Bull breeding soundness and cryopreservation of semen

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The Society for Theriogenology (SFT) adopted the current form for the bull breeding soundness evaluation (BSE) in 2018. The BSE consists of four 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. A bull deemed to be a satisfactory potential breeder should unequivocally meet or exceed these four 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 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. The preparation and interpretation of the morphology slide must be proficient and consistent 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 must 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".

Key words: bull, breeding soundness exam, morphology, semen evaluation

Physical examination

The breeding soundness evaluation should begin with a general physical examination. The bull must obviously be able to see, hear, smell, eat, and walk if he is to perform satisfactorily as a breeding sire. The overall condition of the bull should be noted. We expect bulls to lose two body condition scores during a controlled breeding season. The bull that goes into breeding under conditioned will not produce quality semen by the end of the season. The feet and legs, especially the rear legs, should be examined for signs of injury or conformation flaws. The age of the bull and his permanent identification markings should be recorded during the physical exam. Any conformation of developmental defect such as umbilical hernia, microphthalmia or screw claw should be noted on the form.

The sheath should be palpated for evidence of hematomas, lacerations, abscesses, adhesions or scars. The preputial orifice should also be examined for signs of defects. The penis is usually examined during electroejaculation. The penis should be closely observed and palpated for lacerations, scars, abscesses or signs of infection. Papillomas, hair rings and persistent frenulum are more likely to be found in young (12-24 months) bulls.

Test mating is not included in the routine breeding soundness examination. If the history indicates unsuccessful copulation by the bull, a test mating should be arranged. The presence of penile deviations, anesthesia of the glans penis, and evaluation of libido can only be made by a test breeding.

Rectal examination

The internal reproductive organs are examined by rectal palpation. A gloved hand is inserted into the rectum to wrist depth. The first structure identified on the pelvic floor is the urethralis muscle covering the pelvic urethra. Massaging the cylindrical urethralis muscle will stimulate muscle contractions that are felt as strong pulsations of the organ. By sliding the hand forward along the urethralis muscle, a firm transverse ridge will be located on the midline that feels like a wedding band around the urethralis muscle. This structure is the prostate.

Just cranial and lateral to the prostate on each side, the vesicular glands will be located. The vesicular glands are lobulated, somewhat flattened structures that are rather soft to the touch. Size varies between 6 and 14 cm in length and 2 and 6 cm in width, depending upon the size and age of the bull. Cystic or abscessed areas are occasionally palpated in the vesicular glands, especially in young bulls. In the bull with acute seminal vesiculitis, considerable pain may be evidenced when these structures are palpated. A fibrotic consistency palpated in the vesicular glands is indicative of present or past inflammatory disease.

The examiner should advance the hand along the ventral midline 3 to 6 cm cranial to the prostate to encounter the ampullae. The ampullae can be identified as paired tubular structures, 5 to 12 mm in diameter. These structures are firm and resemble the size of a No. 2 pencil.

The internal inguinal rings are the next structures that should be examined. The hand is turned such that the fingertips contact the lateral body wall in a perpendicular manner when the wrist is at the brim of the pelvis. The finger tips are swept ventrally along the body wall until a horizontal slit in the body wall is encountered approximately 45 ° below a horizontal plane. The palpation of the spermatic cord entering the horizontal slit ensures that the internal inguinal ring is positively identified. As palpable per rectum, the inguinal ring is abnormally large if more than three fingers can be admitted.

These internal reproductive organs should be carefully palpated for size, symmetry, pain, heat and consistency. Any deviations from normal should be recorded on the evaluation form. The urinary bladder, kidneys, lymph nodes, peritoneum, omentum and other internal organs should also be quickly examined

before concluding the rectal examination. The rectal palpation should have the bull stimulated for semen collection, either by electroejaculation or artificial vagina.

Scrotal examination

Examination of the scrotum should begin with a visual appraisal for shape, symmetry, dermatitis, scars or other signs of injury. Following visual examination, the scrotal contents should be carefully palpated.

Testicles

The testicles should be palpated for firmness, relative size, symmetry, evidence of pain or swelling. Each testicle should be gently palpated first in examining for symmetry, firmness and pain or swelling. Following this, more pressure should be applied so that the testicle is palpated deeply and firmly for evidence of fibrosis. The testicles should be squeezed individually up and down in the scrotum to detect evidence of adhesions.

