Azoospermia in Stallions: Determining the Cause

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Abstract: Determining the cause of failure to ejaculate sperm can be a diagnostic dilemma. The first diagnostic step is to ascertain whether the stallion is ejaculating. If the stallion appears to ejaculate, but there is azoospermia (absence of sperm in the seminal fluid), testing alkaline phosphatase (ALP) activity in seminal plasma can determine whether testicular and epididymal fluids are present. If ALP activity is low, the possibility of either blockage to sperm outflow in the excurrent duct system or retrograde ejaculation should be pursued diagnostically. If ALP activity is high, the possibility of a testicular defect should be pursued diagnostically. In some cases (notably plugged ampullae or transient, thermally induced testicular degeneration), treatment or the passage of time may restore a stallion’s fertility.

In a review of ejaculatory dysfunction, McDonnell1 reported that approximately one-fourth of stallions referred to a fertility clinic had evidence of ejaculatory problems. Most of these cases were associated with anejaculation (ejaculatory failure). Less than 1% of the horses were truly azoospermic. Azoospermia can be difficult to accurately diagnose and to correct.2,3

Once anejaculation is ruled out, diagnostic efforts can be directed at determining the cause of azoospermia (the absence of sperm in seminal fluid). Some disorders, notably ampullary obstruction of sperm outflow (plugged ampullae), can be corrected, restoring a stallion’s fertility.4 Likewise, some disorders (e.g., transient, thermally induced testicular degeneration) that result in failure of spermatogenesis (sperm production in the testes) self-correct with time, resulting in restoration of sperm output and therefore fertility.5 Some disorders resulting in obstruction of sperm outflow (e.g., chronic epididymitis with blockage of tubules) or failure of spermatogenesis (e.g., age-related testicular degeneration) are not correctable, causing permanent infertility and necessitating retirement of the affected breeding stallion.

This article discusses diagnostic modalities for some of the causes of azoospermia in stallions (FIGURE 1). Case summaries further illustrate diagnostic approaches.

Confirming Ejaculation

Clinical evaluation of stallions that seem to be infertile should begin with determining whether ejaculation is occurring. Lack of secondary signs of ejaculation (i.e., flagging the tail, treading on hind feet, and strong urethral pulsations, usually followed by dismount with the glans penis still fully or partially engorged), in conjunction with azoospermia, suggests that the stallion did not ejaculate.6 Several articles1–3 describe therapies for anejaculation, which are beyond the scope of this article. These therapies include the following (FIGURE 1):

- Breeding and/or pharmacologic management to increase sexual stimulation before and during breeding
- Treatment and/or breeding management to minimize potential musculoskeletal pain that could interrupt emission and ejaculation
- Pharmacologic manipulation to lower the threshold to emission and ejaculation

Techniques for managing repeated anejaculation can be arduous and time-consuming.3

When breeding behavior and ejaculation appear to be normal, but sperm are not present in seminal fluid, azoospermia should be suspected. Secondary signs of ejaculation during collection may convince a clinician that ejaculation occurred. However, these signs can occasionally occur without seminal emission (i.e., semen does not move into the urethra before ejaculation). Confirming that ejaculation did occur by demonstrating the presence of gel (which is produced in the seminal vesicles and appears near the end of the ejaculatory process) and a high alkaline phosphatase (ALP) concentration in seminal fluid devoid of sperm indicates failure of spermatogenesis in testes. Similar findings with a low ALP level (i.e., values below serum concentration and similar to the pre-ejaculatory fluid concentration) in seminal
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Fluid suggests obstruction of sperm outflow from the testes and epididymides. ALP levels of 6913 to 22,180 U/L are found in ejaculates of clinically normal stallions. In our laboratory, values <100 U/L are typical when complete obstruction of sperm outflow or anejaculation occurs. Retrograde ejaculation into the urinary bladder should be ruled out in stallions that exhibit behavioral signs of ejaculation but yield low-volume ejaculates that contain few or no sperm. Collecting and microscopically examining centrifuged urine sediment immediately after ejaculation can confirm whether retrograde ejaculation occurred. Brinsko described a stallion with recurrent retrograde ejaculation that had several azoospermic ejaculates. We have noted that some stallions experiencing retrograde ejaculation deliver essentially no sperm into the semen receptacle, with the entire ejaculate moving retrograde into the urinary bladder, whereas other stallions deliver ≤5% of available sperm into the semen receptacle, with the remainder moving retrograde into the urinary bladder.

