Bull BSE and semen analysis for predicting bull fertility
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Summary

There are many factors that affect bull fertility. Although few bulls are completely sterile, in an unselected population, 20 to 40% of bulls may have varying degrees of reduced fertility. Breeding soundness refers to a bull’s ability to get cows pregnant. The purpose of a standard breeding soundness evaluation (BSE) is to identify bulls with substantial deficits in fertility, although it does not consistently identify subfertile bulls. In that regard, the use of commercial frozen-thawed semen that meets minimum quality standards can result in pregnancy rates with a substantial range in fertility. Although no single diagnostic test can accurately predict variations in fertility among bulls producing apparently normal semen, a combination of laboratory tests may be predictive of fertility. This review is focused on a standard BSE, with some consideration of laboratory tests that can augment the prediction of bull fertility.

Keywords: Bull breeding soundness evaluation, BSE, bull, fertility, semen

Introduction

The term “breeding soundness” refers to a bull’s ability to get cows pregnant. Although few bulls are completely sterile (unable to impregnate a female), unless a bull is capable of getting a reasonable number of cows pregnant during a limited breeding season, he is not an efficient breeder. In an unselected population of bulls, 20 to 40% may have reduced fertility.1

High reproductive efficiency is the most economically important factor for success in cow-calf enterprises; it has greater economic impact than growth rate, feed efficiency, or carcass quality. Subfertile bulls delay conception, prolong the calving season, reduce calf weaning weights, and increase the number of cows culled due to failure to become pregnant or conceiving late in the breeding season. With multiple sire breeding groups and low bull-to-female ratios, reproductive performance may be adequate, despite the presence of some bulls with poor breeding performance. However, single-sire mating groups and high bull-to-female ratios increase the importance of fertile bulls.

Traditional BSE

The two general methods of evaluating the reproductive potential of bulls are: 1) breeding a large number of normal, fertile females and assessing pregnancy; or 2) conducting a breeding soundness evaluation. Although a breeding trial is the ultimate test of fertility, it is expensive, particularly if reproductive performance is poor. Therefore, it is strongly recommended to conduct a BSE prior to breeding and eliminate bulls expected to have poor fertility. As no single measurement or criterion is a reliable predictor of fertility, several criteria are usually evaluated.

In 1992 after several years of discussion among members of the Society for Theriogenology, the current standards for evaluating beef bulls for breeding soundness were adopted.2,3 These standards provide a uniform method of assessing a bull’s likelihood of establishing pregnancy in 25 or more healthy, cycling cows in a 65-70 day breeding season. Prolonged (or unlimited) breeding seasons preclude opportunities to optimize labor, health maintenance procedures, and marketing opportunities. Furthermore, there are important animal welfare issues, including cows being repeatedly mounted and bred, increased risk of injury to bulls with excessive breedings, and calves born in environmental conditions that threaten their health and survival.

Breeding soundness evaluation is done for three general purposes; prior to the breeding season, prior to sale, and in cases of poor reproductive performance. A bull judged unsuitable for breeding can cause considerable concern to the owner, as well as criticism of the person conducting the evaluation.
Conversely, failure to identify subfertile bulls can result in poor reproductive performance. Therefore, it is essential that veterinarians have the knowledge and skills required to perform BSEs.

Although a BSE is NOT just a semen examination, the latter is a critical component of a BSE. Furthermore, a BSE does NOT guarantee that a bull is highly fertile, nor does it rank reproductively sound bulls regarding their relative fertility, or ensure that bulls are free of viruses or other infectious agents. However, a breeding soundness evaluation generally DOES identify bulls with undesirable heritable traits or bulls unlikely to achieve a high pregnancy percentage in a limited breeding season.

Breeding soundness includes satisfactory general health, physical soundness, libido, and semen quality. The classification is based on physical evaluation and the bull’s ability to meet minimum thresholds for testicular development, sperm motility, and normal sperm morphology. Despite the requirement for adequate libido, it is not routinely evaluated. Therefore, the person using the bull for breeding should be reminded that they have a responsibility to observe bulls to ensure that they are identifying, mounting, and successfully breeding estrous cows.

