

# Case-based studies of infertility in bulls

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## Abstract

The primary purpose of analysis of the spermogram is to form an opinion on a bull's potential fertility. When spermograms are abnormal, often a prognosis for recovery to normal sperm production is also a consideration. Semen quality can change quite rapidly for the better or worse depending on whether a bull is suffering from, or just recovering from, a process detrimental to spermatogenesis. Therefore, the spermogram must be interpreted in light of the bull's history, the results of the physical examination, and in some cases the semen traits of volume and concentration. Some sperm defects appear to have a genetic basis and knowledge of recessive conditions, or genetic predispositions, will also help to decide on a course of action when abnormal spermograms are encountered. Cases highlighted in this presentation demonstrate some examples of interpretation of abnormal spermograms.

## Résumé

Le but premier de l'analyse du spermogramme est de se faire une opinion sur la fertilité potentielle d'un taureau. Souvent, quand les spermogrammes sont anormaux, il est également possible de considérer un pronostic de retour à une production normale de sperme. La qualité du sperme peut changer très rapidement et s'améliorer ou se dégrader, selon que le taureau souffre d'une condition préjudiciable à la spermatogénèse, ou qu'il se remette à peine d'une telle condition. Il faut donc interpréter le spermogramme en tenant compte des antécédents du taureau, des résultats de l'examen clinique et, dans certains cas, des traits du sperme relatifs au volume et à la concentration. Certaines anomalies du sperme semblent avoir un fondement génétique, et la connaissance de conditions récessives, ou de prédispositions génétiques, contribuera également à la décision quant aux mesures à prendre en présence de spermogrammes anormaux. Les cas mis en évidence dans cet exposé présentent certains exemples d'interprétation de spermogrammes anormaux.

## Effect of Nutrition and Body Condition on Semen Quality

In early April at a wintering station for 60 bulls in western Canada, bulls were becoming more aggressive

with each other and were breaking down the holding pens. In frustration the manager turned them out to pasture. The bulls were in good body condition (3 out of 5), 2-7 years of age, and included approximately equal numbers of Charolais and Angus. At this location pastures were still in winter dormancy and the dead forage was very short. The April weather conditions were mainly cold (about 17 to 41°F or -8 to +5 °C) and windy with frequent days of snow or rain. There was no feed supplementation for the bulls at pasture. On April 21 we traveled to the wintering station to do breeding soundness evaluations (BSEs). It began to snow as we started semen collection. Semen quality was unsatisfactory in nine of the first 13 bulls, therefore, a decision was made to abandon semen testing until a later date. The manager was advised to take hay bales into the pasture for the bulls. On May 18, we returned to the station and started over with the BSEs. Of the nine bulls that failed earlier, eight now passed. The one bull that failed had a case of 'sperm accumulation'.<sup>5</sup> In total, 52 of 60 (87%) bulls passed their BSE on May 18. The main feature of abnormal spermograms on April 21 was elevated percentages of distal midpiece reflexes, detached heads and proximal droplets, a typical stress picture. It appeared that the reason for poor semen quality at the first BSE attempt was related to weight loss and possibly a lack of shelter from the cold winds and wet conditions.

The adverse effects of stress caused by hunger and poor weather was also recorded at another time. During the month of May 1982, 606 western Canadian range bulls at seven wintering stations had been evaluated for breeding soundness with routine results. However, an unusual snowstorm occurred on May 28 and 29 which halted bull testing temporarily. The storm left about 12 inches (30 cm) of snow and freezing temperatures were reached at night. The bulls were at pasture during and after the storm. On June 03 and 04, 117 bulls at one location were evaluated for breeding soundness. The gross motility of a large number of semen samples was poor. Semen smears were made for later evaluation. The cause for the poor motility was found to be a high incidence of distal midpiece reflexes (Tables 1 and 2). These sperm defects were believed to be caused by stress due to a lack of available grazing caused by the deep snow. The sudden appearance of high levels of distal midpiece reflexes only three to four days after the storm implicated the cauda epididymis as the site of development of the defect. On June 15, six bulls affected by

**Table 1.** Incidence of distal midpiece reflexes (DMRs) in 606 range bulls during May 1982 prior to a snowstorm.

Level of DMRs (%)	15-20	26-50	51+	Total
Affected bulls (n)	40	22	4	66
Affected bulls (%)	6.6	3.6	0.7	10.9

**Table 2.** Incidence of distal midpiece reflexes (DMRs) in 117 range bulls three to four days after the snowstorm.

Level of DMRs (%)	15-20	26-50	51+	Total
Affected bulls (n)	13	25	15	53
Affected bulls (%)	11.1	21.4	12.8	45.3

distal reflexes after the storm were re-evaluated. Their semen then had good gross motility and the incidence of distal midpiece reflexes had decreased greatly.

