

## **UNDERSTANDING A BREEDING SOUNDNESS EVALUATION AND FACTORS THAT IMPACT BULL FERTILITY**

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### **Introduction**

The bull breeding soundness examination (BBSE) has evolved from its infancy from a group of practitioners in the western United States in the early 1950s following a period of severe cold weather in the area that left many range bulls succumbing to scrotal frost bite prior to spring turn out. BBSE criteria were first considered by the group known as the Rocky Mountain Society for the Study of Breeding Soundness in Bulls. This group was instrumental in the creation of initial fertility standards in bulls and promoting research in this area of veterinary medicine. The group evolved over time to what is now the Society for Theriogenology (SFT), and an allied group of specialists known as the American College of Theriogenologists (ACT). Bull BSE standards were adopted and have continued to evolve as peer reviewed research and new technology has permitted the exam to progress. In 2016 the SFT requested that a group of veterinarians both private practitioners and those in academia to review the current standards considering the peer reviewed research that had been published since the standards were last adopted in 1993. The goal of the committee that was formed was to advise the SFT on changes that would improve the current exam and produce a practical manual that would prove to be resource for our profession that has not been produced since 1983.

The overall goal of the exam has not changed through time in that we strive to discover those bulls in a population that are sub-fertile or sterile and eliminate those animals from the bull battery. Understanding that there are few sterile bulls that exist and that most bulls if given sufficient time will settle cycling, healthy females. However, such unlimited breeding seasons deny the producer the opportunity to take advantage of labor, health maintenance procedures and marketing opportunities. Equally important is the considerations for animal well-being such as cows being repeatedly mounted and bred before becoming pregnant, calves born in suboptimal environmental conditions that compromise their health and survivability, and the potential for injury in bulls with low breeding efficiency. The true measure of breeding soundness is the ability and evidence of getting cows pregnant and having a live calf born. However, performing a BBSE prior to the breeding season under the guidelines set forth by the SFT will allow practitioners a uniform method of assessing bulls and designating those that will likely settle 25-30 cows in a 65–70-day breeding season.

A complete BBSE is NOT a semen exam. However, it is true that semen evaluation is a component of a breeding soundness evaluation. A BBSE does NOT guarantee that a bull is highly fertile. A BBSE does NOT rank bulls with respect to fertility. A BBSE will NOT ensure that bulls are free

of a virus or other infectious agents in their semen. A BBSE DOES identify those bulls that possess undesirable heritable traits or that are not likely to achieve a high pregnancy percentage in a limited breeding season. A BBSE considers the physical characteristics of a bull necessary for mobility and athleticism in the pasture, his structural soundness, his overall and reproductive development, the size and health of his testes, and the quality of his semen.

### **Bull Breeding Soundness Evaluation**

The BBSE consists of examination for physical soundness and evaluation of semen quality. Classification is determined by physical evaluation and the bull's ability to meet minimum thresholds for testicular development, sperm motility and normal sperm morphology.

#### **Physical Examination**

A BBSE begins with assessment of a bull's conformation, body condition and overall physical health. He should be of sufficient size for his age, free of obvious disease and should carry adequate muscling and body fat to ensure he is capable of freely walking within the herd to identify females in estrus then mounting and completing coitus. The feet and legs should be free of defects that limit this mobility. Acute or chronic laminitis, post-legged conformation, and screwclaw are some of the more common musculoskeletal conditions that limit a bull's agility and athleticism. Post-leg and screwclaw are potentially heritable conformation attributes with undesirable consequences in the herd.

#### **Scrotum and Testes – Examination**

Bull testes are maintained in the scrotum, a protuberance of the ventral abdominal skin. The scrotal wall consists of skin, sweat glands, the tunica dartos, and is lined by the tunica vaginalis parietalis which is an extension of the parietal peritoneum. The testes are maintained in this location to assist with thermoregulation that is essential for normal spermatogenesis. The testes must be cooler than core body temperature and there is a temperature gradient of 4 to 6<sup>0</sup> C from base to apex of the scrotum. Elevated core body temperature such as may occur with febrile conditions may elevate core testicular temperature with resultant failure of normal sperm production. Likewise, any condition of the scrotum that interferes with normal thermoregulation may also impair spermatogenesis. Such conditions include wounds, scars and ischemia of the scrotum which might result from frostbite in severely cold environments. The tunica dartos functions in conjunction with the cremaster muscle, part of the spermatic cord, to elevate the testes toward the abdomen to reduce heat loss in periods of cold, or to relax and provide greater surface for heat loss during times of normal or excess heat load within the scrotum.

