How to Investigate Azoospermia in Stallions

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1. Introduction
In a review of ejaculatory dysfunction, McDonnell reported that ~25% of stallions referred to a fertility clinic had evidence of ejaculatory problems. The vast majority of cases were anejaculatory (failure to ejaculate). Less than 1% of horses in that survey were truly azoospermic (i.e., ejaculated seminal fluids devoid of sperm). Failure to ejaculate sperm can be a troublesome problem that requires accurate diagnosis, determination of prognosis for correction (sometimes necessitating retirement as a breeding stallion), and arduous treatment and/or breeding management to correct. Figure 1 represents an attempt at a schematic overview of an approach to diagnosis of lack of sperm in ejaculates.

2. Confirming Ejaculation
Clinical evaluation of stallions failing to produce sperm in ejaculates should begin with determining whether or not ejaculation is occurring. Lack of secondary signs of ejaculation (i.e., flagging of the tail, treading on hindlimbs, and presence of strong urethral pulsations) followed by dismount with the glans penis still partially engorged and an absence of sperm in seminal fluids suggest that the stallion did not ejaculate. A number of reports describe therapy indicated for ejaculation failure, but they are not the subject of this report. Briefly, they include breeding and/or pharmacological management to increase sexual stimulation before and during the breeding process, treatment and/or breeding management to minimize potential musculoskeletal pain that could interrupt the emission and ejaculatory process, and pharmacologic manipulation to lower the threshold to emission and ejaculation. Techniques used to manage repeated ejaculatory failure can be arduous and time consuming, and they are reviewed by Varner et al.

When breeding behavior and apparent ejaculation seems to be normal but no sperm are present in the seminal fluids collected, azoospermia should be suspected. Secondary signs of ejaculation (e.g., flagging the tail, urethral pulsations, etc.) that occur during a collection process may convince the clinician that ejaculation occurred. However, these signs can occur without seminal emission (i.e., semen does not move into the urethra before ejaculation). Confirming that ejaculation did occur by showing the presence of gel (i.e., gel is produced in the seminal vesicles and appears near the end of the
ejaculatory process) and a high alkaline phosphatase concentration in seminal fluids devoid of sperm indicates failure of sperm production in testes. Alkaline phosphatase levels of 6913–22,180 units/l are found in ejaculates of clinically normal stallions. The same findings with low levels of alkaline phosphatase (i.e., below serum value and similar to pre-ejaculatory fluid) in seminal fluids suggest that obstruction to sperm outflow from the testes and epididymides is present. Retrograde ejaculation into the urinary bladder should also be ruled out for stallions that exhibit behavioral signs of ejaculation but yield ejaculates that are of low volume and devoid of sperm. Collecting and microscopically examining centrifuged urine sediment immediately after ejaculation will confirm whether or not retrograde ejaculation occurred.

3. Relationship to Testicular Size and Texture
If signs of ejaculation and high alkaline phosphatase levels are present in seminal fluids, examination of testes size, shape, and texture by palpation and ultrasonography is indicated. If testes are quite small and soft, advanced testicular degeneration or hypoplasia should be suspected. Young stallions in which testes size has never been large are most likely to be hypoplastic, whereas older stallions that previously had normal testes size are most likely to have advanced degeneration. However, testicular degeneration or hypoplasia also occur in testes of normal size.

4. Relationship to Hormone Concentrations
If an adequate population of Leydig cells exists in the interstitium, resting and stimulated (i.e., after administration of gonadotropin releasing hormone (GnRH) or human chorionic gonadotropin (hCG)) concentrations of testosterone may be within normal limits. However, in advanced cases of testicular degeneration, resting concentrations of testosterone and estrogen in the circulation will be lower than normal (sometimes similar to those of geldings).
In response to the testicular defect culminating in low production of testicular steroids, circulating concentrations of gonadotropins (particularly follicle stimulating hormone (FSH)) can be higher than normal. In cases of advanced testicular degeneration, administration of GnRH or hCG may fail to elicit a normal increase in circulating testosterone concentration as well.

5. Testicular Biopsy

Testicular biopsy may be indicated to assess status of spermatogenesis. To perform testicular biopsy, the stallion is sedated (e.g., detomodine hydrochloride and butorphanol tartrate). After aseptic preparation of the scrotal skin and donning of sterile gloves, the testis is grasped and stabilized ventrally in the scrotum. If desired, an anesthetic (e.g., lidocaine or carbocaine) bleb may be injected subcutaneously at the intended biopsy site, but the authors do not usually employ local anesthesia. A sterile, spring-loaded biopsy instrument (Fig. 2) is pushed laterally to medially through the scrotal skin, tunica dartos, testicular tunics, and tunica albuginea into the cranial mid-testis (Fig. 3). This instrument is 14 gauge × 16 cm in length with a 22-mm penetration depth and a 1.7-cm sample notch. The biopsy
punch is triggered, and the instrument is removed from the testis and scrotum. Digital pressure is maintained for 1–2 min on the tunica albuginea and scrotal skin over the biopsy site to control any hemorrhage. The testicular parenchyma is gently removed from the exposed notch of the biopsy instrument (Fig. 4), and it is transferred to Bouin’s solution or 4% paraformaldehyde for 24 h. The fixed tissue is then transferred to alcohol and submitted to a histology laboratory for processing and mounting. Preferred stains are PAS-Hematoxylin or PAS-Toluidine Blue. To the trained observer, examination of testicular parenchyma under light microscopy will reveal individual cell types and whether or not spermatogenesis is proceeding to completion. Although insufficient tissue is present to determine accurate percentages of stages, the presence of several Stage VIII tubules (Fig. 5) reveals that sperm are being released into the seminiferous tubule lumina.13,14 If straight tubules (tubules connecting seminiferous tubules with the rete testis) are present in the biopsy, the presence or absence of released sperm within their lumina can be assessed. The diagnosis of testicular degeneration or hypoplasia can be made by the presence of high numbers of degenerating germ cells, the absence of more advanced germ cells, and the presence of numerous basilar vacuoles 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.8

6. Examination for Evidence of Obstruction to Sperm Outflow

Physical examination of scrotal contents and internal genitalia are required to diagnose the location of an obstruction to sperm outflow.3,5 Thorough palpation and ultrasonographic examination can reveal a number of abnormalities that may contribute to the obstruction of sperm outflow. The presence of space-occupying lesions within testicular or epididymal tissue (such as tumors or extensive fibrosis, sometimes with calcification) has been observed.6,7,13 Firm, enlarged epididymides, sometimes with dilated ducts as with chronic obstructive epididymitis, can be adhered to the testicular tunics.13,15 Extensive adhesions between vaginal and parietal tunics can result from hematocele, orchitis, periorchitis, or testicular rupture (Figs. 6–9).7,13,15 Ultrasonographic examination may reveal dilated ampullae, vas deferens, or ducts of the cauda epididymides with sperm stasis or ampullar blockage (Figs. 10 and 11).7 Although rare, stallions with congenital aplasia of the epididymides,10 pelvic vas deferens or ampullae,116 and prostatic adenocarcinoma resulting in blockage of excurrent duct openings into the pelvic urethra have also been identified.

References and Footnotes