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Case Report

Monorchidism in a Phenotypic Mare With a 64,XY, SRY-Positive Karyotype

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ABSTRACT

Disorders of sexual development (DSD) are associated with atypical chromosomal, gonadal, or phenotypic sex. It is likely that the number of cases of DSD are underestimated in the equine population. Monorchidism in the horse is very rare. This case report describes the clinical assessment of a phenotypic mare with stallion-like behavior which led to the diagnosis of a DSD. A 4-year-old Quarter Horse mare presented in good body condition, with normal external genitalia for a mare, and normal mammary glands with two bilaterally symmetric teats. No uterus, cervix, or gonads were detected on transrectal palpation. Transrectal ultrasonography revealed a single gonad in the right dorsal abdomen with the morphologic appearance of a testicle. Presurgical hormonal evaluation revealed elevated serum testosterone and anti-Müllerian hormone (AMH) concentrations. The right gonad was successfully removed via standing exploratory laparoscopy and submitted for histopathology. No gonad was identified on the left side during laparoscopy. Histopathologic examination confirmed that the excised gonad was a testicle. Cytogenetic and molecular analysis revealed a 64,XY, SRY-positive chromosomal constitution. Hormonal evaluation 5 weeks after surgery revealed low serum testosterone and AMH levels. A diagnosis of monorchidism was based on ultrasound examination, laparoscopic exploration of the abdomen, removal of a single gonad, and a subsequent decrease in serum testosterone and AMH concentrations to basal levels. In summary, a combination of clinical signs, endocrine evaluation, chromosomal and molecular analysis, and histopathology can be used in the diagnosis of DSD conditions.

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1. Introduction

Disorders of sexual development (DSD) may result when there is a lack of coordination between chromosomal, gonadal, or phenotypic sex [1]. Historically, terms such as intersex, sex reversal, hermaphroditism, and pseudohermaphroditism were used to describe such cases in human and veterinary medicine. Human medicine was the first to adopt new DSD terminology and more recently veterinary medicine has trended towards classifying cases of abnormal sexual development according to the sex chromosome constitution of the animal [2,3]. Reports investigating DSD in the horse date back to the early 1970s [4–6]. Cases of DSD can have many different clinical presentations including, but not limited to

infertility, subfertility, atypical or ambiguous external genitalia, and behavioral abnormalities [4,7,8].

Cytogenetic and molecular analysis allows for better understanding and diagnosis of a disorder of sexual development. The most common types of DSD associated with abnormal sex chromosome constitution in the horse include 63, XO; 64,XX, SRY-negative; 64,XY, SRY-positive; and 64,XY, SRY-negative [6,9]. Unlike other species, there have been no reported cases of XX, SRY-positive individuals in the equid population to date. In addition to cytogenetic analysis, physical examination, reproductive evaluation, hormonal analysis, and surgical exploration may be utilized to diagnose cases of DSD in the horse.

Monorchidism has been reported in several species including the human, canine, feline, and horse [10–13]. Monorchidism is defined as the complete absence of one testicle [14]. This disorder is rare in the horse [15]. It is thought that the monorchid condition occurs due to either testicular agenesis or testicular degeneration, with the condition in horses most likely due to degeneration [10].

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Animal welfare/ethical statement: The manuscript reports a clinical case and no ethical approval was required.

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Fig. 1. Perineum of the phenotypic mare with 64,XY, SRY-positive DSD.

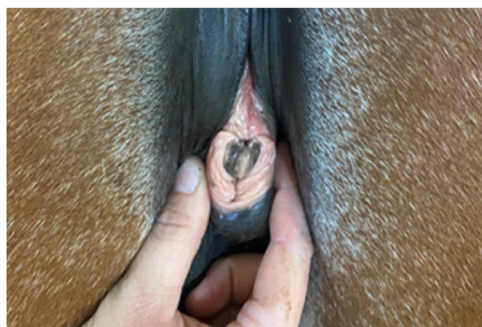


Fig. 2. Clitoris of the mare.

Diagnosis of monorchidism in horses is based on hormonal analysis and exploratory surgery or laparoscopy.

The goal of this case report is to describe the clinical assessment and diagnostic procedures utilized to confirm a disorder of sexual development.

2. Case History

A 4-year-old Quarter Horse mare was initially evaluated by a referring veterinarian for aggressive, stallion-like behavior around other mares. A blood sample was submitted to a diagnostic laboratory^a which reported a serum testosterone level of 3,650 pg/mL (reference range for a mare: <50 pg/mL).