The adverse effects of poor pasture on testis function and the need for protein supplementation was reported for crossbred Brahman bulls in western Australia. Protein supplementation resulted in maintenance of scrotal circumference, whereas, scrotal circumference decreased in non-supplemented bulls. Weights of testes, epididymides, and vesicular glands were greater in supplemented bulls.<sup>25</sup>

In another study, the percentage of bulls with satisfactory semen quality declined in August and September compared to the late spring and early summer months. It was suggested that seasonal factors, such as the decline in photoperiod and a decline in the nutritional quantity and quality of pasture, might have been involved in the decline of semen quality of physically sound bulls.<sup>8</sup>

There is also an effect of body condition score on semen quality. When only physically normal bulls were considered, significantly fewer bulls with a BCS of 2/5 had satisfactory semen quality than bulls with a BCS of 3/5.<sup>8</sup> The effect of wintering conditions on body condition and semen quality was also seen in two neighboring community pastures (Barth, unpublished). At one pasture (Wreford) bulls were bedded in sheds open on the south, and kept in pens with a 20% porosity fence for a windbreak. Bulls were fed hay in round bale feeders and supplemented with rolled barley, minerals, and vitamins. At a second pasture (McCraney) about 50 miles away, bulls were wintered on open prairie without bedding and willow bunches served as wind breaks. Hay from the same source as used at Wreford was fed on hilltops to encourage bulls not to lie on feed, but retreat to the willows in the lowland for wind protection. Water was supplied at both locations. The difference in body condition and semen quality between locations is shown

in Table 3. Semen collection for both groups of bulls occurred a few days apart during the first few days of May. Bulls were of similar breeds and ages ranged from three to eight years at the time of semen testing.

Nutritional signals exert powerful effects on the reproductive system of mature male ruminants. Peripheral metabolic hormones including leptin, insulin, growth hormone, and insulin-like growth factors, signal nutritional status to the GnRH neurons in the hypothalamus which are the primary regulator of fertility.<sup>10,22</sup> These metabolic hormones may also have direct effects on the testes. Receptors for these hormones have been detected in various cell types in the testes.<sup>1,9,15</sup> These hormones affected Leydig cell steroidogenesis in vitro and may be involved in testicular cell multiplication and differentiation.<sup>11,21,33</sup>

### Decision-Deferred Yearling Bulls

A yearling Red Angus bull with a January 25 birth date was purchased for \$6000 at a sale on March 14 and placed with 14 females on March 26. On April 30 the bull was presented for a semen evaluation because all females that were observed to be bred greater than 21 days ago (6 of 6) had returned to heat. The following spermograms were obtained.

	March 07	April 30
Heads	15%	18%
Midpieces	14%	24%
Coiled principal piece	-	4%
Detached normal heads	-	8%
Detached abnormal heads	-	4%
Proximal droplets	-	15%
Normal sperm	71%	27%
Percent staining alive	70%	52%

The scrotal circumference on March 07 was 37 cm. Referring to the March 07 spermogram, what are the chances that a semen sample would have 29% of head and midpiece defects, but no other defects such as detached heads and proximal droplets? The March 07 spermogram looks suspicious as a case of a pubertal yearling bull deliberately passed with the hope that semen quality would improve with a little more time. Producers place a lot of pressure on veterinarians to pass yearling bulls for sales because decision-deferred bulls sell poorly. What are the chances that bulls presumed to be immature will have improved semen quality within the next few months? Table 4 shows some data from a government bull rearing station over a four year period. At this station bulls are purchased in the fall at six to eight months of age and reared for use in community pastures the fol-

**Table 3.** Effect of different wintering conditions on semen quality in two groups of bulls.

Location	n	BCS	Satisfactory semen	Non-significant frostbite	*Significant frostbite
Wreford	41	3-3.5	33/41 (80.5%)	3/41 (7.3%)	1/41 (2.4%)
McCraney	33	2.5-3	23/33 (69.7%)	5/33 (15.2%)	1/33 (3.0%)

\*When inflammation was still present at the site of scabs, frostbite lesions were considered to be significant

**Table 4.** Results of breeding soundness evaluations of yearling bulls tested at 13 to 15 months of age (April 15-21) over four years from 2008-2011. Decision-deferred bulls were retested at 15 to 17 months of age (June 11-15). No retests were done in 2008. Unsatisfactory bulls were culled for such things as small testes, epididymitis, and locomotory abnormalities.