Relationship to Testicular Size and Texture

If a stallion exhibits outward signs of ejaculation and has a high ALP level in an azoospermic ejaculate, examination of testicular size, shape, and texture by palpation and ultrasonography is indicated. If testes are small and soft, advanced testicular degeneration or hypoplasia should be suspected. Young stallions that have never had large testes are most likely to have hypoplastic testes, while older stallions that previously had normal-sized testes are most likely to have advanced testicular degeneration. When the epididymides are large compared with testicular size, atrophy due to testicular degeneration can be presumed. However, testicular degeneration or hypoplasia resulting in azoospermia is possible in testes of normal or near-normal size.

Relationship to Hormone Concentrations

With advancing testicular degeneration, circulating concentrations of estrogen and inhibitin decline and follicle-stimulating hormone concentration increases. If the number of Leydig cells in the interstitium is adequate, resting and stimulated concentrations (2 hours after intravenous administration of 15 to 100 µg of gonadotropin-releasing hormone or 10,000 U of human chorionic gonadotropin) of luteinizing hormone and testosterone may be within normal limits. With more advanced testicular dysfunction, circulating testosterone concentration declines (sometimes to the low levels seen in geldings [i.e., ≤40 pg/mL]) and luteinizing hormone concentration may increase (TABLE 1).
Testicular size had decreased from 220 cc in January 2007 to 91 cc in March 2009, and the stallion failed to produce any pregnancies between February and April.

and the stallion failed to produce any pregnancies between February and April. Testicular size had decreased from 220 cc in January 2007 to 91 cc in March 2009. The following values were obtained in April.

<table>
<thead>
<tr>
<th>Hormone</th>
<th>Concentration</th>
<th>Laboratory-Reported Normal Ranges</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Endocrine Laboratory TAMU Research</td>
</tr>
<tr>
<td>Testosterone</td>
<td>334 pg/mL</td>
<td>800–2000 pg/mL</td>
</tr>
<tr>
<td>Estradiol-17β</td>
<td>28.0 pg/mL</td>
<td>—</td>
</tr>
<tr>
<td>LH</td>
<td>13.12 ng/mL</td>
<td>1–10 ng/mL</td>
</tr>
<tr>
<td>FSH</td>
<td>23.40 ng/mL</td>
<td>2–12 ng/mL</td>
</tr>
<tr>
<td>Inhibin</td>
<td>1.77 ng/mL</td>
<td>2.2–3.4 ng/mL</td>
</tr>
</tbody>
</table>

Interpretation: The hormone concentrations are consistent with a substantial degree of testicular degeneration. Leydig and Sertoli cells are experiencing some dysfunction as indicated by lower-than-normal levels of testosterone, estradiol, and inhibin. The pituitary is compensating for testicular dysfunction by increasing secretion of gonadotropins (luteinizing hormone [LH] and follicle-stimulating hormone [FSH]).

According to unpublished observations, administration of gonadotropin-releasing hormone may fail to elicit a normal increase in circulating testosterone concentration in some cases of advanced age-related testicular degeneration.

**Testicular Biopsy**

Testicular biopsy may be indicated to assess status of spermatogenesis. To perform testicular biopsy, the stallion is sedated (e.g., 8 to 10 µg/kg IV of detomidine hydrochloride and 0.01 to 0.02 mg/kg IV of butorphanol tartrate) and the scrotal skin is scrubbed and disinfected. Sterile gloves are donned, and the testis is grasped and stabilized ventrally in the scrotum. If desired, local anesthetic (e.g., lidocaine, mepivacaine) can be injected subcutaneously at the intended biopsy site, but we do not usually use local anesthesia. A sterile, spring-loaded biopsy instrument is pushed laterally through the scrotal skin, dartos, testicular tunics, and tunica albuginea into the cranial mid-testis. This 14-gauge instrument is 16 cm in length, with a 22-mm penetration depth and a 1.7-cm sample notch. The biopsy punch is triggered to procure the specimen, then the instrument is removed from the testis and scrotum. Digital pressure is maintained for 1 to 2 minutes on the tunica albuginea and scrotal skin over the biopsy site to control hemorrhage. The testicular parenchyma is gently removed from the exposed notch of the biopsy instrument and is transferred to Bouin solution or 4% paraformaldehyde for 24 hours. The fixed tissue is then transferred to alcohol and submitted to a histology laboratory for processing and mounting. Preferred stains are periodic acid-Schiff (PAS)–hematoxylin or PAS–toluidine blue. To the trained observer, examination of testicular parenchyma under light microscopy reveals individual cell types and whether spermatogenesis is proceeding to completion. Although the amount of tissue is insufficient to determine accurate percentages of tubules within each of eight stages, the presence of several stage VIII tubules reveals that spermatogenesis is proceeding to completion and sperm are being released into the seminiferous tubule lumina. If straight tubules (which connect seminiferous tubules with the rete testis) are present in the biopsy specimen, the presence of released sperm within the lumina can be assessed. A diagnosis of testicular degeneration or hypoplasia can be made if high numbers of degenerating germ cells (sometimes sloughed into the tubule lumina) are present, more advanced germ cells are absent, and numerous basilar vacuoles are present within the seminiferous epithelium. Reduced size and number of Leydig cells in interstitial tissue and “Sertoli cell only” seminiferous tubules are hallmarks of advanced testicular degeneration.