Physical examination

At the start of a BSE, bull conformation, body condition, and overall physical health should be assessed. It is important to observe the bull moving, as lameness may not be apparent in the restrained bull. He should be of appropriate size for his age, free of obvious disease, and should have adequate muscling and body fat. Feet and legs should be free of defects that limit mobility or mounting. Acute or chronic laminitis, excessively straight rear legs (post-leg), sickle hocks (excessive curvature of the metatarsal joint), twisted claws (corkscrew claw), and interdigital fibromas are relatively common musculoskeletal conditions in bulls. Post-leg, sickle hocks, corkscrew claw and interdigital fibromas predispose the bull to lameness and are potentially heritable; therefore, bulls with these conditions are not recommended for breeding. Since it is essential that a bull have good eyesight, the eyes should be carefully examined.

Scrotum and testes (weaned bulls)

The first selection (and culling) of bulls is generally at weaning (approximately 7 to 10 months of age). Since few calves clearly display abnormal development and conformational traits at this age, culling is usually based on the breeder’s assessment of the bull’s growth potential. The principal reproductive criteria for selection of bulls at this age is testicular development. Bulls as young as seven months with below average scrotal circumference (SC) are predisposed to have below average SC at one and two years of age. Therefore, starting as early as seven months, any bull with below average SC should be culled. Simmental, Angus and Zebu-derived bulls (predominantly Santa Gertrudis) must have a minimum SC of 23 cm at 198-291 days of age to have a nearly 100% probability of having a SC ≥ 30 cm by 365 days of age. Other continental breeds, predominantly Charolais, and Polled Hereford bulls require a SC ≥ 26 cm to reach ≥ 30 cm by 365 days of age. If minimum requirements for SC are increased to 32 cm at 365 days of age, an additional 2 to 3 cm are needed at weaning. Coe and Gibson evaluated 264 bulls (from 13 beef breeds); they reported that at 200 days of age, calves with a SC > 23 cm had a 95% probability of achieving a SC > 34 cm by 365 days of age, whereas calves with a SC < 23 cm only had a 54% probability of achieving a SC > 34 cm by 365 days.5

Culling at weaning minimizes losses associated with maintaining cull bulls or mistakenly entering them in performance test programs. Furthermore, weaned bulls that do not have both testes well descended in the scrotum should be culled, regardless of SC. Although cryptorchidism is not common in bulls, it is considered heritable and the bull should not be used for breeding.

Scrotum and testes (yearling bulls)

Numerous studies of performance station bulls indicate drastic differences in semen quality according to age (Table 1); a few months can make a tremendous difference. Although most producers are unwilling to accept that less than 50% of their bulls will be judged satisfactory, if standards are rigorously applied, many young bulls will not meet the minimum standards, typically due to inadequate
semen quality. Good communication with producers, preferably in advance of performing BSEs, are essential.

Table 1. Percentage of yearling bulls (n=254) of various beef breeds with satisfactory semen quality.5

<table>
<thead>
<tr>
<th>Age (mo ± 15 d)</th>
<th>No. bulls</th>
<th>Mean (range) SC (cm)</th>
<th>Satisfactory semen quality (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>12</td>
<td>40</td>
<td>33.8 (28.5-39.5)</td>
<td>40</td>
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<tr>
<td>13</td>
<td>100</td>
<td>34.5 (28-41)</td>
<td>55</td>
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<tr>
<td>14</td>
<td>84</td>
<td>34.1 (28-45)</td>
<td>56</td>
</tr>
<tr>
<td>15</td>
<td>30</td>
<td>34.9 (27-41)</td>
<td>73</td>
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Examination of the scrotum and testes

Measure SC by forcing the testes to the bottom of the scrotum and placing a flexible tape around the largest circumference, with sufficient tension so that the scrotal skin is somewhat compressed under the tape. Avoid artificial increases in SC by excessively forcing the testes to the bottom of the scrotum or by forcing the testes to move apart from each other (for example, by wrapping a hand around one testis, with your finger tips on the midline of the anterior aspect of the scrotum). Evaluate the scrotum for scars or other pathology. The scrotum should have a distinct neck; this allows the testes to move away from the body (essential to cool the testes during hot weather). Bulls with little or no scrotal neck, or with an extremely long scrotal neck (bottom of scrotum is ventral to the metatarsal joint) are generally not recommended for breeding.