Age	n	Satisfactory (%)	Decision-deferred	Unsatisfactory
13-14 mo	524	413 (78.8)	85 (16.2)	26 (5.0)
15-16 mo	71	25 (35.2)	-	46 (64.8)

lowing spring and summer. About two-thirds of the bulls were Angus and one-third were Charolais.

We expect about 15% of physically normal mature bulls to have unsatisfactory semen quality in prebreeding season semen tests. If the reason for a decision-deferred classification in physically normal pubertal bulls is inadequate semen quality, one might expect over 80% of them to have satisfactory semen quality at the age of maturity (16 months of age). However, the data in this study indicated that a much lower percentage (35%) of decision-deferred bulls is likely to have satisfactory semen at maturity. Therefore, it would be very unwise for veterinarians to hope that most pubertal bulls will have good semen quality after an additional month or two for maturation.

### **Testicular Degeneration**

A two-year-old Horned Hereford bull was evaluated for sale on March 02. The BSE certificate indicated he had a SC of 35 cm and satisfactory semen quality. He was purchased by a Hutterite colony on March 10 and on April 30 he was re-evaluated. At that time he had a BCS of 4/5, soft testes and a SC of 30 cm. The semen sample had poor concentration, poor motility, and only 35% of spermatozoa stained alive. The spermiogram obtained was as follows:

- 37% head defects (primarily pyriform or microcephalic)
- 15% midpiece defects (fractured and folded)
- 18% detached heads
- 9% proximal droplets

- 10% teratoid forms
- 11% normal sperm

A diagnosis of testicular degeneration was given with a poor prognosis for recovery in time for the breeding season. The bull was returned to the original producer who pastured the bull with an unknown number of heifers for the summer. He then returned the bull to the Hutterites in late September claiming that the bull had impregnated four of the heifers. The bull was immediately brought to the veterinary college for evaluation. The bull was properly identified as the same bull which had suffered from testicular degeneration earlier in the year. The bull now had a BCS of 3/5, he had firm testes, a scrotal circumference of 34 cm, and a satisfactory semen sample. We evaluated the bull on three successive springs and he continued to have satisfactory breeding soundness classifications.

In a somewhat similar case, a Charolais bull was purchased for \$20,000 in November at 18 months of age. He had a satisfactory BSE certificate and his SC was 38 cm. In the following March he was taken to an artificial insemination (AI) center for semen cryopreservation, but due to poor semen quality the AI center sent the bull home. The bull was brought to our veterinary college on March 28 for evaluation. At that time, the bull had a body condition score of 4/5 and a scrotal circumference of 34 cm. Due to the way Continental European breeds deposit fat between muscle groups rather than subcutaneously like the British breeds, the bull appeared to be very muscular. His testes felt soft and the neck of scrotum felt spongy due to fat deposition in the scrotum. There were no other abnormal physical findings. The

semen sample had poor concentration, poor motility, and only 28% of spermatozoa stained alive. The spermogram obtained was as follows:

**Spermogram March 28**

- 36% malformed heads (mainly small pyriforms)
- 17% midpiece defects (mainly swollen mitochondria and bent or coiled midpieces)
- 5% abnormal detached heads
- 41% teratoids
- 21% proximal droplets
- 9% knobbed acrosomes
- 1% normal
- 28% stained alive

The diagnosis was testicular degeneration most likely due to abnormal scrotal thermoregulation because of obesity. Based on past experience, we predicted the bull had a 70% chance of recovery to normal semen quality by late summer if he could lose the excess weight. Over the summer the owner allowed the bull to roam at pasture with the rest of the herd, including other breeding bulls.

**Spermogram September 01**

- 2% pyriform heads
- 2% midpiece defects
- 2% detached heads
- 1% proximal droplets
- 93% normal
- 88% stain alive

The bull's testes were now firm and the SC had increased to 38 cm. Unfortunately a breeding season was lost, but the owner was pleased that this valuable bull was saved. Early in the following year the owner started to use the bull for breeding, but the cows he bred were returning to estrus. At first the owner thought perhaps it was due to cows being to early post partum for good fertility, but as time went on it became apparent that there was once again a problem with the bull. The bull was re-evaluated and it was found that his scrotal circumference had decreased again to 36 cm and semen quality was poor (55% normal sperm). At this point the owner made a claim on his insurance policy and received a payout. The cause for the second decline in semen quality could not be determined.

