

The pathogenesis of abnormal sperm production in bulls

Albert Barth
Western College of Veterinary Medicine
Saskatoon, SK

Veterinarians in food animal practice often evaluate hundreds of bulls for breeding soundness annually. When there are no abnormal findings and the semen traits are good, the decisions are not difficult. However, many questions arise when physical or seminal abnormalities are found. The underlying causes of abnormalities and the prognosis for improvement are generally of particular concern. An understanding of normal spermatogenesis, the nature of abnormal spermatogenesis, and the underlying causes of abnormal spermatogenesis is important to improve the accuracy of diagnosis and prognosis for bulls of questionable breeding soundness.

Most veterinarians have had exposure to the details of spermatogenesis and the chart of the cycle of the seminiferous epithelium in their undergraduate curriculum and the subject has been covered in many textbooks. A very brief review follows. The seminiferous tubules are lined internally with 5-7 layers of germinal cells forming the seminiferous epithelium. During each cycle of germ cell replication, the A and B spermatogonia undergo six mitotic divisions forming the spermatocytes. The primary and secondary spermatocytes then go through meiosis and form spherical spermatids. The spermatids, which are the haploid product of meiosis, go through a metamorphic process in which the acrosome develops, the nucleus flattens and elongates as its chromatin condenses, and the sperm tail develops. After release of spermatozoa into the tubule lumens, the spermatozoa move through the rete testis and exit at the dorsal pole of the testis via the efferent ductules. The efferent ductules are long and convoluted making up about a third of the caput epididymis. The epididymis is a single tube that is approximately 120 feet long in the bull and epididymal passage takes approximately 9 days. During passage through the epididymis, further maturation of the spermatozoa occurs.

Spermiogenesis, the last 18 days of spermatogenesis, is the period of metamorphosis of spherical spermatids into fully formed sperm. This is the period during which sperm defects develop. Different defects develop at different times of spermiogenesis and during epididymal maturation. For example, the mitochondrial helix develops during the last 4 days of spermiogenesis, thus when taking into account 10 days for epididymal passage, sperm with defects of the mitochondrial helix will appear in the ejaculate about 2 weeks after the defects develop.

An experiment was done to monitor the sequential appearance of various sperm defects in response to 4 days of scrotal insulation or 7 days of dexamethasone injections to mimic stress (1). The various types of defects appeared in a definite order according to their developmental position in spermiogenesis at the time of the insult. Peak numbers of different defects occurred in the following order: 7 to 11 days - distal midpiece reflexes and proximal droplets, 11 to 15 days - mitochondrial sheath disruptions and detached heads, 20 days - knobbed acrosomes, 18 days - nuclear vacuoles, 23 days - coiled principal pieces, and 22 to 24 days - pyriform heads. Recovery was nearly complete in all bulls within 6 weeks. Since many adversities are of much longer duration than those induced experimentally, defects developing in the epididymis and testis would appear in the ejaculate simultaneously rather than in sequentially. Nevertheless,

spermiograms often have one predominant type of defect and when the history of the bull is considered, the cause of that type of defect can be determined. There are many causes of abnormal spermatogenesis, but in bulls with previously normal spermiograms, most can be placed into the categories of stress or heat. In northern areas, photoperiod likely has an important effect on gonadotrophin secretion and spermatogenesis. Other less common causes of abnormal spermatogenesis include heredity, toxicity, infections, and nutritional deficiencies.

Effect of stress

Stress due to illness, injury, pain, hunger and a variety of other problems, affects testicular function through endocrine mechanisms. Normal function of the epididymis and the seminiferous epithelium is dependent on high local levels of testosterone. Stress results in excess adrenal production of cortisol which in turn reduces pituitary LH and FSH secretion and consequently testosterone production. Many people underestimate the impact on spermatogenesis of seemingly minor problems such as grain overload, foot rot, sole abscesses, and dehorning.

Effect of heat

Testicular temperature is maintained at about 4 °C cooler than body temperature. There are many ways in which testis cooling can be compromised. It appears that the mechanism of heat damage is through tissue hypoxia. Normally, because of the peculiar anatomy of the blood supply to the testis, its tissue operates on the brink of hypoxia. Raising testicular temperature increases the metabolic activity, but there is no corresponding increase in blood supply and tissue hypoxia ensues (2). A common problem in beef bulls is fat deposition in the neck of the scrotum. Recent work has shown that when the neck of the scrotum is insulated scrotal temperature below the neck increases resulting in an increase in sperm defects (3). Body condition scores that were above or below average were shown to adversely affect semen quality (4).

