The sequential appearance of sperm abnormalities after scrotal insulation or dexamethasone treatment in bulls

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Abstract

Scrotal insulation and dexamethasone treatment were used as a model to compare the effect of testicular heating and stress on spermatogenesis. Insulation was applied to the scrotum of eight bulls (insulated) for a period of four days, eight bulls were treated daily for seven days with 20 mg dexamethasone injected intramuscularly, and four bulls were untreated controls. Semen from four bulls in each group was collected and evaluated over a six-week period after treatment. Blood samples for testosterone analysis were taken hourly for eight hours at the beginning and the end of the six-week period from the control bulls and before and after treatment from the four insulated and four dexamethasone-treated bulls that were not used for semen collection. At the end of the last blood sampling period, the four bulls in each group were castrated for the collection of testicular tissue for the determination of testosterone concentrations.

Basal, peak episodic, and mean serum testosterone concentrations among control bulls, pre and postinsulated bulls, and pretreatment samples of dexamethasone-treated bulls were not different ($p>0.05$); however, bulls that had received dexamethasone treatments had significantly lower basal, peak episodic, and mean testosterone concentrations ($p<0.05$). Tissue concentrations of testosterone in control, insulated, and dexamethasone-treated bulls were not significantly different but tended to be lower in dexamethasone-treated bulls ($p>0.13$).

The spermiograms of the control bulls varied insignificantly over the six-week sampling period; however, there was a marked increase in sperm defects in insulated and dexamethasone-treated bulls. The types of sperm defects and the temporal relationships of rises and declines of sperm defects were quite similar for both treatments. All bulls recovered to approximately pretreatment levels of sperm defects by six weeks after the initiation of treatment. Results indicate that two of the most common types of insults to spermatogenesis in bulls, heat and stress, result in similar spermiograms.

Résumé

Évaluation séquentielle des anomalies de la semence chez le bovin suite à un traitement à la dexaméthazone ou après isolation du scrotum

L'isolation du scrotum et un traitement à la dexaméthazone ont été choisis comme modèles pour étudier l'effet de la température testiculaire et du stress sur la spermatogénèse. L'isolateur a été appliqué sur le scrotum de huit bovins pour une période de quatre jours. Le traitement à la dexaméthazone a été administré à un autre groupe de huit bovins à raison de 20 mg DIE pour sept jours par voie intramusculaire. Quatre autres animaux ont servi de groupe témoin. Dans chacun des groupes, la semence de quatre animaux a été récoltée et évaluée pour une période de six semaines posttraitement. Des échantillons sanguins ont été prélevés pour doser la testostérone à toutes les heures pour une période de huit heures au début et au terme de l'étude pour le groupe témoin, alors que les échantillons sanguins ont été prélevés avant et après traitement sur les quatre animaux qui n'ont pas été utilisés pour la récolte de la semence dans les groupes traités par isolation ou dexaméthazone. Après l'obtention des échantillons sanguins, les quatre bovins de chaque groupe ont été castrés pour analyser le taux de testostérone tissulaire.

Les résultats démontrent qu'il n'y avait pas de différence significative ($p > 0.05$) pour le taux de base, le pic épisodique et la concentration sérique moyenne de la testostérone entre le groupe témoin, le groupe pré- et post-isolation et le groupe prétraitement à la dexaméthazone. Toutefois, le groupe posttraitement à la dexaméthazone a présenté des valeurs plus basses de façon significative ($p > 0.05$) pour le taux de base, le pic épisodique et la concentration sérique moyenne de la testostérone. La concentration tissulaire en testostérone n'était pas différente pour aucun des groupes, mais elle avait tendance à être plus basse pour le groupe traité à la dexaméthazone ($p > 0.13$). Alors que les spermogrammes provenant du groupe témoin ont varié de façon non significative durant la période d'observation, les spermogrammes des groupes traités par isolation ou par dexaméthazone ont montré une augmentation marquée d'anomalies du sperme. Le type d'anomalies et l'occurrence temporelle de l'augmentation et du déclin des anomalies du sperme étaient semblables entre les groupes traités. Tous les bovins ont recouvré approximativement leur fonction de spermatogénèse prétraitement après six semaines.

Les résultats indiquent que deux des plus fréquents facteurs néfastes à la spermatogénèse, la chaleur et le stress, présentent des spermatogrammes semblables.

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Introduction

The testis is very sensitive to a variety of adverse influences including heat, stress, toxicity, hypoxia, radiation, and genetic abnormalities (1). The most com-

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mon of these affecting western Canadian range bulls would seem to be heat and stress.

Fever, climatic heat, fat deposition in the neck of the scrotum, scrotal frostbite, and trauma may all interfere with thermoregulation of the testicles. The internal scrotal temperature of bulls is normally maintained in a range of 33°C–34.5°C, and even small increases in testicular temperature, though still below core body temperature, cause a major disturbance in spermatogenesis (2). It appears that the testicles of scrotal mammals normally operate on the brink of hypoxia (3,4). In the bull, the testicular artery, coiled within the vascular cone and surrounded by the venous pampiniform plexus, is 3.5–4.5 m in length (5); the coiling produces a countercurrent heat exchange mechanism and also results in a slowing of blood flow, so that the pulse is almost obliterated in the arterial supply to the testicular tissue (6). Increases in local temperature result in an increased metabolic rate and a corresponding increase in oxygen demand; however, blood flow does not increase and thus the testis becomes hypoxic. Interestingly, birds and mammals with intra-abdominal testicles have no problem with testicular hypoxia, since the blood supply comes directly off the aorta without arterial coiling, which results in normal blood perfusion and oxygenation even at body temperature (4,7).