In a community pasture study of 61 obese two-year-old Angus, Hereford, and Charolais bulls (body condition score of 4/5), only 34% had satisfactory semen quality in May. By late September these bulls had a body condition score of 3/5 and 69% had satisfactory semen quality.<sup>7</sup> The unsatisfactory bulls were then culled. The retained bulls still had satisfactory semen quality the following spring.

### **Azoospermia**

In March of 1996, a 14-month-old Charolais bull with a scrotal circumference of 36 cm received a satisfactory BSE classification; however, an evaluation of

sperm morphology was not reported. He was placed with 14 cows from May 15 to July 31 and was seen to mate normally. None of the cows were pregnant at autumn pregnancy testing. He was retested in October 1996 by the same veterinarian and was classified as 'decision-deferred' due to inability to obtain a semen sample. On December 05, 1996, the bull was brought to our veterinary college for a breeding soundness examination.

The bull was physically sound, his testicles felt firm, and the scrotal circumference measurement was 37 cm. A 2-mL semen sample was collected and it had an opaque, white appearance. Wet mounts and stained semen smears revealed that there were virtually no normal sperm. This finding might lead one to suspect severe testis degeneration; however, in this case the scrotum and testes felt normal. The semen consisted almost entirely of severely malformed sperm with very small, shriveled nuclei, often with the tail coiled around it (teratoids). This may be called a case of azoospermia or severe oligospermia. Since the bull received a satisfactory evaluation in the spring, it would appear that the bull produced normal sperm and then deteriorated becoming azoospermic. On the other hand, knowing that particular practitioner, it's likely that morphology was not evaluated.

Over the past 25 years, we have seen similar spermograms in 18 bulls; four Charolais, nine Aberdeen Angus, two Hereford, one Simmental, one Holstein, and one Braford. We've also seen similar spermograms in a ram and a dog. Two of the Charolais bulls were maintained for more than a year from the time of diagnosis without improvement in semen quality. The other bulls were known to have azoospermia for at least three months. In all cases, there were no clinically detectable physical abnormalities and the testes were of normal size and consistency. In all cases, semen samples were white, but had few, or no normal spermatozoa.

One of the Charolais bulls was maintained and studied over a period of 1.5 years. A karyotype was done and it was normal. Serial blood samples taken every 30 minutes over an eight-hour period showed normal levels of LH and testosterone. Two types of treatment were tried based on a hypothesis that the cause of azoospermia might be a deficiency of gonadotrophin, or a lack of receptors to gonadotrophins in target cells. In the first summer, experimental GnRH implants were placed subcutaneously. In the second summer, 2 grams of depot testosterone was injected subcutaneously every two weeks for three treatments. Neither of the treatments resulted in improvement in the spermogram. Testis histology was done on two bulls after slaughter and in both cases, spermatogenesis progressed up to the spermatid stage and no further.

Three of the azoospermic Charolais bulls were closely related. Two were half-brothers and the sire of



those bulls was the grandsire of the third bull that had azoospermia. All three bulls also had a close relative on the female side of the pedigree. This suggests a hereditary cause for azoospermia/oligospermia.

Recent reports have identified the existence of spermatogenesis loci on the long arm of chromosome Y in men. It appears each locus is active during a different phase of male germ cell development.<sup>32</sup> Cytogenetically detectable deletions of this region are associated with azoospermia in men. Thus, one or more “deleted in azoospermia” (DAZ) genes may be implicated in the pathogenesis of azoospermia or severe oligospermia in men. Others have isolated a homologue of the human Y-linked DAZ gene from mice. The gene in mice is autosomal, located on chromosome 17 and apparently has a single copy.<sup>12</sup>

### Accumulation of Senescent Spermatozoa

A three-year-old Horned Hereford bull that appeared fertile in a previous multi-sire breeding situation was presented to a veterinarian for evaluation. The veterinarian collected a good semen sample, but apparently did not examine it microscopically. The bull was placed by himself with 20 cows on May 1 and was observed to have good serving capacity. Less than one month later the owner became concerned when he saw that the first six cows the bull bred had returned to estrus. At this point, he took the bull to a second veterinarian who could not find any abnormalities to explain the apparent infertility, but the owner removed the bull from breeding. The bull was brought to our veterinary college about three weeks after his last semen test. The testes and epididymides felt normal and his scrotal circumference was 37 cm. An 8-mL semen sample obtained was very concentrated, but had no gross wave motion. Four additional samples, collected in succession immediately, were similar. Stained semen smears revealed that in all samples most sperm were dead and there was a high percentage of detached heads. It appeared that the infertility observed was due to an accumulation of senescent sperm.

