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Semen evaluation and overview of common sperm abnormalities
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Abstract
A veterinary breeding soundness examination as directed by Society for Theriogenology (SFT) standards provides information that is vital for the cowman and in turn the beef cattle industry. An incomplete exam, specifically the omission of a microscopic evaluation of sperm morphology compromises the results and thus its value. This is also the case with a poorly performed morphology exam whether due to sample handling, equipment (microscope) quality or competency of the veterinarian.

From the standpoint of specific sperm abnormalities, when identified in significant numbers, there is utility in assigning prognosis if possible. Likewise, a suspected etiology can also differentiate bulls that might be held for re-testing versus immediate culling. Thus the category of abnormality, suspected etiology, and age of the bull represent information useful in providing both a prognosis and the scheduling of a date for re-test.

Keywords: Bull, breeding soundness, morphology, sperm abnormality

Introduction

The economic justification for a veterinary breeding soundness examination (VBSE) is based on the identification and the subsequent culling of individuals that will potentially have a negative effect on cowherd productivity. Basically, this effect can be expressed both by decreases in pregnancy percentage and sale weight, with secondary resulting effects being increased culling of open cows and those that appear to be less productive, and also the insidious propagation of low fertility offspring. While percent pregnant has an obvious and easily observed impact on profitability, decreased sale weights due to calves being conceived late in the breeding season might be less readily identified by a producer, as is the decrease in fertility of retained females. Specifically, low pregnancy rates and/or a wide distribution of calvings are the hallmarks of a subfertile bull and as the completely sterile bull is rare, it is the identification of this individual (subfertile bull), which in the general population can represent 20-40% of all bulls,¹ that is the focus of our efforts. For a variety of reasons, evaluation of sperm morphology is the single best tool for identifying these bulls. Morphology and indeed every aspect of the VBSE must be performed conscientiously, consistently and competently in order for the results to be valid. The purpose of the following narrative to provide guidance toward a consistent approach with regard to semen evaluation.

Sample collection, handling and slide preparation

A detailed review of semen collection is beyond both the scope and mission of this manuscript, but needless to say a suitable sample is most efficiently obtained via electroejaculation, following rectal palpation in which the urethralis muscle is stimulated and the rectum fatigued. Additionally, a good quality, uncontaminated (absence of dirt/hair/debris) sample is more easily obtained when the penis is extended and gross observation indicates a color change of the ejaculatory fluid from clear to milky and preferably creamy white. As sperm motility must be assessed, careful handling from collection to microscopic evaluation is crucial and can be accomplished by utilizing a disposable, Styrofoam™ coffee cup as a receptacle followed by examination within 3-5 minutes with a pre-warmed slide. The process described works fairly well as long as the environmental temperature is greater than 40° F and duration of time to evaluation is short. When ambient temperatures are below 40° F utilization of a warmed collection vial is recommended.

The evaluation of semen begins with a gross examination, following this visual assessment, motility is evaluated microscopically, and following preparation of a suitably stained semen smear morphology is performed. Because of both the importance of the morphology examination and the need,
when evaluating large numbers of bulls, to proceed quickly, the microscopic evaluation of morphology can be postponed until a later time.

Evaluation of sperm morphology is facilitated by the use of a good semen smear. It is crucial to start with a clean, warm slide. Some “new” slides may have detergent coatings that interfere with staining. India ink is an acceptable stain but does not actually stain the sperm cells and is considered a “background” stain. Eosin-nigrosin (E-N) is a vital stain and is the currently recommended (SFT) stain. It is easy to use and has consistent staining properties. The eosin portion will penetrate dead sperm cells, staining them pink (red is dead) while leaving live cells unstained (white) against a dark background provided by the nigrosin component. A smear stained with Diff Quik™ is also adequate for visualizing sperm morphology and provides the advantage of allowing easy visualization of white blood cells. Another useful staining procedure is the Feulgen staining method. This technique begins with preparing a smear and then allowing it to dry for an hour. Next place the slide in 5 N HCl for 30 minutes. Wash the slide by running water into the corner of a staining dish containing the slide for two minutes. Then place the slide in Schiff’s reagent for 30 minutes. Wash again as before and air dry. The Feulgen technique is superior for identifying the nuclear vacuole (crater) defect and because the process removes fat globules, it is an excellent staining technique for smears from extended semen.

