

## **What's known about selected sperm abnormalities in the bull?**

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### **Abstract**

The morphological evaluation of spermatozoa is an important and often overlooked aspect of the bull breeding soundness examination and it is imperative that practitioners not only be able to recognize defective spermatozoa but also to understand the origins of observed defects and provide information related to their effects on the bull's fertility. This review describes the common causes of altered spermatogenesis. It details many of the morphological abnormalities frequently encountered during routine bull breeding soundness exams. Insight is given into their causes as well as the potential impact of each defect on the fertilization process.

**Keywords:** Bull, spermatozoa, acrosome, proximal droplet; breeding soundness examination

### **Introduction**

The testicle of a bull is a very sensitive organ which is capable of responding to an assortment of insults including heat, hypoxia, radiation, toxicity, and stress as well as the influence of genetic disorders. The response of the testis to these insults often leads to the formation of spermatozoa with observable defects that can be seen during a routine spermogram. Disturbances in spermatogenesis result in the formation of a wide range of defective spermatozoa which can be observed clinically. Attempts to make both an etiologic diagnosis as well as provide a prognosis for the bull's potential recovery of normal spermatogenesis is one of the goals of a complete breeding soundness examination when excessive numbers of abnormal spermatozoa are observed. This paper

will review a select population of sperm defects which could be encountered in veterinary practice and provide their common causes as well as the prognosis for fertility when known.

### **Causes of altered spermatogenesis**

The two most common insults which affect bulls on pasture appear to be heat and stress.<sup>1</sup> The affects of trauma, elevated ambient temperature, fever, excessive deposition of fat in the scrotal neck and scrotal frostbite can all lead to increased heat in the scrotum. The impact of the excessive heat in the scrotum leads to altered spermatogenesis and the eventual appearance of increased numbers of morphologically abnormal spermatozoa in the ejaculate. The temperature of the scrotal contents in normal bulls generally varies between 33.0 and 34.5 °C with a temperature gradient of 6 °C from that of core body temperature.<sup>2</sup> Numerous studies have shown that even very small increases in testicular temperature can have dramatic impact on spermatogenesis leading to the subsequent appearance of abnormal spermatozoa in the ejaculate.<sup>1,3,4</sup> The testicles of bulls appear to function in a nearly hypoxic state which is in part due to the actions of the pampiniform plexus which essentially eliminates the pulse pressure and slows the blood entering the testicle.<sup>2</sup> When scrotal temperatures increase, the metabolic demands of the testicles increase as well, but there is no corresponding increase in testicular blood flow ultimately leading to testicular hypoxia and subsequent alterations in spermatogenesis.<sup>1</sup>

Stress in the form of illness, pain, herd or social interactions, transportation, and weather conditions can be experienced by bulls. There appear to be disturbances in the endocrine pathways associated with normal spermatogenesis in bulls under stress.<sup>1</sup> The affect of stress has been measured by examining the relationship between cortisol,

luteinizing hormone (LH) and testosterone. High cortisol levels in bulls have been associated with reduced levels of LH and testosterone when compared to bulls with normal cortisol; the elevated cortisol levels may interfere with normal spermatogenesis.<sup>5</sup>

The toxic effects of gossypol on spermatogenesis are well-recognized and the characterization of the of abnormal spermatozoa has been the subject of several studies.<sup>6,7</sup> The spermatotoxic effects of gossypol appear to be related to both the dose and duration of consumption of the phenolic compound produced by the cotton plant.<sup>8</sup> The incidence of gossypol-induced altered spermatogenesis in bulls is likely low due to the rumen's ability to detoxify the gossypol. However, high intake of free gossypol, albeit rare, can overwhelm this system and create toxicosis.<sup>7</sup>

Genetic causes of sperm abnormalities are not as common as environmental causes but are becoming more recognized because of improved diagnostic tools.<sup>9</sup> These defects have been shown to either consistently or occasionally have a genetic mode of transmission. With the ability to scrutinize large numbers of AI sires and their progeny a number of sperm defects have been classified as genetic in nature.

