Beef Session

Moderators: *Arn Anderson, Tom Portillo*

Expanding the BSE to become indispensable

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Introduction

The Society for Theriogenology (SFT) adopted the current form for the bull breeding soundness evaluation (BSE) in 1992. The BSE consists of 4 parts: a physical exam, a minimal scrotal circumference by the age of the bull, minimum progressive motility of 30%, and minimum morphology of 70% normal cells.[6] A bull deemed to be a satisfactory potential breeder should unequivocally meet or exceed these 4 requirements. These standards as set forth by the SFT give the veterinarian an objective approach to evaluating a bull. It has been said that, "rarely are bulls infertile, but there are a lot of sub-fertile bulls". Identification of these sub-fertile bulls by veterinarians allows cattle producers to negate a potential negative impact on the overall productivity of the herd due to poor reproductive efficiency.

Each part of the exam should be of equal importance to the veterinarian, and no part should be omitted if an accurate assessment of the bull is to be determined. Carson et al, in a review of BSE trends for the past 20 years, deemed that overwhelmingly the most common cause of unsatisfactory or deferred classification of bulls was due to unacceptable semen morphology.[5] The preparation and interpretation of the morphology slide must be proficient and consistent in order for the results of the exam to be valid.

The challenges that food animal practitioners encounter regarding remaining relevant in the livestock industry continue to surface, and the profession has to find means to maintain their position in a changing world. Promoting ourselves as experts and the benefits of a professional exam by a veterinarian are essential to suppressing emerging imitators that provide a mediocre version of this exam sometimes referred to as the "semen check".

Sample Handling and Slide Preparation

A quality semen sample can reliably be obtained with proper preparation of the bull followed by collection via electroejaculation. The urethralis muscle can be stimulated during the rectal examination that is part of the physical exam. It is during this time that the accessory sex glands (ampullae, prostate, vesicular glands) are evaluated for pathology. Ideally, the bull will extend his penis outside of the sheath before a semen sample is taken, producing a sample that is devoid of contaminants (organic material, hair, etc.). The sample should be protected from extremes in environmental temperatures that may cause changes in sperm morphology that may not be a true representation of the sperm population.

The collection device should allow for stabilization of the ejaculate temperature from time of collection to microscopic examination. Several collection devices are available, with a Styrofoam cup or a water jacket being the 2 types most commonly used by practitioners. The Styrofoam cup is an acceptable means of holding the semen if environmental temperatures are above 40°F (4.4°C).[11] Warm water jackets offer the advantage of maintaining semen at an acceptable temperature when ambient temperatures are less than 40°F (4.4°C) and can be made from an empty 1 liter intravenous fluid bag that is taped to a standard collection handle and filled with 98.6°F (37°C) water. A 10 mL vacutainer red top tube that is constructed from glass is placed inside a plastic collection cone and taped tightly at the neck of the tube. The tube is then placed inside the water jacket in the collection handle. The water should be maintained at the aforementioned temperature or this will become counterproductive to the goal of maintaining a consistent temperature post-ejaculation. The maintenance of the water temperature can become tedious for the practitioner in field conditions, but can be overcome with the use of a water incubator.

Samples should be processed within minutes of collection for the most accurate assessment. Initial gross examination of the ejaculate can provide several crucial pieces of information. Concentration can be estimated by the opaqueness of the sample. With highly concentrated ejaculates appearing similar to heavy cream and low concentrations similar to skim milk. A yellow color to the sample may be an indication of urine contamination. Urine contamination of semen is of concern as it can significantly reduce the motility of the semen sample. A red or brown discoloration of the semen is an indication of blood contamination and the patient should be further evaluated for the source.

An accurate assessment of sperm morphology starts with preparation of a smear of semen. There are several methods that have been proven effective. It is the author's preference to place a small drop of neat semen on the frosted edge of a warm, clean slide after drawing a thin line of eosin-nigrosin stain (ENS) distal to the frosted edge. ENS is recommended by the SFT and is a live/dead stain. Cells that have a

compromised plasma membrane will take up eosin and will appear red during microscopic evaluation. The live cells with an intact plasma membrane will appear white against the purple background provided by the nigrosin component of the stain. ENS provides consistent results, is readily available to the practitioner, and is economical to use in clinical practice. Following placement of neat semen and stain, a clean slide is held at a 30 to 40° angle and is used to push the stain into the drop of semen and then to draw the sample out along the slide in the same manner as a blood smear. An alternative is to place the stain and drop of semen side-by-side on the frosted edge. The mixture is then mixed with another slide and drawn to the opposite end of the slide using the method described above. Alternately, some clinicians prefer a start/stop method. This method offers different densities of cells along the slide to evaluate.