The spermatic cord exits the inguinal ring and consists of the testicular artery, vein and nerve, the ductus deferens, and the cremaster muscle. A few centimeters dorsal to the testicle the testicular artery forms an extensive pampiniform plexus with the testicular vein. The function of this plexus is for venous blood to cool arterial blood before the artery enters the testicle proper. After leaving the pampiniform plexus the testicular artery continues ventrally to enter the testicle at its distal pole.

Measure the scrotal circumference by firmly forcing the testes ventrally in the scrotum and placing a flexible centimeter tape around the largest circumference. Evaluate the scrotum for presence of scars or other pathology. The testes should be freely moveable within the scrotum and no more than 10% difference in between the paired testes. Palpate each testicle gently for texture that should be firmly resilient. Palpate each testicle deeply to assess areas of firmness that might indicate granulomas or fibrosis or calcification. Softness or a mushy feel are consistent with testicular degeneration. Extreme firmness along the mediastinum testes is consistent with irreversible testicular damage. The vaginal cavity is the potential space between the tunica vaginalis parietalis lining the scrotum and the tunica vaginalis visceralis covering the testis. There should be no adhesions or fluid accumulation in this space. Palpate the head, body, and tail of the epididymides for completeness and for the presence of pain or granulomas. Palpate the spermatic cord for aneurisms or other abnormalities.

The minimum threshold for scrotal circumference is based upon age of the bull. Those thresholds are as follows:

<u>AGE</u>	<u>THRESHOLD</u>
≤ 15 months	30 cm
>15 to 18 months	31 cm
>18 to 21 months	32 cm
>21 to 24 months	33 cm
>24 months	34 cm

### **Internal Reproductive Organs - Examination**

Evaluation of internal reproductive organs is an integral continuation of the physical examination. Evacuate the rectum and by rectal palpation identify the urethralis muscle and prostate gland. Bulls rarely develop prostatic disease, and the normal organ is palpable as a somewhat firm transverse band at the cranial extent of the urethralis muscle approximately wrist deep along the ventral midline of the bull's pelvis. Immediately cranial and dorsolateral to the prostate are the paired vesicular glands. Normal glands are resilient to palpation and the glands are somewhat tear-drop shaped, 2 to 4 cm in diameter and 10 to 15 cm long. Just cranial and medial to the vesicular glands lie the paired ampullae which are the termination of the ductus deferens. The paired ampullae narrow and pass under the prostate to empty into the urethra at the colliculus seminalis. The ampullae are 1 to 1.5 cm in diameter, thick walled and 10 to 12 cm long. Their function is to store mature sperm ready for ejaculation.

The internal inguinal rings are palpable openings in the abdominal wall a few centimeters cranial and approximately 45 degrees ventral to the brim of the pelvis. Examine for fat, omentum or intestines entering the ring. Bulls with inguinal rings larger than 5-6 cm may be more prone to development of inguinal hernia.

## **Penis, Prepuce and Sheath – Examination**

The sheath is an extension of the ventral abdominal skin and should be of appropriate size for the bull. Examine the preputial hairs on the end of the sheath for accumulation of blood or exudates which might indicate penile or preputial injury. The presence of sandy or gritty material on the preputial hair may indicate the presence of urolithiasis. Palpate the entire sheath and penis for areas of swelling or fibrosis. Palpate the dorsum of the distal bend of the sigmoid flexure for evidence of prior hematoma of the penis.

The penis and prepuce are usually examined during semen collection. Examine closely for lacerations, penile hair rings, persistent frenulum, urethral fistulae, or scarring of the prepuce.