The testes should be freely moveable within the scrotum, with ≤10% difference in size between testes. Palpate each testis gently for texture (it should be firm and resilient) as well as deeply, to assess areas of firmness that might indicate granulomas, fibrosis, or calcification. Testes that are excessively soft are consistent with testicular degeneration, whereas excessive firmness along the mediastinum testes is consistent with irreversible testicular damage. There should be no adhesions or accumulation of fluid within the vaginal cavity. Palpate the head, body and tail of the epididymides for completeness and for the presence of pain or granulomas. Palpate the spermatic cord for varicoceles or other abnormalities.

There is a rapid increase in SC around the time of puberty. As SC increases with increasing age, the minimum SC (Table 2) varies according to the age of the bull.2,3 It is noteworthy that these are minimum standards; ideally, bulls should be exceed these standards. Furthermore, some persons use breed-specific minimum standards for SC.

Table 2. Minimum SC in bulls, according to age.2,3

<table>
<thead>
<tr>
<th>Age (mo)</th>
<th>Minimum SC (cm)</th>
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<tr>
<td>&lt;15</td>
<td>30</td>
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<tr>
<td>15-18</td>
<td>31</td>
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<td>18-21</td>
<td>32</td>
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<td>21-24</td>
<td>33</td>
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<tr>
<td>&gt;24</td>
<td>34</td>
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</table>

It is well known that SC is highly correlated with paired testis weight, which is correlated with daily sperm production and semen quality traits. Furthermore, SC is a more accurate predictor of age at puberty than either age or weight, regardless of breed. Bulls with a large SC have half-sib heifers and
daughters that reach puberty earlier and are more fertile. Since the heritability of SC in bulls (from one to two years of age) is approximately 0.5, substantial progress can be made in selecting for this trait.

**Examination of the internal reproductive organs**

Evaluation of internal reproductive organs is an important aspect of a physical examination. Remove feces from the rectum and identify the urethralis muscle and prostate gland. Bulls rarely develop prostatic disease; the prostate is palpable as a transverse band at the cranial extent of the urethralis muscle (approximately 15 to 20 cm from the anus), along the ventral midline of the pelvis. Immediately cranial and dorsolateral to the prostate are the paired vesicular glands. Normal glands feel resilient and are approximately 2 to 4 cm in diameter and 10 to 15 cm long. Just cranial and medial to the vesicular glands are the paired ampulla; they are the termination of the ductus deferens and store mature sperm ready for ejaculation. Each ampulla is 1 to 1.5 cm in diameter, thick walled and 10 to 12 cm long. The ampullae pass under the prostate to empty into the urethra at the colliculus seminalis.

The internal inguinal rings are palpable openings in the abdominal wall, approximately 15 to 20 cm anterior to the pelvic brim and 5 to 15 cm lateral to the midline. Examine for fat, omentum or intestines entering the ring. Bulls with inguinal rings larger than 5-6 cm may be predisposed to development of inguinal hernia.

The most common abnormality detected on transrectal examination is enlargement, excessive firmness or loss of surface lobulation of the vesicular glands. Vesicular adenitis (vesiculitis) was detected in 0.85–10% of yearling bulls during routine breeding soundness evaluations, but is uncommon (<1%) in older bulls. Although both glands may be affected, it is more likely to be unilateral. In semen smears stained with eosin-nigrosin or eosin-aniline blue, neutrophils with intact membranes appear as white, somewhat irregular structures, approximately three times larger than a sperm head. Positive identification can be made by using a blood stain (e.g., Wright’s Giemsa). As few as one neutrophil per three high-power fields (1000 x) suggests inflammation somewhere in the reproductive tract (not necessarily due to vesiculitis).

**Examination of the penis, prepuce and sheath**

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 small particles (similar to sand) on the preputial hair may indicate 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 a penile hematoma.

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.

**Electroejaculation**

The most common method for collection of bull semen is electroejaculation. Commercial machines are designed to electrically stimulate the pelvic genitalia via a rectal probe. This stimulation induces the bull to achieve penile engorgement which progresses to penile extension, erection and ejaculation. The stimulation is delivered in the form of a modified sine wave and begins with small voltage for a duration of two to three seconds, followed by a rest period of up to three seconds. Electroejaculators may be battery operated or require direct connection to line current. The operator may manually control the magnitude of current applied and the frequency and duration of pulses, or they may be programmed to gradually increase the intensity and/or frequency of pulsations leading to ejaculation. There are apparent breed differences in how readily bulls ejaculate with electroejaculation; some breeds are more prone to vocalize during the stimulation procedure.

