) concentrations in peripheral blood and to determine the relationships between blood AMH concentration and testicular development during the early postnatal and prepubertal periods in beef bull calves. A total of 17 Japanese Black bull calves were enrolled in this study. The wk in which the calf was born (within 6 d after birth) was defined as M 0. Blood samples were taken once in every mo from M 0 to M 6 from each bull calf, and plasma AMH and T concentrations were determined. Of the 17 calves, 10 were castrated at 6 mo of age (prepuberty) and the right testis was histologically examined. Plasma AMH concentration (means ± SE) at M 0, 1, and 2 were 123.5 ± 9.8, 189.6 ± 18.7, and 254.6 ± 14.1 ng/mL, respectively. From M 0 through M 2, plasma AMH concentration was significantly greater each mo than in the previous mo ($P < 0.05$); however, plasma AMH concentration significantly decreased over the last 3 mo of the study ($P < 0.05$). The average age at which plasma AMH concentration was the highest was 2.3 ± 0.1 mo of age. Plasma T concentration significantly increased from M 0 (0.18 ± 0.02 ng/mL) until M 6 (6.52 ± 1.41 ng/mL). Plasma AMH and T concentrations at M 4, 5, and 6 were significantly negatively correlated ($P < 0.05$). Linear regression did not reveal a significant relationship between Sertoli or Leydig cell numbers and plasma AMH or T concentrations, respectively. In conclusion, blood AMH concentration peaks at 2 mo of age and is negatively correlated with blood T concentration from 4 to 6 mo of age. Although prepubertal blood AMH or T concentrations did not reflect Sertoli or Leydig cell numbers at the end of the prepubertal period, blood AMH concentration may be indicative of abnormal Sertoli cells function.

1. Introduction

Anti-Müllerian hormone (AMH) is a glycoprotein hormone belonging to the transforming growth factor β family and is specifically secreted by immature Sertoli cells in male mammals [1]. Its primary role is to induce Müllerian duct regression during early male sexual differentiation [2]. During postnatal life, AMH inhibits Leydig cell differentiation from precursor cells in the testis and thus suppresses androgen production [3]; however, the details of the role and regulation of AMH in the testis are controversial.

Postnatal changes in peripheral blood AMH concentration in male humans have been described in detail [4].

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In brief, blood AMH concentration is low in the first wk after birth, increases to peak at 2 to 3 yr of age, and then remains high until the onset of puberty. As in humans, equine AMH concentration before puberty was greater than after puberty [5]. To the best of our knowledge, only one previous study has examined blood AMH concentration in cattle [6]. In that study, which reported data from 5 normal male Holstein-Friesian calves, blood AMH concentration peaked around 5 mo of age; however, the data reported from that study were not statistically analyzed.

Testicular function is generally evaluated by digital palpation and determination of testosterone (T) concentration after hCG challenge. However, the most active components of the prepubescent testis are the seminiferous tubules, which contain proliferating immature Sertoli cells that synthesize and secrete AMH and inhibin B [7]. In humans, measurement of serum AMH concentration allows estimation of the activity and number of prepubertal Sertoli cells, as well as testicular function, especially FSH and androgen activity [4]. To date, there are no reports that clarify the relationship between AMH concentration and Sertoli cell number and function in cattle.

The objectives of this study were: (1) to determine the time-course of changes in peripheral blood AMH and T concentrations during the early postnatal and prepubertal periods in cattle; and (2) to elucidate the relationship between blood AMH concentration and testicular development in prepubescent calves.

2. Materials and methods

All experimental procedures were approved by the Animal Care and Use Committee of the University of Miyazaki (Approval No. 2013–016) and were conducted in accordance with EU Directive 2010/63/EU.

2.1. Animals

A total of 17 Japanese Black bull calves were enrolled in this study. All of the calves were clinically healthy and reared at Sumiyoshi Livestock Science Station, Faculty of Agriculture, University of Miyazaki, Japan. The animals were fed twice daily at 9:00 a.m. and 4:00 p.m., and water was available ad libitum.

