

BREEDING SOUNDNESS EXAMS

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Introduction

The stated objective of a breeding soundness examination (BSE), as currently defined by the Society for Theriogenology (SFT), is to identify individual bulls possessing the potential of being satisfactory breeders when subsequently used in natural breeding situations. The process by which bulls are identified as potentially satisfactory breeders must not only be simple, unambiguous and repeatable, but it must also possess a reasonable probability of being accurate. This accuracy aspect is important in that the BSE is reasonably accurate in identifying sub-fertile or sterile bulls, but is unable to differentiate among bulls as to their potential level of fertility when used as sires. The examination consists of four-parts: (1) a physical exam, (2) a minimum scrotal circumference measurement based on age, along with two seminal quality assessments which are (3) minimum progressive sperm motility estimates of 30% and (4) a minimum normal sperm morphology score of 70%. Bulls are classified as being: (1) a satisfactory potential breeder, (2) an unsatisfactory potential breeder or (3) classification deferred. If the classification is deferred, then a subsequent examination date is suggested. The SFT established this standardized procedure which is periodically updated (Chenoweth et al., 1993; Carson and Wenzel, 1997; Chenoweth and Spitzer, 1997; Chenoweth, 1997; 2000; 2004a; 2004b; Hopkins and Spitzer, 1997). It is designed to be used for a variety of breeds in different environments. One should also take into consideration that these evaluation guidelines are minimum standards and that individual examiners may prefer to use other procedures or higher standards if he or she believes that such will increase the predictive value of the BSE system.

The objectives of this review are (1) to examine the parameters that are currently used in breeding soundness examinations and (2) to examine the strengths and weakness of such examinations as they are currently being applied.

Physical Examination

A unique identification of each bull to be examined must be established first. Permanent identification marks such as tattoos, brands or electronic devices are preferred because ear tags tend to get torn out and lost. The animal's owner, breed and apparent age should be recorded. Bull age is a major consideration when making judgments relative to seminal quality because some yearling bulls have not matured adequately to achieve classification as satisfactory breeders by the BSE. Most of the physical examination of the bull can take place only after proper restraint in an appropriate squeeze chute.

The physical attributes involved in the production and delivery of functional gametes in a bull must be examined carefully. Production of functional gametes in the bull takes approximately 75 days considering both the sperm production process of 61 days and the sperm maturation process as they transit the epididymides in approximately 10 to 14 days. Once gametes have been produced in the testes and matured in the epididymis, their delivery requires rather precise coordination of the animal's neuro-muscular-skeletal system. The entire reproductive system of a bull must be carefully examined to determine if any inflammation, fibrosis, adhesions or severe scarring is present. This requires thorough visual inspection along with palpation. One must determine if any single aspect of the bull's anatomy and reproductive system or even combinations of these various attributes could impair his ability to produce and deliver viable sperm to the proper site within the female reproductive tract.

Body condition score

Body condition should be evaluated carefully. In most situations body condition is not a major problem. It is the extremes, either too thin or too fat, which are of concern if the animal is to be a successful breeder. Extremely thin or fat bulls are more likely to have libido or mating problems. Also their semen tends to be substandard. For beef bulls, a numerical ranking 1 through 9 is used. A numerical score of 1 is an extremely thin animal while a body condition score of 9 would be obese. Most importantly, a bull must feel well if he is to seek out receptive females so that he can be an effective breeder.

Feet and legs

While examining a bull for body condition, it is prudent to look at how the bull moves (Ott, 1976). Problems with lameness and stiffness, which could limit mating success, are more likely identified when the bull is moving. Some common problems are structural problems with the feet and legs as well as cracked hooves, swollen joints and foot rot. In more mature sires, arthritis and interdigital fibromas (corns) can be problems. The bull must be able to move freely if he is to keep up with sexually active heifers and cows. The bull's claws should be examined for possible malformations as well as overgrowth which suggest trimming will be necessary. Furthermore, semen quality can be compromised if a bull stays in a recumbent position long enough time to increase his testicular temperature. In any event, when a physical defect that could reasonably impair the bull's ability to mate successfully is found then the bull should not be classified as satisfactory. Some conditions are temporary and, thus, a deferred classification becomes a very useful option.