Despite the method used to make a sperm morphology slide, the goal is to prepare a smear in which sperm cells don't overlap. This allows the examiner to effectively view each sperm cell individually allowing for efficient and accurate classification. It is the author's preference to make 2 morphology slides at the beginning of the semen evaluation process. This not only ensures a quality slide is available, but also allows for efficiency during the process as another slide is ready if the quality is lacking with the first slide, and the slides can dry during the examination of the sample for motility.

Causes of Altered Morphology

The most common causes of abnormal spermatogenesis are altered thermoregulation, effects of hormonal imbalances caused by stress, harmful effect(s) of genes or toxins.[3] Normal spermatogenesis occurs when there is a 36 to 43°F (2 to 6°C) temperature gradient from the testicle and that of the core body temperature.[14] Environmental factors are a major cause of abnormal spermatogenesis in the southeastern United States. Extremes in environmental temperature cause increased scrotal temperatures and thus increase the metabolic rate and oxygen demand of the testicle. However, blood flow does not increase to compensate for the increased demands, resulting in hypoxia of the testicular parenchyma. Hypoxia of the testicular parenchyma results in alterations of spermatogenesis that can be detected during a spermiogram. Increased scrotal temperature and subsequent effects are often noted in young bulls. This increase in scrotal temperature is the result of an insulating layer of fat found in the neck of the scrotum. This condition is most common in those bulls which are being marketed for production sales and are heavily conditioned. In cold climates bulls may suffer from frostbite of the apex of the scrotum, leading to scarring of the scrotum. Scarring can lead to the testicles being held unnaturally close to the body due to the loss of ability by the bull to lower the testicles away from the body through relaxation of the dartos and cremaster muscles.

Stress originating from numerous causes, including environment, sickness, or injury, leads to increased cortisol concentrations in the bull. This elevation in cortisol leads to a decrease in luteinizing hormone and a subsequent drop in testosterone. This drop in intratesticular testosterone concentrations leads to altered spermatogenesis.

Common Morphologic Abnormalities

Pyriform and Tapered Heads

This is the most commonly reported head abnormality of the bovine spermatozoa.[4] The shape of the head is largely decided by the nucleus and is species-specific. The head is shaped like a pear and has a narrow post-acrosomal segment. Different degrees of this defect have been reported from slight to severely tapered through the post-acrosomal segment of the cell. The negative impact on fertility has been documented in both *in vivo* and *in vitro* studies. Thundathil et al reported that ejaculates containing high numbers of sperm with pyriform heads have a reduced fertilization rate compared to control ejaculates with much lower incidence of this abnormality, 68.5 vs 84.4%, respectively.[20] Those pyriform sperm that are capable of binding to the oocyte and initiating fertilization have been shown to have reduced ability to initiate cleavage, and embryonic death ensues.[20] Pyriform heads can be seen secondary to heat stress, testicular insulation and testicular hypoplasia.[4] Bulls that are suspected to be suffering from heat stress can be deferred and re-tested in 60 days to allow time for recovery. Bulls that have no history of environmental insult or display testicular hypoplasia carry a poor prognosis for improvement. Young bulls that are over-conditioned upon examination often produce a high percentage of sperm with this defect, but can often recover after weight loss.[4]