2.2. Experimental design

The wk in which the calves were born (within 6 d after parturition) was defined as M 0. Blood samples were taken once in every mo from M 0 until the calves were 6 mo old (M 6). The mean sampling interval was 29.8 ± 3.1 d (min: 26 d, max: 36 d). Of the 17 calves, 10 were castrated at 6 mo of age (prepuberty) [8]; in cattle, immature Sertoli cells have finished proliferating by 6 mo after birth [9].

2.3. Blood sampling

All blood samples were collected in the morning (9:00–10:00 a.m.) into heparinized tubes. Samples were centrifuged at 1,408 × *g* for 15 min at 4°C to obtain plasma, which was stored at −30°C until use.

2.4. Hormone (AMH and T) assays

Plasma AMH concentration was measured using a commercially available ELISA (AMH Gen II ELISA #A73818 and A73819; Beckman Coulter Inc, Brea, USA) and plasma T concentration was measured using a commercially available Enzyme-Linked Fluorescent Assay (ELFA; VIDAS testosterone, SYSMEX bioMérieux Co, Ltd, Tokyo, Japan). Both assays had been previously validated for cattle [10–12]. The sensitivity and intra-assay and interassay coefficients of variation for the AMH assay were 0.11 ng/mL, < 6.8% and <9.4%, respectively, as established in our laboratory and for the T assay were 0.10 ng/mL, 5.7% and 4.7%, respectively [12]. Samples that did not meet the threshold for assay sensitivity were assumed to be the nadir for each assay.

2.5. Testes collection and measurements

Of the 17 calves in this study, 10 were castrated at 6 mo of age, and their testes collected for examination of testicular structures. Xylazine (0.04 g/100 kg; Celactal 2% injections, Bayer Yakuhin Ltd, Tokyo, Japan) was administered intravenously before castration. Each calf was then held in dorsal recumbency and the scrotum was washed and cleaned. The testes were pushed firmly against the skin, and 2 scrotal skin incisions were made on either side of the median raphe. The testes were then exteriorized and removed using scissors after ligation of the pampiniform venous plexus. After castration, each calf received a single intramuscular injection of 3 million units of procaine penicillin G (Meiji Seika Pharma Co, Ltd, Tokyo, Japan). The right testis was fixed in 10% phosphate-buffered formalin after its weight and major and minor axis lengths were recorded. The mid-section of the fixed testis was embedded in paraffin, cut into 4 μm-thick sections, and stained with hematoxylin and eosin. The methods used to measure testicular structures have been described previously [13–16].

2.5.1. Testicular volume, seminiferous tubule, and Sertoli cell calculations

The following calculations were made based on the testicular measurements: (1) testicular volume (V) was calculated as $V = \pi \times (\text{minor axis length}/2)^2 \times \text{major axis length}$. (2) The average diameter of a seminiferous tubule (D_{ST}) and the average number of Sertoli cells in 10 seminiferous tubules (N_{10ST}) were calculated based on 5 randomly chosen fields of view at magnification (200×). (3) The ocular was fitted with a quadratic grid micrometer. The percentage of V occupied by seminiferous tubules (%ST) was determined as the percentage of seminiferous tubules in the 600 grid fields in fields of view (200×) taken at random within the mid-section of the testis. The volume of ST in the testis (V_{ST}) was calculated as V × %ST. (4) The volume of 10 seminiferous tubules in 1 field was calculated as $V_{10ST} = \pi \times (D_{ST}/2)^2 \times 4\ \mu m \times 10$. The number of Sertoli cells in a testis was determined by $N_{10ST} \times V_{ST}/V_{10ST}$.

2.5.2. Leydig cell volume, number, and structure calculations

In 3 of 10 calves, Leydig cell number in the testis was not determined because of sample loss. The volume and