Effect of season

Although cattle do not have distinctly seasonal reproductive activity, there is evidence of a seasonal basis in bovine reproduction. Breeding soundness evaluation records of 2110 *Bos taurus* beef bulls were obtained from the medical records of the Western College of Veterinary Medicine. The percentage of bulls with satisfactory semen quality increased as time progressed from the colder months of January and February toward the warmer months of May and June. This suggests that a combination of a short photoperiod, cold stress, and reduced feed quality may be detrimental to spermatogenesis. Feed quality is less likely to be a factor since significantly more bulls had satisfactory semen quality in March and April than in January and February despite being on the same supply of stored feed (4). The percentage of bulls with satisfactory semen quality declined in August and September, perhaps due to a decline in photoperiod and in the nutritional value of late season pasture. Our studies of testosterone levels at the equinoxes and solstices in 15 bulls over 3 years, as determined by serial blood sampling for periods of 10 hours, showed lowest levels of testosterone in September and highest levels in June.

Inherited sperm abnormalities

Several sperm abnormalities have been shown to have a hereditary basis. An examination of the pedigrees of 16 virtually sterile Friesian bulls with the Knobbed Sperm defect showed that all were related to 2 bulls. Pedigree analysis showed that the knobbed defect was likely also inherited as a recessive condition in Charolais cattle (5). The Dag defect was first described in 2 full brothers of the Danish Jersey breed. A normal fertile bull that had produced 2 sons and 1 daughter's son with the defect was mated through artificial insemination to 120 of his daughters. Of 38 bull calves resulting from these matings, 6 had the Dag condition indicating inheritance via a recessive gene (6). Recently we have seen the Dag defect in >90% of sperm in two Polled Hereford bulls that were half brothers and 4 Galloway bulls derived by embryo transfer from one flush. The Tail Stump defect, Decapitated Head defect and the Rolled Head-Nuclear Crest-Giant Head syndrome are other examples of sperm defects inherited via recessive genes. In addition, there likely are more complex forms of inheritance for many other types of sperm abnormalities.

Effect of toxins

Although there are reports in the literature of a wide variety of toxins that affect spermatogenesis, there are few reports of naturally occurring cases. Naturally occurring cases of gossypol and zearalenone toxicity have been reported. The effects of feeding Brahman bulls a cottonseed meal containing gossypol were examined during an 11-week period. Bulls consuming 8.2 g gossypol daily had a significantly higher incidence of abnormal sperm midpieces from weeks 3-11 than bulls whose diet was free of gossypol (7). However, in some studies feeding cottonseed containing free gossypol at levels exceeding 8 g daily did not result in disturbed spermatogenesis. In these studies it appeared that bulls ingested sufficient minerals in the water to bind with the free gossypol ingested, thereby detoxifying it (8). In addition, supplemental Vitamin E at 400 IU per bull daily prevented the damaging effects of gossypol (9). The semen of 2 bulls, fed maize containing 20 mg/kg zearalenone for 72 days, was found to be unfit for insemination after 21 days. Histological examination of the testes showed marked degeneration of the germinal epithelium of the testes (10). Producers and veterinarians have often expressed concern about the safety of various therapeutic agents on semen quality. Treatment with steroid hormones may depress pituitary gonadotrophin production leading to abnormal spermatogenesis. The effects of treatment with several antibiotics and phenylbutazone were determined in an experiment utilizing normal bulls (11). There was no adverse effect of treatment with tilmicosin, oxytetracycline, dihydrostreptomycin and phenylbutazone on semen quality.

Infectious organisms

The adverse effects on spermatogenesis seen in many illnesses may be due not only to fever, but also to bacterial toxins. Suppression of LH surges after intravenously administered endotoxin in rams was followed by an increase in abnormal sperm (12). Viral infections with a direct effect on the testes other than those mediated through fever have not been reported for bulls. However, there are reports of fibrotic lesions in the testis apparently caused by paramyxovirus infection in boars (13). The porcine reproductive and respiratory syndrome virus was found to replicate in germinal cells of boars leading to deterioration in semen quality (14).

