How to Obtain a Stallion Testicular Biopsy Using a Spring-Loaded Split-Needle Biopsy Instrument

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1. Introduction

Testicular biopsy in the stallion is an important yet underused technique in equine practice. The two major reasons that practitioners hesitate to obtain a biopsy when indicated is unfamiliarity with the technique and the risk of complications.

The primary indications for biopsy of the stallion testis are for diagnostic evaluation of azoospermia/oligozoospermia or to histologically assess changes within testicular parenchyma detected on ultrasonography or palpation such as in the case of degeneration or neoplasia.

Stallions that are diagnosed with azoospermia often present to the veterinarian for evaluation of infertility, either because mares that were bred live cover return to estrus, or because collection via artificial vagina yields an ejaculate with no spermatozoa. Azoospermia must be characterized on the basis of the horse’s spermatogenic capability versus ejaculatory disorders, including retrograde ejaculation. Azoospermia may be due to bilateral obstruction along the length of the epididymal ducts, epididymis, ductus deferens, or ampullae. A thorough examination will include palpation and ultrasonography of the external and internal genitalia, collection by artificial vagina to determine presence of urine in the ejaculate, and assay of enzymatic activity, including alkaline phosphatase and serum endocrine assays. Results of these examinations will help the clinician to determine if a testicular biopsy is indicated to assess normal seminiferous tubule architecture and presence of proper germ cell differentiation.

Similarly, stallions with testicular degeneration may present for infertility, although in some cases the clinician will note soft testes with decreased dimensions on palpation. In many cases, prior breeding soundness examination results will be available for comparison of testicular length, width, and height, as well as total scrotal width, and can be used to monitor stallions over time and especially with advancing age. In some cases, however, infertility is a presenting complaint and/or previous records are not available for comparison. In these cases, testicular biopsy can provide a histologic diagnosis to the clinician of degenerative changes within the testicular parenchyma and disruption of spermatogenesis, which may have significant effects on fertility and longevity of that stallion’s breeding career.

On the contrary, stallions with testicular neoplasia most commonly present to the veterinarian for...
nonpainful testicular enlargement.\textsuperscript{6,7} In these cases, ultrasonography of the affected testis may identify abnormal regions of the testicular parenchyma that may be biopsied for histologic diagnosis.\textsuperscript{8} Histological confirmation of neoplasia supports the clinician's decision to perform hemilateral or bilateral castration and pursue other diagnostics for potential metastasis.

Several methods of testicular biopsy have been reported for use in the stallion, including fine needle aspiration, open surgical biopsy, punch biopsy, and use of a split-needle spring-loaded biopsy instrument.

The easiest and least diagnostic method to assess the testicular parenchyma is to obtain a fine needle aspirate using a small-gauge needle and syringe.\textsuperscript{9} The aspirated material is smeared on a slide and evaluated after staining with the usual cytology technique. Some authors advocate that with a trained eye, the specific stages of germ cell development can be counted from slides made from fine needle aspirates and that the percentage of each cell type can help diagnose stallions with infertility.\textsuperscript{9,10} Although this method is inexpensive and least invasive to the testis, it does not provide the diagnostician with any information about the architecture of the seminiferous tubules and may not reflect the entire cell population of the testis.

Open biopsy of the testis, also commonly referred to as a wedge or surgical biopsy, results in the highest risk of complications. The horse is placed under general anesthesia and the scrotum is aseptically prepared. An incision is made through the scrotum and a wedge biopsy of the testis is obtained.\textsuperscript{11} The scrotal incision is apposed with suture. A study on open biopsy in the stallion demonstrated transient inflammatory infiltrate and formation of granulation tissue in biopsied testes as well as transient decreases in testicular size and local areas of testicular degeneration after biopsy.\textsuperscript{11} However, these effects all resolved over time. Although the tissue obtained affords excellent histopathologic assessment of the seminiferous tubular architecture and any degenerative or neoplastic conditions present, procurement of this type of biopsy may increase the risk of hemorrhage, infection, adhesion formation, and, most significantly for breeding stallions, a negative effect on spermatogenesis that can range from decreased spermatozoa production to the production of morphologically abnormal spermatozoa or degenerative changes within the seminiferous tubules. This technique also requires general anesthesia.

Punch biopsy or biopsy using a Tru-Cut style needle is similar to the technique using a spring-loaded biopsy instrument, except that the needle or biopsy punch is introduced by hand into the testicular parenchyma, which can lead to increased rates of hemorrhage and parenchyma damage, depending on the size of the biopsy obtained.\textsuperscript{12,13} Samples are adequate for assessment of tubule architecture.