Illness, starvation, severe and prolonged climatic cold, transportation, and environmental changes (e.g., show circuit stress) and pain (e.g., sole abscesses, laminitis, arthritis, dehorning) are common stressful conditions for bulls. Stress most likely interferes with spermatogenesis through an endocrine mechanism. Very high local levels of testosterone are necessary for normal testicular (8,9) and epididymal function (10). Pituitary luteinizing hormone (LH) stimulates the secretion of testosterone by the Leydig cells (11), and follicle stimulating hormone (FSH) optimizes testosterone concentration in the seminiferous tubules by increasing the production of androgen-binding protein in the Sertoli cells (12). Studies have shown that high cortisol levels profoundly affect LH and testosterone production. In resting bulls, elevated levels of cortisol are associated with basal levels of LH and testosterone (13,14). A stress induced rise in cortisol decreases the secretion of LH and testosterone in bulls (15) and, thus, may interfere with spermatogenesis. The administration of dexamethasone also results in a decrease in plasma LH and testosterone (16). Dexamethasone treatments have been shown to increase the incidence of the crater defect in bovine spermatozoa (17).

Since heat and stress apparently affect testicular function through different mechanisms, it was postulated that they may result in different types of sperm abnormalities. Thus, a bull's spermiogram might indicate the cause of abnormal spermatogenesis. This paper reports the results of an experiment that was designed to compare the effects of scrotal insulation (heat) and dexamethasone treatment (stress) on spermatogenesis.

Materials and methods

Over two consecutive summers, 31, two-year-old, bulls of mixed *Bos taurus* breeds were placed in feedlot pens and allowed to acclimatize to their new environment for at least three weeks before further selection for the experiment. During the acclimation period, semen was collected by electroejaculation at approximately weekly intervals to establish baseline values for semen quality parameters. Bulls that were difficult to handle, had poor semen quality, or had small testicles were eliminated, and 20 bulls that maintained normal semen quality were selected for the experiment and randomly assigned to treatment groups. Nine bulls were used the first summer and 11 bulls the second summer. Bulls were divided evenly among treatments each year; however, in the first year, there was one control bull and, in the second year, there were three control bulls. Overall, insulation was applied to the scrotum of eight bulls for a period of four days, eight bulls were treated with 20 mg dexamethasone (Dexamone "2", rogar/STB, BTI Products Inc., Ajax, Ontario) intramuscularly (IM) daily for seven days, and four bulls were untreated controls. The treatment methods were based on a small pilot study (unpublished observations) and on a report in the literature (17) that indicated that treatments would cause a disturbance of spermatogenesis with the likelihood of recovery in 6–8 wk.

Scrotums were insulated by wrapping them with two layers of flannelette, a 1 cm thickness of glass wool, and two layers of gauze bandage, all covered with a denim bag. The bag was laced closed sufficiently tightly over the neck of the scrotum to prevent the bull from pulling a testicle through the top, but not so tightly as to pinch the neck of the scrotum. Temperatures inside the scrotal bags on the caudal surface of the scrotum were measured on the second day of insulation with a glass rectal thermometer.

Beginning on the first day of insulation or dexamethasone treatment (day 0), semen was collected from four insulated bulls, four dexamethasone-treated bulls, and four untreated control bulls approximately three times a week for the first 25 days and then twice a week until day 42. Semen traits recorded were: volume, concentration (visual estimate), motility (gross wave motion), percent alive (percent of sperm not taking up eosin stain), and a differential count of sperm abnormalities (spermiograms). Semen smears were stained with eosin-nigrosin stain and Feulgen's stain for DNA (18). Sperm defects were listed under the following 22 categories: knobbed acrosomes, pyriform heads, microcephalic sperm, single nuclear vacuoles, multiple nuclear vacuoles, abnormal DNA condensation, rolled heads, nuclear crests, giant heads, detached normal heads, detached abnormal heads, distal midpiece reflexes, Dag-like midpiece defects, mitochondrial sheath disruptions, bent principal pieces, coiled principal pieces, abaxial tails, accessory tails, tail stumps, proximal droplets, distal droplets, and teratoid forms (18). If a sperm cell had more than one defect, each defect was recorded; therefore, each defect category equaled percent of all cells. Three hundred spermatozoa were counted differentially for each type of stain. All sperm defects were enumerated with the eosin-nigrosin stain; however, the Feulgen stain was required to more accurately enumerate nuclear abnormalities. Increases in sperm defects of five percent or more above baseline values were arbitrarily considered to be due to treatment and were called marked increases.