Eyes

Identification of females in heat requires good sight as well as a sense of smell. Sight problems can interfere with a bull's ability to find sexually receptive females. Some common eye problems are infectious keratoconjunctivitis and the resulting scars as well as squamous cell carcinomas.

Vesicular glands /ampullae / prostate

A careful rectal examination should include manual palpation of both the vesicular and ampullary glands using the urethralis muscle and prostate as reference points. The urethralis muscle lies on the floor of the pelvis about wrist deep. It surrounds the pelvic urethra with the prostate forming a transverse ridge across its dorsal cranial aspect. Abnormalities have not been reported for either the urethralis muscle or the prostate of the bull. The vesicular glands of the

bull are paired lobulated structures that are situated cranial and lateral to the prostate gland. Each gland is about 6 to 12 cm long, 2 to 4 cm wide and 1 to 2 cm in thickness. Inflammation of these glands is not uncommon in yearling bulls resulting in a diagnosis of vesiculitis. In this case, the glands are enlarged, swelling to the point that the lobulation is undetectable. The glands tend to become very firm and are painful upon palpation. In this situation, the diagnosis of vesiculitis is confirmed by the finding of a purulent exudate or identification of large numbers of neutrophils (>5 per 100 X field) in the ejaculate. Care must be taken in collecting the ejaculate from questionable bulls because samples collected from the sheath tend to be contaminated with increased numbers of neutrophils potentially yielding a false positive diagnosis. Other detrimental conditions may be abscesses or aplasia of the vesicular glands.

The paired ampullary glands lie between the vesicular glands. They are the thickened portion of the posterior terminus of the ductus deferens. If the elongated ampullae enlarge becoming rounded and firm then a diagnosis of ampullitis is appropriate. In most cases, ampullitis is associated with inflammation of other parts of the reproductive tract.

Inguinal rings

In the bull, the paired inguinal rings lie slightly below the pelvic floor being 1 to 2 cm ventral from the exterior. They are 2 to 5 cm lateral to each side of the midline forming ventromedial slits less than 6 cm in length. Elongation of the inguinal rings beyond 6 cm is a problem that can result in herniation. If the scrotal neck is enlarged, then one should re-examine the bull for a possible inguinal hernia.

Penis / prepuce

The penis and sheath should be examined both visually and by palpation so that any inflammation, adhesions, severe scarring or fibrosis can be detected. The presence of exudate indicates an abnormal inflammation. One should carefully palpate the penis through the sheath to determine if trauma has caused enlargement, excessive firmness and/or asymmetry. The penis should not only be symmetrical, but should be freely moveable within the sheath. No abnormal masses should be present. Inversion of the sheath is common with polled bulls although rarely noted in horned animals. Some judgment is required in this case because inversion of the sheath without lesion or scarring does not automatically cause a bull to be classified as unsatisfactory. Most importantly the penis must be totally extended from the sheath to eliminate the possibilities of a persistent penile frenulum, fibropapillomas, hair rings and/or abscesses. In the more mature bulls, the penis should be examined for the presence of hematomas (broken penis). Broken penises are not common in younger bulls that have not been used for breeding purposes.

Testicles / spermatic cord

The testis, the primary organs of reproduction, should be examined for symmetry, consistency and size. The scrotum and its contents must be free of adhesions and scarring so that internal contents move freely within the outer sack. Notable differences in the size and shape of the testicles suggest the possibility of unilateral testicular hypoplasia, which can arise also from other congenital, traumatic or infectious conditions. The consistency of both testes should be uniformly firm and resilient. Bulls having soft, flaccid testes or those with fibrosis or calcification are unsatisfactory. Classification can be deferred in those with flaccid testes but that are otherwise normal. Certain types of testicular degeneration, such as that caused by

excessive heat, can be temporary. A cryptorchid bull must be classified as unsatisfactory. The condition of the genitals is paramount. Only bulls with two normal testes that are positioned symmetrically within the scrotum can be classified as satisfactory.

