The effects of cold stress on boar fertility

by

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I. INTRODUCTION

The effects of environmental temperature on the reproductive function of male domestic animals have been the subject of a number of studies. These studies have dealt mainly with the effects of high ambient temperatures on the male reproductive function in domestic and laboratory animals.

It has been conclusively shown that high environmental temperatures are detrimental to the spermatogenic process. Furthermore, several studies on boars have confirmed the negative effect of high environmental temperatures on reproductive performance.

In the midwestern states, a number of boars have experienced infertility when exposed to prolonged cold stress during inclement weather. The physio-pathological explanation of this phenomenon is yet to be illuminated. It has been speculated that cold stress alters the normal thermodynamics of the testis leading to spermatogenic arrest.

Information is scarce relative to the effects of low environmental temperatures upon the reproductive function of domestic or laboratory animals; studies have been limited mainly to laboratory animals.

This study was undertaken to evaluate the effects of cold environments on the thermodynamics of the testis, the

germinal epithelium of the testis, and the motility and morphology of sperm in boars. It was hoped that this information would provide an explanation for the cold stress infertility frequently seen in boars and some insight for the prevention of this condition.

II. REVIEW OF LITERATURE

A. Thermoregulation of the Testis

One of the first descriptions of the activity of the scrotum was made by Cooper (1830). "The scrotum varies greatly in its appearance and size, under the influence of cold, it is contracted and wrinkled; under heat, it is smooth on its surface and greatly extended." In eutherian mammals with scrotal localization of the testis, the scrotum has its embryological origin in the genital swellings that appear on either side of the urethral plate in the perineal region of the early embryo (Setchell 1978a). The shape of the scrotum, in eutherian mammals, varies from the sub-anal pouches of rodents and swine, to the very pendulous one of the ungulates (Waites 1970, Setchell 1978a).

The scrotum is comprised of a thin skin, scarce subcutaneous tissue and a muscular layer, the tunica dartos that lies mainly in the distal portion of the scrotum (Setchell 1978a). Sweat glands are present in the scrotal skin. Their number and function differ among species. In the rat, there are fewer of these sweat glands as compared to sheep (Waites and Voglmayr 1962, Waites 1970). According to McNitt et al. (1972b), the number of these sweat glands appears to be adequate. However, they do not show thermal

sweating despite having active secretion.

The existence of a temperature gradient between the body temperature and the scrotal system has been shown. McNitt et al. (1972b) defines the scrotal system as the scrotum, testis and other adjacent areas, that may affect directly the thermoregulation of the testis. This gradient varies from 2 to 7°C with the scrotal system being lower than that of the body temperature (Brooks 1973, Godinho and Cardoso 1980, Stone 1981, Damber and Janson 1978, Lazarus and Zorgniotti 1975, Fowler 1968, McNitt 1971, Waites and Moule 1961, Waites 1970, Setchell 1978a, Robinson and Rock 1967).

The development of the rectum-testis temperature gradient in the rat has been studied by Kormano (1967). During the first 35 days of life, the gradient undergoes certain changes. It increases from 1.05°C at 20 days to 2.04°C at 30 days. The characteristic temperature gradient of the adult is reached at 35 days, when it is 2.5°C. At this time the scrotum has reached its full development. The finding of this gradient has been confirmed by Damber and Janson (1978) and Brooks (1973).

This gradient varies among the different species. It is 5.2°C, 5.2°C, 2.5°C and 2.38°C, respectively, in the bull, ram, pig and man (Godinho and Cardoso 1980, Waites and

Moule 1961, Stone 1981, Rock and Robinson 1965). This gradient can be altered by many different factors, such as scrotal heating, fever, cryptorchidism, high environmental temperature and varicocele (Waites 1970, Setchell 1978a, Rock and Robinson 1965, Zorgniotti and MacLeod 1973, Lazarus and Zorgniotti 1975).

Within the scrotal system, the temperature varies according to species and organ structure. For instance, the testicular temperature of the rat is 3°C to 4°C lower than body temperature; in the caput of the epididymis, it is one degree warmer than the testes; and in the cauda epididymis, it is 3 to 4°C below testicular temperature (Brooks 1973). Stone (1981) reported that in the pig the temperature differences between the rectum and the testes, caput of the epididymis or cauda of the epididymis were 2.5°C, 1.5°C and 1.9°C, respectively. The testicular temperature ranges between 35.0°C and 36.5°C (Stone 1981, McNitt 1971, McNitt et al. 1972a) while the temperature in the tunics were 34.1°C and at the scrotal surface 33.8°C (McNitt et al., 1972a).

Waites and Moule (1961) found that testicular temperatures in the ram were 33.4-34.7°C and 33°C at subcutaneous scrotal levels.

The scrotum, in itself, plays a very important role in thermoregulation of the testis. Setchell (1978a),

reviewing the thermoregulation of the testis, said that there was sufficient evidence, from studies in sheep, pig, and rat, of the presence of temperature receptors - cold, heat - in the scrotal skin.

Scrotal convection heat loss is regulated by the tunica dartos (Zorgniotti 1982). The smooth muscle fibers of this structure are inserted in the scrotal skin (Setchell 1978a). Its nervous control is from the nerves of the lumbar sympathetic trunk in the case of the cat, dog and rabbit (Langley and Anderson 1895, cited by Setchell 1978a).

The tunica dartos relaxes when scrotal skin temperature increases (Waites 1970, Shaffik 1977). The scrotum of merino rams starts to relax and extend, when the scrotal temperature reaches 35°C. At this temperature, the tunica dartos is completely relaxed, the surface area of the scrotum has increased 20% more than normal (Fowler 1967, cited by Waites 1970). Rathore (1975) exposed a group of rams to an increase in environmental temperature from 26.7°C to 46.1°C (30-40% humidity), starting at 5 AM and ending at 5 PM. As the ambient temperature increased, there was a progressive increase in scrotal length. This increase in the scrotal length continued until environmental temperatures reached 32.2°C, beyond this point, no further increase occurred.

Stimuli, such as cold, touch and circulating

catecholamines, make the tunica dartos contract and form skinfolds. This contraction draws the testicle closer to the body wall. The tunica dartos is capable of substained contraction in cold environments (Setchell 1978a). This contraction was once attributed to the cremaster muscle, but this seems unlikely because the cremaster is striated muscle and therefore incapable of sustained contraction.

Shaffik (1977) suggests that the cremaster muscle, by its sphincteric action on the cord veins, plays an important function in the temperature regulating mechanisms of the testicle. Its compression of the cord veins, resulting in decreased blood flow and less exposed cord surface, prevents heat loss at low environmental temperatures. Meanwhile, the testes are brought closer to the warmer temperature of the body wall. Relaxation of the cremaster muscle leads to a greater blood flow through the testicular vein and much more exposure of the cord surface. The testicle is in a lower position which favors heat loss. This operative mechanism in combination with the tunica dartos is capable of maintaining the testiculo-rectal temperature gradient at physiological levels in the face of changing environmental temperature. This cremasteric-dartos component regulates the blood flow within the cord and scrotal vessels. It also changes the surface area of both scrotal skin and spermatic cord. In a hot environment, relaxation of the

cremaster muscle and tunica dartos increases the blood flow in the scrotum and the spermatic cord, and increases their exposed surface areas. This facilitates heat radiation. Contraction of these structures in response to cold, preserves the testicular temperature by diminishing the cord and scrotal blood flow and reducing their surface area.

The temperature of the scrotal testis is determined by several factors which create a balance between the heat that is transported into the testicle by the arterial blood, the amount of heat that the intrinsic testicular metabolism generates and the heat loss through the scrotal surface (Waites 1970, Setchell 1978a).

Waites and Moule (1961) demonstrated the existence of a vascular countercurrent heat exchange mechanism in the spermatic cord of the ram. They showed that the blood flowing through the testicular artery cools by as much as 5.2°C between the aorta and the dorsal pole of the testicle. Most of the cooling takes place in the coiled segment of the artery in the spermatic cord, where it passes in intimate contact with the returning venous blood flow that passes through the pampiniform plexus. The venous blood is warmed by a similar amount. Two conclusions were drawn from this study. Firstly, this mechanism regulates the temperature of the testicular tissue at a level close to that inside the scrotal skin. This is brought about by the returning

venous blood which governs the temperature of the arterial blood that reaches the testis. Secondly, the thermoregulatory mechanisms of the countercurrent system are effective only as long as the magnitude of the temperature gradient between body temperature and scrotal temperature allows heat exchange. It is not autoregulatory. When the testes were cooled below 34°C, the rate of arterial cooling in the spermatic cord did not keep pace with the rate of cooling of the testis.

There are anatomical features that favor this physiological phenomenon. The testicular artery that emerges from the inguinal canal becomes coiled, and the veins of the pampiniform plexus intimately surround it. Another characteristic that facilitates this heat exchange is that the tunica adventitia of the veins merges with that of the artery (Setchell 1978a).

Blood flow through the testis and scrotum seems to vary according to scrotal temperature (Waites 1970, Setchell 1978a). Waites et al. (1973) immersed the scrotum, epididymis, and testis of the rat in water at temperatures from 28° to 45°C. The mean blood flow in the testicular tissue when the bath was at 33°C, which is the normal scrotal temperature, was 21.8 to 24.4 $\pm$ 1.2 ml/100 g/min. Increasing the temperature to 37°C for 20 minutes, did not

significantly affect testicular blood flow. The testicular blood flow underwent a sharp increase when the scrotal temperature was elevated to 43°C or further to 45°C. This change was mainly due to an increase in cardiac output and to local effects. In the scrotal skin, the blood flow doubled when the temperature was elevated from 33°C to 37°C.

Damber and Janson (1978) showed that when only one rat testicle was exposed to local heat (37°C), no effects on blood flow were observed in the treated or untreated testicle. When both testes were exposed to 41°C, there was a sharp decrease in vascular resistance. This effect was more pronounced at 43°C.

In the ram, when the scrotal temperature was increased to 40°C, there was no increase in testicular blood flow (Waites et al. 1968). Contrasting with these findings, Dutt et al. (1977) reported that blood flow to the testis, measured with the 133-Xenon isotope clearance technique, increased after exposure of ram testis to 32°C. After 5 and 7 days of exposure, blood flow decreased significantly. This occurred either when the animals were exposed to this temperature or only local heat was applied.

The scrotal skin in sheep contains arteriovenous anastomosis (Molyneux 1965). According to Waites (1970) and Setchell (1978a), vasodilation in the scrotum and elevated blood flow can be brought about by reversal of

vasoconstriction by direct effect and by a reflex removal of the sympathetic tone because of stimulation of afferent fibers in the cutaneous receptors.

The vascular responses in the testicular tissue to the cooling of the scrotum have been little studied. Setchell (1978a) suggests that it can be assumed that cooling would result in vasoconstriction. It has been shown that cooling of isolated arteries of pigs at 20°C results in temporary cold-induced vasoconstriction. In small vessels, the constriction persisted for hours and indefinitely at lower temperatures. This indicates that change in blood flow in the skin, exposed to cold environment, is not necessarily mediated by nerve impulses (Folk 1974).