Distal Midpiece Reflex

This is the most commonly reported defect of the midpiece region of the bovine spermatozoa. It is an indication of a problem within the cauda epididymis.[4] This defect is best described as a sharp bend in the tail at the distal midpiece. Some authors may describe this abnormality as a bend starting in the distal midpiece in the shape of the letter "J".[4] These cells are often detected as moving backwards or in tight circles when assessing the progressive motility of the ejaculate. The natural form of this defect often possesses a cytoplasmic droplet within the bend. The lack of a cytoplasmic droplet trapped in the bend is an indication that the defect may be iatrogenically induced.[4] If the ENS becomes hypotonic or morphology smears are allowed to dry slowly, a significant proportion of cells with distal midpiece reflexes may be noted. It is suggested that a new morphology slide be made should this defect be noted without a cytoplasmic droplet in excess of 20 to 25% of sperm in the ejaculate. Epididymal function is highly dependent upon testosterone concentrations. Causes of reduced testosterone concentra-

tions include temperature extremes, exogenous estradiol, stressful events, and induced hypothyroidism.[4] This defect can be detected within a week of a stressful event such as severe winter weather.[17] These sperm lack progressive motility and therefore can be compensated by normal sperm in the ejaculate. The prognosis for return to breeding soundness is generally good if the stress is not prolonged. Practitioners should expect to discover other morphologic abnormalities in the spermiogram following a more prolonged stressful event. The spermiogram will not recover for 6 to 8 weeks following a prolonged stressor to the bull.

Abnormal Midpiece

There are many morphologic abnormalities of the midpiece that can placed in this generalized category. It is the author's opinion that within this broad category of midpiece abnormalities we find the "swollen" midpiece or pseudodroplet and Dag defect most commonly. The other defects reported that can be placed under this category are the "corkscrew" or bent midpiece. Abnormalities of the midpiece are characterized by major disruptions in the axonemal fibers and mitochondrial sheath. This segment of the cell develops within the seminiferous tubule and is important to fertilization because the mitochondria is housed here. Abnormalities of the mitochondria are considered compensable as these sperm lack motility and therefore the ability to traverse the female reproductive tract. The Dag defect, named after a Jersey bull whose ejaculate contained 100% of the specific abnormality, is believed to have a genetic predisposition.[10] Disturbances in spermatogenesis can also cause this defect in small percentages of sperm in the ejaculate. The Dag defect is characterized by a figure eight appearance of the midpiece and principal piece. Some forms of this group of defects can be caused by gossypol toxicosis if fed in high quantities. Gossypol is a phenolic compound found at different levels in whole cottonseed that causes damage to sperm structure during spermatogenesis. Gossypol-induced defects of the midpiece can be reversed 28 days post elimination from the diet.[9] Limit feeding whole cottonseed can avoid the negative effects on fertility while allowing utilization of this by-product feedstuff.

Proximal Droplets

These spherical cytoplasmic condensations should move down the tail during the epididymal transit and eventually will be shed when the sperm cell is exposed to seminal plasma during the ejaculatory process.[15,18] The proximal droplet is 2-3 μm in diameter and is characteristically located at the implantation fossa of the midpiece.[15] It is common to discover significant numbers of proximal droplets in peripubertal bulls.[4,12] This defect is a major reason for 12 to 15 month old bulls failing to meet SFT semen quality standards. The droplets should diminish as the bull matures and carries a good prognosis for gaining satisfactory semen quality.[4] Mature bulls demonstrating high percentages of proximal droplets carry a poor prognosis as the droplets are an indication of a degenerative process within the seminiferous tubules. The proximal droplet is considered a noncompensable defect. The justification is that the cell has the ability to traverse the female tract and compete with normal sperm at the level of the uterine tube for the right to bind with the oocyte. *In vitro* studies indicate poor zona binding and cleavage rates in ejaculates containing significant numbers of proximal droplets.[1,18]

Detached Heads

This defect is commonly encountered to some degree in most ejaculates. The detached but otherwise normal heads are likely due to prolonged storage in the epididymis or ductus deferens during extended periods of senescence. Detached abnormal heads are evidence of problems arising during spermatogenesis. This defect is often noted in young bulls approaching puberty or bulls that have recently endured a stressful event.[2] This defect may be also be an indication of a problem during the 9 to 11 day period of epididymal transport. Detached heads are considered a compensatory defect because of the lack of the tail to provide linear motility. It is suggested that bulls be recollected within the same visit if a significant amount of detached heads are noted on the initial spermiogram. Some bulls may have to be collected multiple times in 1 day to deplete the epididymal reserve and achieve a satisfactory sample. The key is that on subsequent samples the number of detached normal heads become less with each collection to give the practitioner a clue that they are seeing a so called "rusty load". If the defect is determined to be due to a stressful event, the bull can be deferred and rechecked in 2 to 3 weeks if the insult is deemed to be eliminated at the time of the exam. It may take several months for the bull to improve if the animal is peripubertal.