The split-needle spring-loaded biopsy instrument\textsuperscript{a} (Figure 1), first used in equine practice in 1994,\textsuperscript{14} is an inexpensive tool that has distinct advantages over other testicular biopsy methods in stallions. The 14-gauge diameter is adequate for histologic assessment of seminiferous tubule architecture without causing significant loss of parenchymal tissue and without negative effects on spermatogenesis, although one study demonstrated a transient decrease in percentage of progressively motile spermatozoa for 3 weeks after biopsy.\textsuperscript{15} However, this decrease in motility did not have detrimental effects on fertility.\textsuperscript{15} The rapid-fire mechanism of the biopsy instrument not only shortens the time to procure the biopsy but also minimizes trauma to the tissues. Additionally, the biopsy can be obtained under standing sedation without general anesthesia.\textsuperscript{16}

Because many practitioners hesitate to obtain a testicular biopsy due to inexperience using the technique and because of the risk of complications, the objectives of this study were (1) to evaluate the ease of use and diagnostic validity of biopsy samples obtained with the spring-loaded split-needle biopsy instrument in the hands of novice users and (2) to assess the complication rate in stallions after testicular biopsy using a split-needle spring-loaded biopsy instrument.

2. Materials and Methods
Thirteen mixed-breed stallions ages 3 to 12 years were acquired by the Washington State University Veterinary Teaching Hospital for training veterinary students techniques in stallion handling, medicine, and surgery. Twelve of the stallions had 2 descended testes, and 1 stallion was unilaterally cryptorchid with the left testis located in the ingui-
nal canal. All animal use was approved by the Institutional Animal Care and Use Committee at Washington State University.

The study was conducted over 2 breeding seasons, with 6 stallions enrolled in the first year and 7 stallions in the second year.

The stallions in the first group were placed in stocks and sedated, using intravenous detomidine HCl (10 μg/kg) or xylazine HCl (0.5 mg/kg) and butorphanol tartrate (0.01 mg/kg). Testicular ultrasonography was performed; none of the stallions had observable testicular pathology. Location of the central vein was noted but did not influence the selected biopsy site. The central vein appeared normal in all testes. Pulsed wave color Doppler was used to assess the peak systolic velocity (PSV) and end diastolic velocity (EDV) of the testicular artery at the marginal aspect of each testis, as previously described.17 Semen was not collected from any of the stallions before biopsy.

Testicular biopsies were obtained by senior veterinary students with no prior experience. Students were instructed on a standard technique in a 30-minute seminar before biopsy sampling.15,16,18 The testes were washed using warm water and 5% povidone iodine. The left testis was biopsied in all stallions, using aseptic technique (Fig. 2). Operators used the left hand to apply gentle traction to the left spermatic cord to prevent retraction of the cremaster muscle. This was best accomplished by introducing the hand between the testes and grasping the caudomedial aspect of the testis and spermatic cord. The other hand was used to inject the cranialateral aspect of the scrotal skin and dartos with 2 to 3 mL of 2% lidocaine at the cranialateral aspect of the testis. This location is preferred to avoid accidental puncture of the head of the epididymis or arcuate branches of the testicular artery.

Fig. 2. Supplies needed to obtain a testicular biopsy from a stallion: a, 10% neutral buffered formalin; b, 2% lidocaine; c, flunixin meglumine; d, syringe and small-gauge needle for injection of lidocaine; e, No. 15 scalpel blade; f, sterile examination gloves; and g, split-needle spring-loaded biopsy instrument.

Through the incision against the testicular parenchyma perpendicular to the testis and fired (Fig. 4). The small incision was left open. No stallion reacted adversely to the procedure. Minor hemorrhage occurred at the sampling site but ceased within 10 minutes. Biopsy samples obtained with this instrument measured 17 mm long with 14-gauge diameter and were placed in 10% neutral buffered formalin and processed for histopathology to assess diagnostic validity.

All stallions received 1 dose of flunixin meglumine (1.1 mg/kg; IV) after the biopsy. Antimicrobials (trimethoprim sulfamethoxazole, 25 mg/kg; q 12 h; PO) were administered for 4 days starting the day before the biopsy. Ultrasonography of the testes, including Doppler assessment of blood flow, was performed for 3 consecutive days after biopsy. Data obtained before and after biopsy were compared through the incision.
within and between testes, using general ANOVA/AOCV after log transformation. Stallions were monitored twice daily for 10 days after biopsy for complications, at which point castration was performed. Gross examination of the testes was performed, and samples of the biopsy tract within the testicular parenchyma were submitted for histopathology.