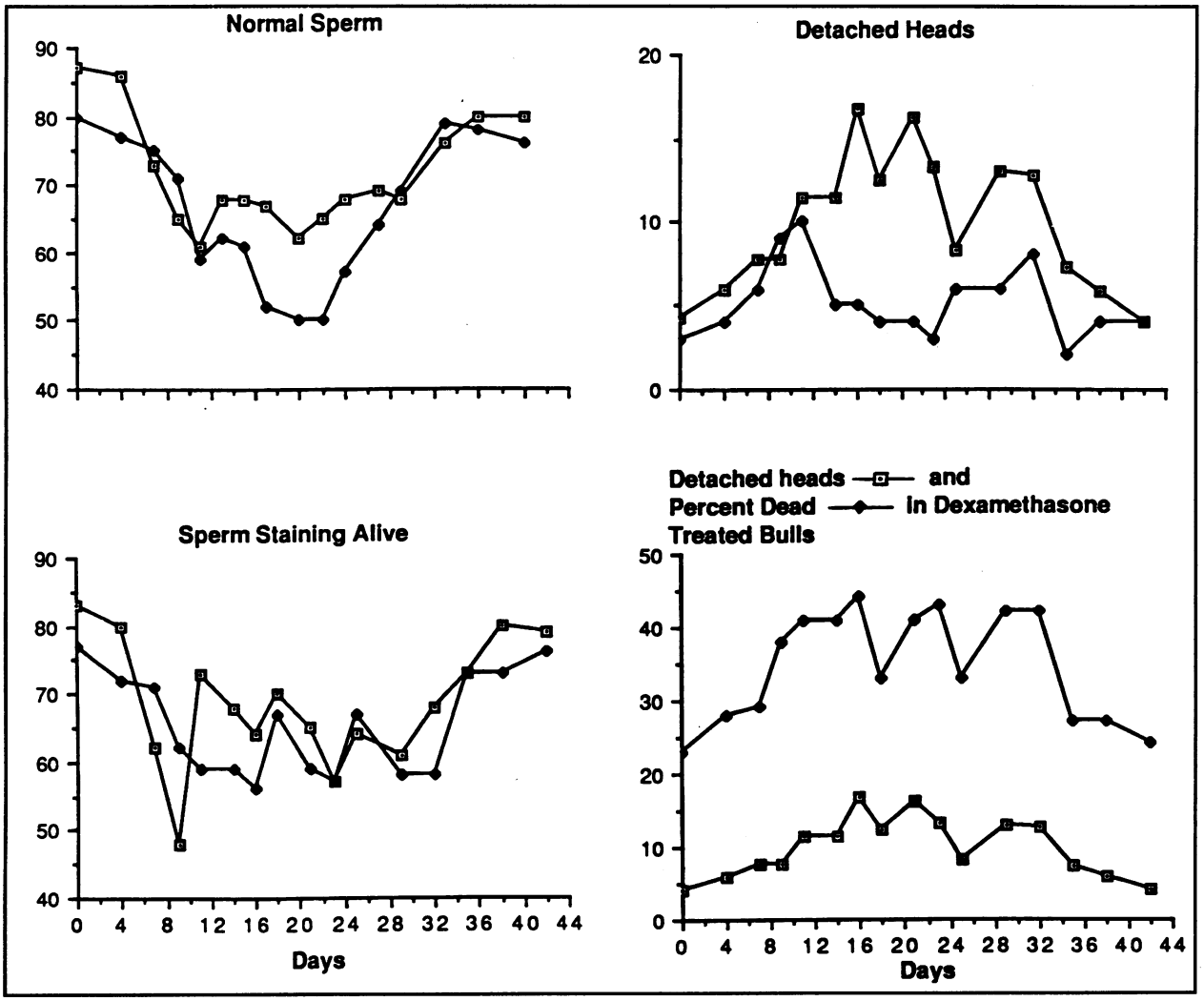

Figure 1. Changes in mean percent of normal sperm, percent "alive" or "dead" on eosin-nigrosin stain, and detached heads in four insulated (——◆——) and four dexamethasone-treated (——□——) bulls. The Y axis is in percent and the X axis is in increments of two days with day 0 being the day of initiation of treatment.

Eight other bulls (four insulated for four days and four dexamethasone-treated for seven days) were used to determine the effect of treatments on blood and testis tissue concentrations of testosterone. In these bulls semen was not collected, but blood samples were taken hourly for eight hours before treatment and during the last day of insulation or dexamethasone treatment. At the end of the last blood sampling period, the bulls were castrated for collection of testicular tissue for determination of the effect of treatments on testis tissue concentrations of testosterone. The control bulls were used for both semen collection and blood and testis tissue sampling. Blood was collected from four control bulls at the beginning of the six-week semen collection period and blood and testicular tissue samples were collected at the end of this period. Testosterone was extracted from 200 μL serum samples with 3 mL of ether. Tissue samples were ground in a predetermined quantity of water, tissue was removed by centrifugation, and testosterone was extracted from the supernatant with ether. Testosterone concentrations were determined by radioimmunoassay (19).

Testosterone is secreted in an episodic manner (13,14) and, therefore, the number and amplitude of hormone episodes, basal levels, and mean eight-hour levels were measured. An episode was defined as an increase of at least five times the baseline value. Baseline values were determined by calculating the mean hormone concentration of the two lowest values for an eight-hour period. Statistical comparisons between groups were done by analysis of variance of mean basal values, mean peak value of episodes, and the mean eight-hour testosterone levels (20). Bulls that received dexamethasone had no episodes of testosterone secretion, but the highest value over the eight-hour bleeding periods was used to compare amplitude of hormone secretion between groups.

Mean tissue testosterone concentrations for insulated and dexamethasone-treated bulls were compared to control bulls by the *t* test (20).

Results

The insulating bag came off the scrotum of one bull on the first day of insulation. The bag was off for a maximum of six hours before it was replaced and no extra insulating time was allotted to the bull. The seminal quality changes in this bull were similar to those of the other insulated bulls and, therefore, the data from this bull were not eliminated. The scrotal surface temperatures inside the insulating bags ranged from 35.1°C to 35.6°C.