Waites and Voglmayr (1963) studied the thermoregulatory activity of the apocrine sweat glands in the scrotum of rams. They observed that when scrotal temperature was raised above 35.3°C using dry hot air, the scrotal temperature began decreasing along with an increase in the humidity of the scrotal skin, which was due to excretions from the sweat glands. The glands are activated by receptors in the scrotal skin, which are responsive to temperatures above 35°C (Waites 1970).

The scrotum of the boar displays a great number of scrotal sweat glands that are not responsive to thermal changes (McNitt et al. 1972b). The surface sweating of the pig

is not effective in causing a reduction in temperature (Ingram 1965, Mount 1968). Stone (1981/1982) showed that at air temperatures greater than 32°C, the scrotum of the boar could not decrease its temperature by convection or conduction because the environmental temperature of the surrounding moving air exceeded the scrotal temperature.

Hales and Hutchinson (1971) studied effect of scrotal heating in wooly rams. The heating of the scrotum by a warm bath at almost 40°C causes a dramatic increase in the respiratory rate from 35 breaths/min. to 192 breaths/min. Within five minutes of scrotal heating, the frequency declined slightly as the heating continued, reaching a value of 165 breaths/min., with a range of 127-210 breaths/min. If the scrotum is cooled, then there is an immediate fall in the frequency to a mean 28 breaths/min. in 5 minutes. Fowler (1968) showed that scrotal heating of rams at 39.5°C to 40.3°C for 120 minutes results in a drop in rectal temperature. This drop in the rectal temperature is related to the severity of the scrotal heating.

When scrotal temperature on pigs was raised to 42°C, keeping ambient temperature at 25°C, there was no increase in respiratory frequency. If the ambient temperature was increased to 30°C and scrotal temperature was maintained the same, the respiratory frequency increased. This increase in panting was inhibited by exposure to cold ambient

temperature (Ingram and Legge 1972).

In pigs, when the scrotal heating was applied, it caused panting that was not accompanied by a fall in body temperature. At the most, the increased ventilation rate prevented the temperature from rising (Ingram and Legge 1972). This is in contrast to the response in sheep where the temperature may be lowered 2°C (Hales and Hutchinson 1971). Pigs exposed to low temperature and heating of the scrotum stopped shivering, and decreased oxygen consumption (Ingram and Legge 1972).

B. Temperature Effects on Testicular Function

Testicular function is affected by a number of conditions that alter the temperature regulation of the testis. VanDemark and Free (1970) reviewed the effects of cryptorchidism, pyrexia, season and humidity, varicocele, exposure to high environmental temperature and direct application of heat on the scrotum. Cold exposure and other changes concurrent with it, they concluded based on the evidence at that time, would alter spermatogenesis in some species.

Piana and Savarese (1891) cited by Setchell (1978b) and Crew (1922) suggested that the tissue changes observed in cryptorchid testis were due to the higher temperature in

the abdominal cavity.

Induced cryptorchidism in 3-4 month-old bull calves, using elastrator bands to push the testicle into the abdominal cavity, caused aspermia. This effect was evident when semen samples were collected at 12 months of age. Histological examination of the testis revealed atrophied seminiferous tubules and small testicular size. The internal temperature was 3°C higher in the cryptorchid testis than in the scrotal testis. When cryptorchidism was induced at 12 months of age, it did not result in complete sterilization (Kellaway et al. 1971).

Degeneration of the germinal epithelium, caused by induced cryptorchidism in the rat, appears within two weeks (Hagenas and Ritzen 1976). When cryptorchidism was induced in guinea pigs (Hoschoian and Andrada 1975) at prepuberal stages, cryptorchid testes were not altered in comparison to scrotal testis. At puberty, there was a great difference in the structure of testicular tissue. The cryptorchid testis only had a few spermatogonia and vacuolated Sertoli cells.

Reflection of the epididymis into the abdominal cavity in rats brings about a reduction in the sperm number in its cauda. This is caused by exposure to abdominal temperature. Foldesy and Bedford (1982) found a reduction of 28% after two days and a 50% reduction of sperm after four days.

This trend continued until after 16 days, when it reached the 25% level of the reserve in the untreated scrotal cauda of the epididymis. The increase in temperature apparently causes a change in the physiological activity of the epididymal epithelium. This is seen as a reduced ability to reabsorb water, Na^+ and Cl^- and to secrete K^+. These changes are detectable within two days and by seven days, there is a dramatic alteration of Na^+ and K^+ levels in the tubular fluid (Wong et al. 1982). The mechanism by which this alteration of the epididymal function takes place has not been elucidated. It was speculated that exposure to body temperature decreases apical permeability of the plasma membranes of epithelial cells and inhibited active transport (Rasweiler and Bedford 1982).

Pyrexia has also been shown to affect testicular function. Pattabiraman et al. (1974) induced pyrexia in rabbits by injecting a yeast suspension. The rectal temperature increased from 103.4°F to 105.6°F and persisted for 16-21 hours. Examination of the germinal epithelium, after two days, showed normal spermatogenesis. However, after 10 days, the epithelium showed desquamative changes, early spermatids were absent, and in a few tubules, giant cells were observed. This damage appeared to be temporary because regeneration was observed 100 days after the body temperature increase.

MacCleod and Hotchkiss (1941) investigated the effect of hyperthermia on human spermatogenesis. Body temperatures were artificially increased to 41°C in a fiber cabinet for 30 minutes. The effects on testicular tissue were observed in nearly three weeks and it was evidenced by a drop in sperm count.

Bowler (1967, 1972), using anesthetized rats, immersed the testis in a hot bath at 43.5°C for 20 minutes. This treatment was done one to seven times at 6 week intervals. Mating tests performed after exposure were negative. They regained fertility within 65 to 170 days after the last treatment. The rats that were heat treated seven times recovered in 170 days. The testicular tissue of infertile rats had seminiferous tubules that were severely damaged. This damage varied with the number of heat treatments. The percentage of severely affected tubules, in which there was destruction of the architecture, increased from 8% in rats treated twice to 28% in those treated seven times. In these tubules, spermatogenesis was arrested.

The length of exposure time at high temperature and its effect on testicular tissue was the subject of a study by Collins and Lacy (1969). They exposed the scrotal region of rats to 43°C. This was done in a progressive lengthening of time, ranging from 10-30 minutes in steps of five minutes. Exposure for 10 minutes caused little

damage. After exposure for 15 through 30 minutes, adverse effects in germ cells were evident and this was greatest in the extended treatment periods. The damage was observed at 3 days, 7 days, 3 weeks and 6 weeks. Setchell and Waites (1972) observed a decrease in sperm concentration in the testis of rats after exposure to a warm water bath at 41°C for 1.5 hours. This decrease in sperm cells production began between 6 to 10 days and lasted for 39 days. This was associated with a decrease in testis weight, which persisted even after the number of spermatozoa returned to normal.

Bedrak et al. (1980), studying the chronic exposure of male rats to high environmental temperatures (33-35°C) for 3 weeks, observed a reduction in sperm production and damage to the Sertoli cells.

Ruminants seem to be highly susceptible to high ambient temperature. Lindsay (1969) used rams of three different breeds, which were exposed to increments of environmental temperature, from 27°C to 43°C in a period of 4 weeks. A decrease in semen motility was observed in rams exposed to high temperatures.

Prolonged exposure to high ambient temperatures (32°C) causes a decrease in testicular weight of rams (Gomes et al. 1971).

Brown-Woodman et al. (1976) observed a reduction in

sperm motility and a significant increase in sperm with morphological abnormalities, such as detached heads and abnormal tails after two weeks of local heat application (40-40.5°C) to the scrotum of rams for three hours.

Seasonal changes in semen production and the occurrence of summer sterility in sheep have been reported (McKenzie and Berliner 1937). Moule (1948) studied fertility of sheep in Australia and observed that fertility was lower in the hottest areas.

Kumi-Diaka et al. (1981) checked the spermiogram of Bos indicus and Bos taurus bulls for 12 months in Nigeria. Seasonal variation did not have a significant effect in sperm cell concentration, sperm cell number, percentage live sperm and sperm cell abnormalities in the indigenous Bos indicus bulls. However, a significant seasonal variation in Bos taurus bulls was found. This seasonal variation was characterized by significantly higher sperm abnormalities, lower percentage of live sperm, and lower sperm cell numbers in the hot periods. Godinho and Cardoso (1980) suggested that the different abilities between Bos taurus and Bos indicus breeds to cope with high environmental temperature may not be associated with the recto-testicular gradient, but to the functionality of the individual components of the scrotal system such as density and morphology of sweat glands.

DeAlba and Riera (1966) studied the sexual maturity and spermatogenesis in cattle under elevated environmental temperatures. Jersey bull calves with an average age of 26 weeks were subjected to 35-36°C ambient temperature and 80-90% relative humidity from 7 AM to 3 PM daily for 303 days. They did not show lack of libido, or delayed puberty, but at an older age, the ejaculate showed very low motility, the highest being 20%. The percentage of abnormal spermatozoa was 80% in treated cattle, compared to 42% in control animals. The former also had smaller testicles and spermatogenesis was arrested. The accessory glands had an increased size.

Skinner and Louw (1966) established that even a short period of 12 hours of exposure to high environmental temperature (40°C) was sufficient in bulls to affect optimum spermatogenesis. The degree of damage varied between Bos indicus and Bos taurus cattle, being higher in Bos taurus. There was an increase in the number of primary abnormalities as well as a decrease in sperm motility.

According to Swierstra (1970b), temperature is one of the most critical factors of the physical environment for pigs, because pigs have a relatively inefficient thermoregulatory system.

Seasonal variation in semen quality has been observed in boars. Lawrence et al. (1970) observed an increase in

abnormal semen after the summer. Kazantseva (1971) showed that ejaculate volume, sperm concentration and conception rate are adversely altered by the high temperature during the summer months.

Mazzarri et al. (1968) kept boars at constant elevated temperatures (35°C) for 4-8 weeks or applied local heat to the scrotum until the testicular temperature reached 40.5°C for 3 hours. The ejaculate volume was not affected in either case. However, sperm count, percentage of motile spermatozoa and fertility were negatively affected. The damage was greatest, when heat was applied directly to the scrotum. The fecundating capacity of the sperm was absent between days 37 and 57 post treatment, a period of minimal sperm motility. This adverse effect was magnified when daylight was increased from 10 to 16 hours.

McNitt and First (1970) found in a study on the effects of short term exposure to hot environments, that exposure of boars to 33°C and 50% relative humidity for 72 hours produces a reduction in sperm motility. In conjunction with this reduction in overall motility, a drop in progressive motility was observed between 20 and 28 days post treatment. These two parameters returned to control levels by day 44 post treatment. The percentage of primary abnormalities began to rise by day 12 and reached a maximum by day 28 and appeared to decrease by day 44. The most

frequent abnormalities observed were coiled tail and abnormal heads. Secondary abnormalities such as detached heads were very frequent. Along with these findings, Einarsson and Larson (1982), using a singular temperature (35°C) and 40% relative humidity for 24 hours or 100 hours, reported that the percent of motile sperm decreased in boars exposed for 100 hours starting day 16 afterwards and returning to control values by day 41. No change in sperm motility was observed in boars exposed for 24 hours. Morphological abnormalities were not significant in 24-hour exposed boars, but were significantly increased in boars that were exposed 100 hours. The main abnormalities were abnormal heads and proximal droplets with maximal numbers occurring by 3 weeks after exposure. Recovery was observed by 6 weeks after exposure.