## **Sperm Motility Evaluation**

The semen should be protected from temperature shock and a drop should be placed on a clean, dry microscope slide for estimation of motility. Although mass motion may be observed at low power, high power observation under a cover slip is preferred to assess the percentage of progressively motile sperm in the ejaculate. Concentrated samples should be sufficiently diluted with warm, fresh physiologic saline to visualize individual sperm under high power. The threshold for Satisfactory Potential Breeder is a minimum of 30% progressively motile sperm.

## **Sperm Morphology Evaluation**

Sperm morphology should be evaluated under oil immersion to adequately evaluate individual sperm. Prepare a slide by mixing diluted sperm with eosin-nigrosin like a blood smear for evaluation with a light microscope. Alternatively, dilute a drop of the ejaculate with 10% neutral buffered formalin and prepare the slide like a blood smear for examination with a phase contrast microscope. Count a minimum of 100 sperm cells classifying them according to normal morphology or those with head, midpiece, or tail abnormalities. When sperm morphology is good, counting 100 spermatozoa is adequate to determine a spermiogram. However, when there is a high percentage of abnormalities a few hundred spermatozoa may need to be counted to determine an accurate spermiogram.

Sperm evaluation provides a noninvasive method to evaluate testicular and epididymal function, providing information like that gained by a testicular biopsy. Spermiograms are defined as a description of sperm morphology during evaluation. An abnormal spermiogram with supporting evidence from the history and physical exam can give insights into reasons for abnormal testicular function, and consequently allow formation of a prognosis for recovery or potential treatment. When an abnormal spermiogram is found, the types and number of abnormalities combined with history regarding environment, nutrition, and health status can be used to compile a reason for spermiogram disturbances noted. The veterinarian can then use that information to make a diagnosis and prognosis for recovery.

The most common causes of abnormal spermatogenesis in males include: abnormal testicular thermoregulation; hormonal imbalances, particularly those associated with stress; and effect(s) of toxins or expression of deleterious genes.

## **Immaturity**

Immaturity is often recognized in the spermogram by the observation of a high numbers of immature sperm cells also known as spheroids combined with high levels of sperm with proximal droplets and distal droplets. Peripubertal bulls often have a high percentage of sperm with proximal droplets in the ejaculate. As bulls mature, the number of proximal droplets in the spermogram should decrease.

Immature sperm cells are quite variable in size, depending on whether the cell is a primary or a secondary spermatocyte or a spermatid. Immature sperm cells must be differentiated from white blood cells in semen. This differentiation can be accomplished by staining a dried semen smear in Diff-quick®. Once the stain is dried the round cells can be evaluated and a final diagnosis of immature sperm cell or white blood cell can be made by the evaluator. If a diagnosis of immaturity is made the bull should be reevaluated in 4-6 weeks to allow for maturation.

## **Testicular Degeneration/Regeneration**

Testicular degeneration is an acquired condition which often follows impairment of the thermoregulatory processes of the scrotum and testes. Diagnosis of testicular degeneration is based on physical examination findings coupled with evaluation of the spermogram. Testes are often palpably softer than normal, and evaluation of an ejaculate reveals a low concentration of sperm, with a high percentage with morphological defects. Immature germ cells and medusa formations often increase in the ejaculate.

Testicular degeneration can be associated with systemic illness, prolonged increases or decreases in ambient temperatures, excessive fat in the neck of the scrotum, scrotal dermatitis, scrotal frostbite and insulation of the scrotal contents due to trauma, inguinal hernia or hydrocele. Local inflammatory processes, such as periorchitis or orchitis, and prolonged recumbency associated with lameness may also impair normal testicular thermoregulation. Progressive degeneration that accompanies aging is likely caused by multiple insults over time. A high proportion of older bulls with testicular degeneration have distal fibrosis of the testis, likely due to vascular lesions.