3. Clinical Examination

On presentation to the Johnson Family Equine Hospital at Colorado State University, the mare's general physical examination was considered normal for a mare. Oral examination revealed four small canine teeth typical for that of a mare. Examination of the perineum revealed a normal vulva and clitoris (Figs. 1 and 2). In addition, two small bilaterally symmetric teats were evident (Fig. 3).

Transrectal palpation failed to identify uterine, cervical, or gonadal structures. Transrectal ultrasound revealed a structure in the right abdomen with the uniformly echogenic appearance of a testi-

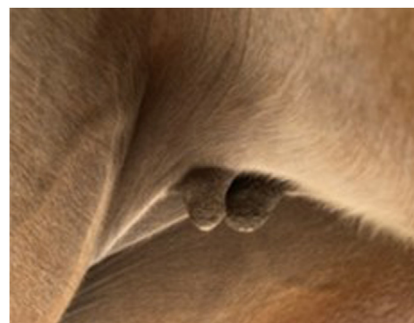


Fig. 3. Two bilaterally symmetric teats.

cle. No gonad was detected on the left side of the abdomen. Vaginal speculum examination revealed a shortened vaginal cavity with no external cervical os.

3.1. Initial Endocrine Evaluation

A serum sample submitted to an endocrine laboratory^b revealed markedly elevated levels of testosterone and anti-Müllerian hormone (AMH), while concentrations of inhibin B and estrone sulfate were slightly elevated above the normal range of values for a non-pregnant mare. Interestingly, a human chorionic gonadotropin (hCG) stimulation test did not result in an increase in serum testosterone levels in blood samples collected 2 and 15 hours after intravenous administration of 10,000 units of hCG. (Table 1)

4. Laparoscopic Surgery

The mare was allowed access to water, but held without food for 12 hours prior to laparoscopic surgery. The mare was placed in standing stocks and sedated with 5 mg of detomidine hydrochloride (Dormosedan) and 5mg of butorphanol tartrate (Torbugesic) intravenously. An intravenous catheter was placed in the left jugular vein to facilitate administration of a detomidine hydrochloride CRI (constant rate infusion) of 20 mg of detomidine in 1-liter lactated Ringer's solution (LRS). The CRI was titrated to effect with a total of 800 mL administered over the course of the laparoscopic procedure. The right and left paralumbar flanks were clipped and aseptically prepared. Mepivacaine hydrochloride (2%), USP (Carbocaine-V) was injected at each laparoscopic portal site bilaterally. The surgical site was aseptically draped in a normal manner. Laparoscopic exploration of both sides of the abdomen were performed using standard technique. A stab incision was made through the skin and external abdominal oblique fascia, on the left side of the abdomen, midway between the last rib and the ventral aspect of the tuber coxae. A 10 mm blunt trocar cannula was inserted in the incision and gently advanced through the body wall into the abdominal cavity. After abdominal entry was confirmed, the abdomen was insufflated with CO₂ to a pressure of 10 to 15 mm Hg. Two additional portal sites were created in the same manner as the first. The second portal was made approximately 7 cm dorsal and cranial to the first and the third portal approximately 7 cm ventral to the first.

A 30-degree laparoscope was inserted into the most dorsal portal on the left side. A left gonad was not identified, and no distinct uterus was visualized. The right side was approached using the same portal locations as on the left. On the right side, a gonad, with the gross appearance of a testicle, was visualized in the dorsal abdomen (Fig. 4). A laparoscopic injection needle was inserted into the middle portal and utilized to inject 20 mL of 2% mepivacaine hydrochloride, USP (Carbocaine-V) into the tissue surrounding the testis. Traumatic grasping forceps were inserted into

Table 1
Initial endocrine evaluation in a mare with 64,XY, SRY-positive DSD.

Sample	Testosterone (pg/mL)	Inhibin B (pg/mL)	Estrone Sulfate (ng/mL)	AMH (ng/mL)
Pre-Hcg	731	76	11	96
2 hr Post-hCG	762	-	-	-
15 hr Post-hCG	773	-	-	-
Laboratory Reference Range ^b				
Non-pregnant Mare	20–45	2–60	0.1–6	0.1–6.9
Gelding	< 50	n/a	< 0.1	n/a
Cryptorchid	100–500	n/a	35–60	> 0.15
Stallion	800–2,000	50–300	140–200	30–200

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Fig. 4. Gonad present in the right abdomen, consistent with the appearance of a testicle.

the most ventral portal and firmly grasped the testis. A LigaSure (Medtronic, Minneapolis, MN) device was inserted into the middle portal and was used to separate the vascular attachments dorsally. Once the gonad was excised it was maneuvered to the abdominal wall. The ventral portal was dilated, and the testis was removed from the abdomen.