My approach utilizing the SFT E-N stain is to first place a small droplet of stain on the slide, then add a drop of semen and mix. Placing the semen drop first followed by the stain can result in contamination of the stain solution if the tip of the bottle or dropper inadvertently touches the semen. Once the stain and semen is mixed a second slide is used to push (spread) the semen across the slide in the same manner a blood smear is prepared. Because the feathered edge is the best area for evaluation, an alternative method is to create several thickness gradients by stopping and starting as the mixture is spread. It is also often a good idea to make a second slide at the same time, as it is faster to make two, than to come back later and make another slide on the occasion that the first slide was not of diagnostic quality.

Equipment

The type and quality of microscope utilized is likely to be one of the causes of the inconsistency in results cited as a problem with VBSE’s and indeed there seems to exist among theriogenologists a strong preference for the use of phase contrast microscopy. A study compared the evaluation of sperm morphology with a wet mount (fixed with isotonic formol saline) sample utilizing differential interference phase contrast (DIC) or an E-N stained dry-mount bright field (BF) microscopy provides insight on this issue. In this study the sample slides were examined at 1000X. The DIC was superior in identifying morphologic abnormalities of the sperm head such as the presence of vacuoles but the percentage normal was the same with both types of microscopy. Since the percent normal did not differ, clinical results should not be affected. So based on these findings, for the routine VBSE the use of BF microscopy and the E-N stain is adequate. Another study comparing the use of the E-N stain with BF microscopy, Feulgen-stained with BF microscopy, or phase contrast microscopy revealed that the Feulgen identified more head defects and the phase contrast revealed more distal cytoplasmic droplets. Again, the authors felt that the differences were not enough to be clinically important.

With respect to microscope quality if maintenance and cleaning are assumed and the oil immersion objective utilized to evaluate a properly prepared sample a simple guideline follows: if you can readily identify the diadem defect in a spermogram, the microscope you are using is of adequate quality. If this defect is never observed, you should likely upgrade your microscope.

Gross evaluation of semen

After collection the semen should first be observed grossly. A rough estimation of the concentration can be made based on the opacity or lack of and the color of the semen. Very concentrated samples look like heavy cream while very dilute samples have the appearance of watered down skim milk. Yellow tinted semen can result from urine contamination and this can be substantiated by smell or the use of a blood urea nitrogen test strip. Additionally, semen contaminated with urine will not be motile
or at least will display rapidly declining motility when examined microscopically. Conversely a gold appearance is also associated with very highly concentrated semen and the presence of riboflavin. This is a common finding in many Jersey and some Angus bulls. Red or brown colored semen indicates the presence of blood or blood pigments and the source should be determined.

Evaluation of sperm motility

A small “standing” drop is placed on a pre-warmed slide and evaluated under low power microscopy (40X-200X) for gross motility. Thick, dark, rapidly oscillating swirls are indicative of excellent motility (defined as a high velocity or high speed motility), a high percentage of sperm that are progressively motile, and a sample of high concentration. That sample would typically be classified as Very Good. A sample that displays slower moving swirls is classified as Good. A Fair sample displays no swirls, but significant individual sperm movement. A Poor sample has no or very little movement/oscillation. Because the concentration of a sample impacts the gross motility designation, individual motility should be assessed if there is any question about the validity of a motility rating based on gross motility. Individual motility can be assessed utilizing 200X-400X microscopy and depending on the concentration either a cover slip over either the previously examined droplet or a diluted droplet (dilute w/ warmed sodium citrate solution). Individual motility greater than 70% is Very Good, 50-69% is Good, 30-49% is Fair, and if less than 30% the sample is categorized as Poor.

Based on our current standards (SFT), bulls must have a minimum of Fair sperm motility based on either individual or gross assessment. While this may seem to be low, it is a minimum threshold and while there is a positive correlation between motility and fertility with the use of artificial insemination, this is not reported to be the case with natural service bull fertility.

Evaluation of sperm morphology (the spermogram)

There is no aspect of the VBSE in which there are greater concerns with respect to the delivery of accurate and repeatable results than the portion dealing with sperm morphology. Specifically, inconsistency among veterinarians due to differences in their ability to evaluate a spermogram and this does not take into account veterinarians or others that perform VBSEs or the euphemistic “semen check”, fertility examination, etc. but do not include the evaluation of sperm morphology. This underscores the need for adequate training for veterinarians who wish to provide this service and the protection of the public (cattlemen) from those that pass off a substandard service as a VBSE.