**Examination for Evidence of Obstruction of Sperm Outflow**

Physical examination of scrotal contents and internal genitalia is required to determine the location of an obstruction of sperm outflow. Thorough palpation and ultrasonographic examination can reveal a number of abnormalities that may contribute to obstruction of sperm outflow. Space-occupying lesions (e.g., tumors or extensive fibrosis, sometimes with calcification) have been observed within testicular or epididymal tissue. Firm, enlarged epididymides, sometimes with dilated ducts due to chronic obstructive epididymitis, can adhere to the testicular tunics. Extensive adhesions between vaginal and parietal tunics can result from hematocele, orchitis, periorchitis, or testicular rupture.
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(UFIGURE 3; FIGURE 4; FIGURE 5; FIGURE 6). Ultrasonographic examination may reveal dilated ampullae, vas deferens, or ducts of the cauda epididymides with sperm stasis or ampullar blockage18 (FIGURE 7). Although rare, congenital aplasia/hypoplasia of the epididymides,11 pelvic ductus deferens or ampullae,18,19 or prostatic neoplasia as an extension of an ampullary adenocarcinoma20 can block the egress duct system, preventing sperm from entering the ejaculate.

The following case summaries illustrate procedures and findings for determining the cause of azoospermia in stallions.

**Case 1**
A 33-month-old, 450-kg (990-lb) Paint stallion was examined because of azoospermia. Historical review indicated that the stallion had bred two mares by natural mating the previous summer, but neither mare became pregnant. Two weeks before admission to our clinic, semen had been collected and examined by a referring veterinarian. Sperm were not detected in the ejaculate.

After arrival at our clinic, two ejaculates were collected from the stallion, using a Missouri-model artificial vagina and an ovariectomized mount mare. The stallion displayed normal libido and breeding behavior and appeared to ejaculate on both collections. Indirect evidence for ejaculation included engorgement of the glans penis, urethral pulsations (four or five pronounced pulsations of the urethra palpable on the ventral aspect of the base of the penis), flagging of the tail, and the presence of gel (60 cc in the
first ejaculate; 23 cc in the second ejaculate). After removal of the gel from each ejaculate, <10 mL of transparent liquid remained in the collection receptacle. Examination of the gel-free liquid using a phase-contrast microscope (magnification: ×200) did not reveal sperm. The ALP activity of the gel-free liquid was 43 U/L, a value consistent with ejaculates that do not contain fluid derived from the testes and epididymides.

Palpation of the scrotum and its contents revealed small, slightly soft testes. Length, width, and height of each testis were measured by ultrasonography; results were used to estimate testicular volume according to the formula of an ellipsoid. Volumes of the left (103 cc) and right (107 cc) testes yielded an estimated combined testicular volume of 210 cc, which is similar to that reported for 2.5- to 3-year-old stallions (175 to 200 cc). The epididymides were quite small, particularly where the body (corpus epididymis) joins the tail (cauda epididymis). With the use of ultrasonography, the corpora epididymides were estimated to be 0.3 cm in diameter, and the cauda epididymides were estimated to be only 1 cm in diameter. The epididymides were uniformly echogenic, without the “Swiss-cheese” appearance of normal epididymides attributed to collection of fluid from the convoluted epididymal ducts. Per-rectum palpation revealed normal ductus deferens, ampullae, seminal vesicles, and lobes of the prostate gland.