Rectal probes should be of appropriate diameter for the size of the bull and the stimulating electrodes should be confined to the ventral one-third to one-half of the probe. Avoid probes with circular electrodes, as they will stimulate dorsal and/or dorsolateral pelvic musculature, with obvious discomfort to the bull. Smaller hand-held finger electrodes are also available; the operator introduces the hand
bearing the electrode into the rectum and positions the electrodes over the seminal vesicles, prostate and
ampullae to induce the bull to ejaculate. Advantages of these electrodes are that the stimulating current is
directed directly to the target tissues, with minimal undesirable stimulation of the other pelvic
musculature or nerves. However, these electrodes required a skilled palpator to maintain electrode
contact with the pelvic organs during stimulation and ejaculation.

Before beginning electroejaculation, palpate the bull’s pelvic genitalia by transrectal palpation.
Gently massage the urethralis muscles, the vesicular glands and the ampullae. In that regard, transrectal
massage of the ampullae may decrease the duration of stimulation required to collect semen by
electroejaculation.10 Although there is wide variation among bulls, most bulls begin to achieve penile
engorgement after eight to 15 electrical stimulations, followed by penile extension and erection. As
stimulation increases, three to five jets of clear pre-seminal fluid will be ejaculated, followed by four to
eight jets of the opaque, sperm-rich fraction. Most operators collect a minimum of three ml of ejaculate
for microscopic evaluation. The volume of the ejaculate and sperm concentration are largely determined
by how much prostatic and vesicular gland fluid is collected before collecting the sperm-rich fraction.
Occasionally a bull will release urine during the electrostimulation; typically, sperm motility will be
absent or negligible and the sample will smell like urine. In the authors’ experience (unpublished),
withholding water for several hours prior to semen collection apparently reduces the incidence of urine
contamination. Furthermore, if urine contamination occurs, we typically attempt a second semen
collection (within a few minutes after the first sample is collected), with an accelerated ‘ramp up’ phase to
reach maximum stimulation. Regardless of the method of stimulation used, the operator may wish to
grasp the free portion of the penis with a dry surgical sponge in order to hold the penis in extension for
thorough examination of the penis and prepuce.

Although electroejaculation is a convenient and reliable method of semen collection, there are
frequently concerns that it may have adverse effects on bulls. Pharmaceuticals used to facilitate semen
collection by electroejaculation or to decrease pain associated with electroejaculation have generally not
been efficacious enough to warrant use.10 Pain associated with electroejaculation may be influenced by
operator technique; therefore, operators of electroejaculator equipment must strive to apply electrical
stimulation as gently as possible.10 Although a significant increase in vocalization and plasma cortisol
and progesterone concentrations in bulls following electroejaculation was attributed to acute stress,11 the
lack of a difference in plasma concentrations of substance P after electroejaculation was interpreted as a
lack of pain associated with nociception.11

Alternative methods of semen collection

The ampullae are the dilated termination of the ductus deferens and serve as a reservoir for the
next ejaculate. Massage of the ampullae, prostate (with occasional massage of the urethra; massage of the
seminal vesicles is not essential) will cause stored semen to be released from the ampullae, resulting in
emission (dripping) of semen from the urethra.12 Disadvantages of this method are that a skilled palpator
is required, the procedure is tiring for the operator, care must be exercised to avoid rectal irritation, and
there can be no assessment of libido, mating ability, erection ability, or ejaculation. Additionally, the
penis usually does not extend and the semen sample dribbles down the preputial cavity and off the
preputial hairs. It must be emphasized that the sample is an emission, not an ejaculation and
consequently, semen samples are often contaminated with bacteria and smegma from the prepuce.
Furthermore, transrectal massage was not as efficacious as electroejaculation for obtaining a semen
sample.12

Although an artificial vagina is the method of choice for collecting semen at an artificial
insemination center, it is rarely used in the field. Alternatively, an intravaginal artificial vagina has been
recently developed.13 Finally, semen can be collected from the anterior vagina of an estrual female after
breeding; the vagina should be flushed with saline prior to breeding (to minimize mucus).

