Male Ruminant Breeding Soundness Exams: New Ways to Approach an Old Topic

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Introduction

Breeding soundness examinations (BSE) in male ruminants (bulls, rams, bucks) is by no means a novel topic. However, that doesn't mean we can't introduce some novel techniques along the way to improve our diagnostic and prognostic potential. Not only is this procedure crucial for ensuring a profitable breeding season for our clients, but it is also a great way to generate income for ourselves. When you think about the benefits of performing a BSE, not only is it a relatively quick procedure, but it doesn't cost a lot to perform aside from the initial investment. The best tools you can have on hand are good physical examination skills, a reliable electroejaculation unit, and a decent microscope… all of which can last for many years and will pay for themselves if well cared for. Some other techniques that will be described may involve equipment you already have in your clinic, including ultrasonography and thermography.

Traditional Examination

Obtaining a history should be the first thing done before even touching the animal. Most importantly, you should focus on why the BSE is being performed in the first place. I tend to be a lot more stringent on my recommendations when it is a pre-purchase BSE, because you never know what will happen after that animal is sold, so you should have it all in writing! Also, this allows you to work with the owner to determine if a health certificate and/or any testing (i.e., Trichomoniasis testing for bulls; *Brucella ovis* for rams, etc.) is required. If it is simply a pre-breeding season BSE, I definitely want to pay attention to the specific sperm defects seen and, if the animal is deferred, determine if it is a case of "rusty load" versus something that might be permanent in order to make recommendations. Additionally, determining whether the bull is going to be used for natural cover or collected for artificial insemination may be another important factor when it comes to compensable sperm defects, which will be discussed later.

There is absolutely no technique that will ever surpass a good physical examination. This begins with a lameness exam when the animal is walking into the clinic. If lameness is observed, can you identify and correct the problem or is it more chronic? It is perfectly acceptable to defer or even fail an animal if an owner will not allow you to address a lameness issue. Something as simple as a foot trim can work wonders on a male's ability to mount and breed a female. Evaluating the overall health (BCS, attitude) and eyes (pinkeye? cancer?) of the animal is crucial, especially in those that are being sold for natural cover. Though we still struggle with libido evaluation in our examinations, there is no doubt that a very sick or partially blind animal will not be able to effectively mount and breed a female. This is another parameter where veterinarians tend to feel guilty deferring a bull/buck/ram on; but, again, the goal of the exam is to make sure the animal is reproductively sound, so these issues need to be addressed. One last physical exam technique not

commonly used anymore is pelvic size in bulls, which is based off of data that showed the sire's pelvic size is heritable and can affect the daughter's pelvic size [1]. This seems to be of less concern anymore with the advent of genomics and increased information available from bull studs (i.e., expected progeny differences for maternal calving ease), which is much more reliable. However, in smaller herds, acquiring a pelvic measurement may be of some value.

Evaluation of the reproductive tract is equally as important as the general physical examination. The testes should palpate symmetrically, be freely moveable within the scrotum, and have a firm, but resilient consistency, like a tennis ball [1]. The epididymides should also be palpable and symmetrical. The scrotum should be free of any thickening (i.e., from frostbite), adhesions (i.e., from trauma), bug bites, etc. In general practice, scrotal circumference (SC) measurements is still the preferred method of assessing sperm production. For mature bulls ($\geq$ 24 months), a minimum scrotal circumference of 34 cm is required to pass a BSE [1]. However, you should always combine your SC with your visual and palpable assessment of the scrotum because bigger is not always better, especially if the size is due to fat accumulation [2]. If you suspect that the shape of the scrotum seems to be abnormal, calipers can be utilized to measure each individual testicle or ultrasound to assess the scrotal fat content [3]. In small ruminants, it is extremely crucial to take season into account because testicular volume can vary by up to 30% [4]. Many owners may request BSEs outside of the breeding season, and it is not abnormal for the scrotal circumference and semen quality to be subpar during this time. Some bucks and rams seem to be more affected by season than others (depending on the breed/genetics). However, while some small ruminants can pass a BSE outside of the breeding season, I will never fail one until it can be rechecked in the fall. There is no general consensus for minimum recommended SC in small ruminants, but it seems that mature rams with a SC of < 34 cm or bucks with a SC of < 25 cm tend to not perform well [4].

The prepuce should always be visualized and palpated to ensure there are no adhesions, masses, etc. that may prohibit exteriorization of the penis. Some bulls are genetically predisposed to having a prolapsed prepuce. While this may not directly affect reproductive potential, it makes them more prone to injury and inflammation, which will affect the ability of the penis to protrude. Depending on the severity, there should be a discussion with the owner about whether to surgically correct or just monitor the prolapse. The penis also needs to be exteriorized and examined. In bulls, this usually occurs during the electroejaculation (EEJ) process. However, in small ruminants, the penis generally needs to be manually exteriorized for evaluation. In both small and large ruminants, manual exteriorization can be facilitated by having someone place pressure on the sigmoid flexure, caudal to the scrotum, and another person stabilizing the prepuce and trying to "walk" the penis out. In bulls, a pudendal block may be a more feasible option for facilitating exteriorization of the penis, though this technique is not routinely performed by general practitioners.