Epididymides

Although epididymal problems are rare, each epididymis must be carefully examined. The head, body and tail of the epididymides should be palpated for aplasia, tumors, abscesses and possible granulomas. Epididymitis can be a fairly common problem in yearling bulls. Inflammation of the epididymis can occlude the duct and, if bilateral and severe, results in aspermia. Male genital tract infections such as vesiculitis, ampullitis and epididymitis are usually more generalized.

Scrotum shape

Notable differences in the size and symmetrical shape of the scrotum and its contents are not only abnormal themselves, but usually indicate the presence of other genital problems.

Scrotal circumference measurements

An extremely important part of the BSE is measurement of the scrotal circumference. This highly heritable trait is positively related to sperm output. Care must be taken when making scrotal circumference measurements. Both testes should be carefully massaged downward until they are snugly in the bottom of the scrotum. The measurement, in centimeters, should be taken at the area of greatest scrotal width. The accuracy of the measurement should be confirmed by a second measurement. Bull age is a consideration when assessing scrotal circumference because testicular growth in some yearling bulls is such that they have not achieved their mature size and, therefore, would not be classified as satisfactory breeders by the BSE. The minimum recommended scrotal circumference for bulls from 15 to 24 months or more are given in Table 1.

Table 1. Minimal Scrotal Circumference Measurements for the Breeding Soundness Exam¹

Bull Age (mo.)	≤15	< 15 ≤ 18	< 18 ≤ 21	< 21 ≤ 24	> 24
Minimum Scrotal circumference (cm)	30	31	32	33	34

¹Society for Theriogenology

Semen Examination

Semen is collected from bulls that are to be used in natural service mostly by electroejaculation. The new, automated electroejaculation systems simplify collection. Each ejaculate must be collected into a clean, warm, dry receptacle that is free of toxic agents. Temperature is of prime importance in avoiding the irreversible damage that cold shock inflicts upon sperm. The semen should be collected and maintained at 30 to 35°C until examined. Two ejaculates should be collected and each examined for volume and sperm concentration. These attributes by themselves, however, have not been shown to be crucial potential fertility estimates due to a variety of variability factors involved in the collection process. This initial gross examination of the ejaculate should include determination of color because it may indicate urine contamination or the presence of large numbers of red or white blood cells. Yellow color by itself, however, may only indicate the presence of higher levels of riboflavin

that is seen in the semen of some bulls. Nonetheless, the ejaculate should be examined as soon as possible following collection because the longer it takes to complete the examination the greater the chance of modifying the attributes of the sperm in the sample.

Sperm motility estimates

Sperm motility may be evaluated either as gross motility or by assessing the percentage of sperm exhibiting progressive motility patterns. Gross motility is the amount of swirling action exhibited by the semen sample. This attribute is influenced by both the concentration of sperm and the proportion of progressively motile sperm. This can be done visually or by using the microscope at low magnification after carefully mixing the sample. The particular characteristics by which gross motility is evaluated and rated is given in Table 2.

Table 2. Evaluation of Gross Semen Motility¹

Gross Motility	Rapid swirling	Slow swirling	Generalized oscillation	Sporadic oscillation
Rating	Very good	Good	Fair	Poor

¹Society for Theriogenology

Evaluation of the percentage of progressively motile sperm requires careful mixing of the sample prior to placing a drop on a warmed glass slide and covering it with a warm cover slip. A highly concentrated sample may require dilution in a warm 2.9% sodium citrate solution or some other suitable diluent such as a warmed sterile saline solution (0.85% NaCl) to adequately see individual sperm. Samples should be examined immediately following dilution on a microscope at 400X and the percentage of individually progressive sperm is estimate using several microscopic fields. In the field approximation by 10% are adequate. Properly conducted individual cell evaluations of a sample tend to reflect more closely the true motility of a sample (Table 3).