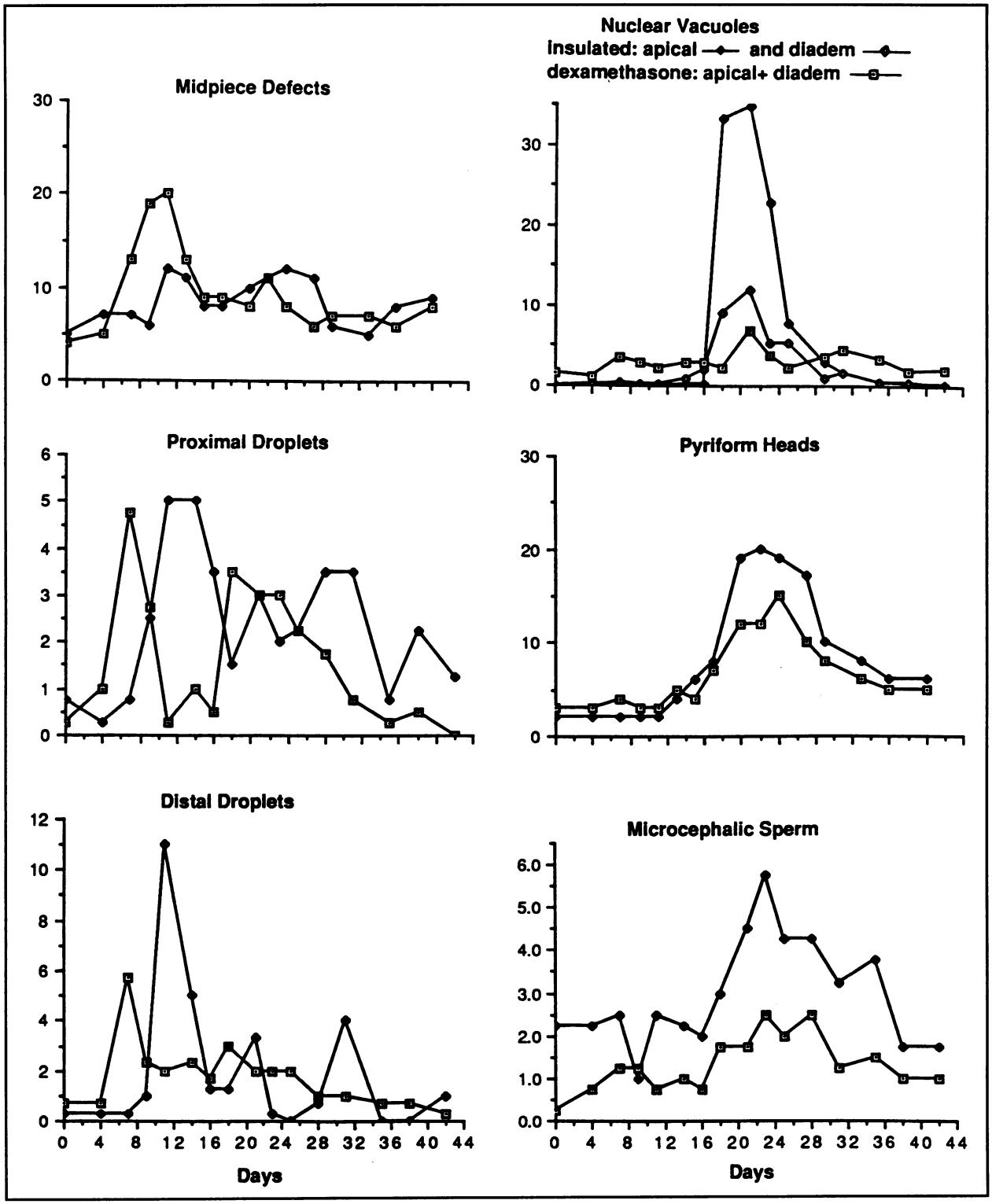

Figure 2. Changes in mean percent of midpiece defects, proximal droplets, distal droplets, nuclear vacuoles, pyriform heads, and microcephalic sperm in four insulated (——◆——) and four dexamethasone-treated (——⊡——) bulls. The Y axis is in percent and the X axis is in increments of two days with day 0 being the day of initiation of treatment.

The bulls tolerated the frequent electroejaculation schedule extremely well, becoming easier to handle and showing fewer and fewer signs of apprehension of the semen collection chute as the experiment progressed.

Semen volume, concentration, and gross wave motion were highly influenced by the ease of electroejaculation of individual bulls, dilution with preseminal fluid, and, occasionally, urine contamination. In some cases, poorly concentrated samples were collected prior to treatment and samples with good concentration and gross wave motion were occasionally collected at times coinciding with maximal numbers of sperm abnormalities due to treatment. Therefore, these semen traits were not considered reliable measures of the effect of treatments. However, in general, semen samples became less concentrated and had depressed motility in response to scrotal insulation or dexamethasone treatment.