Utilizing "New" Old Techniques

As mentioned, NOTHING can outperform a good physical examination. However, there is some value in employing more advanced techniques when performing a BSE. The majority of clinics have ultrasound units, which can have huge prognostic potential in the case of an unsatisfactory examination. One objective means of predicting breeding potential is through the use of

computerized-image-analysis, which measures pixel heterogeneity of testicular parenchyma. Though much more work needs to be done to validate for clinical use, this technique has been shown to correlate well with normal sperm morphology and progressive sperm motility at 60 days prior to semen collection [5]. Additionally, increased testicular pixel intensity has been found in early- versus late-maturing bulls [6], which may provide a useful screening method in the future. The most applicable use of ultrasound that can be used in clinical practice currently is for objective evaluation of pathologies. Testicle palpation can be an extremely subjective examination method. Using ultrasound to evaluate the testicular parenchyma is much more sensitive at detecting subtle abnormalities that may include orchitis, testicular degeneration, hematocele, etc. Additionally, some studies have looked at the fat cover thickness over the testicular vascular cone and found that it can be associated with scrotal temperature and, subsequently, semen production [7]. This finding may help to provide evidence to owners that a new nutritional plan may be necessary. Lastly, ultrasound can be used to pick up subtle changes in accessory sex glands that may not be palpable [8,9]. In small ruminants, this method is the only way that the accessory sex glands may be evaluated due to their size. Pathologies, such as seminal vesiculitis, can have a major impact on fertility in both small and large ruminants. While the reported prevalence may vary, it seems to be more common in either young, peripubertal bulls or aged bulls (>9 years); with spontaneous recovery possible in young bulls but a grave prognosis for older bulls [10]. Testicular degeneration is another fairly common abnormality that can be picked up on ultrasound by the presence of mineralization within the testicular parenchyma. Very often this is a temporary sequela to an underlying issue, such as abnormal thermoregulation, nutritional excesses/deficiencies, infectious diseases, or trauma, and can be reversed with good management [11]. Less commonly, it may be a congenital or inherited problem that may be permanent. Obtaining a good history and re-evaluating the animal may be crucial in determining the prognosis for any of these conditions.

Another technique I have become interested in using is thermography to evaluate abnormalities in scrotal temperature. While in our referral clinic we have access to a thermal camera, this procedure could easily be performed using a cheap, handheld infrared thermometer. In general, the testes should be 2-6°C cooler than the core body temperature. On evaluation of scrotal temperature, there should be symmetry from the left to right with a 4-6°C decrease in temperature from the top to the distal aspect of the scrotum [12]. Major deviations from the normal can adversely affect thermoregulation and may present an underlying problem with semen production, which will likely also be detected in sperm morphological analysis. However, being able to determine the underlying cause will assist in developing recommendations for the owner.

Semen Evaluation

Of course, determining semen quality is vital for determining breeding potential. Semen collection for BSE is most commonly performed in ruminants using electroejaculation, as it is easier and more convenient than using an artificial vagina. In my opinion, the best unit to invest in is a Lane Pulsator IV (Lane Manufacturing, Inc., Denver, CO), especially for those of us who work with multiple species, because there is a good variety of probe sizes available. The main disadvantage for using electroejaculation is the inability to accurately assess sperm output; however, a scrotal circumference measurement can help to estimate this parameter.

We like to prepare our collection cones and tubes ahead of time and put them in a warm incubator (37°C). This helps to prevent cold shocking the sperm at collection and producing iatrogenic sperm defects (i.e., bent tails). When preparing to collect the semen, we keep the tube in our hand to continue to keep it warm prior to ejaculation. The semen should be evaluated grossly for color, clarity, and consistency. A normal ejaculate should appear creamy and white, indicating that there is appropriate concentration. A very dilute sample will appear only mildly cloudy and, while enough sperm may be present for evaluation, we recommend attempting a recollection as it will likely contain many secondary defects since true ejaculation did not occur. In small ruminants, a dilute sample is not an uncommon finding outside of the breeding season due to seasonal effects on sperm output. A yellowish color in the ejaculate should be tested for urine by looking for the presence of blood urea nitrogen with a test strip since urospermia may cause the motility to be falsely decreased [11]. However, increased riboflavin concentrations in the ejaculate (presumably a hereditary trait), may also produce a light-yellow color and not affect sperm quality at all [13].

