Evaluation of sperm morphology: What the spermiogram is telling us

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Abstract
Evaluation of sperm morphology is a critical component of complete male breeding soundness examinations in all species. Through the development of spermiograms, veterinarians can develop a diagnosis for the visualized disruption in spermatogenesis and the likely probability for recovery. Consequently, the veterinarian and producer are able to engage in more meaningful conversation regarding realistic expectations for the future breeding status of a bull. These conversations add value to the common diagnoses that are associated with breeding soundness exams such as satisfactory potential breeder, deferred potential breeder or unsatisfactory potential breeder.

Key words: bull, breeding soundness exam, sperm, sperm morphology

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

The most common causes of abnormal spermatogenesis in males include: abnormal testicular thermoregulation; hormonal imbalances, particularly those associated with stress; and effect(s) of toxins or expression of deleterious genes. Stress typically elevates systemic cortisol concentrations, profoundly decreasing release of luteinizing hormone (LH) and testosterone. Stress has many origins, including environment, illness or injury, causing changes in the spermiogram similar to those induced by disruption of thermoregulation. The primary spermatocyte is extremely sensitive to alterations in the hormonal milieu secondary to stress or illness. For example, in cases of testicular degeneration, the changes appear first in the cytoplasm, centrosomes, and spindles at the level of the primary spermatocyte which predispose to disturbances in the developing spermatid. In this manuscript, we will discuss different reasons for abnormal spermatogenesis and common finding associated with those clinical conditions.

Immaturity
Immaturity is often recognized in the spermiogram by the observation of a high numbers of immature sperm cells also known as spheroids combined with high levels of sperm with proximal droplets and distal droplets. Peripubertal bulls often have a high percentage of sperm with proximal droplets in the ejaculate. As bulls mature, the number of proximal droplets in the spermiogram should decrease. Immature sperm cells are quite variable in size, depending on whether the cell is a primary or a secondary spermatocyte or a spermatid. Immature sperm cells must be differentiated from white blood cells in semen. This differentiation can be accomplished by staining a dried semen smear in Diff-Quik®, new methylene blue, or Wright’s giemsa. Once the stain is dried, the round cells can be evaluated and a final diagnosis of immature sperm cell or white blood cell can be made by the evaluator. If a diagnosis of immaturity is made, the bull should be reevaluated in 4-6 weeks to allow for maturation.

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

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

Testicular degeneration and subsequent regeneration are associated with a variety of morphological abnormalities. The time from the initial insult and the length of the testicular insult will determine the types of morphological abnormalities noted on the day of evaluation. For example, an increase in proximal droplets can be encountered as early as 9 days following an insult while acrosome abnormalities are first observed in the ejaculate 30 days following an insult. This coupled with the fact that we know that spermatogenesis is 61 days in the bull with epididymal transport taking approximately 9-11 days, we can note a wide variety of defects in an ejaculate at any given time (Figure 1). It may take repetitive spermiogram analysis to determine where in the stages of degeneration or regeneration the bull is currently at.
Stress

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

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

Genetic predisposition

Although adverse environmental influences are the most common cause of abnormal spermatogenesis, an increasing number of sperm defects are recognized as having a genetic origin. A table of sperm defects that have known genetic modes of transmission is listed below. One should have suspicions of a genetic influence when a high proportion of the ejaculate is affected by the same morphological abnormality with minimal other morphological abnormalities in the ejaculate (Table 1).

Toxins and nutritional changes

Many toxins have potential to affect spermatogenesis, although few naturally occurring cases have been documented. Gossypol, a phenolic toxin in the pigment glands of cottonseed, impairs sperm production in several species, including ruminants. Free gossypol in cottonseed and cottonseed meal can disrupt spermatogenesis, leading to increased numbers of morphologically

Table 1: Heritable morphologic abnormalities in bovine sperm

<table>
<thead>
<tr>
<th>Category</th>
<th>Abnormality</th>
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<tbody>
<tr>
<td>Acrosome defects</td>
<td>1. Knobbed acrosomes</td>
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<td></td>
<td>2. Ruffled, incomplete acrosomes</td>
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<tr>
<td>Head defects</td>
<td>1. Abnormal DNA condensation</td>
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<td></td>
<td>2. Decapitated sperm defect</td>
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<td></td>
<td>3. Round head</td>
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<td>4. Rolled head</td>
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<td>5. Nuclear crest</td>
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<tr>
<td>Midpiece abnormalities</td>
<td>1. Dag defect</td>
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<td></td>
<td>2. Corkscrew defect</td>
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<td></td>
<td>3. Pseudodroplet</td>
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<tr>
<td>Tail defects</td>
<td>1. Tail stump defect</td>
</tr>
<tr>
<td></td>
<td>2. Primary ciliary dyskinesia</td>
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abnormal sperm and decreased sperm motility. Bulls fed diets containing free gossypol at levels as low as 8 mg/kg per day for 56 days produced increased numbers of sperm with segmental aplasia of the mitochondrial sheath and other midpiece defects, proximal droplets, strongly folded or coiled tails, tailless (detached) heads, simple bent and terminally coiled tails (coiled principal pieces). Production of defective sperm induced by gossypol exposure is reversible within 28 days after removal of gossypol from the diet.

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

Tests mass and consequent spermatogenesis fluctuates based on metabolic cues, and appropriate nutritional management remains important throughout life. Bulls should be maintained on a diet with adequate amounts of balanced dietary protein and energy sources to ensure proper endocrine development to support spermatogenesis. Vitamin A deficiency has deleterious effects on germinal cell populations, resulting in loss of all cells in seminiferous tubules other than spermatogonia and Sertoli cells. There is minimal evidence that deficiencies of B vitamins, vitamin C, D or E, calcium, manganese, zinc, iodine, potassium or selenium directly cause bull infertility. Deficiencies of cobalt, iron, zinc and copper may be associated with anemia, lack of appetite and weight loss, thereby having negative effects on male reproduction. Copper deficiency reportedly must be severe to affect spermatogenesis.

Iatrogenic changes

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

References