Epididymis

The head of the epididymis is readily palpable on the proximal pole of the testicle on the craniolateral surface. Grossly, the structure is a somewhat flattened U-shape band of up to 5 mm thick that extends ventrally toward the body of the epididymis.

The body of the epididymis lies on the caudal-medial aspect of the testicle and is less readily palpable than the head or tail. The body of the epididymis can be palpated by separating the testicles and carefully palpating the medial surface of the testicle. The normal body is recognized as a band of tissue, up to 1 cm wide and 3-4 mm thick.

The tail of the epididymis is readily palpable on the distal pole of the testicle. There is considerable variation in the size and degree of adherence to the testicle among bulls. The tail of the epididymis folds upon itself and continues dorsally on the medial aspect of the testicle as the ductus deferens.

Throughout its length the epididymis should be palpated for sperm granulomas and evidence of pain. The head of the epididymis is an area where sperm granulomas are frequently discovered.

Ductus deferens

The ductus deferens is palpated as a 2-3 mm cord-like structure ascending on the medial surface of the testicle. The ductus deferens continues dorsally into the spermatic cord and should be palpated as far-toward the external inguinal ring as possible.

Scrotal circumference

Scrotal circumference, testis size and semen production are highly correlated, especially in young bulls. Scrotal circumference is readily quantitated.

To measure scrotal circumference, the veterinarian should grasp the scrotum at its base and firmly push the testes into the lower part of the scrotum. In cold weather, special care should be taken to ensure that the testes are forced down into the scrotum enough to eliminate scrotal wrinkles. Care should also be taken not to press the thumb and fingers between the testes in order to avoid a faulty measurement. The scrotal tape is formed into a loop and slipped over the scrotum and pulled up snugly around the widest point of the scrotum.

Title 1: Minimum recommended scrotal circumference

Age	SC (cm)
≤ 15 months	30
> 15 ≤ 18 months	31
> 18 ≤ 21 months	32
> 21 ≤ 24 months	33
> 24 months	34

Motility

Gross and progressive motility estimates are subjective evaluations which may differ with time and the person making the observations. Repeatability of motility estimates is variable under field conditions due to the influence of environmental factors that may alter motility. The glass ware (slides, cover-slips) and microscope stage should be at 37° C for optimal results. The microscope should be located in an area free of sunlight, dust and drafts.

Gross motility

Estimations of gross motility are made by microscopic evaluation of a drop of undiluted semen. A single drop of semen is placed on a warmed slide and evaluated at 100X magnification. Gross motility is affected by the concentration of the sample. The 1993 classification scheme for gross motility was discontinued in 2018. The Society for Theriogenology recommended evaluation of progressive motility.

Progressive motility

Estimations of progressive motility are made by microscopic evaluation of a single drop of diluted semen. Physiologic saline or 2.9% sodium citrate warmed to the same temperature as the semen sample may be used as diluents. These solutions should be fresh in order to avoid problems with diluent toxicity to the semen sample.

A single drop of semen is placed on a warm microscope slide. The warm diluent is added to the semen at the rate of up to five drops. A warm coverslip is placed on the diluted semen and placed on the microscope stage to be observed at 400X magnification.

A numerical progressive or individual motility score is attained by determining what percentage of sperm cells per high power field are moving in a forward, linear direction.

Several fields should be observed when determining the progressive motility scores. White blood cells, red blood cells, epithelial cells, and spheroids (immature sperm cells) should also be appraised on this slide. The presence of these cells is noted on the form. The average number of spheroids per high powered field will be added to the number of abnormal sperm cells when determining a morphology score. The minimum motility score is 30% progressive or individually motile sperm.

Morphology

The percent normal morphology is determined utilizing a stained semen slide. A single drop of semen is placed near one end of a microscope slide and diluted with stain and second

slide is drawn across the first slide in the same manner as a blood smear is prepared. The recommended stain is Eosin-Nigrosin, available from the Society for Theriogenology. The slide is allowed to air dry on the slide warmer or heated microscope stage. The slide is evaluated at 1000X magnification. One hundred individual sperm cells are counted and classified as normal, head abnormality, mid-piece abnormality, or tail abnormality. Only cells that are clearly visible are counted. That is, if several cells are clumped together, these should be ignored and only those cells that can be clearly and totally identified are counted. Additionally, only sperm heads are counted. Loose sperm tails on the slide are ignored. The minimum accepted morphologically normal cells is 70%. It is important to gain an understanding of the cause of the most common sperm abnormalities to aid in diagnosis and prognosis for future fertility.