The second group of stallions included the stallion with the left inguinally retained testis. In that stallion, the right testis was used for biopsy. Stallions in this group were sedated as described, and the testes were assessed for pathology via ultrasonography. No abnormalities were identified. Biopsies were obtained by students as described and processed for diagnostic validity. Stallions received 1 dose of flunixin meglumine (1.1 mg/kg, IV) and antimicrobials (trimethoprim sulfamethoxazole, 25 mg/kg q 12 h, PO) for 3 days starting the day of the biopsy. Stallions were monitored twice daily for 10 days after biopsy for complications, at which point castration was performed. The left inguinal testis of the cryptorchid horse was removed via a left parainguinal approach. Gross examination of the testes was performed.

All castrated testes were used for collection of spermatozoa from the cauda epididymis. Progressive motility and spermatozoa morphology were assessed; however, statistical analysis comparing biopsied and nonbiopsied testicles has not yet been completed.

3. Results

No systemic effects of testicular biopsy were observed in any stallions in both years (Table 1). Four stallions developed transient scrotal edema that resolved within 3 days (Fig. 5). In the first group, 3 stallions developed a subcutaneous/albuginea hematoma, diagnosed by ultrasonography, which resolved within 7 days. At the time of castration, the biopsy tract was seen as a pin-point on all biopsied testes in both groups. Gross abnormalities noted at castration included a small amount of fibrinous material between the albuginea and tunica vaginalis in 1 stallion.

The pulsed wave color Doppler studies did not demonstrate any significant effect of biopsy on blood flow between paired testes (p > 0.05). There was, however, a significant difference over days sampled (p = 0.011 for PSV and p = 0.02 for EDV).

![Fig. 5. Mild scrotal edema may occur after testicular biopsy. Most cases resolve within 3 days with no treatment.](image-url)
matogenesis and seminiferous tubule architecture. The biopsy sample was diagnostically valid and this sample demonstrates normal spermatogenesis and seminiferous tubule architecture.

All biopsy samples (6/6; 100%) from the first group of stallions and 6 of 7 (85.7%) biopsy samples from the second group of stallions were of acceptable diagnostic quality as assessed by histopathology (92.3% overall) (Fig. 6). Histopathology samples of the biopsy tract from the first group of stallions demonstrated no evidence of disruption of spermatogenesis or changes within the testicular parenchyma.

4. Discussion

Obtaining a testicular biopsy from a stallion can seem daunting for inexperienced or recent graduate veterinarians. This study demonstrates that novice users of the split-needle spring-loaded biopsy instrument can produce samples of diagnostic quality with minimal complications in the stallion after instruction regarding the technique.

One key to success of the novice operators was ensuring that the environment was safe for the procedure. Stallions were placed in stocks in a quiet examination room of the Veterinary Teaching Hospital. Similarly, practitioners on the farm may find that a certain locale or time of day will facilitate sample collection. Avoiding times when farm equipment may be operating nearby, as well as established feeding or exercise/collection times, will minimize excitement in the stallion. Avoiding movement or presence of other horses can also decrease the possibility of distraction. If stocks are not available, cross-ties and/or an experienced handler are a must. Additional physical restraint such as a lip chain or nose twitch may be necessary in some situations. In the present study, intravenous sedation was adequate restraint in the stocks for all stallions. The operators in the study found that a stool with wheels worked well for access and visualization of the testis during the biopsy procedure and afforded the ability to push back from the horse if necessary. On the farm, practitioners may find the easiest and safest method is to stand at the level of the front legs or barrel of the horse and crouch down to access the testis.

Placement of the tip of the spring-loaded biopsy instrument is important due to the proximity of major blood vessels and the head of the epididymis. The craniolateral aspect of the testis is an ideal sampling location because it avoids both of these potential complications (Fig. 4). It is recommended that the scrotum be incised prior to biopsy procurement so that the tip of the biopsy instrument does not drag the scrotal skin deep within the testicular parenchyma. Operators found that use of a small-gauge needle facilitated transfer of the biopsy sample from the tip of the instrument to the formalin jar, although care must be taken not to create an artifact in the tissue.

The one biopsy that was not diagnostically valid on histologic assessment was obtained by an operator who pulled away from the horse at the time that she fired the instrument. When the sample was transferred to formalin, it was noted to be very small and contained scrotal skin. Because of the experimental design of this study, the sample was processed for histopathology as planned, to determine the diagnostic validity. However, if the horse was being evaluated for testicular pathology or azoospermia, a second biopsy sample would have been obtained at the time, knowing that first sample acquired would most likely not yield a diagnosis. The novice operator was again instructed on technique and subsequently became proficient.

