Azoospermia associated with bilateral segmental aplasia of the ductus deferens in a stallion

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- Although complete azoospermia is uncommon in horses, a frequent cause for this condition is blockage at the ampullary region caused by stasis of spermatozoa.
- Low alkaline phosphatase activity in seminal fluid may be indicative of blockage of the efferent portion of the duct system.

A 3-year-old Quarter Horse halter stallion that weighed approximately 730 kg (1,606 lb) was referred to the **Kansas State University Veterinary Medical Teaching Hospital (KSU-VMTH)** for routine semen evaluation. The history given by the owner indicated that the stallion had never been sick and had not been exposed to exogenous hormones. From the age of 6 months, the horse had been vaccinated regularly against tetanus, eastern and western equine encephalomyelitis, rabies, influenza, and rhinopneumonitis. As a 4-month-old colt, a left-sided inguinal hernia was diagnosed and successfully repaired at the KSU-VMTH. The limited reproductive history included a natural mating as a 2-year-old to a mare that did not become pregnant. That same year, the owners attempted to collect semen once but did not observe spermatozoa in the recovered fluid.

The stallion was in excellent body condition, and no conformational abnormalities were found. A physical examination was performed, and all vital signs were within reference ranges. Prior to semen collection, the stallion's scrotum, testes, epididymides, and spermatic cords were palpated; no abnormalities of these structures were detected. Although the testes were considered to be large, their tone and consistency were normal, with no signs of adhesions, swelling, or pain. Epididymal tails were considered slightly enlarged but were in a normal caudal position.

For semen collection, the stallion was exposed to a mare in estrus, and the horse achieved a full erection within 2 minutes. The penis was palpated along its length, and no abnormalities were found in the body or the glans. The urethral fossa, urethral process, and diverticulae were considered normal. After the penis was palpated, the stallion was allowed to mount a phantom mare, and an artificial vagina[a] was used for collection. Sixty-five milliliters of fluid was collected, and the fluid was yellow and had a urine-like odor. On microscopic evaluation, abundant, crystal-like material was observed, but no spermatozoa were found. Within 1 hour, a second semen collection was attempted, and 55 mL of yellowish fluid containing numerous crystals but no spermatozoa was obtained. Two hours later, a third collection was performed, and 28 mL of a transparent fluid was obtained. Microscopic evaluation of the third sample also revealed the absence of spermatozoa. During all 3 semen collection attempts, the stallion thrusted normally, had a normal engorgement of the glans penis, and had multiple short and rhythmic urethral pulsations indicative of ejaculation. Immediately after, the stallion had rapid penile detumescence and a change of attitude indicating that the stallion had fully ejaculated. Fluid from the second and third semen collection attempts was centrifuged at 500 × *g* for 15 minutes. Microscopic analysis of the resulting pellet confirmed the absence of spermatozoa.

An ultrasonographic evaluation of the testes, spermatic cords, epididymides, and accessory sex glands was performed. Ultrasonographic examination of the testicular parenchyma revealed no abnormalities, with the exception of mild distension of the central vein of the left testis. Ultrasonographic measurements of the testicular **length** (**l**), **width** (**w**), and **height** (**h**) were taken from both testes to calculate the combined testicular volume by use of the formula $4/3\ \pi(h/2 \bullet l/2 \bullet w/2)$. The volume was determined to be 378 mL, which was considered large.[1] Images of the head, body, and tail of the epididymis were normal, as were both spermatic cords. Rectal palpation and ultrasonographic evaluation of the accessory sex glands included the bulbourethral glands, the prostatic lobes, seminal vesicles, and longitudinal and cross-sectional views of the ampullae. All the organs and structures were considered normal at palpation and ultrasonographically, except for the ampullae, which were considered small at palpation relative to the size of the horse. Cross-sectional measurement (diameter) of the ampullae was 1.1 cm, which was considered small for a stallion this size.[2,3] Furthermore, we were not able to identify fluid in the lumen on the cross-sectional views of the ampullae.

Although the stallion had small ampullae, an initial tentative diagnosis of azoospermia caused by stasis of spermatozoa or ampullary blockage was made. During the following week, the stallion's ampullae were massaged vigorously transrectally at least twice daily, and alternating IV injections of 20 U of oxytocin or an IM injection of 7.5 mg of prostaglandin $F2_{\alpha}$ were given 15 to 20 minutes prior to each collection. Twelve semen collection attempts were performed, resulting in volumes ranging from 15 to 25 mL. All collected fluids

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were transparent and did not have any urine contamination. Two of the collected samples had a small amount of gel that, together with the stallion's behavior, strongly suggested that the stallion was having complete ejaculations. All samples were centrifuged at 1,000 × *g* for 10 minutes, and microscopic evaluation of the resulting pellets confirmed the absence of spermatozoa, spermatazoal heads, or round spermatogenic cells. The pH in the 12 collected samples ranged from 7.43 to 7.83. Because high activities of alkaline phosphatase are detected in the epididymal fluid and may be indicative of complete ejaculation,[4-6] alkaline phosphatase activities were analyzed in the first, fifth, and tenth samples, which yielded activities of 11, 13, and 24 U/L, respectively (reference limit, > 10,000 U/L[6]).

