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NOTE

Theriogenology

A case of equine cryptorchidism with undetectable serum anti-Müllerian hormone

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ABSTRACT. Serum anti-Müllerian hormone (AMH), a marker of equine cryptorchidism, is detectable in intact and cryptorchid stallions but not in geldings because it is secreted from Sertoli cells. A 4-year-old uncastrated Thoroughbred racehorse had no visible testes; therefore, the horse was considered a bilateral cryptorchidism. However, the serum AMH was undetectable (<0.08 *n*g/m*l*). Human chorionic gonadotrophin (hCG) stimulating test result indicated that the horse was a gelding. The results of sex chromosomal analysis and sequence analysis of *SRY* gene suggested that the horse was a genetically-intact stallion (X/Y). Only one small degenerative testis was present in the abdominal cavity. The reasons of undetectable serum AMH levels and negative response to hCG stimulation might be low numbers of Sertoli and Leydig cells. This study reports a case of serum AMH-undetectable cryptorchid stallion.

KEY WORDS: anti-Müllerian hormone, cryptorchidism, monorchidism, stallion

Cryptorchidism is estimated to affect 5–8% of male foals, and most of these are unilateral [2]. For easy handling, male horses are often castrated rather than sterilized. Occasionally, some unilateral cryptorchid horses undergo removal of the only normal testis in the scrotum and are erroneously considered geldings, in spite of retaining one testis. These hemi-castrated unilateral or bilateral cryptorchid horses are male, but it is difficult to distinguish these from geldings by appearance. In addition, some intact colts' testes are easy to raise temporarily by excitement and difficult to be visible externally. Thus, differentiation between male and gelding is typically based on appearance; however, objective methods are also required.

Differentiation between cryptorchid and gelding horses is accomplished by measuring concentrations of blood basal testosterone [3, 6] and basal estrone sulfate [3] or by using human chorionic gonadotropin (hCG) stimulation tests [3, 5]; however, the diagnostic accuracy is inadequate, particularly in prepubescent animals. Recently, the usefulness of serum anti-Müllerian hormone (AMH) was reported [4, 11]. In humans, cryptorchidism and anorchidism can be distinguished by measuring serum AMH [7, 9, 10]. Serum AMH is detectable in intact and cryptorchid stallions, but not in geldings; this is because AMH is secreted from only Sertoli cells [4, 11]. Here we report a case of serum AMH-undetectable cryptorchid Thoroughbred stallion. This study was approved by the Animal Care and Use Committee at JRA Hidaka Training and Research Center.

A 4-year-old Thoroughbred racehorse was uncastrated but had no visible and non-palpable testes in the scrotum; consequently, the horse was considered a bilateral cryptorchidism. The appearance of external genitalia was normal male.

hCG stimulating test and serum AMH measurement were performed to reveal the undescended testes. Serum testosterone concentrations before and after (1, 2, 3, 24, 48, and 72 hr) hCG administration (9,000 IU, intramuscularly) were measured by chemiluminescent enzyme immunoassay (PATHFAST, LSI Medience Corporation, Tokyo, Japan) and were found to be consistently low (<0.1 *n*g/m*l*) [12]. The serum AMH concentration was undetectable (<0.08 *n*g/m*l*) by enzyme-linked

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Table 1. Segregation data of the respective alleles of X- and Y-linked markers indicating sex chromosome aberrations

Chromosome	Y	X						Y/X	Y
Marker type	Microsatellite DNA							Gene	
Marker name	Eca. YH12	Eca. YM2	Eca. YA16	LEX003	TKY38	TKY270	LEX026	AMEL gene	SRY gene
	98	119	157	200	129	168	297	AMELY/AMELX	SRY

The respective allele sizes of microsatellites are designated based on the measured size by a capillary DNA sequencer. SRY, sex-determining region of the Y chromosome gene; AMEL, amelogenin gene.

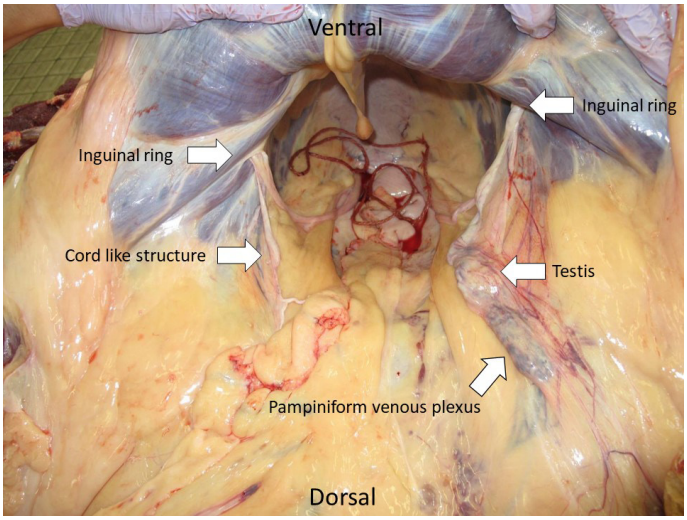

Fig. 1. Intra-abdominal cranial-caudal view by removal of digestive tracts. Small testis and pampiniform venous plexus were observed on right side. Only cord-like structure without any object were observed on left side.

immunosorbent assay (AMH Gen II ELISA, #A73818, Beckman Coulter, CA, USA) [11].