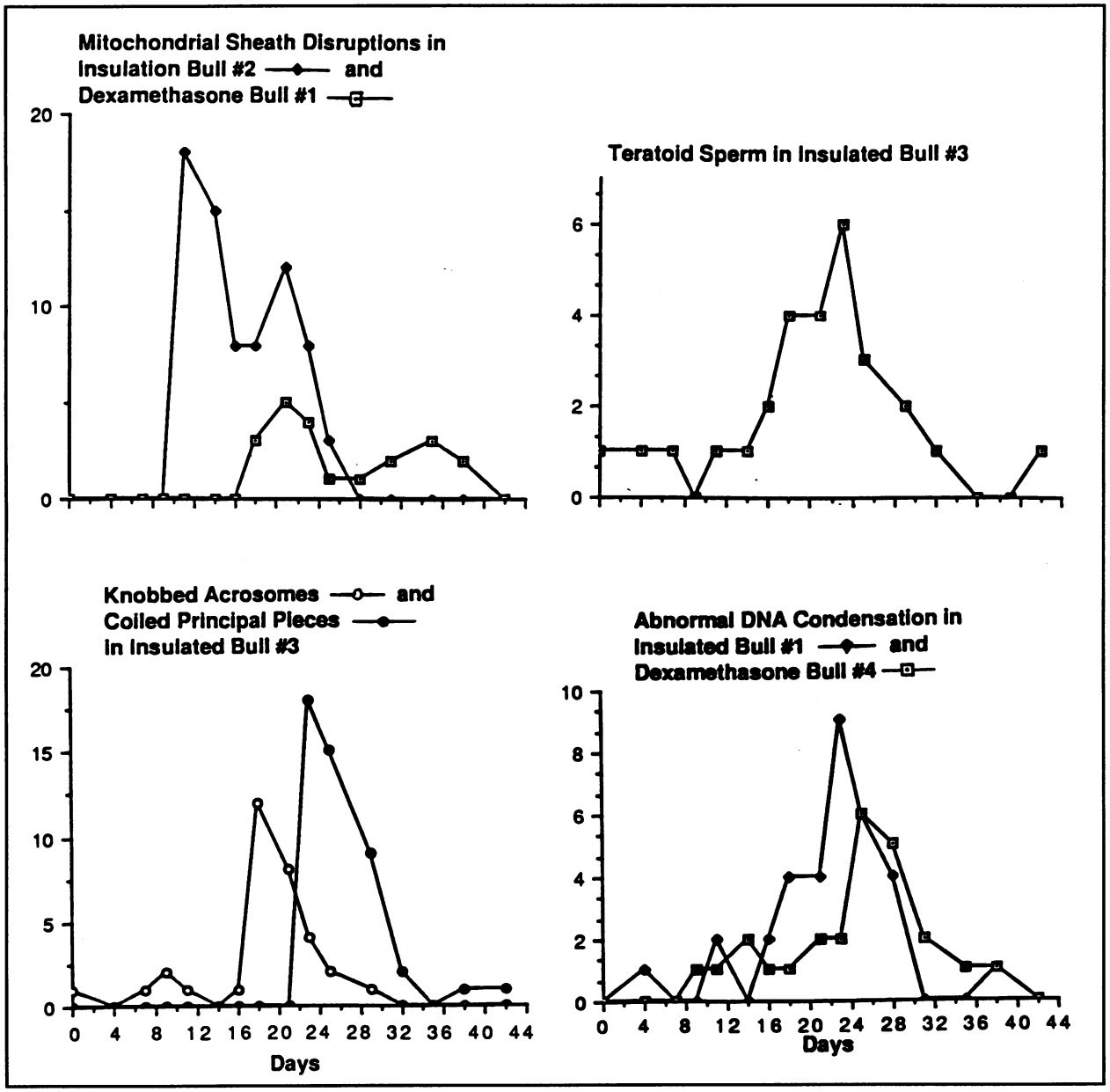

Figure 3. Specific types of sperm defects that were markedly increased in only one insulated and/or one dexamethasone-treated bull. The Y axis is in percent and the X axis is in increments of two days with day 0 being the day of initiation of treatment.

Sperm morphology

The number of normal spermatozoa among control bulls ranged from 78% to 94% over the entire semen collection period. In spite of the frequent electroejaculation regime, there were no marked increases in individual sperm defects within control bulls that could be attributed to the stress of handling or electroejaculation. However, there were marked increases in many types of sperm defects following scrotal insulation and dexamethasone treatment. In all bulls in both treatment groups, there was a marked decline in the percent of normal sperm within seven days after the start of treatment. The percent of normal sperm reached the lowest point at approximately three weeks and returned to pretreatment levels by six weeks after the start of treatment. The percent of sperm staining "alive" (sperm not taking up eosin stain) roughly paralleled the decline and rise in normal sperm. The percent of sperm with detached heads appeared to be inversely related to the percent alive sperm (Figure 1).

All insulated and dexamethasone-treated bulls had marked increases in distal midpiece reflexes, detached heads, pyriform heads, and nuclear vacuoles. Two or three bulls in each treatment group had marked increases in proximal droplets and distal droplets. Three insulated bulls and one dexamethasone-treated bull had marked increases in microcephalic sperm. Some bulls seemed predisposed to respond to treatment by producing a type of defect not seen in the other bulls. For example, one insulated bull had a marked increase in coiled principal pieces and knobbed acrosomes, but these defects were rarely seen in any of the other bulls (Table 1).

Sperm defects that showed marked increases in two or more bulls within or among treatment groups occurred in a similar temporal relationship. Therefore, the per-

Table 1. Sperm defects that had an equal to or greater than 5% increase (+) over pretreatment values in four scrotally insulated (Ins) and four dexamethasone-treated (Dex) bulls

	Ins1	Ins2	Ins3	Ins4	Dex1	Dex2	Dex3	Dex4
Pyriform heads	+	+	+	+	+	+	+	+
Distal midpiece reflex	+	+	+	+	+	+	+	+
Detached heads	+	+	+	+	+	+	+	+
Apical vacuoles	+	+	+	+		+		+
Diadem vacuoles	+	+	+	+	+		+	
Proximal droplets	+	+	+		+		+	
Distal droplets	+	+			+			+
Microcephalic		+	+	+	+			
Abnormal DNA	+							+
Mitochondrial sheath		+			+			
Knobbed acrosomes			+					
Coiled principal piece			+					
Teratoid			+					

Table 2. Time of occurrence and duration (days) of peak incidences of sperm defects after the beginning of scrotal insulation or dexamethasone treatment