Common morphologic abnormalities

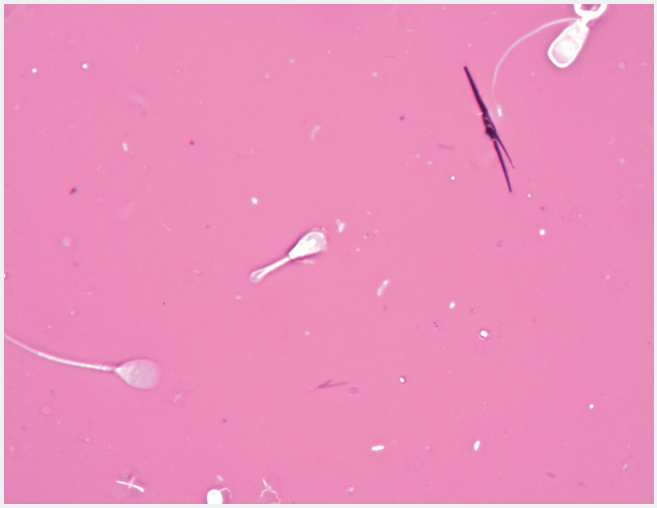
Pyriform and tapered heads

This is the most commonly reported head abnormality of the bovine spermatozoa. 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% versus 84.4%, respectively. 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. Pyriform heads can be seen secondary to heat stress, testicular insulation and testicular hypoplasia. 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 (Figure 1).

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. 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" 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. 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 20-25% of sperm in the ejaculate. Epididymal function is highly dependent upon testosterone concentrations. Causes of reduced testosterone concentrations include temperature extremes, exogenous estradiol, stressful events, and induced hypothyroidism. This defect can be detected within a week of a stressful event such as severe winter weather. 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 6-8 weeks following a prolonged stressor to the bull (Figure 2).

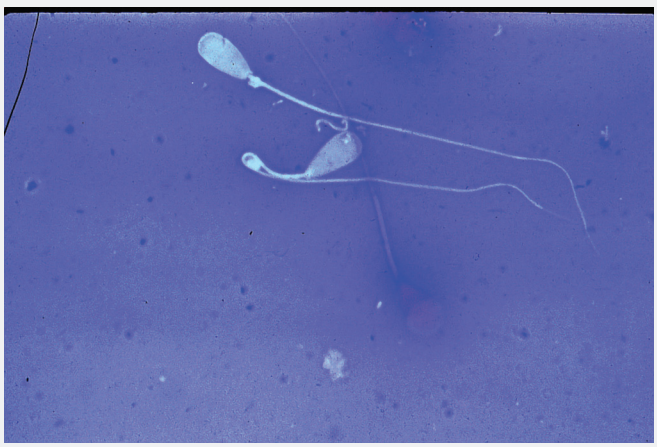
Figure 1: Pyriform head



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 pseudo-droplet 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. Disturbances in spermatogenesis can

Figure 2: Distal midpiece reflection



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 (Figure 3).

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. The proximal droplet is 2-3 µm in diameter and is characteristically located at the implantation fossa of the midpiece. It is common to discover significant numbers of proximal droplets in peripubertal bulls. This defect is a major reason for 12-15-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. 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 non-compensable 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 (Figure 4).

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. 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 number of detached heads are noted on the initial spermiogram. Some bulls may have to be collected multiple times in one 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 stressful event the bull can be deferred and rechecked in two to three 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 (Figure 5).