Cameron and Blackshaw (1980) heated boars 6 hours/day (33.4 $\pm$ 3.1 - 37 $\pm$ 2°C and relative humidity 40-80%) for 4, 5 or 7 days. Ejaculate volume, gel volume, sperm concentration and sperm output were not affected in any of the different time exposures. Although a significant increase in morphologically abnormal sperm cell was seen in all groups at the end of week two.

Wettemann et al. (1976) reported that boars, which were heat stressed at 34.5°C for 8 hours per day for 90 days, showed an increase in mean rectal temperature (39.2°C)

during the first week, then it dropped to 38.7°C for the remainder of the time. Ejaculate volume and gel weight per ejaculate were similar to the controls kept at 20°C. There was a significant alteration of the morphological characteristics of the semen. During the first week of treatment, motility (79.5%) and acrosomal structure abnormalities (12%) were similar between control and heat stressed animals. Within two weeks, control animals showed 83.3 $\pm$ 4% sperm motility and in heat stressed boars it dropped to 65.8% $\pm$ 19.3. From week three through 6 weeks, sperm motility in control boars was 85.3 $\pm$ 3.7% and 46 $\pm$ 3.7% in heat stressed ones. Abnormal sperm cell numbers increased by the second week and sperm output was reduced from week two through six. In fertility tests, only 28.6% of 77 gilts bred with semen from heat stressed boar conceived, compared to 42% of 88 gilts bred with control boars.

Stone (1981/1982) used a different approach to experimental heat exposure. He exposed boars to a gradual increasing thermal treatment. The air temperature was increased (1°C) per day during 20 days, from 20°C to 40°C. Sperm motility dropped when air temperature reached 30°C. It was significantly lower than pretreatment levels at 32°C, remaining low for a further 7 weeks and was lowest (19%) at 3 weeks after heating. The proportion of abnormal sperm types increased in response to an increase in air

temperature but returned to normal by 7 weeks after treatment. The most common abnormalities were proximal droplets, narrow heads and tail abnormalities. They were mainly seen two to four weeks after treatment.

Thermolability of germinal cells seems to vary according to the species and to the stage in the spermatogenic process. B-type spermatogonia of rams, accumulated in mitotic division and died in metaphase or late prophase after 12-48 hours of heat exposure (40 $\pm$ 0.2°C for 140-150 minutes). (Waites and Ortavant 1967). In the rat, spermatogonia are thermoresistant when they are at rest (spermatogonia A), although very sensitive when in mitotic division.

Mazzarri et al. (1970) cited by VanDemark and Free (1970) reported that spermatogonia in the pig were unaffected by heat treatment. This finding was confirmed by Wettemann and Desjardins (1975, 1979) who reported that A-type spermatogonia were not the sensitive cells. Spermatogonia were the most resistant cells in testicular tissue of bulls, in which cryptorchidism was induced (Kellaway et al. 1971). Bowler (1972) observed that repeated application of a hot bath at 43°C to the rat scrotum can cause destruction of stem cells.

Certain cell stages of the meiotic process seem to be thermosensitive. VanDemark and Free (1970) in a review suggested that the prophase appears to be specially

thermolabile. Collins and Lacy (1969), in a thorough study of the cell damage in the seminiferous tubule of rats caused by heat, varied the length of time the scrotal region was exposed to heat. Application of 43°C for 10 minutes did not cause damage, whereas, treatment for 15 minutes produced adverse effects that progressed as the time of exposure was extended. With an exposure time of 15 minutes, the early pachytene spermatocytes showed cytoplasmic alteration by 24 hours but by 3 days later these alterations had disappeared. This damage was more obvious as time of exposure was increased up to 30 minutes. Waites and Ortavant (1967) observed that pachytene spermatocytes were affected by temperature exposure in the ram.

In the case of the boar, McNitt and First (1970) deduced that according to the spermatogenic cycle, the damage caused by heat probably occurred in late primary and secondary spermatocytes. Wettemann (1975, 1979) reported that preleptotene spermatocyte as well as pachytene spermatocyte was not affected by exposure to 34.5°C for 8 hours or 31 $\pm$ 1°C for 16 hours. Spermiogenesis is readily affected by elevated environmental temperature and by direct application of heat. Other effects of elevated temperature are seen in spermatids and ultrastructure of the spermatozoa. Acute thermal exposure of the rat scrotum (43°C) causes damage in the Golgi phase of spermatids disappearing

at 3 days after exposure. If exposure time was increased, the spermatids could not reach the acrosomal phase. When exposure lasted 30 minutes, they failed to progress into the Golgi, cap and acrosomal phases (Collins and Lacy, 1969).

Chowdhury and Steinberger (1970) showed that young spermatids are affected very early after heat exposure. They observed irregular nuclear size and shape within 1 hour, with no visible change in the cytoplasm. This damage was progressive with time. By four hours after exposure the nucleus was smaller and had a "ring" appearance and at 24 hours, young spermatids had disappeared.

Voglmayr et al. (1971) studied the metabolic activity and ultrastructure of the ram spermatozoa after heating of testis at 40.6°C for 3 hours. Alteration of the morphology of testicular spermatozoa, characterized by dearrangement of the cytoplasmatic droplet, was observed; the cytoplasmic droplet fine tubular elements disappeared and displayed a great number of vesicles. The mitochondrial matrix was more homogenous and the membranes were difficult to define. The cell membrane in the neck and mitochondrial region showed many discontinuities. The metabolic activity of the spermatozoa experienced a severe depression. Glucose utilization and O_2 uptake were depressed the first day. As a result, respiratory activity was impaired.

Sperm lipids and inositol decreased after heating. Since they seem to be the source of energy, their depletion may lead to mortality of the sperm cell in the epididymis. Using a similar treatment, Brown-Woodman et al. (1976) found a reduction in O_2 uptake and no effect on glycerol utilization. However, within one or two weeks, glycerol breakdown and lactic acid production were greatly inhibited. The morphological changes in the spermatozoa appeared after two weeks. The plasma membrane was rolled by 1 week after treatment, by 3 weeks it was broken, and by 5 weeks, it was absent from the nucleus, tail and mitochondrial region.

Williamson (1974a,b), in order to study the ultrastructural changes in the spermatozoid, immersed the ram's scrotal testis in a hot bath at 40.5°C for 2 hours. At 72 hours post treatment, round spermatids showed separation of the flattened acrosome vesicle from the nucleus. The elongated spermatids showed vacuolation of the nucleus, and a twisted acrosome that degenerated. At 96 hours, a large proportion of spermatids had vacuolated and distended rostral extension of their acrosomes. By 10 days after treatment, most seminiferous tubules showed extensive damage. Testicular spermatozoa were present in large numbers, but half of them with distended acrosomes. When the

acrosomal damage was studied in the ejaculate, three main acrosomal disruptions were detected. These included swelling, vesiculation and eventual disintegration of the acrosome, pronounced swelling of the rostral acrosome and distorted and misshaped acrosome that was separated from the nucleus. They were mainly observed 14-15 days post treatment with the first type being the most common abnormality. Before 14 days, the sperm fertility was not depressed.

Wettemann et al. (1976) observed that as a sequel of exposure to high environmental temperature 34°C for 90 days, the number of spermatids was decreased. This was in the order of 50% (Wettemann and Desjardins 1979). Stone (1981/1982) utilized the time of spermatozoid sojourn through the epididymis to conclude that the changes observed in the ejaculated sperm appearing at 9 to 10 days of exposure, reflect the effect of heat on the epididymal spermatozoa. Moreover, the high incidence of cytoplasmic droplets is consistent with heat effect on the maturation of the spermatozoa in the epididymis.

A complete elucidation of the mechanisms wherefore the spermatogenic activity is disrupted has not been provided. The internal temperature of the testis *per se* seems to be one of the factors that causes direct damage to the germinal cells. In some of the studies previously mentioned, internal testicular temperature was observed

when the scrotal testes were heated or the whole body was exposed to experimental hot environments. Rock and Robinson (1965) recorded a shift in the scrotal-rectal temperature gradient from -1.6 to +1.2 when humans were immersed in a bath at 38-40°C. In rams exposed to scrotal heat at 39.5-40.3°C for 120 minutes, testicular temperatures rose to about 37.5°C (Fowler 1968).

Stone (1981) subjected boars to increasing environmental temperatures from 23 to 34°C. This caused a decrease in scrotal-rectal temperature gradient along with a testicular temperature of 37°C.

VanDemark and Free (1970) analyzed the different experimental heat treatments and concluded that direct application of hot water which would prevent evaporative cooling, resulted in a rapid change in deep testicular temperature. Whereas, a slow change usually takes place under conditions where environmental temperature is elevated or where heat is applied by a warm air current.

Temperature exerts a direct effect on testicular macromolecular synthesis. In vitro incubation of seminiferous tubules of mice testicles at 38.5°C for 48 hours did not affect protein synthesis. RNA synthesis was stimulated, and it was greater at higher temperatures, with optimal synthesis at 37°C; however, DNA synthesis was inhibited when temperatures were increased. Maximal DNA synthesis occurred

at 32°C. As a result of these observations, Nishimune and Komatsu (1972) suggested this characteristic of the testicular germ cells could be responsible for the thermal inhibition of germ cell differentiation. Lee and Fritz (1972) demonstrated that germinal cell from hypophysectomized and induced cryptorchid adult rats incubated in physiological pH at 37°C, released large quantities of hydrolytic enzymes from lysosome-rich particles, when compared to liver cells in the same in vitro conditions. They proposed this as a mechanism responsible for cellular degeneration and damage that occurred in cryptorchid and heat stressed males.

These thermally sensitive lysosomes are found in advanced seminal cells. Lee (1974) determined that spermatocytes and spermatids, but no other cells, incubated at the same conditions, released their lysosome content into the medium. This release was due to alterations in the permeability of plasma membrane of these cells. These results strongly suggest that this alteration of permeability account for some of the damage and disruption of spermatogenesis.

The effect of heating on blood flow in the scrotum and testicle has undergone some investigation. Waites and Setchell (1964) observed that there was no consistent

response in blood flow after heating ram testis at 39°C; in 75% of the animals, the blood flow was hardly affected. Waites et al. (1968) reported no change of testicular blood flow in rats at 40°C. Waites et al. (1973) in a more thorough experiment found no change in rat testis blood flow when exposed to 37-40°C but an increased flow at 43°C.

When chronic exposure was applied to rams, testicular blood flow decreased after one week at 32°C. This was accompanied by a thickening of the testicular arterial wall in the middle region of the pampiniform plexus which decreased the luminal space. PgF_2 alpha content of the testis was elevated. It was proposed that the reduction in blood flow could be a consequence of the vasoconstrictive properties of the PgF_2 alpha (Dutt et al. 1977).