	Time of peak incidence		Duration of rise > 5% above baseline	
	Insulation	Dexamethasone	Insulation	Dexamethasone
Distal midpiece reflex	11	11	11–13	7–13
Proximal droplet	11	7	10–13	7
Distal droplet	11	7	11	7
Detached heads	11	15	9–11	11–22
Mitochondrial sheath	14	21	14–23	21
Knobbed acrosome	18	—	18–21	—
Nuclear vacuoles	21	21	18–25	21
Pyriform heads	22	24	20–29	20–29
Microcephalic	21	23	21	23
Teratoid	23	—	23	—
Coiled principal piece	23	—	23–29	—
Abnormal DNA	30	28	28–30	28

centages of different types of sperm defects on each sampling date were averaged for all bulls within a group, including bulls that had less than a 5% increase for a specific defect, and compared graphically (Figure 2). In general, the response was more severe to insulation than to dexamethasone treatment in the percent of pyriform heads, nuclear vacuoles, and microcephalic sperm. On the other hand, dexamethasone treatment resulted in an earlier and more severe response in distal midpiece reflexes as well as an earlier response in proximal and distal droplets. Midpiece defects that occurred 7–11 days after the beginning of treatment, were predominantly distal midpiece reflexes; whereas, those occurring more than 11 days after either insulation or dexamethasone treatment also included gaps in the mitochondrial helix, rough swollen mitochondrial sheaths, and midpiece fractures in various locations. Proximal and distal droplets increased in association with the increase in midpiece defects. Bulls that were insulated or treated with dexamethasone showed marked increases in nuclear vacuoles 18–25 days after onset of treatment. Single vacuoles located at the apex of the nucleus were more common than vacuoles of the diadem arrangement in both insulated and dexamethasone-treated bulls. Insulated bulls showed a more severe response for vacuoles than did dexamethasone-treated bulls (Figure 2). Sperm with nuclear vacuolation reached a very high level (25%) in only one bull in the dexamethasone treatment group; for the remainder of the bulls in that group nuclear vacuolation was at the 5%–10% level.

The spermiograms of individual bulls that seemed predisposed to producing certain types of sperm defects that were absent or rare in the other bulls are shown in Figure 3. Only one bull (insulated) showed a marked increase in both coiled principle pieces and knobbed acrosomes. These defects appeared randomly on rare occasions at less than 2% in the remainder of the bulls. An increase in abnormal DNA condensation occurred in one insulated and one dexamethasone-treated bull; however, the maximum number of affected sperm was 6%–9%. Mitochondrial sheath disruptions and abnormal DNA condensation (clumped appearance on Feulgen stain) were markedly increased in only one bull per treatment group. Although teratoid spermatozoa usually remain below 5% in disturbances of spermatogenesis in bulls (18), in one of the insulated bulls, teratoid sperm reached the level of 6%, 23 days after the start of insulation. Teratoid sperm occurred occasionally at 1%–3% in the other insulated bulls and were always under 1% in dexamethasone-treated bulls.

Table 3. Mean (± SEM) serum testosterone concentrations (ng/mL) in control, insulated, and dexamethasone-treated bulls (n = four bulls per group)

Treatment group	Basal concentration	Peak amplitude	Mean concentration
Control day 0	0.54 ± 0.10[a]	5.29 ± 1.61[a]	2.73 ± 0.35[a]
Control day 42	0.69 ± 0.09[a]	5.66 ± 1.17[a]	3.32 ± 0.68[a]
Preinsulation	0.65 ± 0.13[a]	4.87 ± 1.04[a]	2.03 ± 0.61[a]
Postinsulation	0.57 ± 0.12[a]	4.05 ± 0.70[a]	1.95 ± 0.27[a]
Predexamethasone	0.63 ± 0.08[a]	6.31 ± 1.12[a]	3.08 ± 0.10[a]
Postdexamethasone	0.16 ± 0.03[b]	0.32 ± 0.14[b]	0.22 ± 0.08[b]

[ab]Means in columns with different superscripts are significantly different ($p<0.05$)

For all bulls, the various sperm defects increased 5% or more above baseline values in the following order: distal midpiece reflexes, proximal droplets and distal droplets at 7–13 days, mitochondrial sheath disruptions at 14–23 days, knobbed acrosomes at 18–21 days, nuclear vacuoles at 18–25 days, coiled principal pieces at 23 days, pyriform heads at 20–29 days, microcephalic sperm at 21–23 days, teratoid sperm at 23 days, coiled principal pieces at 23–29 days, and abnormal DNA condensation at 28–30 days (Table 2). All bulls recovered to approximately pretreatment levels of sperm defects six weeks after the initiation of treatment.

Serum testosterone

One or two episodes of serum testosterone secretion occurred during the bleeding periods in control bulls, pre and postinsulated bulls, and in pretreatment samples of dexamethasone-treated bulls with concentrations ranging from 0.54 ± 0.10 ng/mL to 6.31 ± 1.12 ng/mL ($\bar{x}$ ± SEM). After insulation for four days, testosterone concentrations still varied widely over the eight hour bleeding period, ranging from 0.57 ± 0.12 ng/mL to 4.05 ± 0.70 ng/mL, suggesting that insulation suppressed testosterone production; however, the difference was not significantly different from control bulls ($p>0.14$). In bulls that had received dexamethasone treatments, there was very little variation in serum testosterone, with concentrations ranging from 0.16 ± 0.03 ng/mL to 0.32 ± 0.14 ng/mL.

There were no significant differences in mean basal, mean peak episodic, or mean eight-hour testosterone concentrations among control bulls, pre and postinsulated bulls, and in pretreatment samples of dexamethasone treated bulls ($p>0.05$). However, after dexamethasone treatment, mean basal, mean peak episodic, and mean eight-hour testosterone concentrations were significantly lower than in control, insulated, or predexamethasone treated bulls ($p<0.05$, Table 3).

Tissue testosterone

Tissue concentrations of testosterone in control bulls and in bulls after insulation or dexamethasone treatment are shown in Table 4. Although the mean tissue testosterone concentrations in control bulls appeared higher than in insulated and dexamethasone-treated bulls, the differences were not significant ($p>0.13$).