Figure 3: Dag defect

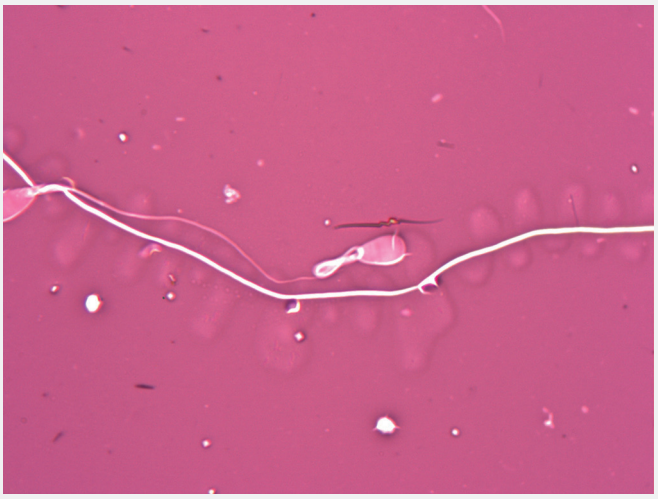
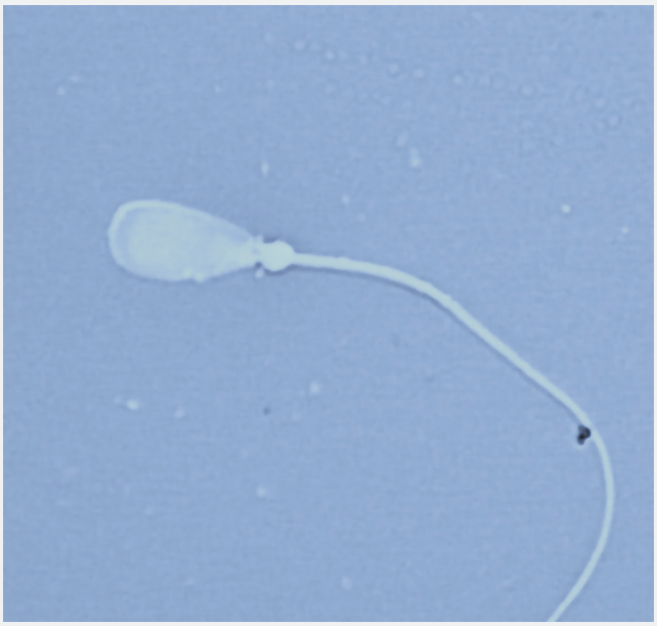


Figure 4: Proximal cytoplasmic droplet



Coiled principal pieces

This defect appears like a rolled-up rope at the very distal end of the principal piece. Impaired thermoregulation due has been implicated as the etiologic agent. In the author's experience, an increased incidence of this defect is detected in months with

Figure 5: Detached normal heads

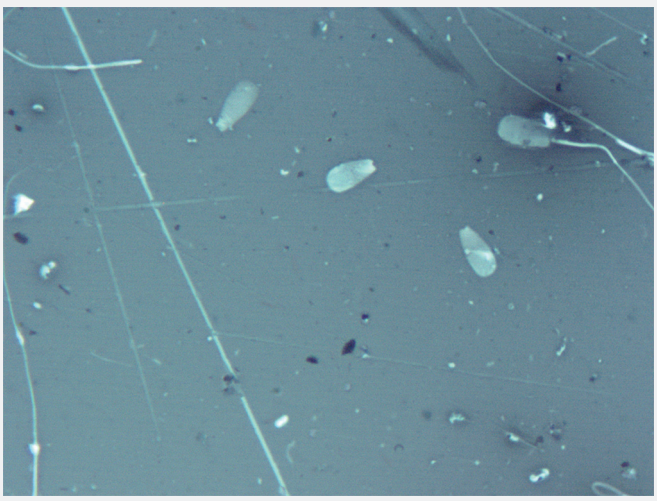
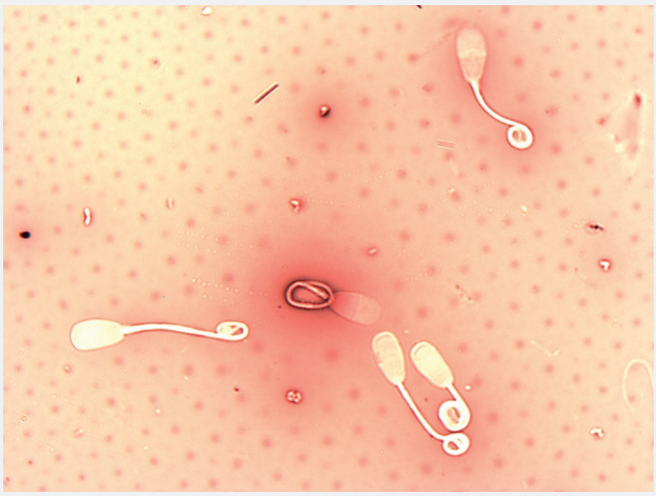


Figure 6: Tightly coiled principal pieces



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. It should also be considered a compensable defect due to the lack of motility. This defect has been reported in gossypol feeding trials (Figure 6).