Due to the expense of computer-automated sperm analyses (CASA) equipment, subjective motility evaluation continues to remain a topic of interest. In an appropriately concentrated sample (i.e., cloudy and creamy), the traditional gross assessment at low magnification (10×) is still my preferred method as it provides some objectivity. The classifications for gross assessment in bulls set by the Society for Theriogenology are “Very Good” for rapid swirling, “Good” for slow swirling, “Fair” for generalized oscillation with no swirling, and “Poor” for no movement [1]. In small ruminants, a scoring system for mass motility has been described using a scale from 0 (no motion) to 5 (numerous rapid waves) [14], with lambing rates reported to have increased by approximately 10% when using semen with a score of 5 versus a score of 4 for fresh, transcervical AI in sheep [15]. If you wish to evaluate individual or progressive motility, you can dilute out the sample with an isotonic solution (saline, sodium citrate, extender, etc.) and perform a motility count in several fields at 40×. In my experience using 0.9% saline can result in a falsely decreased motility, even if pre-warmed; however, this is the fluid that most practitioners have readily available that is cheap. So in a sample that looked “Very Good” or “Good” on gross motility, I hesitate to fail on a poor individual motility that was diluted this way, especially because a good morphological analysis will be much more diagnostic. In fact, I tend to reserve doing an individual motility for those samples that are less concentrated (and hence won’t produce a good swirl) or those that were “Poor” to “Fair” on gross motility.

Determining sperm morphology requires the same techniques as always. In our clinic, we use a phase-contrast microscope, which eliminates the need for staining. However, the eosin-nigrosin based stain is still commonly used for those performing evaluations with a light microscope. The most important point to remember is that you should always evaluate using the oil-immersion lens at 100×. While many of the tail abnormalities are apparent at lower magnifications, this will be the only way to diagnose some head abnormalities that may have a major impact on infertility.

There is an article available online, written by Dr. Michael Jelinski, that provides great images and information on commonly observed sperm defects that I highly recommend (http://vahs.net/wp-content/uploads/Breeding-Soundness-Exam-Summary.pdf) [16]. Figure 1 of this document contains a list of commonly observed sperm defects and their classifications. Sperm defects have

traditionally been categorized as primary or secondary, with the former indicating a spermatogenesis defect and the latter a storage (epididymal) defect. Another classification system is determined by the specific defect's significance on fertility being classified as either major or minor. Most of these defects fall into the same classification scheme as primary or secondary. The newest classification system is one to be aware of because it is likely to replace the primary/secondary classification system in the future [11]. This system classifies defects as compensable vs. uncompensable. The significance of this classification system is based on whether the defect can be "compensated" for with the addition of normal sperm cells [11]. As mentioned previously, it is important to perform a good morphology and be able to assess head defects, as many of these are classified as uncompensable (e.g., knobbed acrosome, pyriform head, nuclear vacuole). The reason for this is because the sperm cells with uncompensable sperm defects still have adequate motility and can make it to the egg and begin the sperm-egg binding process. This process will block normal sperm from being able to bind [11], and fertilization will either not be successful or the resulting embryo will be non-viable [16]. This is in contrast to tail defects, which will have poor motility and be unable to travel to the egg. Therefore, increasing the dose of normal sperm (i.e., additional frozen semen doses) will compensate for those defects, and the normal sperm will be able to make it to the oocyte and initiate fertilization [11]. This classification system is especially useful for those using semen for artificial insemination, as you do not want to cryopreserve semen with a high number of uncompensable defects, but you may adjust the breeding dose if there are many compensable defects. For live cover males, we still follow the 70% minimum rule for passing a BSE [1]; though you should give attention to those samples that have a high number (25-30%) of any specific defect, especially if uncompensable. Many of these defects are associated with stressors that affect thermoregulation or disrupt hormone production, so an attempt should always be made to diagnose the underlying cause (good physical exam, history, etc.) so that an appropriate prognosis for return to fertility can be determined. For ruminants with high numbers of secondary defects, especially at the beginning of the breeding season, a 2 week recheck should be recommended; whereas those with a high number of primary defects may require a recheck in 60 days to see if the problem has resolved.

Conclusions

As a reminder, there is no new techniques available that can replace a thorough physical examination. However, an examination can be aided with the use of ultrasound or thermography for diagnosing the underlying cause and determining prognosis of a fertility issue in male ruminants. Sperm morphologic assessments is also crucial, as it can also assist with diagnosis and providing recommendations to the owner. The last point I'd like to make is to not be afraid to keep up with the times. The Society for Theriogenology has implemented electronic BSE software that has some wonderful features: it's easy to use, economical (cheaper than the old forms), provides an attractive print-out for clients, and is efficient, allowing you to retain individual owners and bulls within the system. This system also allows all of this information to be accumulated into a national or international database, which may produce useful data for future studies that can improve our BSEs. It can be found at: http://www.therio.org/?page=eBSEinformation.

Figure 1

Defect	Primary/ Secondary	Major/ Minor	Compensable/ Uncompensable
Proximal droplet	Primary	Major	Uncompensable (?)
Abnormal midpiece	Primary	Major	Compensable
Strongly folded tail	Primary	Major	Compensable
Knobbed acrosome	Primary	Major	Uncompensable
Pyriform head	Primary	Major	Uncompensable
Nuclear vacuole	Primary	Major	Uncompensable
Stump tail	Primary	Major	Compensable
Distal droplet	Secondary	Minor	Compensable
Distal midpiece reflex	Secondary	Minor	Compensable
Micro/macro-cephalic	Secondary	Minor	Compensable
Detached head	Secondary	Minor	Compensable
Terminally coiled tail	Secondary	Minor	Compensable

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