Bulls that produce large volumes of concentrated semen containing mostly dead sperm are encountered quite commonly. In one study of 1331 range bulls, 1.1% of bulls were affected.<sup>7</sup> In the normal male, sperm are continuously moved out of the reproductive tract by peristalsis ensuring a steady supply of new sperm.

The characteristics of a sperm accumulation are:

1. the ability to collect  $\geq 20$  mL of concentrated semen at one time
2. usually most sperm are morphologically normal
3. nearly all sperm are dead; the percentage of detached heads may be elevated
4. depletion-electroejaculation every second day until the semen density is dilute for a about one week (three collection days) results in a return to normal percentages of live normal sperm

Two controlled breeding trials were done using feedlot heifers to determine the effect of this condition on first service pregnancy rates. The bulls used were a two-year-old Charolais bull and a four-year-old Polled Hereford bull. The spermograms of these bulls before, during, and after the trials are shown in Tables 5 and 6.

Breeding observations for at least one-hour periods were made in mornings and evenings and were assisted by tail painting the heifers. Pregnancy was determined by ultrasonography 27 days after the end of the breeding period. From these observations it was possible to determine the order in which heifers were bred and whether pregnancy occurred. Pregnancy rates were compared to control bulls and are shown in Tables 7 and 8.

These results indicate that it takes about a week of breeding for bulls to rid themselves of senescent sperm and then pregnancies begin to occur a normal rate. The results also help to explain the history of the case presented at the beginning of this section. Additional experiments with four other bulls showed that accumulation occurs in the ampullae and cauda epididymis.

After depletion-electroejaculation, re-accumulation occurs within about one month. Yearling bulls as well as older bulls may accumulate senescent sperm.<sup>5</sup>

**Table 5.** Spermograms of a two-year-old Charolais bull before, during, and after a 21-day breeding period (day 0 to 21).

Date	Volume mL	% motile	% alive	Head	Sperm defects			Normal
					Mid piece	Detached heads	Other	
04 May, day -9	10	5	9	5	2	52	2	39
13 May, day 0	10	5	10	3	3	46	1	47
24 May, day 11	2	60	67	5	9	4	2	76
03 June, day 21	2	90	90	3	2	4	3	91

**Table 6.** Spermograms of four-year-old Polled Hereford bull before, during, and after a 21-day breeding period (day 0 to 21).

Date	Volume mL	% motile	% alive	Head	Sperm defects			Normal
					Mid piece	Detached heads	Other	
13 May, day -18	10	5	5	3	12	45	0	40
28 May, day -3	2	15	20	2	13	13	2	70
10 June, day 10	2	45	48	4	5	5	3	83
23 June, day 23	2	85	80	2	1	2	1	94
22 July, day 52	10	5	12	2	39	12	0	46

**Table 7.** Proportion of females becoming pregnant in a three-week period when bred by a Charolais bull with sperm accumulation vs a control bull.

	Charolais	Control bull
Week 1	2/12 = 16.7%	5/8 = 62.5%
Week 2	6/8 = 75%	6/6 = 100%
Week 3	9/10 = 90%	9/12 = 75%
21 day pregnancy rate	17/30 = 56.7%	20/26 = 76.9%

**Table 8.** Proportion of females becoming pregnant in a three-week period when bred by a Polled Hereford bull with sperm accumulation vs a control bull.

	Polled Hereford	Control bull
Week 1	0/6 = 0%	3/5 = 60%
Week 2	6/8 = 75%	16/20 = 80%
Week 3	9/10 = 90%	2/4 = 50%
21 day pregnancy rate	15/24 = 62.5%	21/29 = 72.4%

### Knobbed Acrosomes

A Charolais bull was examined for breeding soundness in the spring at 16 months of age. Although his scrotal circumference was only 30 cm, he was classified as satisfactory. The bull was placed with 36 purebred cows and was observed to have good serving capacity. After a breeding season of 105 days, the cows were pregnancy tested in late November and 53% of cows were found to be open. In December, the bull was re-evaluated. His scrotal circumference was 32 cm, a semen sample had good concentration and good gross motility, but 76% of his sperm had flattened or indented acrosomes. There were few other sperm abnormalities.