### **Distal midpiece reflex**

The distal midpiece reflex (DMR) is the most common tail abnormality encountered when evaluating the morphology of bull sperm.<sup>10</sup> The typical appearance of a DMR is that of a distinct hairpin bend of the tail at the location of the distal midpiece, however there can be varying bending patterns noted in the tail giving the affected spermatozoa several different appearances. A consistent finding is the presence of a cytoplasmic droplet noted within the bend of the tail.<sup>10</sup> When this defect is observed in

live samples, the spermatozoa will appear to be swimming in a reverse motion often in a circular pattern.<sup>10</sup>

The DMR defect can be induced experimentally when spermatozoa are exposed to hypotonic solutions or when cooled very rapidly.<sup>10</sup> This is critically important when preparing a slide for staining. Many of the morphology stains used today (ie. eosin nigrosin) are hypoosmotic and will create a similar defect if spermatozoa are exposed for an extended time.<sup>10</sup> It is critical that the slide be prepared properly and dried quickly so as to reduce the chance of creating this defect iatrogenically. One striking difference in the defects which occur naturally and those that are caused in vitro is the presence of a cytoplasmic droplet within the reflex of the tail. When a large number of DMR defects are noted without the concurrent presence of a cytoplasmic droplet the possibility of an artificial cause should be investigated.<sup>10</sup>

The DMR defect is produced in the corpus and cauda epididymis where the spermatozoa still have a distal cytoplasmic droplet which is present in the bending of the tail.<sup>10</sup> The evidence which supports the epididymis as the origin of this defect is based on appearance of this defect after known testicular insults. Semen evaluation of 606 bulls prior to a severe snowstorm showed the percentage of bulls with greater than 15% DMR to be only 10.9% whereas of the 117 bulls examined 3-4 days after the snowstorm 45.3% had greater than 15% DMR defects.<sup>10</sup> A significant increase in the DMR defect was observed 6-12 days after a brief period of scrotal insulation.<sup>10</sup>

Other recognized causes of this defect include treatment with estrogens, induced hypothyroidism and fever. All of these conditions have been shown to reduce

testosterone levels, which appears to adversely affect the epididymal environment leading to the formation of the DMR defect.<sup>10</sup>

The fertility of ejaculates containing high numbers of spermatozoa with DMR defects has not been critically evaluated however it has been observed that the defect could be found in normal fertile bulls with a prevalence up to 25%.<sup>10</sup> Owing to the fact that this defect is of epididymal origin, it would seem that any effect on fertility would be short-lived provided the inciting insult was removed. Since affected spermatozoa would swim in a reverse fashion it is unlikely that they would be able to participate in fertilization thereby could be compensated for by additional spermatozoa. There is no evidence that spermatozoa with the DMR defect are capable of regaining normal function.<sup>10</sup> Semen evaluation in affected bulls should be performed often to detect changes in prevalence of the DMR defect that could help identify an inciting cause.

### **Knobbed acrosome**

The knobbed acrosome defect has been described as a refractile or dark-staining area or eccentric thickening often giving a beaded appearance to the apex of affected spermatozoa.<sup>10</sup> More commonly however, it appears with the apex of the spermatozoa having a flattened or indented acrosome.<sup>10</sup> With the availability of electron microscopy, studies have shown that with both the beaded and indented forms the abnormal acrosome folds back on the sperm apex and a few affected spermatozoa may show a bead-like protrusion from the apex of the spermatozoa. The folding back of the acrosome may also result in a bending back of the apex of the nucleus which causes the apex to appear to have an indentation.<sup>10,11</sup>