To determine whether the perceived blockage was at the level of the colliculus seminalis, an endoscopic evaluation of the urethra was performed with a 1-m-long, 8-mm-diameter video-endoscope.[b] The penile and pelvic portions of the urethra were normal. Bulbourethral, prostate, and urethral gland ducts were viewed and considered normal. The colliculus was grossly normal, and patency of the seminal vesicle openings at the level of the colliculus was evaluated by canulating both sides with a 1-m polyurethane tubing with a bevel shape through the endoscope; no abnormalities were detected.

The results of 12 semen collection attempts preceded by ampullary massage and oxytocin or prostaglandin treatment, together with clinical findings and the low activities of alkaline phosphatase, suggested a tentative diagnosis of complete azoospermia caused by an obstruction or blockage in the efferent duct system. However, the possibility of azoospermia caused by spermatogenic arrest at the testicular level or epididymal blockage was still plausible. To rule out the possibility of azoospermia caused by primary testicular dysfunction, testicular biopsy, fine needle aspiration from the epidydimal tail, and fine needle aspiration of the ductus deferens were considered. Spermatozoa in the ductus deferens would rule out the possibility of primary testicular dysfunction, as well as an epididymal blockage.

Ductus deferens cannulation was performed with general anesthesia. The horse was premedicated with xylazine (0.4 mg/kg [0.18 mg/lb], IV), and general anesthesia was induced with guaifenesin[c] (60 mg/kg [27.2 mg/lb], IV) and thiopental[d] (5 mg/kg [2.3 mg/lb], IV) administered to effect. Anesthesia was maintained with isoflurane.[e] With the stallion in dorsal recumbency, surgical preparation of the inguinal area was performed. A 10-cm skin incision on the left side over the spermatic cord was made, the subcutaneous tissue was bluntly dissected, and the common vaginal tunic was incised. A Penrose drain was wrapped around the cremaster muscle to have better retraction and exposure of the spermatic cord. The ductus deferens was exposed dorsal to the epididymal tail just before it entered the external inguinal ring and prior to becoming part of the spermatic cord. The ductus deferens was considered engorged, and approximately 3 mL of creamy fluid was aspirated from the lumen of the ductus deferens with a 25-gauge needle. Evaluation of this sample revealed spermatozoal concentration of 1.5 × 10^9 cells/mL with approximately 30% motility. Morphologic evaluation of spermatozoa suspended in phosphate-buffered 10% formalin via phase contrast microscopy revealed detached heads (56%), proximal protoplasmic droplets (19%), coiled tails (2%), midpiece abnormalities (6%), and morphologically normal spermatozoa (17%).

During surgery, the ductus deferens was cannulated with a 12-gauge catheter.[f] An attempt was made to force sterile saline (0.9% NaCl) solution into the ductus deferens in an effort to determine whether the fluid could be observed ultrasonographically at the ampullary level; we were unable to infuse the saline solution, and none was seen in the ampullae. Intraoperative endoscopic evaluation of the urethra also revealed no evidence of fluid draining from the colliculus. A 6-F, 70-cm urethral catheter[g] was used in an attempt to cannulate the entire length of the ductus deferens. After threading the catheter approximately 15 to 20 cm into the ductus deferens, resistance was met that made it impossible to continue the cannulation. The incision on the ductus deferens was closed with 4-0 **polydioxanone (PDS)**[h] suture, and the common vaginal tunic and subcutaneous tissues were closed with 3-0 PDS suture.[h] The skin incision was closed with 2–0 PDS suture.[h] After surgery, the stallion was administered procaine penicillin[i] (11,000 U/kg [5,000 U/lb], IM, q 12 h), gentamicin sulfate (5 mg/kg [2.3 mg/lb], IV, q 24 h), and phenylbutazone (2.8 mg/kg [1.3 mg/lb], PO, q 12 h).

On the third day after surgery, another attempt was made to collect semen from the stallion. The stallion did not have any signs of discomfort and mounted and seemed to ejaculate normally. The recovered fluid was azoospermic, and alkaline phosphatase activity was 3 U/L.