Another possible damaging mechanism through which temperature may affect testicular functions is alteration of seminal fluid. However, local heating of rat testis did not decrease the secretion of seminal fluid per unit of weight, nor did it alter the concentration of inositol, glycine or potassium in the rat testis fluid. Total seminal secretion decreased after heating but this was probably due to a decrease in testis weight (Setchell and Waites 1972). Setchell et al. (1971) found that the flow of the fluid collected from merino rams when the testes were

heated at 40.5°C for 3 hours dropped during heating, but recovered afterwards. There was a decrease in sperm output that seemed biphasic; the initial drop occurred at about 20 days and a secondary drop occurred between 29 and 37 days.

When testis or any other tissue is exposed to high temperature, its metabolic rate increases (Setchell 1978b). The testis takes up half of the oxygen delivered by arterial blood under physiological conditions. The oxygen content of the venous blood falls as the testis temperature rises to 37°C. At higher temperatures, the oxygen content falls no further, therefore, the testis enters a self-induced hypoxia (Waites and Setchell 1964, Setchell 1978b). Since the capillaries do not reach the interior of the seminiferous tubules, the oxygen tension in their inner cell layers may be 7 mm Hg lower than that in the venous blood inside the capillaries. This is caused by oxygen consumption by the tubular cells and a limited rate of diffusion of oxygen through the tissue (Setchell 1978b). Concommitantly, with this increase in oxygen demand by the testis, blood flow through the testicles does not seem to increase (Waites et al. 1973, Damber and Janson 1978, Dutt et al. 1977, Waites et al. 1968).

The interstitial tissue appears hypertrophied after the testis has been heated or made cryptorchid; this is

probably due to a reduction in tubular size (Clegg 1961).

When boars were heat stressed for 90 days (34.5°C), the plasma testosterone concentration experienced certain changes. The first day, levels of plasma testosterone were not affected, however, after seven days they were significantly reduced and after 14 days the levels were only slightly decreased. Plasma testosterone returned to pretreatment levels and were normal from the third week through 13th week of exposure (Wettemann et al. 1976). In the same experiment, in vitro steroidogenesis was assessed. A suppression of androstendione, testosterone and dihydrotestosterone formation along with an increase in androsterone and androstonediol synthesis were the findings. This pattern of synthesis resembles the one observed in prepuberal androgen formation in the mice and rats.

Collins and Lacy (1974) heated rat testis at 43°C for 30 minutes without finding any alteration of the steroid metabolism by the interstitial cells in vitro. There was no difference in the ability of the Leydig (interstitial) cell to metabolize pregnenolone and progesterone to testosterone. Moreover, no change in accessory glands was observed.

Hagenas and Ritzen (1976) induced cryptorchidism in rats and after two weeks, the total amount of ABP (androgen

binding protein) was depressed significantly to a third of the control levels. This might indicate less ABP reaching the germinal cell and points to an alteration of the Sertoli cell function. In heat acclimatized rats (33-35°C for 3 weeks), the testosterone levels were reduced and at histological examination the Sertoli cells were affected (Bedrak et al. 1980).

When cryptorchid-made guinea pigs reached puberty, an accumulation of 4-androstendione similar to prepuberal testis was found (Hoschoian and Andrada 1975).

Gomes et al. (1971) recorded a decrease in testosterone in ram testis. In control animals, the concentration was 1.09 $\pm$ 0.52 mg/gm dry weight and in heat exposed at 32°C for two weeks the concentration was only 0.35 $\pm$ 0.15 mg/gm of dry weight. This was accompanied by a decrease in testosterone levels in testicular venous blood (82 $\pm$ 4.4 to 1.9 $\pm$ 0.6 following treatments). Rhynes and Ewing (1973) subjected bulls to 35.5°C environmental temperature and 50% relative humidity for seven weeks. Plasma testosterone levels were reduced, commencing at 24 hours after initiation of treatment. By the second week, testosterone levels had decreased to 50% and stayed at this level through the following two weeks. From this point there were erratic levels, but by week seven it had returned to 80% of control levels. Cryptorchid induced bulls showed plasma

testosterone levels 5 times lower than normal bulls, but compared with steers, the testosterone levels were higher, indicating some production of androgen by the interstitial cells (Kellaway et al. 1971).

The effects of cold on testicular function, either in humans or domestic animals, have not been studied in depth. Nevertheless, some experimental evidence has been gathered.

Direct cooling of the denuded rat testis in salted water at 2°C-0°C for one hour produced no change in the testis. When the temperature was dropped to -2°C over the same length of time, mild spermatogenic damage in tubules at the periphery of the testis was observed at seven and 14 days after exposure. As the temperature was dropped to -4°C or -5°C, moderate damage to spermatogenesis was observed at 3 hours, two days, four days and 14 days afterwards. Spermatogenic cell destruction only occurred when temperatures were dropped to -6°C and -8°C. Exposure of the scrotal testis, held in situ, to this -6°C or -8°C cold bath caused mild and moderate damage to the germinal epithelium, respectively. It was only after -10°C, for one hour, that severe damage was observed four days later. The testicular temperature was measured in these testes held at this temperature. The testicular temperature was 2°C. The tissue damage, in these experiments, was probably mediated through ischemia (MacDonald and Harrison 1954).

Rats exposed to low ambient temperature (2°C) showed migration of the testis to the abdominal cavity (cold induced cryptorchidism). After 12 months of exposure, there was no evidence of spermatogenesis. In extreme cases, the seminiferous tubules were completely disorganized and the germinal epithelium had disappeared (Dugal et al. 1962).

Perrault and Dugal (1962) exposed rats to -5°C for 25 days. Advanced state of testicular degeneration (alteration of germinal sequence) due to cold induced cryptorchidism was observed at 11 days and it was accompanied by a decreased testicular size. Endocrine function of the testis was apparently inhibited as was evidenced by reduction of the prostate and vesicular gland size similar to castrated animals. From these results, they concluded that the effect of cold affected both cellular and endocrine functions. These two effects were superimposed and additive.

Riar et al. (1979) subjected rats to low environmental temperatures (-5°C) and to simulated altitude of 6.060 m to recreate hypoxic conditions. This exposure lasted 6 hours per day for seven and 21 days. During treatment, the testes were retracted into the abdomen. The testis returned to a scrotal position 10-15 minutes after exposure. After seven days of exposure, the testis, epididymis and

vas deferens showed a significant reduction in wet weight. If the exposure time was extended, the reduction was even greater. The vesicular gland showed a significant drop in weight after 21 days of exposure. The number of viable spermatozoa dropped after 7 days of exposure, while abnormal and decapitated spermatozoa numbers increased. Histologically, the testis showed extensive vacuolation and considerable arrest of spermatogenesis. Metabolism in the testis was also altered. The testis showed an accumulation of cholesterol after 21 days, a fall in sialic acid content and an increase in alkaline and acid phosphatase. This accumulation of cholesterol indicates a drop in steroid synthesis.

Swierstra (1970a,b) studied the effect of low environmental temperature on sperm production, and semen characteristics on 8.5-8.7 month-old boars. Boars were kept in individual outside pens for 11 weeks (January through March). The mean daily temperature for January, February, and March were -18°C, -20°C, and -15°C, respectively. Semen collections were done every 72 hours. The boar exposed to low temperature showed more libido in comparison to the controls kept at 17°C. The boars of the former group produced larger semen volumes, but the sperm concentrations were less. Sperm motility did not differ between control and cold-stressed boars. Since sperm concentration was less but semen volume

was larger, he concluded that total sperm per ejaculate did not differ between the two groups. Testicular growth was not impaired. Quantitative testicular histology showed that daily sperm production per gram of testis was similar between the two groups. There were no reports of a morphological study.

C. Boar Spermatogenesis

The spermatogenic process has been divided into spermatocytogenesis and spermiogenesis. Spermatocytogenesis is the part of the cell division process that reduces the DNA content of the cell to one-half of somatic cells. Spermiogenesis is the progressive series of structural and developmental changes that result in the transformation of spermatids into spermatozoa (Garner and Hafez 1980).

Once spermatogonia have commenced the multiplication process, each step of spermatogenesis passes through a fixed and constant duration. A direct consequence of this is the close relation in which different generations of germ cell are found. In any given area, well-defined cellular associations or stages form a constant succession in time. In other words, in the seminiferous epithelium certain cells are only found in association with certain other cells.

In the boar, spermatogenesis is initiated as early as 60

days and in other animals by 94 days of age. The seminiferous tubules already contain pachytene spermatocytes and some elongate spermatids (Swierstra 1977).

Sperm production is a continuous process, the total number of spermatozoa is a function of testis size. Mature boars produce an average of about 24 million spermatozoa per gram of testis per day (Swiestra 1971).

Swierstra (1968) studied the cycle of the seminiferous epithelium using 19 m Ci of thymidine-methyl H^3. The cycle was divided into 8 stages according to the morphology and cytology of the germ cells and their relative position with the seminiferous tubule. Each stage is composed of a characteristic arrangement of cells, a cross section of the seminiferous tubule usually displays a single stage of the cycle. A review of these stages can be found in Swierstra (1968).

The duration of the cycle of the seminiferous epithelium was determined to be 8.6 $\pm$ 0.1 days. The time that spermatozoid takes to traverse the epididymis ranged from nine to 12 days with a mean of 10.2 days.

Using the assumption that in the boar as in some other species, spermatogenesis extends over four cycles of the seminiferous tubule, an estimation of 34.4 days was made for the duration of spermatogenesis in the boar.

Frankenhuis et al. (1982) used a different approach to the study of the kinetics of the seminiferous cycle. They developed a method in which the cycle of the seminiferous tubule was characterized according to the development of the acrosome or the shape of the nucleus of the spermatids. The cycle consisted of 12 stages. Swierstra (1968) classified spermatogonia into 3 groups based upon nuclear morphology, namely pale type A, and dark type A and B spermatogonia. Frankenhuis et al. (1982) classified the spermatogonia into 4 groups: Undifferentiated and differentiated spermatogonia, intermediate spermatogonia and B spermatogonia. The duration of the cycle was 8.6 days.

D. Electroejaculation in the Boar

The use of electroejaculation in large animals was first described by Gunn (1936). He utilized two different electrodes. One electrode was placed in the rectum and the other in the longissimus dorsi muscle of rams. A maximum current of 160 to 190 milliamperes at a frequency of 50 cycles per second was used. This kind of electrical stimulation provoked a very severe reaction and therefore adequate restraint was necessary.

Dziuk et al. (1954) improved the electroejaculation technique in bulls, goats, boars and rams. One improvement

was the use of a transformer which facilitated gradual changes in the voltage and a rectal probe that was made of a stiff rubber hose with alternate electrodes. The electrical stimulation was given in a progressive pattern, of increasing voltage for five seconds and returning to zero before the next stimulation until semen was obtained.

In the case of the boar, Dzuik et al. (1954) reported also that the collection was a noisy, drawn-out affair, resulting in a very contaminated sample of 1 to 8 ml. A current between 10 to 15 volts and 500 to 1000 milliampers was used.