In a different unrelated case, a Horned Hereford bull was rented from a neighbor who had used the bull

in two previous years in multiple sire breeding groups. Since calf crops were normal, it was assumed that the bull was fertile. The bull was placed alone with 50 cows and was observed to be a good breeder; however, after a 90-day breeding season, only eight cows (16%) were pregnant. The bull was evaluated in November after the pregnancy testing; he had a scrotal circumference of 34 cm, the semen sample had good concentration and good motility, but 77% of his sperm had knobbed acrosomes and only 16% of sperm were normal. In this case the knobbed defect differed in appearance from that of the Charolais bull above. Instead of a flattened or indented apex, the acrosomal apex of sperm from the Hereford bull had a beaded appearance.

Many cases of the knobbed sperm defect have been reported in bulls.<sup>4,14,18,19</sup> There appear to be two main forms of the knobbed defect, the beaded form and the indented form. In the beaded form, very high percentages of sperm are affected and bulls often are sterile. In the indented form, percentages of affected sperm may vary from <10 to 90% and fertility varies accordingly. In the beaded form most sperm are not indented at the apex, whereas, in the indented form most sperm are flat or indented at the apex and usually only small numbers have a bead. In the beaded form, published photomicrographs and electron micrographs of spermatozoa from infertile Friesian bulls showed that the defect was characterized by an enlargement of the apical ridge which became folded back on the apex of the sperm head giving a beaded appearance. Often the swollen apical ridge contained vesicles with inclusions. The beaded form was reported to be inherited via a simple recessive gene,<sup>14,18</sup> and although the indented form also appears inherited as a recessive characteristic most likely there are other modifying genes involved.<sup>4</sup>

Four bulls with the indented form of knobbed acrosomes and two control bulls were used in single sire natural mating.<sup>23</sup> The four affected bulls each consistently produced good quality semen except that 65-85% of sperm had knobbed acrosomes. Pregnancy rates after

21 days of breeding ranged from 61-85% for the bulls with indented acrosomes and 52 to 65% for the control bulls. Frozen semen from two of these bulls producing 61% and 85% knobbed sperm and from a normal control bull was used to artificially inseminate 98 heifers (32-33 per bull). The number of sperm per insemination dose was 11 million and the percent motile sperm per dose was similar for each bull used. Pregnancy rates for the bulls with knobbed sperm were 48.5 and 50%, and 46.3% for the control bull. Therefore, with single-sire natural service and artificial insemination it appears that fertility was unaffected by a high percentage of the indented acrosome defect. One explanation for the unexpected fertility levels was that there were sufficient numbers of sperm with normal acrosomes present in both the natural service and artificial insemination experiments to result in normal fertility.

Two Charolais bulls producing high percentages of indented sperm (KM and CH3) were placed with normal control bulls in competitive mating situations at pasture.<sup>4,23</sup> A competitive mating was considered to be one in which more than one bull bred a cow at the same estrus period. Breeding was observed twice daily and chin ball markers were used to confirm normal breeding activity. Calves produced were identified phenotype and blood-group typing.

In the first competitive mating trial KM, producing about 90% indented sperm, and two normal bulls (Simmental and Brown Swiss) were placed with 49 cows for 52 days. KM produced only two calves winning 8.7% of 23 competitions, compared to 50-55% of competitions won by the control bulls.

In the second competitive mating trial CH3 was placed with four control bulls (two Hereford and two Charolais) and 118 cows for 63 days. CH3 produced only three calves winning 13.6% of 22 known competitions compared to 41-50% of competitions won by the control bulls. It appears that bulls with a high percentage of the indented sperm will leave very few calves when placed at pasture with normal bulls.

In vitro fertilization experiments indicated that sperm with indented and flattened acrosomes generally did not attach to ova and the few that did were unable to penetrate the zona pellucida.<sup>31</sup> The proportion of normal sperm achieved in the oviductal sperm population after a competitive mating would be much lower for the bulls with knobbed sperm than from normal bulls. Thus the chances of fertilizing ova favored the control bulls during competitive mating.

### **The Diadem Defect**

A two-year-old Charolais bull was presented for evaluation prior to a spring breeding season and was found to have satisfactory breeding potential. He was

placed by himself with 30 females and observed to have good service capacity by the owner. However after approximately one month, a second bull was added since many of the cows that were observed to be bred were returning to heat. Based on the time of arrival of calves the following spring, the owner believed that very few calves were born to the first bull.