Discussion

The results of this experiment are similar to previous reports indicating that, after scrotal insulation, skin surface temperatures are increased from approximately 34°C to a range of 35°C–36.5°C (21–23). In one study, the normal subcutaneous scrotal temperature of bulls in ambient temperatures 15°C–28°C was shown graphically to range from approximately 33°C to 34.5°C, whereas these temperatures increased to approximately 35°C–37.5°C after insulation (2). Waites and Moule (24) showed that the deep testicular temperature of rams ranged from 33.3°C to 34.7°C and remained close to the subcutaneous scrotal temperatures over a range of ambient temperatures of 28°C–40°C. It appears therefore, that scrotal insulation raises deep testicular temperatures to similar levels as skin surface temperatures under insulation.

Ross and Entwistle (23) showed that the rate of spermatogenesis and the rate of epididymal passage was not changed by scrotal heating. Using tritiated thymidine to mark germinal cells and autoradiography, they determined that the length of the seminiferous epithelial cycle of insulated bulls was 13.4 days and similar to that reported for noninsulated bulls (25,26). Estimates of sperm epididymal transit times in normal bulls have ranged from 4 to 15 days; however, it appears that a time of 8–11 days is widely accepted for bulls that are ejaculated daily (27–30). The rate of epididymal passage of insulated bulls (23) was 13.5 days; however, in that experiment semen was collected every three to four days, which may have resulted in longer transit times, as well as some additional time spent in storage in the cauda epididymis. It was concluded that testicular heating did not change the rate of epididymal passage.

The effect of stress on rate of spermatogenesis or epididymal passage has not been reported; however, it would appear that dexamethasone (stress) does not affect the rates of spermatogenesis or epididymal passage, since the time of appearance of various defects after insulation or dexamethasone treatment was similar in this experiment. Therefore, spermatozoa appearing in ejaculates up to two weeks after insulation or dexamethasone treatment would have been present in the epididymis at the time of treatment, and sudden large increases in sperm defects would be attributable to abnormal epididymal function caused by the treatment. Spermatozoa

Table 4. Testosterone concentrations (ng/mg) in testicular tissue of control, insulated, and dexamethasone-treated bulls (n = four bulls per group)

	Control	Insulation	Dexamethasone
	0.63	0.55	0.20
	0.62	0.24	0.30
	0.92	0.19	0.15
	2.44	0.52	0.08
Mean (± SEM)	1.15 ± 0.44[a]	0.38 ± 0.09[a]	0.18 ± 0.05[a]

[a]Means are not significantly different (p>0.13)

appearing in ejaculates after two weeks would have been in various stages of spermiogenesis during treatment, and sudden increases in sperm defects would be attributable to abnormal spermatogenesis caused by treatment.

None of the control bulls showed a marked increase in any types of sperm defects. This indicates that the changes in sperm morphology in dexamethasone-treated and scrotally insulated bulls were due to treatment rather than the semen collection procedure. It also indicates that electroejaculation on a frequent basis, at least in bulls selected for ease of handling, does not cause sufficient stress to result in semen quality changes.

The sequential appearance of various defective sperm would most likely be related to their position in spermiogenesis or epididymal transit at the time of cell injury and the remaining time required for the injured cell to be released into the lumen of the seminiferous tubules and/or transported through the epididymis (18,25,26). These data indicate that nuclear defects would most likely develop during nucleus condensation and shaping, a period of 6–14 days before spermiation and, assuming 11 days for epididymal transit, 17–25 days before sperm are in a position to be ejaculated. Mitochondrial sheath defects would develop later, one to four days before spermiation, and spermatozoa injured at this time would require 11–15 days to be in a position for ejaculation. The time at which abnormal spermatozoa appear in the ejaculate would also depend to some degree on the length of storage in the cauda epididymis which, in turn, is partially dependent on the semen collection schedule.

It was postulated that the type of defects produced may depend on the type and severity of the insult to spermatogenesis. For example, heat with a main effect of tissue anoxia may affect spermatid development differently than stress with a main effect of depressed tissue testosterone. Thus, it might be interpreted that knobbed acrosomes and coiled principal pieces, which occurred only in an insulated bull (Figure 3), may not develop due to stress. However, individual differences in response to treatment occurred within groups indicating that some differences in response were due to the predisposition of individual animals rather than the type of treatment. In this regard, in a previous report involving three bulls on a similar dexamethasone treatment regime (17), all bulls developed extremely high percentages of sperm with nuclear vacuoles. However, in this experiment only one of four dexamethasone treated bulls developed a very high percentage of nuclear vacuoles (25%).

Scrotal insulation appeared to cause a greater increase in sperm nuclear abnormalities, including pyriform heads, nuclear vacuolation, microcephalic heads, and abnormal DNA condensation, even though the duration of insulation was shorter than the duration of dexamethasone treatment. There is some evidence that heat depresses tissue testosterone levels (31–33). Thus, the more severe effect of insulation compared to dexamethasone treatment may be due to a combination of tissue anoxia and depression of testosterone rather than the depression of testosterone secretion alone expected with the dexamethasone treatment. Both anoxia and low testosterone may interfere with the same cellular metabolic processes, leading to similar outcomes in defects of the cells affected during spermatid metamorphosis.