Because of spermatozoa in the ductus deferens, primary testicular azoospermia and epididymal blockage were ruled out. However, the inability to pass the cannula for more than 20 cm in the ductus deferens was a clear indication that there was an obstruction of the duct. To determine the exact location and nature of the blockage, abdominal exploratory laparoscopy was performed. The stallion was placed in stocks and sedated with xylazine (0.45 mg/kg [0.2 mg/lb]) and butorphanol (0.015 mg/kg [0.007 mg/lb]). The paralumbar fossae were clipped, scrubbed, and infiltrated with lidocaine. The laparoscope was introduced into the abdominal cavity, and the left internal inguinal ring was viewed first. The dilated ductus deferens was observed, and followed dorsally, it tapered to a thin structure just cranial to the entrance in the urogenital fold, cranial and lateral to the bladder. Both ductus deferentia were similarly affected.

Complete azoospermia, which is the complete absence of spermatozoa in the ejaculate, is not a common condition in stallions.[7] Differential diagnoses for absence of spermatozoa in the collection receptacle include failure to ejaculate, retrograde ejaculation, primary testicular dysfunction, and obstruction of the efferent ducts.[7-10]

Because of the stallion's behavior prior to and after the attempted collections, we felt confident that the stal-

lion was having complete ejaculations. Furthermore, behavior after collection together with the presence of gel in some of the semen samples indicated that the third (last) fraction of the ejaculate was present and ruled out failure to ejaculate as a primary cause of the azoospermia. The gel fraction is the last fraction of the ejaculate and is an indication of complete ejaculation.

The initial 2 ejaculates were contaminated with urine, which is thought to be an ejaculatory disorder caused by failure of the neck of the bladder to close at the time of ejaculation.[7] This can be a transient condition in overly excited stallions during their first few ejaculations. However, in most cases of transient or permanent urospermia, the spermatozoa are easily observed. Because in the stallion reported here only the first 2 semen collection attempts were contaminated with urine, no further diagnostic tests or treatments were performed. Furthermore, the lack of spermatozoa in the urine-contaminated samples was a strong indication that the stallion was not having retrograde ejaculations, which have been reported[8] as a cause of severe oligospermia.

Primary testicular dysfunction caused by testicular degeneration or genetic abnormalities can be a cause of azoospermia.[10] However, it would be expected that on physical examination the stallion would have had small or flaccid testes. Furthermore, round or immature spermatogenic cells in the collected fluid would be expected. The testicular size and consistency, normal ultrasonographic appearance of the epididymides, and low alkaline phosphatase activity led us to the presumptive diagnosis of azoospermia caused by an obstruction of the efferent duct system. When azoospermia is diagnosed, a common cause is the obstruction of the ampullary region caused by stasis of spermatozoa.[7] This condition is frequently resolved by multiple collection attempts, preceded by transrectal massage of the ampullary region and administration of drugs that induce smooth muscle contraction, such as prostaglandin or oxytocin, immediately before collection. In this stallion, the ampullary region was massaged several times per day, followed by attempts to collect semen. Because of the lack of success and small ampullary diameter, it was suspected that stasis of spermatozoa at the level of the ampullae was not the cause for azoospermia. Testicular size and consistency, together with normal epididymides and lack of a visible lumen in the ampullary region prior to and after semen collection attempts, were indicative of an obstruction at the level of the ductus deferens. The inability to pass a cannula more than 20 cm into the ductus, as well as the inability to observe fluid accumulation in the ampullae by ultrasonography or in the urethra by endoscopic viewing even after forceful infusion, confirmed our diagnosis.

The laparoscopic examination was performed to confirm the locations where the vasa deferentia were obstructed. The symmetry and bilateral nature of the abnormalities were strong indications of a possible congenital defect.

Congenital bilateral absence of the vas deferens is a known cause of infertility in humans with cystic fibrosis caused by 1 or several mutations of the **cystic fibrosis transmembrane regulator (CFTR)** gene.[11] In humans, the CFTR gene is located in chromosome 7, which is homologus to chromosome 4 in the horse.[12] Unfortunately, the cause for the abnormality found in the horse reported here has not been identified, and there is no research to indicate the clinical relevance of CFTR mutations in horses.

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[a]Missouri model, Nasco Corp, Ft Atkinson, Wis.
[b]Olympus GIF Type 100, Olympus America Inc, Melville, NY.
[c]Guaifenesin, Butler Phoenix Pharmaceutical, St Joseph, Mo.
[d]Thiopental sodium, Abbott Laboratories, Abbott Park, Ill.
[e]Isoflurane Halocarbon Products Corp, River Edge, NJ.
[f]Mila International Inc, Florence, Ky.
[g]Cook Group Co, Spencer, Ind.
[h]Ethicon Inc, Somerville, NJ.
[i]Pfizer pen, Pfizer Inc, New York, NY.

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