Testicular degeneration and subsequent regeneration are associated with a variety of morphological abnormalities. The time from the initial insult and the length of the testicular insult will determine the types of morphological abnormalities noted on the day of evaluation. For example, an increase in proximal droplets can be encountered as early as 9 days following an insult while acrosome abnormalities are first observed in the ejaculate 30 days following an insult. This coupled with the fact that we know that spermatogenesis is 61 days in the bull with epididymal transport taking approximately 9-11 days we can note a wide variety of defects in an ejaculate at any given time. It may take repetitive spermogram analysis to determine where in the stages of degeneration or regeneration the bull is currently at.

## **Stress**

Stress for any reason (environment, social, or illness) causes a rise in cortisol which has negative feedback to the hypothalamus and pituitary causing a decrease in FSH, LH, and testosterone. This leads to sperm changes not only in the testes but epididymis as well. The most notable defect associated with stress is the distal midpiece reflex (DMR). DMR defects are due to an abnormal environment in the cauda epididymis, specifically, the distal third of the cauda epididymis.

While DMRs are the most characteristic change you may notice a variety of defects can be found following a stressful event including proximal droplets, detached heads and mitochondrial disturbances, knobbed acrosomes, nuclear vacuoles, coiled principal pieces, and pyriform heads. Testicular degeneration due to thermoregulatory issues or stress are hard to differentiate without acquisition of a sufficient history and physical exam.

### **Genetic Predisposition**

Although adverse environmental influences are the most common cause of abnormal spermatogenesis, an increasing number of sperm defects are recognized as having a genetic origin. One should have suspicions of a genetic influence when a high proportion of the ejaculate is affected by the same morphological abnormality with minimal other morphological abnormalities in the ejaculate.

### **Toxins and Nutritional Changes**

Many toxins have potential to affect spermatogenesis, although few naturally occurring cases have been documented. Gossypol, a phenolic toxin in the pigment glands of cottonseed, impairs sperm production in several species, including ruminants. Free gossypol in cottonseed and cottonseed meal can disrupt spermatogenesis, leading to increased numbers of morphologically abnormal sperm and decreased sperm motility. Bulls fed diets containing free gossypol at levels as low as 8 mg/kg per day for 56 days produced increased numbers of sperm with segmental aplasia of the mitochondrial sheath and other midpiece defects, proximal droplets, strongly folded or coiled tails, tailless (detached) heads, simple bent and terminally coiled tails (coiled principal pieces). Production of defective sperm induced by gossypol exposure is reversible within 28 days after removal of gossypol from the diet.

Producers and veterinarians are often concerned about potential effects of therapeutic agents on semen quality. Exogenous corticosteroids (e.g., dexamethasone) depress pituitary gonadotrophin secretions and can adversely affect spermatogenesis. In contrast, several commonly used antibiotics including tilmicosin, oxytetracycline, dihydrostreptomycin, and the anti-inflammatory agent phenylbutazone had no adverse effects on semen quality.

Testis mass and consequent spermatogenesis fluctuates based on metabolic cues, and appropriate nutritional management remains important throughout life. Bulls should be maintained on a diet with adequate amounts of balanced dietary protein and energy sources to ensure proper endocrine development to support spermatogenesis.

### **Iatrogenic Changes**

Iatrogenic changes noted in the spermiogram are mostly associated with slide preparation. The most common change noted are those due to hypo-osmotic changes whether that comes from stain, prolonged drying times, cold slides, or cold shock of ejaculate prior to staining. Hypo-osmotic changes are of high suspicion when there is a high percentage of bent midpieces. Characteristically these midpieces have no retained droplet within the bend which aids in differentiating this iatrogenic defect from DMRs (distal midpiece reflexes). Cold shock may also be noted during evaluation of progressive motility as will be depicted by sperm moving slowly, backwards and circling and in severe cases shimmering in place.