Adams et al. (1969) and Clark (1976) reported the use of electroejaculation on boars. They improved the collection technique by anesthetizing the animals with barbiturate. This enormously facilitated the collection. Another feature was the use of 18-inch-long by 1.5 inches in diameter rubber rectal probe with six annular electrodes.

Basurto-Kuba (1979) and Evans (1980) more fully described the technique. Evans (1980) enumerated the advantages and disadvantages of electroejaculation in boars. Electroejaculation allows collection of semen from boars that are unable or unwilling to mate. It permits a thorough evaluation of the penis and testicles. The relative disadvantages are that the boar's libido and mating ability

cannot be evaluated but this can often be obtained from the boar's handler. Another concern is the possibility of death due to the anesthesia. However, according to Clark (1976), the margin of safety of the barbiturates (thiamylal) is excellent.

The quality of semen obtained by electroejaculation and with the artificial vagina has been shown to be comparable in the bull (Austin et al. 1961, Foote 1980, Gomes 1977). Basurto-Kuba (1979) and Basurto-Kuba and Evans (1981) have shown that the sperm-rich fraction of ejaculates obtained by electroejaculation and gloved-hand technique was similar. The percentage of live sperm, cell motility and number of abnormalities also did not differ when either of the two methods was used in the boar.

III. MATERIALS AND METHODS

Nine 6- to 8-month-old boars of various breeds (3 Duroc, 1 Hampshire and 5 Yorkshire) were used in this study. Their weights ranged between 104.5 kg to 188 kg, with an average of 141.2 kg.

These boars were purchased from a commercial piggery. All the boars were housed in individual pens, for the duration of the experiment, in the isolation area of the Iowa State Veterinary Teaching Hospital in Ames, Iowa. The pens were bedded with either sawdust or woodchips.

The animals were fed a commercial ration containing 14% protein, based on corn and soybean, with appropriate minerals and vitamins added.

Each single experimental subject went through the experiment individually.

A cool room was specially designed to hold one pig at a time during the cold stress period. The floor was covered with rubber footing and a light spread of woodchip bedding.

A. Experimental Groups

The nine boars were randomly assorted into three different groups (Table 1).

Table 1. Summary of the experimental procedures for each experimental group

Group	Thermocouple implanted	Vasectomy performed	Initial period at room T° (23±3°C)	Two days at 0±3°C	Ten days at -15±3°C	Fifteen days at room T° (23±3°C)
1	Yes	Yes	Yes	Yes	Yes	Yes
2	No	Yes	Yes	Yes	Yes	Yes
3	No	Yes	Kept at room temperature during the six weeks			

Group 1. (This group consisted of three Duroc boars):

The experimental subjects of this group underwent surgery to implant a Thermocouple[1] (thermistor) in parenchyma of the right testicle. This testicle was vasectomized to prevent its contribution to the ejaculate, thus, eliminating the reflection in the semen of any damage caused by the thermocouple.

After surgery, the boars were kept at room temperature (23 ± 3°C) for eight days. Then, they were moved into the cool room, pre-set at 0 ± 3°C and maintained at this temperature for two days. This pre-cold stress period was an attempt to allow some acclimation to colder temperatures which were to follow. Then, the temperature was lowered to -15 ± 3°C and held for 10 days. Following cold stress, the boars were returned to the pens and maintained at room temperature (23 ± 3°C) for another 15 days.

Group 2. (This group consisted of two Yorkshire and 1 Hampshire boars):

Subjects of this group were vasectomized on the right testicle but not implanted with a thermistor. The rest of the experimental procedure was the same as for Group 1.

[1]Standard Miniature Thermocouple, Thermo electric, Saddle Brook, NJ 07662.

Group 3. (This group served as a control and consisted of three Yorkshire boars):

This group was subjected to the same vasectomy as those in Group 2. The difference was that the boars of this group were maintained at room temperature throughout the experiment (6 weeks).

B. Temperature Readings

An ElpH[3] digital temperature indicator[1] was plugged into the thermistor cord to register the internal temperature of the testicle (Figure 1).

All the temperature readings were done daily between 9:00-9:30 AM. The animals were fed at the time the temperatures were taken to ensure tranquility of the animal.

The rectal temperature was taken at the same time, using a mercury-in thermometer. It was left in the rectum for 3 minutes.

C. Handling of the Subjects

All the boars in this study were fed twice a day. The amount of feed varied with the environmental temperature at which the animal was exposed during the different periods of the experiment.

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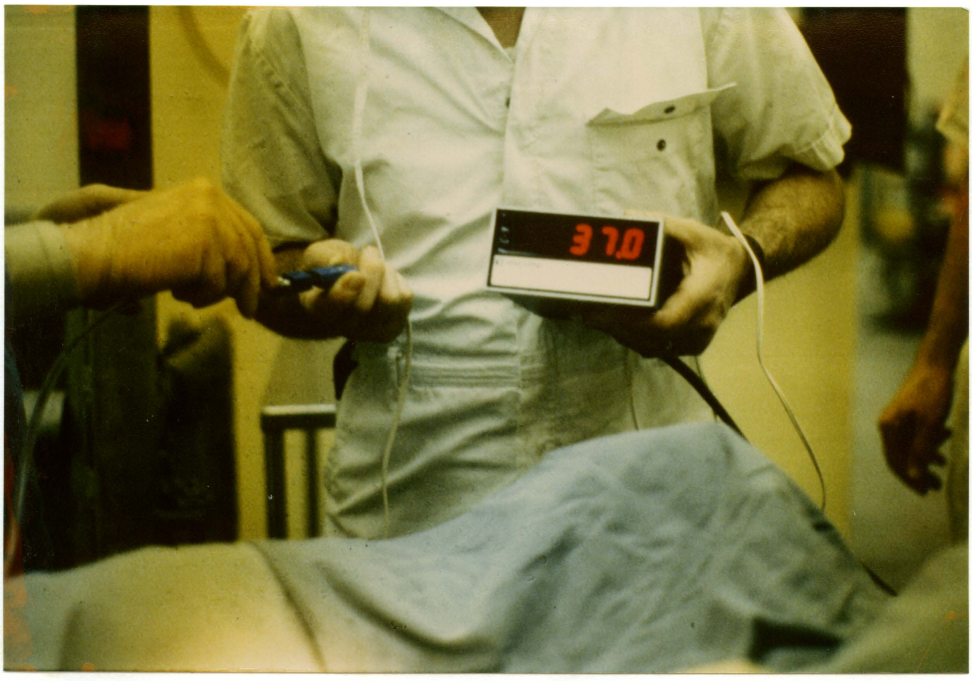
[1]Thermo Electric, Saddle Brook, NJ 07662.

Figure 1. ElpH^3 digital temperature indicator


37.0

While they were at room temperature, they were fed approximately 2.27 kg of the 14% protein ration per day. During the cold stress period, the boars were fed 4-5 kg a day.

Water was provided ad libitum when the animals were held at room temperature. In the cool room, they were watered twice a day.

During the 12 days of cold exposure, the boars were allowed to leave the cool room for 15 minutes to exercise. When boars were electroejaculated while being cold stressed, they were returned to the cold room only after complete recovery from anesthesia.

Artificial lighting was provided from 8 AM until 5 PM (9 hours) during the period of cold stress.

D. Semen Collection

Electroejaculation was used to collect the semen samples. Semen was collected every eighth day for a total of six times. The first one was done just prior to thermistor implantation and/or vasectomy. The duration of the experiment for each boar was six weeks.

The semen collections (electroejaculation) were done under anesthesia (thiamylal sodium[1]). One gram of thiamylal

[1]Surital^{R}, Park Davis and Co., Detroit, Michigan.

sodium was diluted in 10 cc of sterile physiological saline and administered I.V. at a dosage level of 1 gm/115-130 kg body weight. The boars were restrained with a snout snare and the anesthetic was administered either in the marginal ear vein or in the cranial vena cava. The total dose was injected as a bolus to produce a light plane of anesthesia.

Once the animal was anesthetized, a specially designed rectal probe of six electrode rings[1] was lubricated and inserted until the last ring was in the rectum. The penis was exteriorized by using an atraumatic forceps.[2] An electroejaculator machine (Pulsator II)[3] provided the electrical stimulation. The pattern of electrical stimulation was dependent upon the type of response in the individual boar. However, the general pattern was as follows. The lower voltage output was set to start the electric stimulation. The stimulus was held 5 to 6 seconds, followed by 5 to 6 seconds of rest. This allowed the boars to breathe 6 to 8 times between stimulations. A total of 4 to 5 stimulations within each voltage point were used. This was done in a progressive manner until an adequate volume (35-40 cc) of semen was collected.

[1]Boar probe, special order, Tracy Clark, Iowa State University, Ames, Iowa.

[2]Bozeman uterine forceps, Asisto Supply Co., New York.

[3]Pulsator II, Lane Manufacturing Inc., Denver, Colorado.

All materials used to handle the semen were prewarmed at 37°C. A warmed, 250 ml thermos was fitted with a plastic bag and the thermos opening was covered with a 4 x 4 gauze pad to separate the gelatinous material from the sperm-rich fraction.

E. Sample Evaluation

Once the semen was collected, it was taken to the laboratory and examined under light microscope.

Motility (percent live) and morphology of the ejaculate were evaluated.

1. Motility

This parameter was subjectively evaluated and graded in percentages using Basurto-Kuba's scoring method (1979).

Table 2. Scoring used for motility evaluation of the semen

Score	Description	% Alive
1	All spermatozoa nonmotile	0-30
2	Weak oscillatory motion	31-50
3	About equal proportion with progressive and oscillatory motion, 25% nonmotile	51-70
4	Most with progressive motion	71-90
5	Nearly all with high progressive motion	91-100

2. Morphology

The evaluation of this parameter was done by counting 100 sperm cells under 1000X (immersion oil), and recording the result in percentages. The sperm abnormalities were classified according to Hurtgen et al. (1977).

The technique used was to mix a small drop of neat semen and a drop of eosin-nigrosin stain[1] and smear it on a different slide. The slides were marked with the boar number, sample number, date and taped to the record sheet.

F. Surgical Technique

The anesthetic induction was obtained by using thiamylal sodium (Surital^{R}) at the same dose used for semen collection. Once the animal became recumbent, electroejaculation was performed. Then, they were connected to an inhalation anesthesia machine and anesthesia was maintained with halothane (Fluothane^{R})[2]

The right flank, thoracolumbar region and inguinal area were clipped and surgically scrubbed with Betadine[3]. The animal was placed in dorsal recumbency and draped, leaving as the surgical site, the prescrotal area between the hind limbs where the right testicle would be pushed cranially. A

[1]Morphology stain. Society for Thermiogenology Assn., 9th and Minnesota, Hastings, Nebraska 63901.

[2]Fort Dodge Laboratories Inc., Fort Doge, IA 50501.