While standing, the stallion was sedated with xylazine hydrochloride (1.1 mg/kg IV) and butorphanol tartrate (0.022 mg/kg IV). After aseptic preparation of the scrotal skin, a testicular biopsy specimen was procured from the left testis. The biopsy specimen was fixed for 24 hours in Bouin solution, embedded in paraffin, sectioned, and stained with PAS-hematoxylin. Light microscopic examination of the biopsy specimen revealed Leydig cells in the interstitium and normal-appearing seminiferous tubules (basal lamina lined with sustentacular [Sertoli] cells and spermatogonia, with typical early and late primary spermatocytes, round spermatids, and elongated spermatids evident as germ cells progressing toward the lumen of the tubule). Several stage VIII seminiferous tubules (i.e., those with lumina lined with elongated spermatids, which were being released; FIGURE 2) were found in the biopsy specimen. The finding that spermatogenesis proceeded to completion, in conjunction with azoospermia and a low ALP concentration in the gel-free ejaculatory fluid, suggested obstruction of sperm outflow. The extremely small epididymides in this young stallion prompted a diagnosis of bilateral epididymal hypoplasia (failure of epididymides to develop to typical size). The stallion was not castrated, so confirmation of the diagnosis was not possible.

Case 2
A 4-year-old Quarter horse stallion presented to our clinic with a history of azoospermia in two ejaculates evaluated several months apart. When the horse arrived at our clinic, one ejaculate was collected in a Missouri-model artificial vagina using an ovariec-
tomized mount mare. No sperm were visualized in wet-mount preparations of the gel-free ejaculatory fluids, despite the presence of gel, prominent urethral pulsations, and flagging of the tail during ejaculation. Transrectal palpation and ultrasonography revealed no abnormalities of the accessory sex glands. The seminal plasma was assayed for ALP activity, and a value of 1237 U/L — consistent with testicular/epididymal secretions reaching the ejaculate—was obtained. Palpation and ultrasonography of the scrotum and its contents revealed small, soft testes (scrotal width: 6.4 cm) and epididymides, with the right testis being smaller than the left. A biopsy specimen was procured from the left (larger) testis, fixed in Bouin solution for 24 hours, and submitted to the histology laboratory in alcohol for sectioning and staining with PAS-hematoxylin. Examination of the biopsy specimen revealed prominent degeneration of spermatocytes and, in some tubules, round spermatids, with no elongated spermatids in the seminiferous tubules (FIGURE 8). Because there was no history on whether the horse had previously had normal-sized testes, a diagnosis of testicular hypoplasia or degeneration was made, and the owner elected to have the horse castrated.

Case 3
Testicular biopsy specimens were submitted from a 9-year-old Thoroughbred stallion that had reportedly been fertile 2 years previously. Over the past year, the stallion had developed infertility associated with severe oligospermia/azoospermia and small, soft testes (described as golf-ball sized). The referring veterinarian reported occasionally seeing a few dead sperm in wet-mount preparations of raw semen collected in an artificial vagina. The biopsy samples (one from each testis) were transferred from Bouin’s solution to alcohol and submitted to the histology laboratory for...
embedding, sectioning, and staining with PAS-hematoxylin. Examination of the biopsy specimens revealed only a few seminiferous tubules containing round or elongated spermatids (stages V to VII, with elongated spermatid heads embedded deep within the Sertoli cells). No stage VIII tubules were noted in either biopsy specimen. Numerous degenerating spermatocytes were evident in many tubules, some with pronounced epithelial vacuolation (FIGURE 9). A diagnosis of severe testicular degeneration was made. Because of the longstanding history of the condition, the owners elected to castrate the stallion.

**Case 4**

A 6-year-old Thoroughbred stallion had no sperm in dismount samples collected after mating and examined as wet mounts under a microscope. Several semen collection attempts were made using a Missouri-model artificial vagina and an ovariectomized mount mare. All ejaculates were devoid of sperm despite the presence of strong urethral pulsations, flagging of the tail, and gel in the ejaculates. Seminal plasma from several ejaculates was assayed for ALP activity, yielding values <50 U/L. Palpation and ultrasonographic examination of the scrotum and its contents revealed no abnormalities. Transrectal palpation revealed enlarged, turgid ampullae. Transrectal ultrasonography revealed dilated ampullar lumina (FIGURE 7), with a few hyperechoic densities within the distended lumina. Treatment over the next few days included transrectal massage of the distended ampullae before semen collection was attempted; administration of either 20 to 40 U IV of oxytocin or 125 to 250 µg IM of cloprostenol 15 to 60 minutes before semen collection was attempted; and administration of 7.5 mg/kg q24h IV of enrofloxacin for 5 days. Several attempts to collect semen were made each day until “stringy” accumulations of semen containing densely packed sperm with a high incidence of detached heads began to appear in ejaculates. Once the presence of sperm concretions and the percentage of detached sperm heads decreased significantly, the stallion was successfully returned to service. Transrectal ultrasonography when the stallion returned to service revealed ampullae of normal turgidity without distended lumina. The owner was advised to breed the stallion at least twice weekly thereafter (year-round) to help prevent further blockage of the ampullae.