Current SFT- VBSE standards set a morphology threshold of 70% normal with no distinction in regard to classification of abnormalities. The 70% normal metric can be justified by a study in which cows and heifers exposed to bulls with either 70% or 80% morphologically normal sperm had similar pregnancy rates and these pregnancy rates were statistically higher than cows and heifers exposed to untested bulls. An additional argument can be made due to discrepancies among the various classification systems of sperm abnormalities- Primary vs Secondary, Major vs Minor, Compensable vs Uncompensable; and the realization that as more becomes known about a specific abnormality it’s classification status may change.

For comparison sake, the standards set forth by the Western Canadian Association of Bovine Practitioners sets a minimum morphology standard as no more than 30% total sperm defects OR 20% nuclear (head) defects. This approach provides, at least to some degree, a safeguard with respect to the accounting of those defects believed to be more significant in their impact on fertility, specifically by the fact that their occurrence in an ejaculate is predictive of the presence of defective cohort sperm that appear morphologically normal. Therefore, it appears to me that for those of us utilizing the SFT standards, we might at the very least more closely scrutinize those morphology smears that display greater than 20% head abnormalities and take that into consideration when evaluating the marginal bull.

Generally speaking, the important thing to remember when approaching the evaluation of a spermogram is that the presence of a specific sperm abnormality in significant numbers represents the clinical manifestation of a problem occurring during either spermatogenesis or sperm transport. This “problem” could be the pathological response to a transient insult as is seen with stress or exposure to...
environmental temperature extremes of short duration. It can also represent permanent or at least semi-permanent pathology such as found with testicular degeneration. Insult to the seminiferous epithelium resulting in abnormal spermatogenesis and in turn the presence of abnormal sperm also results in the production of sperm that while morphologically normal in appearance undoubtedly have a compromised ability to fertilize an ovum. Thus an ejaculate with 30% abnormal sperm present does not mean that we can say with certainty that there are 70% completely normal sperm, but instead is representative of a threshold by which we can reasonably assure the fertility of that bull to a level that meets the standard described in the introduction.

Commonly encountered sperm abnormalities

Detached head

The normal detached head, which is found in small numbers in virtually all ejaculates, is often present in high numbers in bulls following sexual rest (“rusty load” scenario), in peripubertal bulls, and from bulls that have experienced a recent stress with or without a high fever. It is also found in young bulls with testicular hypoplasia, but these should have already been excluded from further testing based on an inadequate scrotal circumference measurement. It is categorized as both a secondary and minor abnormality and is considered to be compensable due to the obvious lack of motility. Detached heads that are abnormal are categorized based on that abnormality.

If this defect is found in threshold levels, the bull can often be re-collected immediately and the number will be dramatically decreased. When placed in the deferred category, bulls with a history of recent stress etc. can be re-tested as early as two weeks, because it is an abnormality of epididymal origin and epididymal transit is around 11 days. Bulls believed to be peripubertal may benefit from a longer wait time before re-testing.

A very rarely encountered version of the detached head that has a genetic basis and in which affected bulls are sterile is the presence of separated and motile tails. This is a different, distinct abnormality in which 80-100% of sperm in an ejaculate will be affected.16

Distal midpiece reflex

The distal midpiece reflex (DMR) is the most common abnormality of the sperm tail.17 It is considered to be epididymal in origin and therefore a secondary defect. It is also categorized as a minor defect and compensable. This defect is compensable due to the lack of forward, progressive motility. Evidence for its origin is based on its rapid appearance in the ejaculate of bulls within a few days of a thermal insult. This defect appears as a sharp hairpin bend at the distal midpiece18 with a cytoplasmic droplet within the bend. If a droplet is not observed to be present, it is likely that the “bend” is due to contact with a hypotonic solution, presumably the stain that was used. This defect can often be identified during the evaluation of motility as the affected sperm will appear to be swimming backwards.

The etiology is believed to be due to a negative effect on epididymal function due to depressed testosterone levels which can in turn be caused by stress, thermal stress (either high or low), exogenous estrogen, or induced hypothyroidism; although normal, fertile bulls can have up to 25% of this defect in an ejaculate17 due presumably to its compensable nature. However, I have observed that when this defect is present at a level of 20-25% in the ejaculate of a bull that meets standards (>70% normal) when tested at a time of moderate weather and absence of stress, the same bull when re-evaluated during times of environmental temperature extremes will have an increased percentage of this defect in his ejaculate. I now closely scrutinize these bulls and discuss this issue with the owner with respect to the time of year that the bull will be placed into service. Additionally, that this defect could very well have a genetic etiology or at least predisposition in some of the beef breeds is something that should be considered.19 It has definitely been shown to be heritable in Jersey bulls, some of which would have up to 100% DMR defective sperm in an ejaculate.20
Cytoplasmic droplets

The distally located cytoplasmic droplet is thought to be epididymal in origin and its significance or rather status as an abnormality is debatable. As there is no correlation between a high incidence of this sperm type and infertility and also due to the fact that these sperm will often shed their droplets during even short periods of incubation,\textsuperscript{21} these should be re-categorized as a variation of normal.