The knobbed acrosome defect results from altered spermatogenesis caused by either environmental or genetic factors leading to abnormal development of the fine structure of the plasma membrane making it more susceptible to structural and functional changes.<sup>9,12</sup> It has been shown that spermatozoa with the knobbed acrosome defect lack membrane integrity which can lead to premature capacitation and subsequent acrosome reaction however the exact mechanisms involved remain elusive.<sup>11,12</sup>

It is generally accepted that bulls whose semen contains a high percentage of knobbed acrosome defects will have poor fertility.<sup>10</sup> The exact mechanism by which this defect causes reduced fertility has yet to be elucidated, however it has been theorized that spermatozoa with abnormally shaped heads including the knobbed acrosome defect may have altered motility characteristics which could impair the passage of spermatozoa through the female reproductive tract.<sup>13</sup> Spermatozoa containing the knobbed acrosome defect could also have altered sperm-oocyte binding and zona penetration.<sup>13</sup> In vitro fertilization (IVF) models have shown that knobbed acrosome affected spermatozoa have a reduced ability to bind the zona pellucida and could not penetrate the zona pellucida.<sup>11</sup> Thus the effect of the knobbed acrosome defect on fertility appears to be related to both altered passage as well as impaired plasma membrane function.<sup>12</sup> In vitro studies also indicate there could be compromised fertility associated with apparently normal spermatozoa from males with many knobbed acrosome defects.<sup>14</sup> These normal appearing spermatozoa have been shown to undergo premature capacitation and have spontaneous acrosome reaction as well as evidence of chromatin condensation.<sup>12</sup>

This sperm defect has been associated with infertility in a number of species including bulls, boars, rams and stallions.<sup>11</sup> In cattle this defect was first reported in the

Friesian breed in the 1940's however it has since been seen in Charolais, Simmental, Maine Anjou, Salers, Horned Hereford, Angus and Normande.<sup>9,15</sup> This defect is associated with an autosomal sex-linked mode of transmission in the Friesian breed of cattle and in boars.<sup>9</sup> There appears to be evidence to support a genetic cause of this defect in both the Charolais breed and Angus cattle in North America.<sup>9,15</sup>

### **Cytoplasmic droplet**

The presence of a cytoplasmic droplet is common on a small number of spermatozoa in the ejaculate of fertile bulls. The cytoplasmic droplet is a spherical mass of cytoplasm that is typically found in one of two locations on the spermatozoa. It is considered a proximal droplet when located in the proximal midpiece and a distal droplet when surrounding the midpiece just proximal to the annulus.<sup>10</sup> Droplets are rarely observed in an intermediate location due to the rapid migration of the proximal droplet to the distal location prior to shedding.

Cytoplasmic droplets are formed during spermiogenesis when the spermatid changes from its round shape to an elongated shape and cytoplasm is pulled from the head region towards the tail.<sup>10</sup> During this transition the Sertoli cell molds this cytoplasm into a lobule called the residual body.<sup>10</sup> At the time of spermiation the stalk connecting this residual body to the spermatid is severed and leaving the droplet of cytoplasm in the proximal neck region of the spermatozoa.<sup>10</sup>

As spermatozoa enter the caput epididymis almost 85 percent will have a proximal droplet. As spermatozoa move through the epididymis and maturation occurs the proximal droplet moves to a distal location and is eventually shed. By the time the spermatozoa reach the caudal epididymis over 60 percent of the spermatozoa have a

distal droplet.<sup>10</sup> The loss of the cytoplasmic droplet appears to be associated with the gaining of motility in the epididymis.