The bull was presented for evaluation prior to the second breeding season. Again the bull was found to have satisfactory breeding potential. Because of the previous bad experience with the bull, the owner paid close attention to breeding activity and watched for returns to estrus. Five of seven of the first cows to be bred returned to estrus and again a second bull was introduced to the herd. Once again, based on the time of arrival of calves the following spring, the owner believed that very few calves were born to this bull.

Out of curiosity, the producer again presented the bull for evaluation prior to the third breeding season. The examining veterinarian found the semen to be of excellent quality, but forwarded semen samples to our veterinary college for a second opinion. We examined semen smears prepared with morphology stain as well as with the Feulgen stain for DNA.

The following spermiograms were obtained: (each category equals percent of all sperm; some sperm had more than one defect):

#### **Eosin Nigrosin stain:**

- 33% head defects (30% vacuolated nuclei)
- 5% midpiece defects
- 3% detached heads
- 2% proximal droplets.
- 3% knobbed sperm
- 62% normal sperm

#### **Feulgen Stain for DNA:**

- 62% vacuolated nuclei
  - 21% large single apical vacuoles
  - 27% diadems
  - 7% multiple vacuoles
  - 7% large confluent vacuoles
- 2% micro/macro/pyriform
- 4% knobbed acrosomes
- 35% normal sperm.

It appears that the bull's fertility problem was related to sperm nuclear vacuoles. This case is one of many similar cases that we've encountered in bulls with infertility. Vacuoles may be difficult to see on eosin nigrosin smears, but are clearly seen with the DNA stain. This case demonstrates the importance of making good semen smears and the necessity for using microscopes of adequate resolving power.

Sperm nuclear vacuoles, also called craters or pouches, are mainly seen as the diadem defect, single

vacuoles, or large confluent vacuoles. With sperm morphology stains the vacuoles appear as dark spots and must be differentiated from stain crystals or white spots from debris. With differential interference microscopy, vacuoles resemble craters. The archaic nomenclature of nuclear pouches (probably the most accurate description) referred to vacuole formation by invagination of the inner nuclear membrane of spermatids in the early stages of chromatin condensation.

Severe infertility in a Simmental bull used in natural service, artificial insemination, and embryo transfer was described by Miller *et al.*<sup>24</sup> A son of this Simmental bull also produced high numbers of spermatozoa with the diadem defect. This suggests the possibility of heritability for the diadem defect; however, there is clinical and experimental evidence that stressful conditions may also induce the defect in otherwise fertile bulls.<sup>6</sup> Vacuolated sperm were shown to be transported normally to the oviduct,<sup>28</sup> and there is evidence from *in vitro* fertilization that sperm with the diadem defect are able to penetrate ova, but are incompatible with embryonic development.<sup>27</sup>

### Proximal Droplets

Anecdotal information was obtained from a farmer who used frozen semen from a yearling Charolais bull for artificial insemination. Only one of 10 inseminated cows became pregnant, and in addition, when this semen was used to inseminate two superovulated cows, both cows produced 14 unfertilized ova. However, greater than 70% first service pregnancy rates were obtained by this farmer in his own herd and in a neighbour's herd using frozen semen from three other bulls. The semen in question had 24 million sperm per dose, and all traits were normal except that 38% of sperm had a proximal cytoplasmic droplet. This seemed to indicate that the proximal droplets were associated with infertility.

Semen samples were collected from a two-year-old Red Angus bull in southern Saskatchewan on January 05, February 20, and April 11. In all samples there were only few sperm abnormalities except for proximal cytoplasmic droplets affecting 62 to 71% of sperm. Sperm were examined with transmission electron microscopy to confirm that they were structurally normal except for the presence of a cytoplasmic droplet in the sperm neck region. The bull was obtained for a breeding trial to be conducted at the university farm.

On May 15, the bull was placed with 29 females for 21 days. Semen samples that were collected before, during, and immediately after the breeding trial continued to have 63 to 71% of sperm with proximal droplets. Two other normal bulls were placed with similar groups of females at the same time. Breeding was observed twice daily and all bulls were found to be very active breeders. Pregnancy was determined by ultrasound one month

after the end of the trial with the following results:

Charolais control bull	70.4% pregnant
Hereford control bull	81.5% pregnant
Red Angus bull with proximal droplets	17.2% pregnant

These results indicate that proximal droplets were associated with infertility. Others have also shown that high percentages of proximal droplets are related to poor non-return rates on AI and pregnancy rates in natural service.<sup>26,29</sup> IVF studies have shown that sperm with proximal droplets fail to bind to ova. Furthermore, even normal sperm without droplets that are associated with high numbers of proximal-droplet-sperm have an impaired ability to bind to ova.<sup>30</sup>