Table 3. Evaluation of Individual Sperm Motility¹

Progressively Motile Sperm (%)	> 70	50-69	30-49	< 29
Rating	Very good	Good	Fair	Poor

¹Society for Theriogenology

Particular collection problems that may complicate the evaluation of sperm motility are incomplete ejaculates and cold shock. Often when the motility of a sample is low, then collection of another ejaculate and re-evaluation are indicated. Satisfactory breeders should have fair gross motility ratings or have individual sperm motility estimates of at least 30%.

Sperm morphology assessments

Evaluation of sperm morphology involves staining a drop of semen from a well-mixed semen sample. A variety of stains have been used. Most commonly an Eosin Y-nigrosin stain is used to evaluate sperm morphology only. Although this stain can differentiate between membrane-intact and membrane damaged sperm, its use in the field has resulted in ambiguous results as a “live-dead” stain. A drop of the stain and semen are mixed on a warmed slide and then smeared down the length of the slide using the end of a second slide to spread the drop into a thin film. Once the slide has dried, it is examined at 1000X using a good microscope with

an oil-immersion lens. Examination should be limited to the middle third of the slide to minimize preparation artifacts. The required differentiation is the percentages of normal and abnormal sperm. It may be useful to further classify the sperm abnormalities as primary and secondary, but this is not part of the BSE. The sub-classification of sperm morphology as having primary and secondary abnormalities is given in Table 4. An appropriate reference for classifying sperm morphology remains the textbook by Barth and Oko (1989). At least 70% of the sperm in the ejaculate should be normal for the bull to be classified as satisfactory. The recent frequency of ejaculation can markedly affect this parameter because epididymal sperm tend to age and begin degeneration when ejaculation is infrequent.

Classification

As previously noted, a bull is classified as being: (1) a *satisfactory* potential breeder, (2) an *unsatisfactory* potential breeder or (3) the *classification deferred* to a later date. This is important especially with yearling bulls. If the classification is deferred, then a subsequent examination date should be suggested. It is also important to note that the classification, if satisfactory, is for that date only because some physical and ejaculate attributes can change rapidly.

Once the BSE has been completed, then a bull can be classified using this system (Table 5). *Satisfactory* ratings require the animal to be free of significant physical abnormalities that have the potential to reduce fertility. The reproductive system of the bull must be free of any defect that might reduce potential fertility. Scrotal circumference must be at or above that minimum required for his age (Table 1). Gross sperm motility must be at least fair or the percentage of progressively motile sperm must be least 30% (Table 2). A minimum of 70% of the sperm in the ejaculate must be morphologically normal for a bull to be classified as a potentially satisfactory breeder (Table 3).

Table 4. Classification of Bovine Sperm with Primary or Secondary Abnormalities¹

<u>Primary sperm abnormalities</u>	<u>Secondary sperm abnormalities</u>
Under developed	Small normal heads
Double forms	Giant and short broad heads
Acrosome defects	Detached, folded, loose
Narrow heads	acrosomal membranes
Crater / diadem defect	Abaxial implantation
Abnormal contour	Distal droplet
Small, abnormal heads	Simple bent tail
Abnormal midpiece	Terminally coiled tail
Proximal droplet	<u>Other cells</u>
Strongly folded or coiled tail	Epithelial cells
Accessory tails	Erythrocytes
	Medusa formations
	Sperm precursor cells
	Round cells
	White blood cells

¹Bath and Oko, 1989

A bull must be classified as *unsatisfactory* if he fails to meet the criteria for satisfactory in any one of the categories noted above. The assumption is that the bull will not recover to the point that his problems will be resolved with time. If the probabilities exists that one or more of the evaluation criteria could change with time or therapy, then a bull may be categorized as *classification deferred* as shown in Table 5.