Ejaculate volume and sperm concentration

Although the total number of sperm in the ejaculate is used to evaluate sperm production in the
stallion, dog and boar, in bulls the capacity to produce sperm is estimated based on SC. In bulls that are routinely collected with an artificial vagina, ejaculate volume and sperm concentration are meaningful; in bulls collected by electroejaculation, they have little relevance. Regardless, the following terms are frequently used to describe sperm concentration (concentrations are $10^6$ sperm/ml):

Very good: creamy, grainy (750-1000)
Good: similar to whole milk (400-750)
Fair: similar to skim milk (250-400)
Poor: translucent (<250)

**Sperm motility**

Motility is reduced by extremes in temperature (typically low temperature but also excessively high temperature), urine, soap, and other contaminants; therefore, considerable effort should be made to protect semen from these influences. A drop of semen 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 in order to visualize individual sperm under high power. The threshold for Satisfactory Potential Breeder is a minimum of 30% progressively motile sperm.2,3

**Sperm morphology**

Sperm morphology should be evaluated under oil immersion in order to adequately evaluate individual sperm. Prepare a slide by mixing diluted sperm with eosin-nigrosin stain, similar to 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 similar to a blood smear for examination with a phase contrast microscope. Count a minimum of 100 sperm cells; when several abnormalities are present, assessing 300 sperm cells will provide a more accurate count.

There are several systems of classifying sperm.14 The concept of compensable and noncompensable defects is a reasonable approach to interpreting the importance of sperm abnormalities. Compensable abnormalities can be overcome by increasing the dose; for example, sperm with knobbed acrosomes or bent tails would not be able to induce a zona reaction and prevent normal sperm from fertilizing the ovum. In contrast, nuclear vacuoles are considered noncompensable, as regardless of the number of normal sperm present, the probability of the affected sperm fertilizing the ovum is approximately equal to its proportion in the ejaculate.

Regardless of the method used, it is worthwhile to remember that a minimum number of live, normal sperm are required to populate the oviduct. Once in the oviduct, the sperm need normal membrane receptors to bind to the zona pellucida, a normal acrosome and tail to penetrate the zona pellucida, and a normal nucleus for fertilization. As a general rule, fertility will be decreased if there is >30% morphologically abnormal sperm and >20% head defects.14

A common issue during breeding soundness evaluation in yearling bulls is the presence of many sperm with proximal cytoplasmic droplets (one of the most frequent sperm abnormalities in young bulls). This abnormality, often associated with immaturity, can also be present in bulls with testicular degeneration. In immature bulls, the percentage of affected sperm usually declines as the bull completes puberty; most bulls will have satisfactory semen quality in the near future. Therefore, some examiners ignore proximal droplets in yearling bulls, resulting in a higher percentage of these bulls classified as Satisfactory Potential Breeders. However, fertilization rates are markedly lower for bulls with at least 30% sperm with proximal cytoplasmic droplets; as the percentage of droplets decreases, fertilization rates increase. Therefore, according to accepted standards, if the yearling bull is physically sound and meets the other minimum requirements, and due to the presence of proximal cytoplasmic droplets there are <70% normal sperm, the bull should be placed in the Deferred category.
Use of ultrasonography for evaluation of reproductive tissues in the bull

The use of ultrasonography for evaluation of the reproductive tract of the bull has been recently reviewed. Although diagnostic ultrasonography is commonly used for examining the reproductive tract of cows, its use in bulls has been much more limited. A typical clinical ultrasonographic examination of bull testes are unlikely to affect semen quality or sperm production. The ultrasonographic anatomy of bull testes and accessory sex glands have been reported. Although testicular echogenicity increased (i.e., the parenchyma appeared more white) as a bull approached puberty, echogenicity was not superior to scrotal circumference as a predictor of puberty. Ultrasonography can be used to detect and characterize pathology of the testes and accessory glands. It is noteworthy that areas of increased echogenicity (testicular fibrosis) are common, especially in young bulls, but are not associated with decreased semen quality (e.g., proportion of morphologically normal sperm). Neither visual evaluation nor computerized pixel analysis of testicular ultrasonic echotexture were consistently predictive of semen quality in bulls. Therefore, it was concluded that the primary clinical use of ultrasonography for assessment of reproductive function in the bull was characterization of grossly detectable lesions in the testes and scrotum.