It is very common to find a high incidence of proximal droplets in bulls that are approaching puberty, however with repeated collections over the following months the percentage of affected spermatozoa generally drops substantially. In yearling bulls a major cause of failure to pass a breeding soundness examination is often the presence of a large percentage of proximal droplets. In data collection from Colorado, 12-26% of yearling bulls failed to pass an initial breeding soundness examination with 6.3% of these failures attributable to proximal droplets.<sup>16</sup> As bulls mature however, the incidence of cytoplasmic droplets tends to drop dramatically. When over 1500 bulls of various ages and breeds were examined the percentage of bulls whose ejaculates contained proximal droplets was 67 percent, with the number of affected spermatozoa averaging only 2.7 percent.<sup>10</sup> In older bulls a high incidence of spermatozoa with proximal droplets points towards abnormal spermiogenesis likely due to a degenerative process of the seminiferous epithelium.<sup>10</sup>

The prognosis for bulls with a high percentage of spermatozoa with proximal droplets varies depending on the underlying cause and the presence of other defects. Recovery has been seen in bulls with profound disturbances of spermatogenesis leading to ejaculates which contain greater than 50 percent of spermatozoa with proximal droplets, however recovery often requires months.<sup>10</sup> In vitro fertilization was used to evaluate the fertilizing potential of semen from young bulls with a high incidence of proximal droplets. It was concluded that fertility was severely compromised but as the bulls matured the incidence of proximal droplets decreased and fertility increased.<sup>17</sup> This

study also showed the fertilizing potential of a bull whose semen contained  $\geq 30\%$  spermatozoa with proximal droplets will be low until the incidence of proximal droplets decreases.<sup>17</sup>

Distal droplets are not considered to be a major problem and often indicate insufficient maturation in the epididymis. It has been noted that ejaculates containing a high number of spermatozoa with distal droplets when allowed to incubate for several minutes will almost be totally cleared of the defect.<sup>10</sup>

### **Crater or diadem defect**

The crater or diadem defect is seen in spermatozoa which have a nuclear vacuole or invagination of the nuclear membrane into the nucleoplasm which occurs during spermiogenesis.<sup>18,19</sup> This defect can appear as a “string of pearls” at the acrosome-postacrosomal sheath or as round to elongated white spots which often appear to sparkle.<sup>10</sup> The defect often appears as a surface oriented crater and can range from 1 to over 20 in number.<sup>10</sup> This particular defect is often overlooked with light microscopy on routine eosin-nigrosin stained spermograms, whereas phase-contrast microscopy and differential interference microscopy allow one to more easily detect this defect. The use of a nucleus stain such as the Feulgen stain should also allow excellent visualization of the nucleus and associated vacuoles.<sup>10</sup>

The incidence of the crater or diadem defect in bulls is generally very high, often approaching 100%; however the percentage of affected spermatozoa within an ejaculate can vary greatly. In a Czechoslovakian study of young and older bulls, nuclear vacuoles were observed in all bulls with the incidence of affected spermatozoa ranging from 3-

26%.<sup>10</sup> In one study of bulls at an AI center, all bulls had some defects and 28% of the bulls examined had an incidence of crater defects in excess of 20%.<sup>19</sup>

The precise pathogenesis of this defect has not been elucidated however there are several theories put forth in the literature. Coulter suggested a possible viral etiology based on the presence of viral-like particles within the vacuoles with the thought that the virus may attack the developing spermatozoa.<sup>10</sup> Others have shown that certain insecticides when administered to bulls resulted in an increased incidence of nuclear vacuoles.<sup>10</sup> The most common and accepted theory involves the impact of stress on the process of spermatogenesis. It has clearly been shown that administration of dexamethasone to bulls to mimic a stressful event will significantly reduce their LH and testosterone levels and is thought to impair spermatogenesis and lead to the formation of defective sperm. Studies of the sequential appearance of sperm defects after administration of dexamethasone to bulls clearly show there is an increase in the presence of nuclear vacuoles with the peak incidence occurring around 21 days after dexamethasone treatment.<sup>1</sup> Whether the effects of dexamethasone on spermatogenesis are direct or indirect have yet to be determined. When bulls were studied over long periods of time, fluctuations were seen in the incidence of affected spermatozoa indicating a nonheritable etiology. This is supported by the absence of the defect in significant numbers in the ejaculates of bulls which are sons of a known affected bull.<sup>10</sup> However there is reason to believe that there may be bulls which have a heritable predisposition to produce spermatozoa with the crater defect in response to stress.<sup>10</sup>