### **Classification for Breeding Soundness**

Following evaluation according to the SFT criteria bulls are classified according to their suitability for breeding on the day of evaluation. Those bulls that are conformationally sound, free of ocular and musculoskeletal defects and that produce at least 70% morphologically sperm that are at least 30% progressively motile are classified as Satisfactory Potential Breeders (Potentially Fertile). Bulls that do not meet these criteria are placed in one of two categories. Those bulls with temporary conditions which are likely to resolve and allow the bull to meet the above thresholds are placed in the category of Classification Deferred (Subfertile). Bulls in this category are usually juvenile, have an injury or lameness that is likely to resolve or suffer from summer heat induced testicular degeneration. If this classification is used the veterinarian should recommend a date for re-evaluation of the bull. Bulls with undesirable heritable defects, small scrotal circumference that do not meet the minimum for their age, debilitating injury, or disease, or with permanent testicular degeneration should be classified as an Unsatisfactory Potential Breeder (Potentially Sterile).

### **Updates to the Bull Breeding Soundness Exam**

It was the consensus of the committee, that while no one felt that it was likely that sub-fertile bulls were “slipping through” a complete BBSE performed by veterinarians following the current minimum standards, the following changes recommended provide evidence-based improvements of our BSE.

Gross motility will be removed from the SFT BBSE standard form. Mass motion is dependent on three factors: concentration, percentage of progressively motile cells, and velocity of progressively motile sperm. Semen with fair concentration may have 80% rapidly, progressively motile sperm but show no wave motion, whereas highly concentrated semen may have only 50% motile sperm and still show some slow wave motion. Equally, semen with very good concentration and a high percentage of progressively motile sperm may have little or no wave motion if the speed of sperm progression has been diminished by temperature or a prolonged interval from collection to examination. Consequently, gross motility must be carefully interpreted in the light of many factors, and it is the recommendation of the SFT that gross motility is not used for the assessment of semen quality and therefore has been removed from the breeding soundness examination and subsequently the breeding soundness examination form. Individual motility should continue to be assessed and the minimum 30% will remain the standard.

Morphologic classification has focused on placing sperm in the three following categories: normal, primary, and secondary sperm defects. Primary and secondary sperm defects indicate the origin

of the defect not the severity. Adverse conditions may simultaneously affect both epididymal function and spermatogenesis suggesting that primary and secondary defects are of equal importance as indicators of disturbance of reproductive function. Therefore, the time old concept of classifying sperm as primary and secondary, and that primary sperm defects are more important than secondary defects should be questioned. The strong argument to not list sperm defects under the categories of primary or secondary in the future demanded the need for a new classification system. The new morphology classification will now allow for differential counting and then grouping of defects in head, midpiece and tail abnormalities. The prevalent sperm defects should be considered regarding the current understanding of their significance. For good fertility, a minimum of 70% morphologically normal spermatozoa with fewer than 20% head or proximal droplet abnormalities will be needed for a bull to be deemed as Satisfactory Potential Breeder. These levels of tolerable defects are supported by multiple studies.

There are two changes in classification that were adopted following much discussion and evaluation of the literature over the past 34 years. Distal droplets characterized as cytoplasmic droplets just proximal to the annulus have not been implicated in substantial impairment of fertility at high percentages and consequently sperm with otherwise normal morphology retaining distal droplets should not be considered as abnormal. Abaxial tails have found to have no detrimental effects on fertility and therefore they should be considered a normal variation of bovine sperm morphology. Abaxial tail attachment has been reported to be normal feature in the stallion, boar, and dog. A retrospective study was performed to determine the incidence of the defect in Canadian bulls as well as three experiments determining the effect on fertility. The combined results of the experiments indicated that spermatozoa with abaxial tail attachments fertilize ova at a normal rate and are not associated with any increase in embryonic death. In summary, both the distal cytoplasmic droplet and abaxial tail implantation are no longer considered an abnormality.

### **Summary**

A complete BBSE consists of a physical exam, scrotal circumference measurement, progressive motility, and morphologic assessment. The standards adopted in 1993 by the SFT served our profession well and it was the consensus by the BBSE committee that sub-fertile bulls were not slipping through, but these evidenced based changes will improve the exam. However, the committee discovered that we must strive as a profession to be more consistent between exams so that we can trust the results of the exam when classifying bulls. We must also strive to be consistent with this economically important exam between practitioners. The SFT BBSE manual will provide a resource for both current and future veterinarians. The exam will continue to evolve as we discover more through improved technology and research.

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