[3]Betadine. Purdue Frederick Company, 50 Washington St., Norwalk, Conneticut 06850.

small skin incision of approximately 4-5 cms was performed over the cranial end of the right testicle. The subcutaneous tissue was dissected to expose the tunic. The tunic was incised exposing the testicle and epididymis. Once this was accomplished, the vas deferens, which is located dorso-medially to the testis, was exteriorized and ligated with two sutures 3 cm apart.

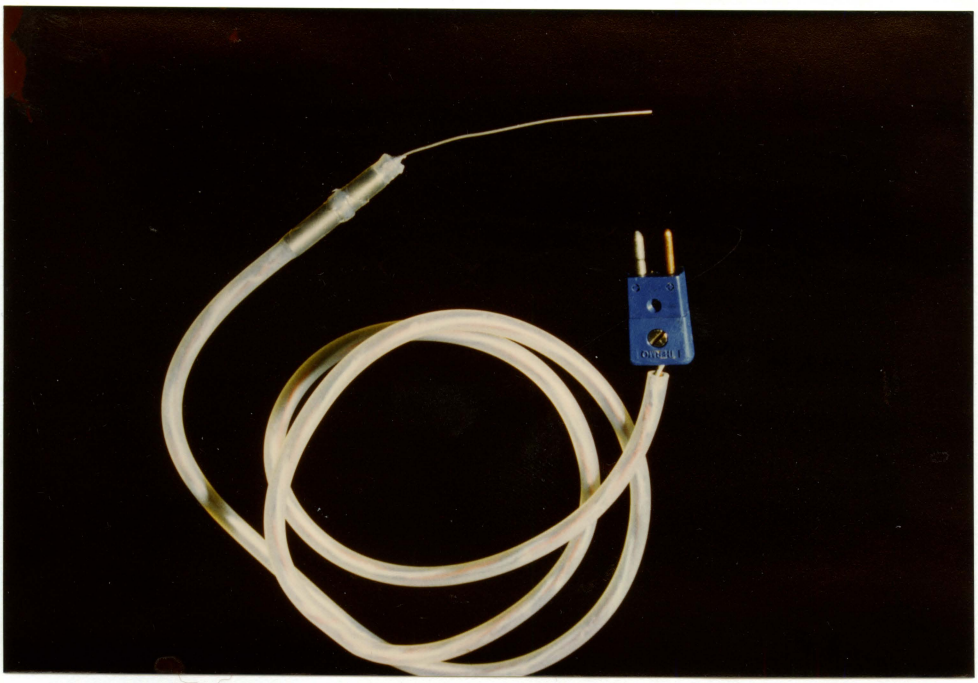
The epididymis-testicular attachment was grasped with an Allis' tissue forceps and the testicle was slightly rotated laterally in order to expose the ventromedial aspect of the testicle which is of lower surface vascularity. A stab-incision with a straight needle was made on this spot to serve as a channel cut for the thermistor. In this way, the thermistor was embedded in the testicular tissue with the tip of the thermistor embedded deep in the testis. The thermistor base was sutured to the epididymo-testis attachment, using two 4.0 stainless steel sutures.[1] The tunic was closed with a continuous suture of 2.0 medium chromic gut.[2]

The electrical cord, which had been coated with silastic tubing in order to avoid tissue reaction (Figure 2),

[1]Surgical steel. Ethicon Inc., Soherville, NJ 08876.

[2]Haver-Lockhart, Shannee, Kansas 66201.

Figure 2. Thermocouple and electric cord coated with sylastic tubing


OMEGA

was placed in a sterile, plastic-obstetric glove and subsequently the animal was turned to a left lateral recumbency so that the corresponding flank and the thoracolumbar area could be surgically scrubbed again and draped. Three small skin incisions were made in this area, in order to make a subcutaneous channel to bring the cord to the thoracolumbar area. A hollow metal tube was passed from the flank skin incision, to the prescrotal area and the thermistor cord was passed through this tubing and then the tube was withdrawn. All skin incisions were closed using Vetafil.[1]

A similar procedure was used to pass the thermistor cord from the flank to the lumbar area.

The thermistor cord was fitted with a terminal connector and the end was protected by a plastic bag glued to the skin.

G. Testicular Sample Collection

All animals were castrated immediately after the last semen sample was collected. The testicles were cut midsagittally and a piece of tissue was removed from a

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[1] Supramid Extra. S. Jackson, Inc., Washington, D.C. 20014.

testicular region that Swierstra (1968) demoninated Locus B, located midway between the poles.

The 5 mm tissue sections were fixed in Bouin's fixative and prepared by routine histological procedures. Sections were stained with Feulgen, in order to facilitate a better visualization of the nucleus (Frankenhuis et al. 1982).

To evaluate the effect of cold stress on testicular tissue, only the tissue sections from the left testicles were used. This testicle remained untouched whereas the right testicle had vasectomy and sometimes thermistor implantation.

The approach to evaluation of testicular damage was to assess fifty cross-sections of seminiferous tubules. Three different cytological characteristics, indicative of alteration of the spermatogenic process, were quantitated.

1. Number of elongated spermatids per cross-section. These elongated spermatids were at a stage of the spermiogenesis which was from the mid-acrosome phase to two-thirds of the maturation phase (Leblond and Clermont 1952).

2. Presence and number of giant cells in each cross section.

3. Evaluation of the histopathological status of the germinal epithelium required a subjective criterion based on the degree of damage to the seminiferous tubule.

Classification according to the damage or integrity:

Type I: The normal structure of the seminiferous tubule is altered, vacuolated cells are present, as well as debris in the lumen. In some areas cells are missing.

Type II: Partial destruction of the architecture of the seminiferous tubule, accompanied by desquamative changes.

Type III: A complete destruction of the architecture of the seminiferous tubule, only one cell layer is commonly present.

Normal: Normal seminiferous tubule. No disruption to the architecture of the tubule.

A light microscope was used to count the cells. A computer program and cell counting device[1] allowed individualization of the cell as well as the recording of the numbers.

H. Statistical Analysis

Much of the experimental data gathered in this study were analyzed statistically. A least-squares analysis of variance was used for analysis of testicular temperatures. Analysis of variance of split plot and contrasts were utilized to compare semen samples in Groups 1 and 2, as well as among Groups 1, 2 and 3. Therefore, thermistor implantation and/or vasectomy, animal (thermistor implantation and/or vasectomy), collection, collection thermistor implantation

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[1]BioQuant II, 6301 Robertson Avenue, Nashville, Tenn.

and/or vasectomy and collection animal (thermistor implantation and/or vasectomy) were evaluated. In the evaluation of histological characteristics of the testicular samples, an analysis of variance and contrast were used.

IV. RESULTS

A. Testicular Temperatures

Daily testicular temperatures were recorded and evaluated according to periods of the experimental process. The first period started the day after surgery and lasted until the experimental subjects were placed in a cooler. The environmental temperature in this period was 23 ± 3°C. The second period comprised 10 days in which the boars were cold stressed at a temperature of -15 ± 3°C. The third period consisted of two weeks post-cold stress at 23 ± 3°C.

All testicular and rectal temperature readings from the three animals in Group 1 are plotted individually in Figures 3-5, for each boar.

In the first period, there was an increase in testicular temperature in Boars 1 and 3 for two days after surgery, but testicular temperatures returned to normal for the remainder of the first period. In the second period, the testicular temperatures of the three boars showed a dramatic increase, reaching an upper value of 43°C in Boar 3 for one reading. In Boar 1, the maximum value reached was 41.9°C and in Boar 5 the maximum temperature was 42.0°C.

The increase of testicular temperature in this period and the remaining of rectal temperature at normal level caused an inversion of the recto-testicular temperature

Figure 3. Testicular and rectal temperatures of Boar 1 at different environmental temperatures

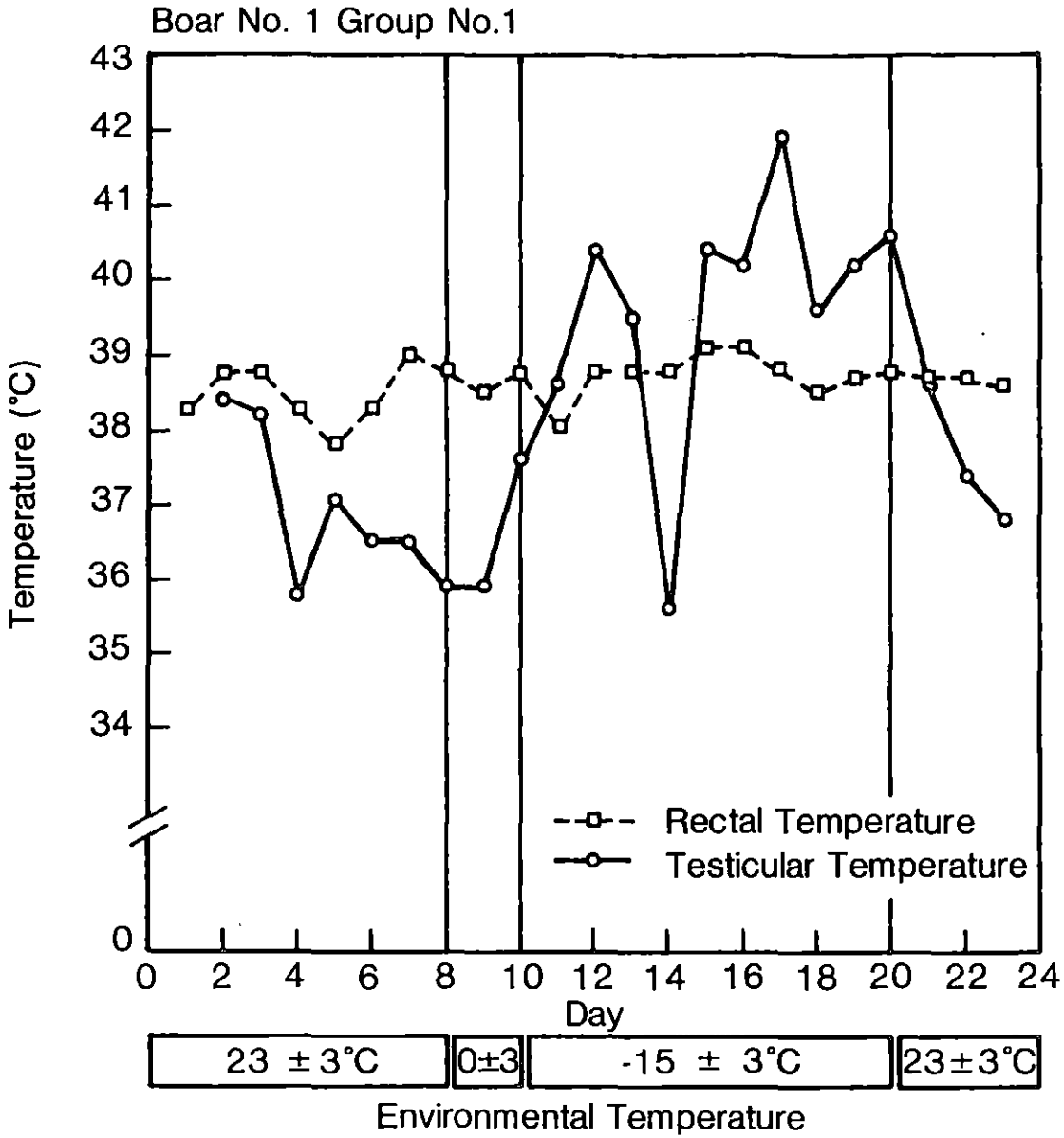

Boar No. 1 Group No.1
Temperature (°C)
43
42
41
40
39
38
37
36
35
34
0
Rectal Temperature
Testicular Temperature
0 2 4 6 8 10 12 14 16 18 20 22 24
Day
23 ± 3°C
0±3
-15 ± 3°C
23±3°C
Environmental Temperature