Table 5. Minimum Criteria for Classification as a Potentially Satisfactory Breeder¹

Physical Examination (Body, feet & legs, eyes, vesicular glands, ampullae, prostate, inguinal rings, penis, prepuce, testes, spermatic cords, epididymides and scrotum)	No significant structural abnormalities No notable abnormalities of the reproductive system
Scrotal Circumference (cm)	Meets minimum circumference measurement for age
Semen Evaluation (Semen normalcy, sperm motility, sperm morphology)	Fair sperm motility (< 30%) Normal sperm morphology (> 70%)

¹Society for Theriogenology

Observations Using BSE

Compilation of data from over 1,100 beef bulls in the Southeast provides some insight into the efficiency of the BSE (Carson and Wenzel, 1997). This study examined bulls consigned to sales, recent purchases, bulls offered for sale by private treaty and herd bulls. Although this initial comparison included 100 Holsteins bulls, these animals were eliminated in following comparisons, where possible, to focus on information from beef bulls. The study utilized a wide range of beef breeds with the varying numbers so that some breeds tended to be over represented and others underrepresented. More than 25% were Angus while 17% were Charolais, 14% Simmental, 8% Limousin, 7% Hereford, 4% Beefmaster, 4% Brangus, with additional breeds representing less than 3% of the beef bulls tested. Approximately 63% of the bulls tested satisfactory while about 29% were unsatisfactory with only about 8% being deferred (Carson and Wenzel, 1997). Most deferred bulls were young. Examination of the age distribution of these bulls indicated that the largest percentage (21%) were between 24 and 36 months of age, but more than half of the tested bulls were less that 24 months of age. The highest percentage (52%) bulls to be rated as unsatisfactory was due to unacceptably high percentages of abnormal sperm. This indicates that the strongest factor with BSE is the percentage of abnormal sperm. Approximately 10% were unsatisfactory due to a physical problem while about 12% failed to meet minimum scrotal circumference measurements for bulls of that age. Approximately 7% of the bulls were over 5 years of age with about 43% of these being classified as unsatisfactory. Data from aged bulls are not likely representative because the owners of the aged bulls may have suspected a problem thereby requesting the examination (Carson and Wenzel, 1997).

Examinations Beyond the BSE

Identifying some unsatisfactory breeding bulls with a simple, unambiguous, and repeatable test such as the BSE is sometimes difficult because some fertility attributes are subtle such that they are not revealed by routine testing (Amann and Hammerstedt, 1993; 2002). Elucidating problems with such animals are normally not cost effective for most clinical practitioners because additional specialized tests are beyond what is readily available to them. Such tests can require highly specialized equipment and/or additional specialized expertise that are available only in dedicated andrology laboratories. These specialized tests, however, are used widely by andrologists and, thus, worthy of consideration (den Daas, 1992; Garner, 1997).

Seminal attributes

It is pertinent to consider whether a particular attribute of a semen sample is compensable or uncompensable. If a particular attribute can be compensated by adding additional sperm, then it is compensable; if not, it is uncompensable (Saacke et al., 1991; 2000). Uncompensable semen traits are those resulting in reproductive wastage due to a genetically-incompetent sperm fertilizing the oocyte. Sperm with abnormally shaped heads and those exhibiting nuclear vacuoles (craters, diadem or nuclear pouches) do yield higher frequencies of low quality embryos. (DeJarnette et al., 1992; Saacke et al., 1994). The presence of uncompensable semen traits also suggests that examination of the ejaculate alone can not detect traits where sperm are able to fertilize but not participate successfully in embryogenesis. These findings emphasize the need to also examine those traits that are expressed only within the female reproductive tract. These include sperm transport and embryogenesis (Saacke et al., 1998a; 1998b; 2000).

Sperm movement can be further evaluated using a variety of methods such as time-lapse photography, optical path measurements, and computer assisted sperm analysis (CASA) equipment such as IVOS (Garner, 1997). Likewise, sperm morphology can be further analyzed using Differential Interference Contrast (DIC) microscopy (Saacke et al, 1991), electron microscopy and computerized image analysis Fourier analyses (Ostermeier et al., 2001a; 2001b).