Dexamethasone treatments appeared to cause an earlier and more severe effect on epididymal sperm than did insulation. Spermatozoa undergo maturation and achieve fertilizing capability during epididymal passage. Although this process is not well understood, it is believed that the epididymis provides a specific environment for maturational processes and that this environment results from secretory and absorptive functions of the epididymis that are regulated by androgenic hormones (10,34–36). These hormones reach the epididymis directly from the blood supply and from seminiferous tubule effluent (37). Dexamethasone has been shown to depress pituitary LH secretion within one hour and testosterone secretion within four hours (16). The epididymis would, therefore, have insufficient testosterone for normal function soon after dexamethasone treatment. On the other hand, scrotal insulation does not depress pituitary LH secretion (32,38). Although not supported by our data, previous reports on cryptorchid rats (31), cryptorchid pubescent bulls (39), and scrotally insulated mature bulls (32) indicate that heat has a direct adverse effect on testosterone secretion by the Leydig cell. However, scrotal insulation appears to have a less dramatic depressive effect on the Leydig cell than dexamethasone. This would explain the delayed appearance of epididymal sperm defects, such as distal midpiece reflexes, and cytoplasmic droplet retention in insulated bulls compared to dexamethasone-treated bulls.

Although distal midpiece reflexes and cytoplasmic droplet retention occurred 10–12 days after insulation, apparently as a result of low testosterone, there also appears to be a direct effect of heat on epididymal spermatozoa resulting in an increased proportion of dead spermatozoa and detached heads by day 8 after insula-

tion (Figure 1). This is in agreement with previous reports that showed an increase in detached heads six to eight days after insulation (2) and a decreased ability to maintain motility and acrosomal integrity following cryopreservation when semen was collected three, six, and nine days after insulation (22). The increase in the numbers of sperm taking up eosin stain ("dead sperm"), indicating loss of cell membrane integrity, was coincident with the increase in the numbers of sperm with detached heads. This suggests that head detachment is directly related to loss of cell membrane integrity leading to cell breakdown (Figure 1).

The large variations in tissue concentrations of testosterone were unexpected. It appears that tissue testosterone concentrations may vary hourly, depending on gonadotrophin stimulus. This would account for one markedly elevated value observed in a control bull (Table 4). In a separate study done later, testosterone concentrations ranged from 0.18 ng/mg to 0.66 ng/mg in testicular tissue samples taken at slaughter from six feedlot bulls aged 15–18 mo (Barth, unpublished observations). Since only one tissue sample can be taken, much larger numbers of bulls than used in this experiment would be required to demonstrate possible effects of treatment on tissue testosterone concentrations.

In conclusion, results indicate that two of the most common types of insults to spermatogenesis in western range bulls, heat and stress, appear to result in very similar spermiograms. Clinical cases of a disturbance of spermatogenesis would likely follow a course similar to that described in these experiments; however, the severity and duration of the abnormal spermiogram would likely depend on the severity and duration of the insult. Nevertheless, the identification of large numbers of specific types of sperm defects combined with a clinical history may help to identify the cause and time of onset of an insult to spermatogenesis, as well as help in the formulation of a more reliable prognosis.

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BOOK REVIEWS

REVUE DE LIVRES

DiBartola SP. **Fluid Therapy in Small Animal Practice.** W.B. Saunders Company, Toronto, 1992. 720 pp. ISBN 0-7216-3182-7. $95.25

This work represents a new undertaking on the part of the editor to bring together in one textbook information relevant to fluid therapy in small animal practice. The preface states that "a sound understanding of the relevant physiology and pathophysiology enhances the clinical approach to any medical problem and ultimately improves patient care." The structure of the book and the information contained within each chapter directly reflect this philosophy.

Overall, the text is comprehensive and well organized, and the information is presented in a clear and logical manner. The print quality is excellent and easy to read. The subject matter in each chapter is reviewed thoroughly. The authors make excellent use of tables, figures, and photographs to aid in the presentation of material. In some instances, however, the quality of the photographs could be improved. The writing style does vary to some extent among chapters, but this is to be expected in a text that has 20 contributors. Each chapter ends with a current reference list containing key, original articles. It is evident that the authors made a significant attempt to review pertinent literature from all disciplines and not restrict themselves to the veterinary medical literature. Despite the extensive review, the authors ensured that the data included in the text are relevant to small animal practice. Finally, the index is extensive and well cross-referenced. Only in one instance was it difficult to find a major topic that was covered in various places within the text.

This book can be divided into three sections. The first section covers body fluid and renal physiology, as well as specific electrolyte and acid-base disorders. The chapter on body fluids in dogs and cats brings together a significant amount of material that is not readily available elsewhere in one source. Four chapters are devoted to electrolyte disturbances and the clinical management of those disorders. Once again, the coverage is extensive. For example, the chapter on calcium disorders is 54 pages long. The section concludes with a series of six chapters on acid-base disturbances that are particularly wellwritten. The authors avoid confusing the reader by clearly defining the terminology to be used prior to discussing acid-base disturbances and their management. The last of these chapters is devoted to a discussion of strong-ion difference as a nontraditional approach to the evaluation of acid-base disturbances. This approach to acid-base analysis is complex, and it remains to be seen if it will gain wide acceptance within the veterinary medical community.

The second section covers the practical aspects of fluid therapy, transfusions, enteral and parenteral nutrition, shock, intraoperative fluid therapy, and peritoneal dialysis. Several chapters are devoted to fluid therapy in the management of various disease processes. The information in this section is written in a style that is easy to read and pertinent information is readily found. The only major topics for discussion that might have been included in a text of this nature are cell-free hemoglobin and perfluorocarbons. In addition, information relating to vascular injuries and interstitial edema deserves more attention in a text of this quality.

The final section consists of a series of clinical cases that provide information on how the material in the text can be applied to clinical situations, and it may be of value to those who prefer to use clinical cases for assimilating new material.

In summary, the text achieves the editor's goal of providing the practitioner with comprehensive information on fluid therapy in small animal practice. However, given the detail in and the cost of the book, I believe that it would best suit the needs of the academic community.

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