The external fascia of the external abdominal oblique muscle was closed utilizing 0 Maxon (Medtronic, Minneapolis, MN) in a simple continuous pattern. The skin at all portal sites was closed with either a simple continuous or simple interrupted pattern using 0 Surgipro (Medtronic, Minneapolis, MN). Recovery from standing sedation was uneventful.

5. Additional Diagnostic Tests

5.1. Histopathology

The excised testicle was submitted to a diagnostic laboratory^c for histopathology. Degenerate seminiferous tubules with vacuolated Sertoli cells were present. There was no evidence of active spermatogenesis. A few small epididymal lumina were observed and noted to be devoid of sperm. Embedded within the testicular tissue was an area of well-differentiated adrenal cortical tissue.

5.2. Follow up Endocrine Evaluation

Five weeks after surgery, blood samples were collected from the horse and submitted to an endocrine laboratory^b for evaluation. Serum concentrations of testosterone pre- and postadministration of hCG, AMH, estrone sulfate, and inhibin B were all within the laboratory reference range for a gelding (Table 2).

5.3. Chromosomal Analysis

Blood samples were submitted to a cytogenetics laboratory^d for analysis, which revealed a normal chromosome number ($n = 64$), normal chromosome morphology, and a mammalian male sex chromosome constitution (XY)(Fig. 5). Chromosomes were stained

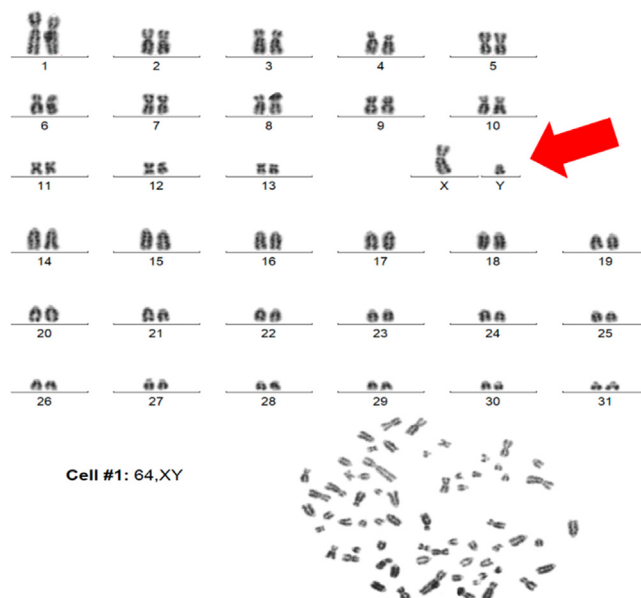


Fig. 5. Karyotype of the horse. Red arrow indicating the X and Y sex chromosomes.

by GTG-banding and once identified were arranged into karyotyping according to the International System of Cytogenetic Nomenclature of the Domestic Horse (ISCHN 1997).

The Y-linked SRY gene was tested by PCR and the X-linked androgen receptor (AR) gene served as a positive control for PCR amplifications. The primers utilized included: SRY-forward 5'-TGCAATCATGGTGTGGTCTC-3', SRY-reverse 5'-ATGGCAATTTTCGGCTTC-3', AR-forward 5'-AGCAGCAACAGGAGACCAAGT-3', AR-reverse 5'-GCTTAAGCCTGGGAAAGTG-3' [16]. Molecular analysis of the sex chromosomes revealed the presence of the male specific sex determining region of the Y chromosome (SRY). The AR gene was detected on the X chromosome.

An additional blood sample was submitted to a second genetics laboratory^e for evaluation of androgen insensitivity syndrome (AIS). The test results did not detect any known mutations in the AR gene.

6. Diagnosis

The phenotypic mare with stallion-like behavior was diagnosed as a monorchid, 64,XY, SRY-positive disorder of sexual development.

7. Discussion

On initial presentation a thorough medical and reproductive history was obtained. The elevated serum testosterone level and report of aggressive, stallion-like behavior lead to differential di-

Table 2
Endocrine evaluation 5 weeks after surgical removal of the cryptorchid testis.