Coiled Principal Pieces

This defect appears like a rolled up rope at the very distal end of the principal piece. Impaired thermoregulation has been implicated as the etiologic agent. In the author's experience an increased incidence of this defect is detected in months with extreme ambient temperatures. This defect is commonly discovered concurrently with defects such as the distal midpiece reflection, indicating an epididymal origin. Barth reported this defect in several breeds, but found that Herefords were commonly represented.[2] It should also be considered a compensable defect due to the lack of motility. This defect has been reported in gossypol feeding trials.[9]

Less Common Morphologic Abnormalities

Knobbed Acrosome

The apical region of normal bovine spermatozoa must possess an acrosome that is tightly adhered and has a smooth, uniform appearance to be considered normal.[13] The acrosome contains many enzymes important to achieving fertilization. The classic beaded appearance of the knobbed acrosome

defect[8] can have other variations characterized by either a flattened or indented apex region.[4] The etiology still remains idiopathic despite many unproven hypotheses. It is considered a heritable condition in cases where no other significant sperm abnormalities exist and the defect is discovered in a significant percentage of the sample.[4,19] These cells show decreased zona binding ability. The cells also show decreased embryonic development beyond the blastocyst stage if the zona is penetrated and the oocyte fertilized,[19] thus suggesting this defect be considered noncompensable. Bulls with significant percentage of acrosomal defects in the absence of other abnormalities carry a poor prognosis for return to breeding status.

Nuclear Vacuoles

These include crater defects and the diadem defect that can be noted at the post-nuclear cap region of the cell. The diadem defect has the appearance of a string of pearls or invaginations in the nucleus.[7] Nuclear vacuoles were induced by both scrotal insulation and administration of dexamethasone, with maximal numbers of sperm containing vacuoles occurring 2 to 4 weeks following treatment.[3] Single or multiple vacuoles can present anywhere in the sperm nucleus. It has been reported that sperm containing nuclear vacuoles can ascend the female reproductive tract, penetrate the zona pellucida, and compete with other sperm for fertilization.[16] However, another study reported a decreased ability of vacuolated sperm to penetrate the zona pellucida in *in vitro-matured* oocytes.[18] The bull could recover to satisfactory semen quality 60 days after removal of the environmental insult.

Integrating the BSE into Your Practice

This exam can be a productive part of the practice and provides a value-added service to the producer. Practitioners often question how they can grow this part of the practice or contemplate means to make it more profitable. We have used a few strategies and feel it could benefit your practice.

The saying that "seeing is believing "is true. As veterinary educators began searching for methods to teach numerous students' motility and morphology of semen samples more efficiently, a camera system was implemented into the microscope. We later discovered unintentionally that clients were also learning from the visualization of what we were seeing under the scope. We found that we had an increase in exams requested from those clients, and many new clients that reported they were referred by such clients that described our new innovation in semen evaluation.

When an abnormality is noted in a bull, whether it is a physical defect or sperm abnormality, open a dialogue with the client about what you are seeing. Explain why it will have a negative effect on either the bull's ability to copulate, produce offspring, or a characteristic that can be passed on to the offspring. By imparting our knowledge to clients they perceive more value has been given to them as they can then take that knowledge and make better decisions regarding their herd in the future and consequently improve the profitability of their herd.

It is more profitable for the practice to perform exams on multiple bulls as opposed to a single bull. We all have small herds that we service, and it would benefit both parties to schedule BSE days at a central location. It is possible for the venue to be at auction barns or livestock arenas as opposed to the practice. This requires some effort on the part of the practice to advertise through social media outlets, feed stores, and extension agents. The marketing of this exam can be enhanced by offering it at a discount or providing a free biological at the time of the exam.