Sperm stasis, with impaired movement of sperm through the excurrent duct system, is apparently associated with plugged ampullae syndrome. Semen collected from a sexually rested, but unobstructed, stallion with this condition contains very high numbers of sperm (sometimes reaching 50 billion in the ejaculate) with a high incidence of detached sperm heads. Repeated ejaculation gradually reduces the incidence of detached sperm heads, while the total number of sperm in the ejaculates declines to normal levels (FIGURE 10).
Case 5

A 4-year-old Quarter horse stallion presented with a history of failing to establish pregnancy in six mares bred by natural service at pasture. Several semen collection attempts were made using a Missouri-model artificial vagina and an ovariectomized mount mare. Although the stallion appeared to ejaculate, the ejaculatory fluids were clear and devoid of sperm. The urinary bladder was catheterized after semen collection was attempted, and the urine was centrifuged. Examination of urine sediment failed to reveal the presence of sperm. Palpation and ultrasonography of the scrotal contents revealed normal-sized testicles with enlarged cauda epididymides. The ducts of the cauda epididymides were greatly distended (FIGURE 11). Transrectal palpation and ultrasonographic examination revealed flattened, hypoplastic or aplastic ductus deferens at the junction of the ampullae, and the ductus deferens proximal to this junction were grossly distended. Numerous attempts to collect semen, which were preceded by administration of either oxytocin (20 U IV) or cloprostenol (125 µg IM) and vigorous massage of the ampullae and pelvic ductus deferens, failed to produce sperm in ejaculates. Exploratory laparoscopic surgery confirmed aplastic segments of the terminal ductus deferens where it coursed over the fundus of the bladder (FIGURE 12), whereas the ductus deferens was distended ventrally to the internal inguinal ring. With the stallion under general anesthesia, needle aspiration of the distended ductus deferens yielded thick, creamy concentrated semen with sperm clumping, and virtually all sperm heads were detached. Attempts to pass cannulas through the terminal ductus deferens into the ampullary lumina were unsuccessful. The diagnosis was bilateral segmental aplasia of the ductus deferens, and the stallion was removed from stud service.

Case 6

A 9-year-old Quarter horse stallion presented to our clinic with a history of neutrophils in ejaculates. The stallion had previously been bred for 2 years to approximately 12 mares per year by natural service, resulting in 90% or better seasonal pregnancy rates. Three months previously, the stallion had been moved to another farm, and the farm manager had noted fluctuation in the stallion’s scrotal size. The following month, the stallion developed a fever of 105°F with prominent swelling of the testes. Culture of semen collected in an artificial vagina yielded *Klebsiella pneumoniae*, and the stallion was treated by the referring veterinarian, who administered systemic antimicrobials, which were not identified in the history. While the scrotal swelling decreased, neutrophils remained in the ejaculate. The stallion was then bred to five mares, producing one pregnancy that was subsequently lost.

On arrival at our clinic, the stallion was teased to erection and the penis washed and dried. Two ejaculates were collected in an artificial vagina. Neither ejaculate contained sperm, but both contained numerous degenerating neutrophils. For bacteriologic culture, the urethra was swabbed before, and immediately after, ejaculation and the filtered ejaculatory fluid was swabbed. Heavy growth of both *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* was recovered from the ejaculatory fluid and postejaculatory urethral swabblings. Palpation and ultrasonographic examination of the scrotal contents revealed firm, small testes with large, firm epididymides. The epididymides could not be moved from the surface of the testes, suggesting adhesion had occurred. The stallion was sedated, and endoscopic examination of the pelvic urethra, seminal colliculus, and seminal vesicles was performed. Prostatic and urethral gland secretions expressed by per-rectum palpation during the examination were clear. The endoscope was passed into the seminal vesicles, which contained thick, mucopurulent debris.

Because of the extent of infection—and epididymitis that apparently blocked sperm outflow into the ductus deferens—the owners elected to castrate the horse and retire it from stud service. After castration, the seminal vesicles were flushed with sterile saline.
and infused with 1 g of amikacin every other day for three treatments, and the horse was discharged from the hospital.

**Conclusion**
When the cause of azoospermia in a stallion is investigated, the first diagnostic step is to ascertain whether the stallion is ejaculating. If the stallion appears to ejaculate, a logical diagnostic examination and testing approach can determine whether blockage of the excurrent duct system or failure of spermatogenesis is the cause of azoospermia. Once a correct diagnosis is made, a prognosis for return to fertility can be offered and treatment options explored.

**References**