Proximally located cytoplasmic droplets are a result of abnormal spermiogenesis and are categorized as uncompensable.\textsuperscript{17,20} However, the placement of the proximal droplet defect in the uncompensable category might be problematic as a study that used ejaculates of either high or low numbers of sperm with that defect for in vitro fertilization revealed that while fertilization was decreased as the percentage of proximal droplets in an ejaculate was increased (this meets the definition of uncompensable), of the ova that were fertilized, cleavage rates were similar.\textsuperscript{22} Indeed the presence of abnormal sperm (proximal droplets) did not impact the fertilizing ability of the normal cohort sperm insinuating that increasing the number of normal sperm could “compensate” for the presence of the defect. This reiterates the problem of becoming overly concerned with the categorization of certain sperm abnormalities instead of focusing on the likely etiology and in turn prognosis. Specifically, in the case of this defect, as previously stated the cause is undoubtedly due to abnormal spermiogenesis with the potentially underlying etiology either immaturity or conversely testicular degeneration. So in the case of the young peripubertal bull we can place that individual in a deferred status with the reasonable assurance that with age (maturity) his spermiogram will improve and in the case of the older bull we can safely assume testicular degeneration and therefore less chance for a return to fertility.

Abnormal midpiece

I will include in this category the “pseudodroplet” defect and the various mitochondrial sheath defects as well as “Dag like” defects. Additionally, the midpiece may appear swollen, “corkscrew”, bent, or asymmetric. These defects are all designated as compensable because of the obvious impact on motility and all are classified as a primary defects in the SFT system. Since the development of this sperm region occurs almost completely during spermiogenesis the specific origin for most of these defects is undoubtedly testicular. It has been shown that some forms of this group of defects can be caused by increased levels of gossypol,\textsuperscript{23} a compound found in the cotton plant and specifically cottonseed, in the diet of bulls. Bulls fed diets high in gossypol appear to be especially sensitive to this compound during puberty.\textsuperscript{24} The etiology of defects caused by gossypol appears to result from damage to sperm structure during spermiogenesis with further damage occurring during epididymal transit.\textsuperscript{23} From a practical standpoint, simply limiting the intake of whole cottonseed to less than five pounds per day for bulls of an age presumed to coincide with the attainment of puberty should be sufficient to avoid this problem. Also, this seems to be more common in Brahman bulls and indeed in the Chenoweth report\textsuperscript{24} the bulls described were Brahman. This or at least a similar defect can be created in rats fed gossypol and also rats deprived of selenium.\textsuperscript{17}

The specific abnormality referred to as a pseudodroplet is actually not common and may have a genetic component.\textsuperscript{17} It is best described as local thickening at and slight thickening of the midpiece.

Pyriform head

This is the most common defect of the sperm head\textsuperscript{17} and is usually found in low numbers even in the ejaculates of fertile bulls.\textsuperscript{18} Because there are bulls of normal fertility that have narrowed sperm and there appear to be variations in the range of “taperedness”, it can be hard to distinguish at what point a designation is made between normal and pyriform.\textsuperscript{17} For example, in the human, sperm formerly categorized as pyriform or pear-shaped sperm are no longer considered as abnormal.\textsuperscript{25} However, there was not a clearly defined distinction with regard to degree of taperedness. In veterinary literature this is a defect and categorized as both a primary and major defect. The evidence for whether or not this abnormality is compensable is equivocal. In general, sperm with misshaped heads do not transverse the reproductive tract, but sperm with this defect apparently do,\textsuperscript{26} although that in fact, appears to be dependent on the level of deformity.\textsuperscript{27} The level of deformity also apparently impacts fertilization rates as
trials evaluating this defect reveal decreased levels of zona penetration, fertilization, and cleavage rates.\textsuperscript{27,28} For example the previously cited work revealed that semen containing this defect at high percentages (85\% pyriform heads) had zona penetration at about half the rate of control (90\% normal) semen. Considering that semen containing a high percentage of pyriform head defects still resulted in some, albeit much lower, fertilization, these authors came to the conclusion that this could be due to the presence of a small number of normal sperm as well as a percentage of less affected pyriform sperm that may be capable of successful fertilization suggesting that this abnormality could be partially compensable.\textsuperscript{28}