Sample	Testosterone (pg/mL)	Inhibin B (pg/mL)	Estrone Sulfate (ng/mL)	AMH (ng/mL)
Pre-hCG	27	7	4	0.06
1 hr Post-hCG	21	-	-	-
2 hr Post-hCG	25	-	-	-
Laboratory Reference Range ^b				
Nonpregnant Mare	20–45	2–60	0.1–6	0.1–6.9
Gelding	< 50	n/a	< 0.1	n/a
Cryptorchid	100–500	n/a	35–60	> 0.15
Stallion	800–2,000	50–300	140–200	30–200

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agnoses of pregnancy, estrus, disorder of sexual development, granulosa-theca cell tumor (GTCT), and administration of androgenic or anabolic steroids [17–21]. The mare had been owned by the same people since birth and there were no reports of iatrogenic hormone therapy. Physical examination and reproductive evaluation revealed a phenotypic mare with the presence of one gonad, consistent with the ultrasonographic appearance of a testicle. These findings led to a clinical diagnosis of a disorder of sexual development.

The normal karyotypes for the male and female horse are 64,XY and 64,XX, respectively. Disparity in the sex chromosome constitution of the horse is one of the main factors resulting in DSD [21]. The most common sex chromosome aberration in the horse is 63,X monosomy and its mosaic forms [6,9,22–25]. The second and third most common chromosomal abnormality are 64,XY, SRY-negative DSD and 64,XY, SRY-positive DSD, respectively [9,26]. Chromosomal analysis and PCR analysis should be performed to definitively diagnose a DSD. In the current case, the chromosomal analysis and PCR analysis indicated that the phenotypic mare was a 64,XY, SRY-positive disorder of sexual development.

In humans, the most common cause of 46,XY DSD is Androgen Insensitivity Syndrome (AIS) [27,28]. AIS results from mutations in the androgen receptor (AR) gene found on the X chromosome [27–29]. This can result in individuals with phenotypic female external genitalia, ambiguous external genitalia, or normal male external genitalia [28]. There are 5 known AR gene variations in the horse, resulting in clinical cases of AIS in comparison to over 1,500 AR gene mutations reported in human cases [7,30–33]. AIS was suspected based on the findings in this clinical case. Evaluation of the five known AR mutations in the horse did not reveal any abnormalities. It is possible that the horse has a mutation in the AR gene that has yet to be identified. Five-alpha-reductase deficiency, resulting from a mutation in the SRD5A2 gene, has also been reported in humans in cases of 46,XY, DSD [34]. This condition has not yet been reported in the horse.

The minimal rise in testosterone level following hCG stimulation was an interesting finding. Inadequate response of testosterone level following hCG administration has been reported in males with AIS and is not completely understood [35]. A multitude of etiologies exist including defective feedback loop and alternative pathways for androgen synthesis, all of which would be difficult to prove in this case [36].

Monorchidism is rare in the horse [10,37,38]. Monorchidism can be diagnosed by a combination of reproductive endocrine assessment and exploratory laparotomy or laparoscopy [39]. In this case, initial serum testosterone and anti-Müllerian hormone concentrations were elevated outside the reference range for a nonpregnant mare or gelding. Ultrasound examination ruled out granulosa-theca cell tumor and pregnancy as potential causes of elevated AMH and testosterone and indicated that the single gonad detected was a testicle. A standing exploratory laparoscopy procedure was performed to remove the right gonad. No left gonad was noted during the procedure. Histopathology confirmed the excised gonad was a

testis. Adrenal cortical tissue was observed in the testis. This was an unexpected finding, but has been reported in the horse and other species [40]. Subsequent endocrine evaluation revealed that testosterone and AMH levels had declined to the reference range for a gelding, confirming the diagnosis of monorchidism.

Monorchidism was previously reported in a pony mare with a rudimentary penis suspected of having a DSD, but no cytogenetic and molecular analyses were performed [8]. Thus, the current case, is the first to report a 64,XY, SRY-positive genotype in a phenotypic mare with monorchidism. A combination of patient history, clinical signs, hormonal analysis, laparoscopy, histopathology, cytogenetic analysis, and molecular analysis were used to obtain a definitive diagnosis.

Authors' Contributions

Brittany Middlebrooks, Patrick McCue, Emily May, Christina Divine were responsible for clinical work-up and sample collection. Brad Nelson and Charlie Barton were responsible for patient care and surgical procedures. Alan Conley was responsible for endocrine analysis. Brittany Middlebrooks was also responsible for manuscript preparation and references. All authors contributed to manuscript editing.

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Referenced Diagnostic Laboratories

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^d Molecular Cytogenetics Laboratory, Texas A&M University; College Station, Texas

^eVeterinary Genetics Laboratory, University of California, Davis; Davis, California

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