Polymerase chain reaction amplification of sex chromosome DNA markers was performed to confirm sex chromosome abnormalities. Four microsatellites on X chromosome (*LEX003*, *TKY38*, *TKY270*, and *LEX026*), three microsatellites on Y chromosome (*Eca.YH12*, *Eca.YM2*, and *Eca.YA1*), *SRY* gene on Y chromosome, and *AMEL* gene on X/Y chromosomes were used. All the DNA markers were genotyped using procedures described by Kakoi *et al.* [8], with minor modifications. All the microsatellites on the X and Y chromosomes as well as the *SRY* gene were genotyped as a single allele (Table 1). The *AMEL* gene was genotyped as AMEL-X/AMEL-Y. These results indicated that the horse had X and Y chromosomes (X/Y-male).

Sequence analysis of the *SRY* gene on the target horse, the sire of the target horse, and two normal stallions as control were performed. The results showed the presence of the *SRY* gene without DNA mutations.

This horse was euthanized because of an unrelated issue, and an autopsy was conducted. No surgical scar was present on the scrotal skin. In the abdominal cavity, bilateral cord-like structures ran through the dorsal aspect to the inguinal rings. The right one had a small testis (3.5 × 2.0 × 1.0 cm) and a pampiniform venous plexus. The left one was cord-like (Fig. 1). These tissues were fixed in 20% neutral buffered formalin and embedded in paraffin wax for histopathological examination. Next, 4-μm sections were cut and stained with hematoxylin and eosin. In the right testis, the number of seminiferous tubules was decreased, and interstitial fibrosis was observed diffusely (Fig. 2A). In the interstitial space, Leydig cells were observed. There was complete absence of spermatogenesis, and the remaining seminiferous tubules contained only Sertoli cells (Fig. 2B and 2C). These tissues were not observed on the left side cord structure.

Our results suggest that the horse was a genetically-intact stallion (X/Y) with monorchidism. Monorchidism or anorchidism in horses is very rare [1]. In monorchid cases, the other testis can be scrotal, inguinal, or abdominal in location [1]. The testis in our case was abdominal, very small compared with common cryptorchid testis, and covered with fibrotic tissues. This was suspected to be agenesis or degeneration, but we could not distinguish these conditions [1].

We have measured more than 50 serum samples in bilateral cryptorchid horses, revealing an average AMH concentration of 35.6 ± 14.1 *ng*/m*l* (mean ± standard deviation). In general, serum AMH concentrations in cryptorchid stallions tends to be higher than that in intact stallions [4, 11], but in the horse described here, AMH concentration was undetectable, possibly due to low numbers of Sertoli cells in the small testis. On the other hand, the accuracy of hCG stimulating test was 94.6% [6], but the result showed false negative in this case. The reasons for this might be immaturity based on abdominal retention and low numbers of Leydig cells. In conclusion, we reported a case of serum AMH-undetectable cryptorchid stallion; therefore. We have to pay attention to the exception like this case, when the serum AMH is undetected in a stallion without surgical history.

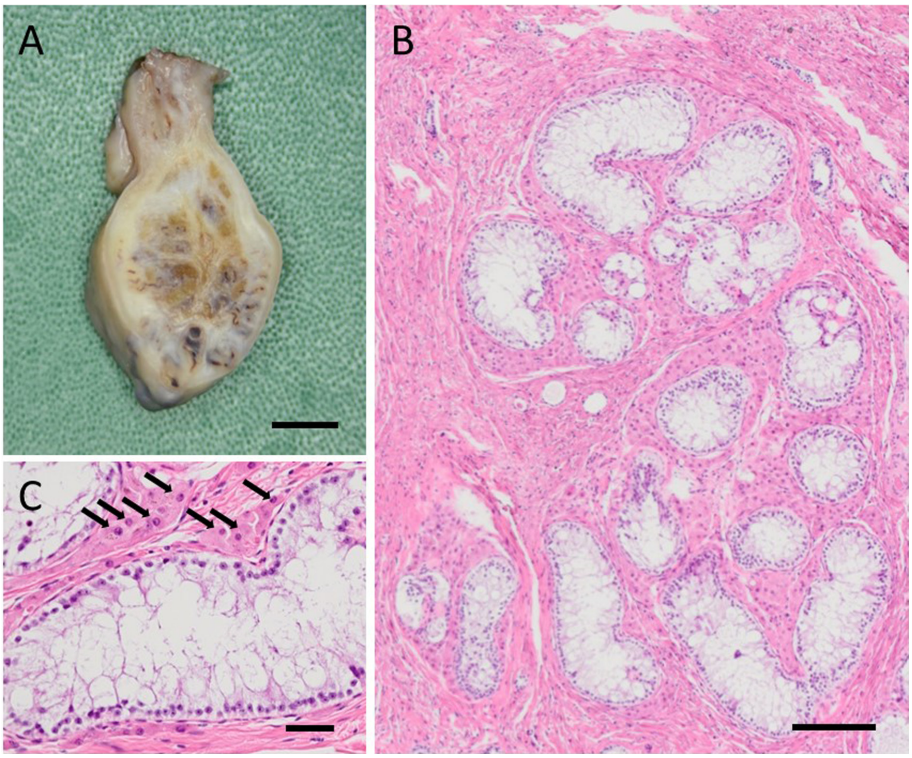

Fig. 2. Right side testis. (A) Cross section of testis. The testis was covered with thick connective tissue. Bar=5 mm. (B) The number of seminiferous tubules decreases with interstitial fibrosis. Hematoxylin and eosin. Bar=200 μm. (C) Seminiferous tube surrounded by Sertoli cells was observed. The spermatocyte was absent. In the interstitial space, eosinophilic Leydig cells (arrow) with round nuclei were observed. Hematoxylin and eosin. Bar=50 μm.

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