Effects of nutritional deficiencies

Although numerous attempts have been made to formulate rations that will increase sperm production and semen quality, it appears that there are no specific nutrients concerned only with fertility. Low feed intake resulting in poor body condition may depress spermatogenesis via an endocrine effect. Protein supplementation of bulls on poor quality forage significantly increased dry matter intake, enabling maintenance of live weight as well as testis size and sperm output (15). Vitamin A deficiency appears to act directly on the germinal cells resulting in a loss of all cells except spermatogonia and Sertoli cells, but there is little evidence that deficiencies of vitamins B, C, D, or E are even occasional causes of infertility in domestic animals. Deficiencies of calcium, manganese, zinc, iodine, potassium and selenium have not been proven to be causes of male infertility. Deficiencies of cobalt, iron, zinc and copper may cause anemia, lack of appetite and loss of weight, and thus have an adverse influence on male reproduction. Generally copper deficiencies must be severe and prolonged to have any effect on fertility (17). Studies in rats and mice have established that Se is required for normal sperm tail development (18) and this element may occasionally be involved in reduced bull fertility.

References

1. Barth AD, Bowman PA. The sequential appearance of sperm abnormalities after scrotal insulation or dexamethasone treatment in bulls. *Can Vet J* 1994; 35: 93-102.
2. Waites GMH, Moule GR. Blood pressure in the internal spermatic artery of the ram *J Reprod Fertil* 1960; 1: 223-229.
3. Kastelic JP, Cook RB, Coulter GH Saacke RG. Insulating the scrotal neck affects semen quality and scrotal/testicular temperatures in the bull. *Theriogenology* 1996; 45 (5): 935-942.
4. Barth, A.D. and Waldner, C.L. Factors Affecting Breeding Soundness Classification of Beef Bulls in Saskatchewan. *Can Vet J* 2002; 43: 274-284.
5. Barth AD. The Knobbed acrosome defect in beef bulls. *Can Vet J* 1986; 27: 379-384.
6. Koefoed-Johnsen HH, Andersen JB, Andresen E, et al. The Dag defect of the tail of the bull sperm. Studies on the inheritance and pathogenesis. *Theriogenology* 1980; 14: 471-475.
7. Chenoweth PJ; Risco CA, Larsen RE, et al. Effects of dietary gossypol in cottonseed meal on aspects of semen quality and sperm production in young Brahman bulls. *Bovine Practitioner* 1995; 29: 51-52.
8. Cusack PMV, Perry V. The effect of feeding whole cottonseed on the fertility of bulls. *Australian-Veterinary-Journal*. 1995, 72: 12, 463-466.
9. Velasquez-Pereira J, Chenoweth PJ, McDowell LR, et al. Reproductive effects of feeding gossypol and vitamin E to bulls. *J Anim Sci* 1998; 76 (11): 2894-2904.
10. Vanyi A; Timar I; Szeky A. Fusariotoxicoses. IX. The effect of F-2 fusariotoxin (zearalenone) on the spermatogenesis of rams and bulls. *Magyar Allatorvosok Lapja*. 1980; 35: 777-780.
11. Barth AD, Wood MR. The effect of streptomycin, oxytetracycline, tilmicosin and phenylbutazone on spermatogenesis in bulls. *Can Vet J* 1998; 39: 103-106.
12. Margareta, W, Kindahl, H, Larsson, K. Clinical, endocrinological and spermatological studies after endotoxin in the ram. *J Vet Med* 1989; 36: 90-103.
13. Ramirez-Mendoza H, Hernandez-Jauregui P, Reyes-Leyva J, et al. Lesions in the reproductive tract of boars experimentally infected with porcine rubulavirus. *J Comp Pathology* 1997; 117 (3): 237-252.

14. Sur JH, Doster AR, Christian JS, et al. Porcine reproductive and respiratory syndrome virus replicates in testicular germ cells, alters spermatogenesis, and induces germ cell death by apoptosis. *J Virology* 1997; 71(12): 9170-9179.
15. Ndama PH, Entwistle KW, Lindsay JA. Effect of protected protein supplements on some testicular traits in Brahman cross bulls. *Theriogenology* 1983, 20: 639-650.
16. Setchell, BP. *The Mammalian Testis*, 1978; Cornell Univ Press, Ithaca, N.Y , 14850, 359-432.
17. Van Niekerk FE; Van Niekerk CH. The influence of experimentally induced copper deficiency on the fertility of rams. *J South African Vet Assoc* 1989; 60: 28-31.
18. Olsen GE, Winfrey VP, Hill KE, Burk RF. Sequential development of flagellar defects in spermatids and epididymal spermatozoa of selenium-deficient rats. *Reproduction-Cambridge* 2004; 127 (3): 335-342.