Infrared thermography for evaluation of the scrotum and testes

Bovine testes must be 4 to 5°C below body-core temperature (38°C) for normal spermatogenesis. Infrared thermography of the bull scrotum, a non-invasive means of assessing scrotal surface temperature, has been used as both a research and clinical tool. A bull is restrained and an infrared thermography camera is held behind the bull and captures the infrared radiation coming from the scrotal surface. The resulting image is composed of a series of pixels, with each pixel having a temperature. The image can be displayed, with colors indicating temperatures, or pixel analysis can be done to objectively characterize temperatures. In one study, infrared thermography was used to assess scrotal surface temperature of 73 yearling beef bulls. Of those bulls, 51, 27 and 22% had a normal, questionable, and abnormal scrotal surface temperature pattern, respectively. Thirty of those bulls, all of which were designated as breeding sound (based on a standard BSE), were each exposed to approximately 18 heifers during a 45-d pasture breeding period (single-sire mating). Pregnancy rate was lower (P < .01) for bulls with abnormal scrotal temperature patterns (68%, n = 8) than for bulls with normal (83%, n = 13) and questionable temperature patterns (85%, n = 9). Therefore, it was concluded that infrared thermography has potential for predicting bull fertility. Although the cost of infrared thermography cameras previously restricted their use, cameras satisfactory for imaging bull scrota now cost less < $5 000 (www.flir.com).

Classification for breeding soundness

Following evaluation according to the criteria described above, bulls are classified according to their suitability for breeding on the day of evaluation. Bulls that have a sound confirmation, free of ocular and musculoskeletal defects and that produce at least 70% morphologically sperm, with at least 30% progressively motile sperm, are classified as Satisfactory Potential Breeders. 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. Bulls in this category are usually juvenile, have an injury or lameness that is likely to resolve or suffer temporary testicular degeneration due to hot weather. If this classification is used, the veterinarian should recommend a date for re-evaluation of the bull. Bulls with undesirable heritable defects, small SC, debilitating injury or disease, or with permanent testicular degeneration should be classified as an Unsatisfactory Potential Breeder.
**Detailed laboratory tests for semen quality**

**Sperm motility**

Computer assisted sperm analysis (CASA) is much more objective than visual appraisal for assessment of sperm motility. Some aspects of sperm motion characteristics assessed by CASA (namely beat cross frequency, linearity, average path velocity, straightness and curvilinear velocity) were significantly correlated with bull fertility, as were average path velocity, total motility, linearly motile sperm, and total number of motile sperm in swim-up preparations. A recent study comparing low- versus high-fertility dairy bulls demonstrated that sperm from high-fertility dairy bulls have a tendency to undergo hyperactivation (a hallmark of capacitation) immediately after thawing. In addition, characteristics of sperm recovered through a sodium hyaluronate swim up medium better reflected the ability of sperm to undergo hyperactivation and reach the site of fertilization, and thus may be more indicative of in vivo fertility.

**Sperm pasma membrane viability**

A functional sperm plasma membrane is essential for fertilization; therefore, the integrity of the membrane should be assessed. Eosin-nigrosin is a ‘live-dead’ or vital stain. In that regards, eosin permeates dead or dying sperm (pink to red sperm heads), whereas viable sperm appear white. The nigrosin simply provides a background to make the sperm easier to see. More recently, various fluorescent probes have been used to identify viable versus nonviable sperm. For example a combination of Syber-14 and propidium iodide is often used. In viable cells (with an intact plasma membrane), Syber-14 makes the DNA bright green, whereas propidium iodide penetrates dead and non-viable sperm and stains them red. The proportion of live and dead sperm (green and red, respectively) is assessed either manually (fluorescent microscopy) or by automation (flow cytometry). Shojaei et al reported that there was a greater percentage of viable sperm after thawing and after swim up in high- versus low-fertility dairy bulls, and that these two end points were positively correlated with fertility. Furthermore, the ratio of sperm recovered after swim-up to viable sperm in post-thaw semen was higher in high- low-fertility bulls. In addition, the proportion of moribund sperm (sperm emit green and red fluorescence due to a compromised sperm membrane) expressed as a percentage of live sperm was low in high-fertility bulls and this end point was negatively correlated with fertility.