It is clear that ejaculates containing high numbers of spermatozoa with nuclear vacuoles are a cause of infertility in the bull. Breeding trials have shown that

spermatozoa with multiple nuclear vacuoles have reduced fertilization characteristics both in vivo and in vitro.<sup>18,20</sup> It appears that affected spermatozoa have a reduced capacity to penetrate the zona pellucida as well as reduced ability to form a male pronucleus.<sup>18</sup> With this knowledge, bulls whose semen contains high numbers of spermatozoa with nuclear vacuoles should be monitored with successive semen evaluations as it has been reported that occasionally bulls recover and regain normal fertility.<sup>10</sup>

### **Dag defect**

The Dag defect traces back to a Jersey bull with this name in which this unique defect was first discovered and reported. This heritable defect which occurs during late spermiogenesis is characterized by severe coiling of the tail with fracture of the distal part of the midpiece.<sup>10</sup> A distal cytoplasmic droplet can also be seen associated with this defect.<sup>9</sup> Other features which may be seen include a roughened appearance to the mitochondrial sheath and fracturing of the axonal elements leading to disruption of the mitochondrial arrangement.<sup>10</sup> This defect has been reported as a cause of infertility in several breeds including the Jersey, Polled and Horned Hereford, and the Swedish Red and White. In these particular infertile bulls the incidence of the Dag defect was generally greater than 50%. This defect can be found in the ejaculates of bulls with normal fertility however the incidence of affected spermatozoa rarely exceeds 5%.<sup>10</sup> When the incidence of this defect approaches 50% there appears to be a profound impact on fertility.<sup>10</sup>

A genetic basis for this defect was proven in the Danish Jersey breed through selected breeding of a suspected carrier to 120 of his daughters which produced 6 bulls

with the typical defective spermatozoa.<sup>10</sup> There may be breed specific differences in the exact pathogenesis of this defect because investigators have noted distinct differences in the microanatomy of affected spermatozoa from various breeds.<sup>10</sup>

### **Pyriform or tapered heads**

The pyriform defect is the most common defect of head shape and it appears as a pear-shaped head with a pronounced narrowing of the postacrosomal area.<sup>10</sup> There are many variations of the pyriform defect that range from almost imperceptible to those with severe narrowing all of which can occur in the same ejaculate. The tapered head shape is slightly different than the pyriform head shape in that the entire nucleus is narrow and the head appears elongated.<sup>10</sup> Both of these head defects can be found in the same ejaculate and affected spermatozoa always appear smaller than their normal counterparts. These two defects appear closely related in origin and may be categorized together.<sup>10</sup>

The incidence of the pyriform or tapered head defect occurring in more than 15% of spermatozoa in an evaluation of over 1300 range beef bulls was determined to be 8.7%. This was lower than the 16.4% incidence found in another study of 216 dairy and beef AI sires.<sup>10</sup> The presence of this defect in low numbers however is a fairly common finding in the ejaculate of many fertile bulls.

The pathogenesis of the pyriform or tapered head defect has not been proven but appears to occur secondary to some disturbance of thermoregulation or an endocrine aberration leading to impaired testicular function.<sup>10</sup> Over-conditioned bulls which have excess fat in their inguinal and scrotal areas commonly have these defects. The appearance of pyriform head defects in experimentally-induced scrotal insulation peaked

at 22 days post insulation and at 24 days in dexamethasone-treated bulls suggesting the damaging effects occurred during nucleus condensation and shaping.<sup>1</sup>