Although sperm concentration is only grossly examined with the BSE, sperm numbers are extremely important when the ejaculate is to be used for other than natural breeding. The determination of sperm numbers in a semen sample is the subject of some concern. Routine hemocytometric, Coulter counter and spectrophotometric methods for determining sperm numbers in bull semen leave much to be desired (Takacs et al, 1987; den Daas, 1992; Evenson et al., 1993). Although relatively restricted in its use, flow cytometry can be used to accurately assess the actual number and concentration of spermatozoa in semen (Evenson et al., 1993; Eustache et al. 2001). Flow cytometric analyses are considerably more accurate than the indirect spectrophotometric methods based on the degree of light scatter (den Daas, 1992). Flow cytometric assessments of the number of fluorescently stained spermatozoa indicated that flow analyses were superior in accuracy to spectrophotometric determinations of sperm concentration in extended bull semen and in counting spermatozoa in straws packaged for insemination (Evenson et al., 1993; Eustache et al., 2001).

Sperm function and chromatin stability tests

It is important to consider that spermatozoa, which are extremely sensitive to environmental insults (Van Dilla et al., 1974), can provide a time-delayed barometer of the health of a bull.

Furthermore, some specialized analyses of sperm function and chromatin stability can provide insight not detected by other tests.

Sperm organelle function can be quantified using organelle-specific, fluorescent staining and flow cytometric analyses. Staining can be either a single membrane impermeant fluorophore or a combination of stains based on differing membrane permeabilities. Some particular applications of particular supravital stains or combinations of supravital and dead cell stains, are viability stains such as SYBR-14 and propidium iodide (Garner et al., 1994; Garner and Johnson, 1995; Christensen et al., 2005; Garner and Seidel, 2005), acrosomal stains including the fluorescent probe chlortetracycline (CTC; Varner et al., 1987) and the lectins *pisum sativum agglutinin* (PSA; Farlin et al., 1992) and peanut (*Arachis hypogaea*) agglutinin (PNA; Graham, 1996, Thomas et al., 1998; Garner et al., 1986; Garner and Seidel, 2005), and mitochondrial-specific stains such as rhodamine 123 (R123; Evenson et al., 1982) and JC-1 (Thomas et al., 1998; Garner et al., 1997; Garner and Thomas, 1999; Garner and Seidel, 2005).

The sperm chromatin, which is comprised of DNA and protamines, can be examined by determining the susceptibility of sperm DNA to in vitro denaturation using the flow cytometric sperm chromatin structure assay (SCSA; Evenson et al., 1980; Ballachey et al., 1987; Karabinus et al., 1997). This assay, which has been used as an independent estimate of mammalian male fertility, measures the in situ resistance of sperm chromatin to degradation as expressed by the relative proportion of double- and single-stranded DNA (Darzynkiewicz et al., 1975; Evenson et al., 1980; 1993). This assay is very useful in assessing DNA integrity, but even some andrologists find it difficult to implement it into their laboratory operations.

Libido and mating ability

Although the BSE does emphasize some on the important factors for male reproduction including animal health, soundness, sperm output and semen quality does not routinely assess the sex drive of bulls. This particular behavioral aspect is independent of the characteristics that are evaluated by the BSE (Chenoweth, 2004b). The best breeding bulls need to be sexual athletes. Assessment of mating ability is extremely important to producers because most modern beef cattle production systems rely heavily on natural breeding. This is unfortunate because most beef bulls do not receive any type of mating ability assessment prior to being sold for breeding purposes. Another behavioral related problem in multi-sire settings is the hierarchy of males within a breeding group. Certainly, some males within groups modify the proportion of females bred by individual sires within that group due to their dominance or subordination (Chenoweth, 2004b).

Summary

The current parameters used for determining breeding soundness provide a means for identifying sterile or sub-fertile bulls. The four parts of the BSE; the physical exam, minimum scrotal circumference measurements, and two seminal quality assessments are simple, unambiguous, repeatable and reasonably accurate. This test, however, does not examine the level of expected mating ability when bulls are to be used as sires. We have to widen the usage of the BSE to more potential sires and to be reasonably satisfied with it until some of the more sophisticated tests become practical enough to be available to clinical practitioners.

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