It is recommended that all practitioners develop a good working relationship with the laboratory to which samples are routinely sent. In addition, it is a good idea to request a slide of every biopsy sample submitted for evaluation. The practitioner can charge the client a small fee to cover the costs of this additional slide, and this will allow the practitioner to fully document the case as well as to corroborate the diagnosis provided by the pathologist. Training one’s eye to evaluate the testicular architecture at the cellular level will not only enhance the veterinarian’s assessment of the case but will also allow him or her to discuss the samples with the pathologist, if ambiguous or notable.

Although significant effects of biopsy were not seen in the stallions in this study, various complications have been reported, including hemorrhage, infection, and formation of scrotal adhesions. In the present study, only normal testes were biopsied, as determined by lack of palpable or ultrasonographically evident lesions. In stallions that present to the veterinarian for azoospermia, it is likely that the testes will also be grossly normal and that the findings of this study in regard to complications would be similar. However, in neoplastic testes, there may be increased vascularity, which may result in increased hemorrhage. Additionally, the insertion of a needle into metastatic tumors may drag neo-

Fig. 6. High-power (×1000) photomicrograph of seminiferous tubules from a stallion testicular biopsy. The biopsy sample was diagnostically valid and this sample demonstrates normal spermatogenesis and seminiferous tubule architecture.
plastic cells through normal parenchyma or into the vaginal cavity. The incidence of testicular tumors in stallions is low\(^6\); controlled studies on biopsy in cases of testicular neoplasia would be incredibly difficult, and reaction of testicular tissue to biopsy would depend on tumor type, size, and systemic health of the animal. Often, testicular tumors are removed by hemilaterial or bilateral castration, and the entire testis is submitted for histopathology rather than taking a biopsy sample.\(^9\) The ultrasonographic appearance of disrupted testicular parenchyma is often obvious, and, because of metastatic risk of some tumor types, owners may elect prompt castration.\(^6\)

After testicular biopsy of the stallions in this study, complications identified were transient scrotal edema, which resolved within 3 days of biopsy; subcutaneous/albuginea hematoma, which was identified via ultrasonography and resolved within 7 days of biopsy; and fibrin deposits within the vaginal cavity, which were identified at the time of castration. No horses demonstrated signs of discomfort after the procedure, and all remained clinically normal. Stallions were not collected before and after biopsy; however, epididymal semen was collected after castration, and preliminary analysis did not show any differences in progressive motility and spermatozoa morphology between biopsied and nonbiopsied testes. On the basis of this study, we conclude that biopsy of normal testicular parenchyma by inexperienced operators of a spring-loaded biopsy instrument can result in minimal, transient gross changes to the scrotum and testis with no effect on extragonadal (epididymal) sperm reserves.

In this study, effects of biopsy were assessed by color Doppler ultrasound evaluation of the testicular artery of the biopsied and nonbiopsied testes. No increases in testicular blood flow were observed. It has been postulated that one of the reasons that biopsy may result in testicular degeneration is the increased temperature within the testicular parenchyma during the procedure.\(^15\) Our examinations did not demonstrate increased blood flow, which probably would be implicated in these temperature changes. Additionally, previous studies of open surgical biopsy demonstrated an influx of inflammatory cells and formation of granulation tissue after biopsy.\(^11\) The histologic sections taken from the testes at castration in the first group of horses did not demonstrate any cellular infiltrate or histologic changes within the testes. This probably is a reflection of the small diameter (14 gauge) of the biopsy compared with surgical biopsy. This study did, however, find a significant difference in testicular blood flow between days that did not follow a trend, which may be reflective of the advancing season, ambient temperature, and individual variation between stallions.

Although this study demonstrates minimal transient complications after testicular biopsy, practitioners who utilize this technique should monitor stallions closely for several days after the procedure. Additional doses of anti-inflammatories and/or antibiotics may be required if scrotal edema or hematoma persists, if the horse shows systemic signs of discomfort, demonstrates lameness in the hind limbs, or if the scrotum becomes warm, painful, and/or produces discharge from the biopsy site. Any signs of complication should be considered an emergency because orchitis and increased testicular temperature can have irreversible effects on spermatogenesis and fertility. Veterinarians who work in reproduction should be experienced in stallion medicine and be able to readily identify and treat or refer complications which may occur after testicular biopsy.

In summary, this study demonstrates that with instruction, novice operators can successfully use a spring-loaded split-needle biopsy instrument to obtain a diagnostically valid testicular biopsy from a stallion with minimal complications. Practitioners should feel comfortable obtaining a biopsy when indicated as part of an advanced reproductive examination in a stallion.

References and Footnote