Summary

The complete bull breeding soundness exam should always include all 4 parts of the exam, but should not be concluded with the simple handing over of a piece of paper declaring that bull "Satisfactory" or "Unsatisfactory". As a profession we must strive to provide a consistent, high quality, professional exam to remain a relevant part of this ever-changing industry. We can accomplish this by showing the value in our service. Open communication, imparting knowledge, and efficiency all harmonize together to project the value we wish to project to our clients. In opening the dialogue of how this 1 exam can affect their bottom line, you will go a long way in helping your efforts to perceive the value of your services.

References

1. Amann R, Seidel G, Mortimer R. Fertilizing potential in vitro of semen from young beef bulls containing a high or low percentage of sperm with a proximal droplet. *Therio* 2000; 54:1499-1515.
2. Barth AD. Evaluation of semen quality. In: *Bull breeding soundness.* 3rd ed. Saskatoon: Western Canadian Association of Bovine Practitioners, 2013; 41-89.
3. Barth AD, Bowman PA. The sequential appearance of sperm abnormalities after scrotal insulation or dexamethasone treatment in bulls. *Can Vet J* 1994; 35:93-102.
4. Barth AD, Oko RJ. *Abnomal Morphology of Bovine Spermatozoa.* Ames, IA: Iowa State University Press: 1989: 130-280.
5. Carson RL, Koziol J, Wenzel JGW, Armstrong C, Edmondson J, Maxwell H. Twenty year trends of bull breeding soundness examinations at a teaching hospital. *Clin Therio* 2014;6:495-501.
6. Chenoweth PJ, Spitzer JS, Hopkins FM. A new bull breeding soundness evaluation form. *Proceedings.* Ann Mtg Soc for Theriogenology, 1992; 63-70.
7. Coulter GH, Oko RJ, Costerton JW. Incidence and ultrastructure of "crater" defect of bovine spermatozoa. *Therio* 1978; 9:165-173.
8. Cran D, Dott H. The ultrastructure of knobbed bull spermatozoa. *J Reprod Fertil* 1976; 47:407-408.
9. Hassan ME, Smith GW, Ott RS, Faulkner DB, Firkins LD, Ehrhart EJ, Schaeffer D. Reversibility of the reproductive toxicity of gossypol in peripubertal bulls. *Therio* 2004; 61:1171-1179.
10. Hellmen E, Ploen L, Settergren I, Nicander L. Middle piece defects of testicular origin in bull sperm. *Nord Vet Med* 1980; 32:423-426.
11. Hopper RM. Semen evaluation and overview of common sperm abnormalities. *Clin Therio* 2015;7:261-268.

12. Johnson KR, Dewey CE, Bobo JK, Kelling CL, Lunstra DD. Prevalence of morphologic defects in spermatozoa from beef bulls. *J Am Vet Med Assoc* 1998; 213:1468-1471.
13. Lunstra DD, Echternkamp SE. Puberty in beef bulls: Acrosome morphology and semen quality in bulls of different breeds. *J Anim Sci* 1982; 55:638-648.
14. Ott RS. Breeding soundness examination of bulls. In: Morrow DA, ed. *Current Therapy in Theriogenology*. 2nd ed. Philadelphia: WB Saunders, 1986; 125-136.
15. Pesch S, Bergmann M. Structure of mammalian spermatozoa in respect to viability, fertility and cryopreservation. *Micron* 2006; 37:597-612.
16. Saacke RG, Dejarnette JM, Bame JH, Karabinus DS, Whitman SS. Can spermatozoa with abnormal heads gain access to the ovum in artificially inseminated super- and single-ovulating cattle? *Therio* 1998; 50:117-128.
17. Swanson EW, Boyd LJ. Factors affecting coiled-tail spermatozoa in the bull. *Am J Vet Res* 1962; 23:300-309.
18. Thundathil J. In vitro fertilizing characteristics of bovine sperm with abnormal morphology. PhD dissertation; Saskatoon, University of Saskatchewan, 2001.
19. Thundathil J, Meyer R, Palasz AT, Barth AD, Mapletoft RJ. Effect of the knobbed acrosome defect in bovine sperm on IVF and embryo production. *Therio* 2000; 54:921-934.
20. Thundathil J, Palasz AT, Mapletoft RJ, Barth AD. An investigation of the fertilizing characteristics of pyriform-shaped bovine spermatozoa. *Anim Reprod Sci* 1999; 57:35-50.