An elevation of proximal droplets is often seen in association with a disturbance of testicular function due to stress or heat in adult bulls, and as a normal part of maturation in pubertal bulls. Prior to the experiment reported above, six other mature bulls with very high percentages of proximal droplets were purchased with the intent of using them in breeding trials. In each of the six cases, recovery to normal spermiograms with very few proximal droplets occurred before breeding trials could be set up. This indicates that often bulls recover quickly from proximal droplets. In experiments involving scrotal insulation (heat) or treatment with dexamethasone (stress), there was a sudden rise of proximal droplets, detached heads, and midpiece reflexes within 5-10 days.<sup>6</sup> Therefore, normally developed sperm may retain proximal droplets due to epididymal malfunction.

The Red Angus bull described above developed a unilateral cauda epididymitis approximately five weeks after the end of the breeding trial. This raises the possibility that a mild undetected epididymitis was the basis of epididymal malfunction leading to the proximal droplets. Although the swelling disappeared within a month, high percentages of spermatozoa were affected by proximal droplets until he was sold for slaughter in June of the following year, 18 months after the initial diagnosis.

### Abnormal DNA Condensation

The DNA in mammalian sperm nuclei is bound to basic nuclear proteins forming chromatin. During nuclear condensation in spermiogenesis, large, lysine-rich somatic proteins called histones are removed from the DNA and are replaced by small, arginine-rich protamines. Protamines form disulfide cross-links between each other on strands of DNA and by doing so electrostatic repulsion between neighboring DNA segments is eliminated and the DNA-protamine complex becomes tightly packed.<sup>2</sup> Incomplete removal of histones would result in a less stable nucleus and increased suscepti-



bility to denaturation of chromatin. Also, incomplete removal of histones (abnormal chromatin condensation) has been linked to infertility of affected sperm.<sup>3,20</sup>

The sperm chromatin structure assay determines the resistance of sperm nuclei to denaturation (the splitting of the double stranded DNA helix into single strands) by heat or acid treatment. Acridine orange staining results in green fluorescence for double stranded DNA and red fluorescence in single stranded DNA. Flow cytometry is used to determine the ratio of green to red staining sperm nuclei and as such, the proportion of sperm nuclei with abnormal structural stability of DNA.<sup>16</sup>

In the Feulgen staining method,<sup>17</sup> aldehyde groups in the deoxyribose of the DNA are unmasked by acid hydrolysis and are then exposed to Schiff's reagent (basic fuchsin in sodium metabisulfite). Normal sperm nuclei stain homogeneous purplish-red, especially when examined with phase contrast microscopy, whereas, abnormal chromatin condensation appears as coarse to fine clumping of nuclear material and local or generalized reduced stainability of the nucleus. The results obtained by the Feulgen method have been shown to be significantly correlated to those obtained with the sperm chromatin structure assay.<sup>13</sup>

Each year approximately 50 cases of bull infertility related to semen quality are reported to the our veterinary college by veterinarians from western Canada. Usually these bulls were evaluated for breeding soundness prior to use in spring breeding programs, but producers became concerned about high numbers of females returning to estrus after observed matings. Of these, one to three cases each year have abnormal chromatin condensation in a high percentage of sperm nuclei as determined with the Feulgen staining method.

An example of a case of infertility due to abnormal chromatin condensation follows. A two-year-old Simmental bull produced semen that had very good density and motility, and sperm morphology on eosin nigrosin stains was >80% normal. The bull was joined with 17 cows for six weeks and all cows returned to heat. Upon joining the cows with a second bull, normal first service conception rates occurred as confirmed by breeding observations and by pregnancy testing in the fall. Feulgen staining revealed that 100% of sperm nuclei of the affected bull had coarsely clumped, chromatin. A year later, the same bull, still producing a high percentage of sperm with a fine grainy chromatin, was used in a controlled natural service breeding trial. In the trial, the bull competed with a Charolais and a Brown Swiss bull in breeding 66 females over 60 days. Twice daily breeding observations, use of chin-ball markers, calf phenotype and blood typing were used to determine the sire of calves born the following spring. Only one calf appeared to possibly be sired by the Simmental bull.

Later a project was done using six bulls that had abnormal chromatin in 2% to 75% of their sperm.<sup>13</sup> The results indicated increasing infertility with increasing levels of abnormal DNA condensation. We were able to show in one bull that scrotal insulation exacerbated the problem; however, clearly some bulls produce high percentages of the abnormality without any known cause for abnormal testis function.

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