Figure 4. Testicular and rectal temperatures of Boar 3 at different environmental temperatures

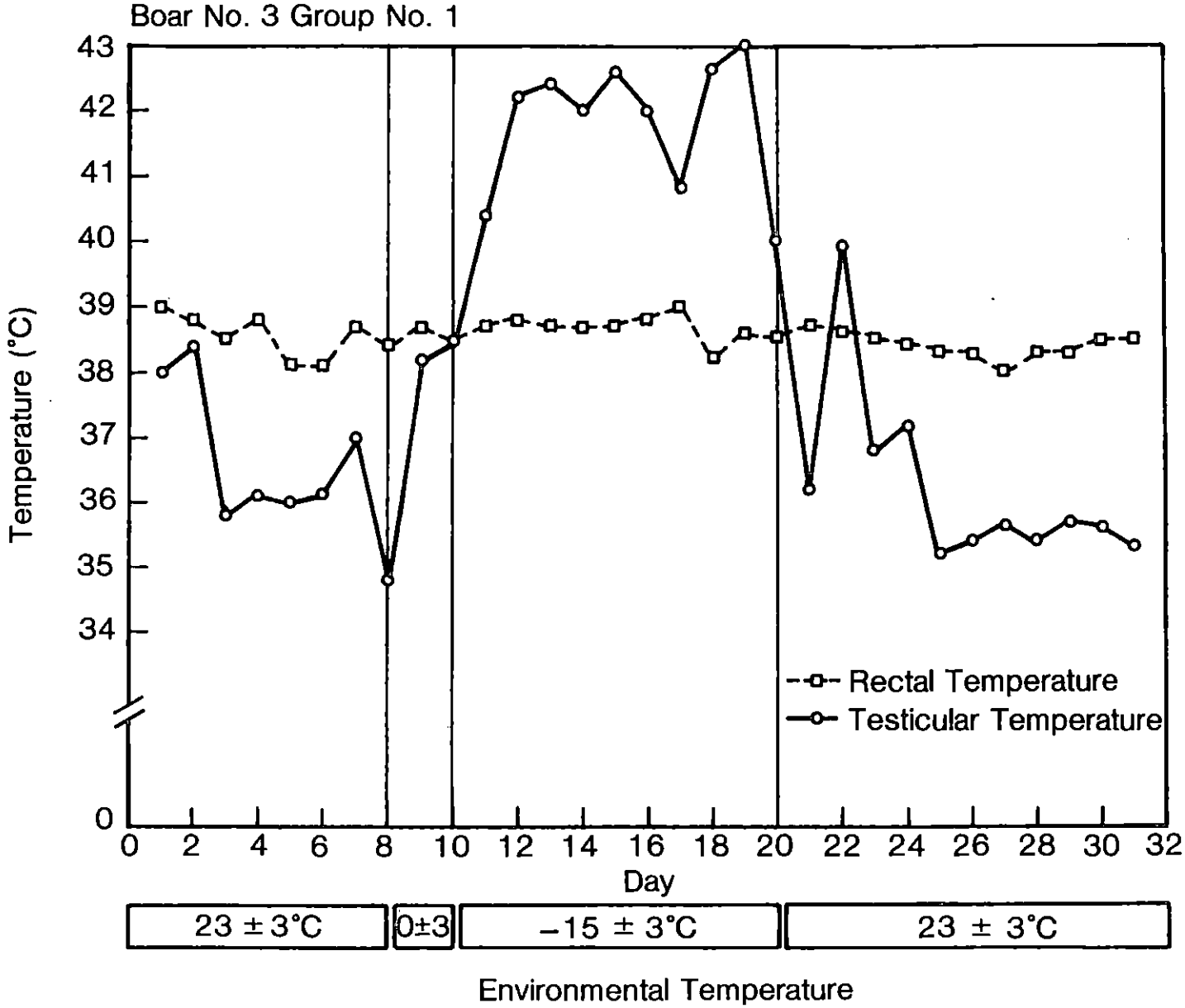
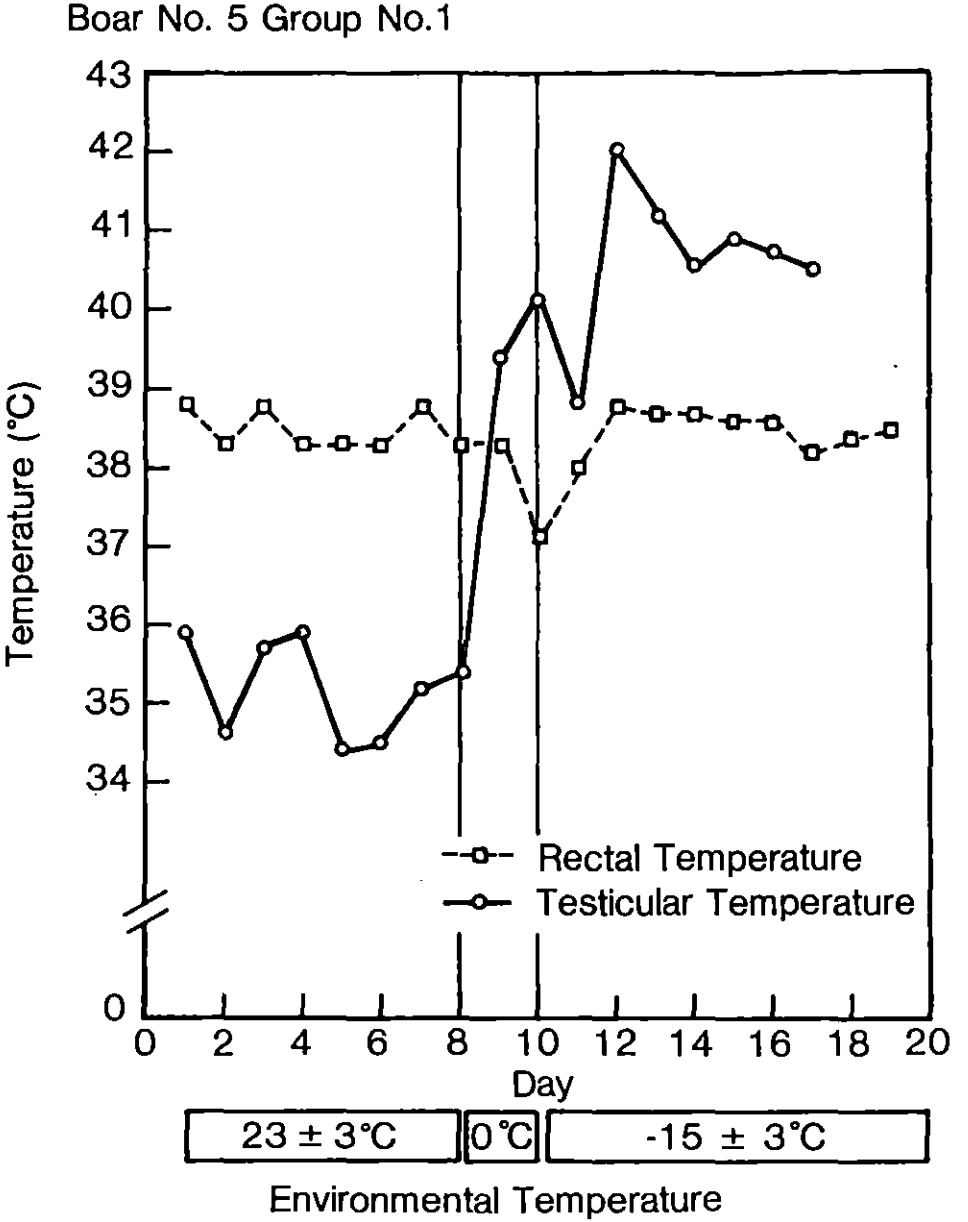

Boar No. 3 Group No. 1
Temperature (°C)
43
42
41
40
39
38
37
36
35
34
0
0 2 4 6 8 10 12 14 16 18 20 22 24 26 28 30 32
Day
Rectal Temperature
Testicular Temperature
23 ± 3°C
0±3
−15 ± 3°C
23 ± 3°C
Environmental Temperature

Figure 5. Testicular and rectal temperatures of Boar 5 at different environmental temperatures


Boar No. 5 Group No.1
Temperature (°C)
43
42
41
40
39
38
37
36
35
34
0
Rectal Temperature
Testicular Temperature
0 2 4 6 8 10 12 14 16 18 20
Day
23 ± 3°C
0°C
-15 ± 3°C
Environmental Temperature

gradient from +2.6°C to -2.1°C.

The testicular temperature in the third period changed towards pre-cold stress values, and returned to normal levels in 2 boars.