The effects of spermatozoa with pyriform or tapered heads on fertility appear to be related to their reduced ability to bind and penetrate the zona pellucida however there does not appear to be an increased incidence of embryo or fetal loss provided fertilization occurs.<sup>10</sup> Work by Saacke investigating the accessory sperm population has also shown that the severity of the head defect may dictate the accessibility of spermatozoa to the ovum thereby limiting the possibility of zona binding.<sup>21,22</sup>

The prognosis for bulls exhibiting large numbers of pyriform or tapered heads in their ejaculates varies depending on the underlying cause. When a cause such as altered testicular thermoregulation can be identified and corrected, the prognosis is generally good provided enough time is allowed to resume normal spermatogenesis. However in bulls which have high numbers of pyriform or tapered heads in which there is no apparent reason for the altered spermatogenesis the prognosis is generally poor for recovery.<sup>10</sup> The percentage of affected spermatozoa can generally be used as a prognostic indicator.

### **Detached heads**

The presence of detached heads on routine evaluation of a bull's spermiogram is relatively common. This defect is easy to identify during evaluation of a sperm morphology slide. The incidence of this defect in bulls of normal fertility and various ages appears to be around 5 percent.<sup>10</sup> A number of conditions have been associated with the presence of increased numbers of detached heads. In one study of eight bulls with testicular hypoplasia the incidence of detached heads ranged between 39 and 93 percent.

However, of these eight bulls, seven were traced back to a common ancestor and the possibility that the high incidence of detached heads was inherited could not be overlooked.<sup>10</sup> The relationship between detached heads and testicular hypoplasia is not consistent among all bulls with this condition. In a study of 141 bulls with testicular hypoplasia the percentage of detached heads was only 6.9 percent.<sup>10</sup>

There appears to be a condition in bulls that mimics the clinical appearance of “plugged ampullae” in stallions. The incidence of this condition was found to be 1.1% when over 1300 bulls were evaluated. The characteristics of the ejaculate consist of a very large volume of highly concentrated semen which contains a high proportion of dead spermatozoa as well as 15-45 percent detached heads.<sup>10</sup> These bulls appeared to improve with frequent repeated collections however the incidence of this defect would be higher after periods of sexual rest. The most common theory is that these bulls have a failure of normal sperm transport with accumulation of spermatozoa within the epididymis and ampullae.<sup>10</sup>

Other conditions that have been shown to cause a transient increase in the number of detached heads include: seminal vesiculitis, epididymitis, or any condition which leads to failure of normal testicular thermoregulation. Lameness which causes the bull to lie down for long periods of time with resultant failure of normal thermoregulation is known to increase the number of detached heads.<sup>10</sup>

### **Abaxial attachment of the tail**

Abaxial attachment of the tail is an infrequently encountered defect of bull spermatozoa. In a retrospective analysis of semen analysis from 1049 range and AI bulls the percentage of bulls found to have the defect was 10.5% and only 0.48% of bulls had

semen with greater than 50% abaxial attachment of the head.<sup>23</sup> It is considered a normal finding in the boar and stallion however its significance in the bull remains unclear.

There have been reports of sterile bulls that had an increased number of spermatozoa with abaxial attachment of the head however there were no controlled breeding trials performed.<sup>23</sup> In three controlled experiments comparing the fertility of bulls with high numbers of abaxial tail attachment to that of known fertile bulls there was no difference in all fertility parameters measured between bulls.<sup>23</sup> The combined results of these experiments indicate that abaxial attachment of the tail does not impact fertility and should be considered a normal morphological variation of bovine spermatozoa.<sup>23</sup>

### **Conclusion**

There has been a strong correlation between morphologically abnormal sperm and some degree of infertility for many years. It is important to not only be able to recognize morphological defects of spermatozoa but also to provide the client with possible etiologic causes of the defects observed and make suggestions as to their impact on fertility. With the use of routine staining procedures most defects can be observed when examined at 1000X with light microscopy. Electron microscopy may more accurately identify the exact defect present in affected spermatozoa allowing a more in-depth understanding of the sperm abnormalities.

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