With respect to etiology this defect is seen following environmental heat stress, validated by scrotal insulation studies,\textsuperscript{13} and also from bulls with testicular hypoplasia.\textsuperscript{17} In addition to environmental causes of heat stress, the scrotal insulation effects of fat deposition around the scrotum that results from heavy feeding during gain tests has the same deleterious effect. Bulls examined after recently coming off a gain test or going through adverse environmental extremes that have this abnormality in numbers that contribute to not meeting the metric for percent normal sperm should be deferred. In the case of bulls with testicular hypoplasia, they typically do not meet VBSE standards for scrotal circumference anyway and older, mature bulls that do not have a history of a transient insult that would provide a reason for a disruption in spermatogenesis carry a poor prognosis.\textsuperscript{17} But remember, slight degrees of taperedness may be normal. Also those young, over-fitted bulls that are deferred, might need more than 60 days to recover and meet standards.

Terminal coiled tail (coiled principal piece)

The terminally coiled tail defect also termed a coiled principal piece has been described to not be as commonly found,\textsuperscript{17} but we seem to encounter bulls with significant numbers of this defect especially following environmental temperature stress. It will be seen with other heat stress related defects and this has been documented by a scrotal insulation trial.\textsuperscript{13} It is also a defect that is increased proportionally following gossypol toxicity [29]. Due to poor motility it is compensable.

Less commonly encountered sperm abnormalities that are important due to their significance

Knobbed acrosome

The knobbed acrosome defect can be identified as an apical swelling that may protrude from or fold over the head\textsuperscript{30} but appears most often as a flattening or indention of the apex.\textsuperscript{17} This defect was identified as having a genetic etiology, specifically being an autosomal sex-linked recessive trait in the Friesian breed.\textsuperscript{16,30} A genetic etiology should be considered when this defect is prominent in the ejaculate over time, but when identified with several other defects an environmental (temperature related)\textsuperscript{13,14,17} or other transient cause is likely. Thus when this defect is encountered with other head defects there is a better prognosis for recovery\textsuperscript{31} and it would be prudent to defer and recheck the bull in 60-90 days. It is considered to be both a major and primary defect; and the best current evidence is that it is uncompensable.\textsuperscript{14} The uncompensable nature of this defect is not straightforward as it actually appears to be compensable based on the fact that sperm with this defect don’t transverse the reproductive tract of cows efficiently\textsuperscript{26} and those that do are unable to penetrate the zona pellucid.\textsuperscript{14} However, this defect is the perfect example of a defect the presence of which denotes the occurrence of normal appearing, but defective cohorts.\textsuperscript{13-15} These defective, but morphologically normal cells although able to penetrate ova, had lower rates of fertilization and reduced cleavage by zygotes.\textsuperscript{14,15} Therefore from a practical perspective we should scrutinize more closely those bulls whose ejaculate displays this defect predominately in large numbers (>20\%) as we know it can have a genetic basis and that it expresses infertility at levels higher than its occurrence within an ejaculate.

Nuclear vacuole

The nuclear vacuole defect is also termed as a crater defect and includes the diadem defect, which is a string or line of vacuoles around the acrosome-nuclear cap junction.\textsuperscript{32} While small numbers (<15\%)
of this defect in an ejaculate can be compatible with fertility, larger numbers suggest a disturbance in spermatogenesis and in fact most instances in which this defect is present at 10% or greater it is accompanied by other defects that reduce semen quality.\textsuperscript{33}

The etiology of this defect is undoubtedly environmental stress as the appearance of this sperm abnormality follows within days of the administration of dexamethasone or the application of scrotal insulation\textsuperscript{34} and the possibility of a genetic etiology has been ruled out.\textsuperscript{35} The prognosis for recovery is good if the inciting cause is eliminated.\textsuperscript{34,36}

Dag

The Dag defect named for the Jersey bull from which it was identified has a genetic etiology. Because up to 100% of the sperm in an ejaculate can be affected\textsuperscript{21} it has proven to be largely self-limiting. “Dag like” defects seem to be an etiologically distinct abnormality and were grouped with the midpiece defects.

References