Brito et al compared several methods for evaluating sperm membrane viability, including eosin/nigrosin, trypan blue, fluorescent probes, and the response of sperm to the exposure of a hypoosmotic solution. In that study, sperm were used for in vitro fertilization, and fertility was assessed on the basis of the proportion of ova that underwent cleavage. Although all of these stains evaluate the physical integrity of sperm membrane, the hypoosmotic swelling test (HOST), which evaluates the functional competence of the sperm membrane, was the only method that contributed to conventional sperm quality tests in predicting the success of in vitro fertilization.

**Sperm DNA decondensation**

Sperm chromatin integrity is critical for fertilization and ongoing embryo development. Oxidative stress was suggested as a major cause of sperm DNA damage, with reduced preimplantation embryo development and pregnancy rates. Furthermore, increased testicular temperature reduced the stability of sperm DNA and the ability of these sperm to undergo DNA decondensation and pronuclei formation. The sperm cell structure assay (SCSA) uses flow cytometry to determine chromatin integrity, based on resistance to acid denaturation. Sperm are exposed to low pH and stained with acridine orange, which emits green or red fluorescence when it binds to double- (intact) or single-stranded DNA (denatured), respectively. The ratio of red/(red + green) fluorescence measures chromatin denaturation, which is significantly correlated with fertility.
Sperm proteins

Heparin binding proteins were proposed as means of predicting fertility differences among bulls producing morphologically normal sperm33,34 and there are associations between specific seminal plasma proteins and fertility.35,36 Sperm proteins differed between low- versus high-fertility Nelore (Bos indicus) bulls with acceptable sperm.37 Similarly, accessory gland fluids from high-fertility Holstein bulls had more bovine seminal plasma protein (BSP) 30 kDa and phospholipase A2, whereas osteopontin appeared to improve the ability of epididymal sperm (from low-fertility bulls) to penetrate oocytes in vitro.38 In a recent study,39 scrotal insulation was used to induce abnormal spermatogenesis and sperm proteins associated with abnormal spermatogenesis were identified; there was differential expression (between normal and abnormal sperm of the same bull) of the alpha 4 subunit of Na+/K+ATPase, tissue inhibitor of metalloproteinase-2 (TIMP-2), angiotensin converting enzyme (ACE), and hexokinase-1.39

Abnormal sperm

Morphologically abnormal sperm failed during gamete interaction or pre-implantation development.40,41,42 In addition, embryos resulting from fertilization of oocytes by morphologically normal sperm, co-existing in the ejaculate along with abnormal sperm, had reduced developmental competence, suggesting that these sperm were functionally impaired. Therefore, increasing the insemination dose to compensate for infertility due to compensable factors43 requires further investigation. Data from commercial embryo production units suggested that bulls differ in their ability to produce preimplantation embryos in vivo (Thundathil and Mapletoft, unpublished data). In that regard, damage to sperm DNA due to oxidative stress, chromosome anomalies and environmental effects, including elevated testicular temperature, time of AI relative to estrus, duration of semen storage, duration of sperm-oocyte interaction, age of males, and infectious agents in semen influence embryo quality (reviewed by Chenoweth, 2007).44

Models utilizing multiple variables

A seven-variable model (post-thaw total motility, post-thaw sperm with a linear motile pattern, sperm concentration, concentration of motile sperm after swim-up, sperm ZP-binding, cleavage rate of total oocytes, and blastocyst rate of total oocytes) accounted for 84.6% of the variation in non-return rates.45 However, this approach may not be sensitive enough to discriminate among highly fertile bulls. Similarly, a model with 30 post-thaw sperm characteristics (including cleavage rate) accurately predicted fertility (based on conception and non-return rates) of both high- and low-fertility bulls.46

Conclusion

A traditional breeding soundness examination will usually identify bulls that are grossly abnormal. However, variations in fertility among bulls classified as satisfactory suggest that submicroscopical differences may exist in the sperm characteristics of these bulls. Therefore, understanding the molecular basis of these differences in sperm characteristics may enable us to develop laboratory assays to complement traditional breeding soundness evaluation and better predict fertility of bulls.

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