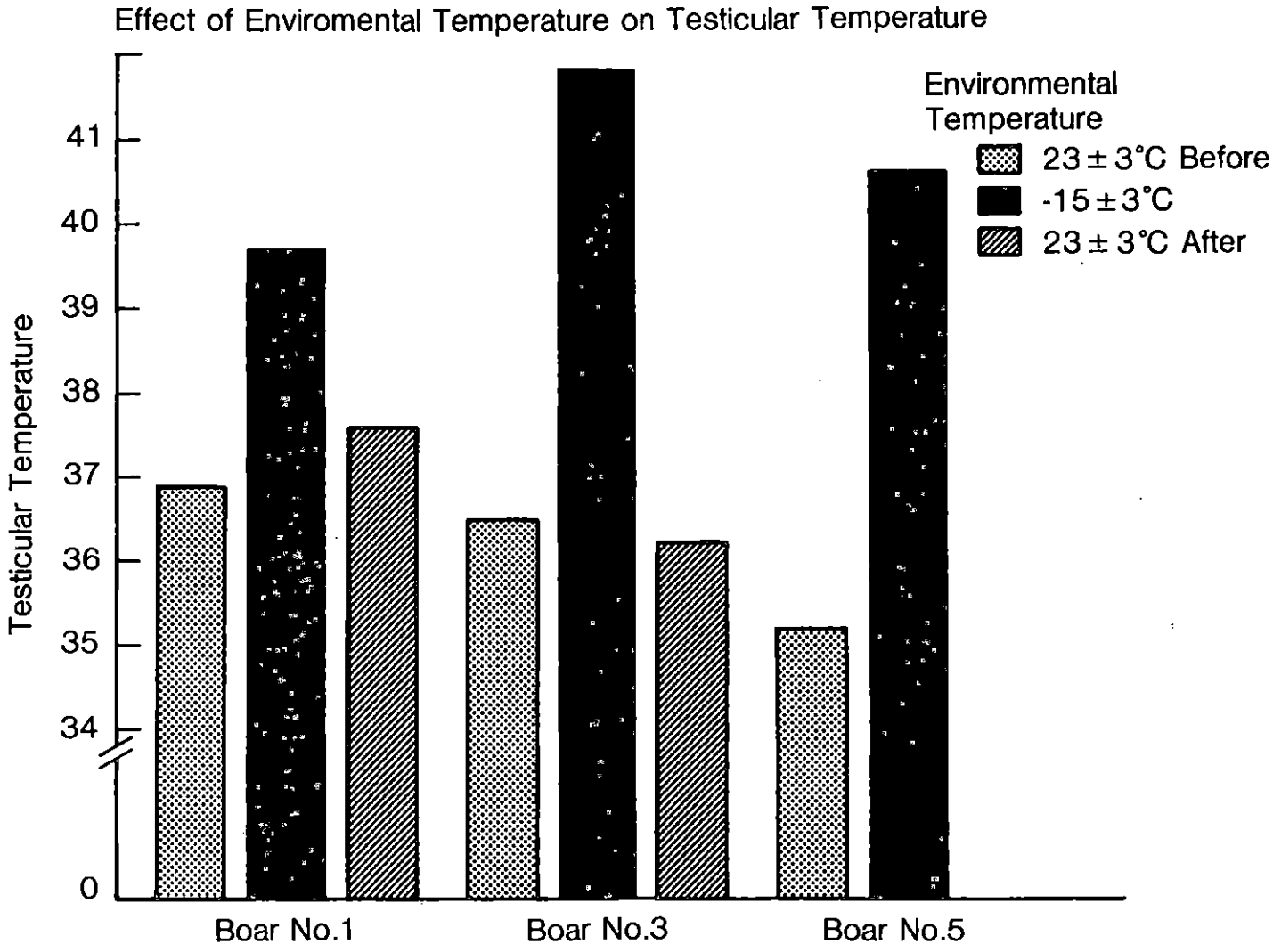
Figures 3 through 5 show the recorded temperature values after cold stress until the thermistor stopped recording due to breakage. Breakage was ascertained at castration.

Figure 6 shows the mean testicular temperatures for Boars 1, 3 and 5. In the first period, the mean testicular temperatures for Boars 1, 3 and 5 were 36.9°C (35.9°C-38.4°C), 36.5° (34.8-38.4°C), and 35.2°C (34.4-35.9°C), respectively. The overall mean for this period was 36.2°C. The mean testicular temperatures for the 3 animals in the second period were 39.7°C (35.6-41.9°C), 41.8°C (40.3-43.0°C) and 40.7 (38.8-42.0°C), respectively. The mean testicular temperature for the 3 animals in this period was 40.7°C. For the third period, the mean testicular temperature for Boars 1 and 3 were 37.6°C (36.8-38.6°C) and 36.3°C (35.3-39.9°C), respectively. Boar 5 did not have thermistor recordings for period 3, but a missing plot value of 36.6°C was assigned.[1]

When the overall mean testicular temperature of the three

[1]This value was obtained as a missing plot by the formula deyeloped by F. Yeats, 1933.

Figure 6. Graphic illustration of the effect of different environmental temperatures on testicular temperature. Comparison among mean testicular temperature in each period were made


Effect of Enviromental Temperature on Testicular Temperature
Testicular Temperature
41
40
39
38
37
36
35
34
0
Environmental Temperature
23 ± 3°C Before
-15 ± 3°C
23 ± 3°C After
Boar No.1
Boar No.3
Boar No.5

periods were compared utilizing a least square mean analysis of variance, significant differences ($P<0.05$) between the first period and the second period and between the second and the third period were found (Table 3). However, there was not a statistically significant difference between the first and the third period ($P<0.05$).

Table 3. Analysis of variance of testicular temperatures at different environmental temperatures

Source of variation	Degrees of freedom	Sum of squares	Mean square	F-value
Animal	2	1.35	0.67	0.52[a]
Treatment	2	36.97	18.48	14.36*
Error	3	3.86	1.28	

[a]This F-value is indicative that there is no individual effect.

*P<.05.

B. Rectal Temperature

Mean rectal temperatures for boars in Group 1 were 38.5°C, 38.6°C and 38.4°C, respectively, over the three temperature periods while the mean rectal temperatures for boars in Group 2 were 38.7°C, 38.4°C and 38.5°C for the same temperature periods.

Rectal temperatures of animals in Groups 1 and 2, which were subjected to cold stress, showed no significant dif-

ferences among the three periods. Rectal temperatures in Group 3 (not cold stressed) were not significantly different from Groups 1 and 2. All the rectal temperatures were within physiological levels, demonstrating the capacity of homeothermia in boars that were cold stressed. Daily rectal temperatures are recorded in the Appendix.

C. Scrotal Behavior

The scrotum of boars in Groups 1 and 2 was completely relaxed and relatively smooth in the first and third periods. In the second period, when the environmental temperature was lowered to $-15 \pm 3°C$, the scrotum underwent a decrease in size and had a contracted and wrinkled appearance. This contraction brought the testicles in closer apposition to the body wall (Figure 7).

D. Effect of Cold Stress on Semen Quality

1. Motility

Results of the motility study are listed in Table 4.

There was a tendency for motility of sperms in the semen samples to decrease from the third collection onwards in Group 1 and 2, which were cold-stressed boars. In Group 3, the trend was different, it showed a tendency of increased motility after the third collection.

According to Hurtgen et al. (1977), a semen sample is re-

Figure 7. Scrotal behavior: the scrotal contraction caused by low temperatures has brought the testicles to apposition with the body wall

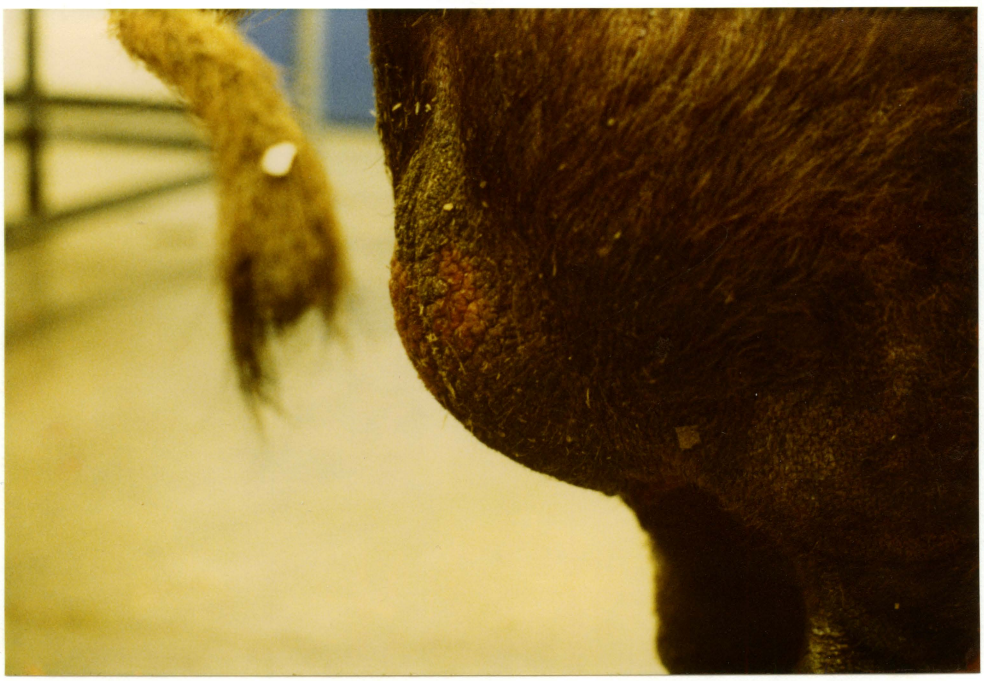


Table 4. Comparison of motility between collections and groups

Group No.[a]	Collection[b]	Motility (%)	Scoring
1	1	83	4
	2	90	4
	3	66	3
	4	68	3
	5	56	3
	6	65	
		$\bar{X}$[c] = 71	
2	1	81	4
	2	80	4
	3	73	4
	4	73	4
	5	81	4
	6	76	4
		$\bar{X}$ = 77	
3[d]	1	76	4
	2	81	4
	3	75	4
	4	86	4
	5	88	4
	6	90	4
		$\bar{X}$ = 83	

[a] 3/group.

[b] pre-cold stress collections: 1 and 2 ($\bar{X}$ = 83.5), post cold stress collections: 4, 5 and 5 ($\bar{X}$ = 69.6).

[c] Std. error = 2.20.

[d] Control group.

garded as poor quality when motility of the sperm is lower than 70%. This occurred only in Group 1.

When the results of sperm motility in Group 1 (thermistor implantation and vasectomy) and Group 2 (vasectomy) were compared, the effect of the surgical procedure on sperm motility was not statistically significant (Table 5). However,

Table 5. Analysis of variance of semen motility between collections of Groups 1 and 2[a]

Source of variance	Degrees of freedom	Sum of squares	Mean square	F-value
Model	35	5118.75	146.25	-
Group	1	306.25	306.25	4.20
Animal (Group)	4	291.66	72.75	-
Collection	5	1539.58	307.11	3.17*
Group - Collection	5	1039.58	207.91	2.14
Animal - Collection (Group)	20	1941.66	97.80	-
Corrected Total	35	5118.75	-	-

[a]Contrast before vs. after cold stress - $P<.05$.
Contrast first two samples vs. last two samples - $P<.05$.

*$P<.05$.

Table 5 also shows that there was a significant difference among collections ($P<0.05$). The comparison of the motility of sperms in the semen samples before cold stress with the

ones after cold stress revealed a significant difference ($P<0.05$).

Comparison of the first two semen samples collected before cold stress and the last two semen samples (after cold stress) also showed a significant difference ($P<0.05$) of sperm motility.

Analysis of the effect of the different treatments in the three groups showed that motility of sperms in the semen samples was significantly lower ($P<0.05$) in the boars that were cold stressed (Groups 1 and 2) than in the ones maintained at room temperatures (Group 3) (Table 6).

Table 6. Analysis of variance of semen motility among groups[a,b]

Source of variance	Degrees of freedom	Sum of squares	Mean square	F-value
Model	23	6006.42	261.15	2.98
Group	2	1167.59	583.15	6.66*
Animal (Group)	6	1669.94	278.24	3.17*
Collection	5	814.81	169.96	1.68
Group - Collection	10	2354.62	235.46	2.69
Error	30	2630.55	87.68	
Corrected Total	53	8637.03		

[a]Contrast cold stress vs. noncold stress - $P<0.05$.

[b]SD = 9.36.

*$P<.05$.

2. Morphology

The morphological characteristics of semen in the different collections are listed in Table 7.

Using the criteria of Hurtgen et al. (1977) to evaluate the alteration of the morphology of the semen, each primary type of abnormality was summarized individually.

Spermatozoa with proximal droplets increased above a level regarded as acceptable for good semen quality in Boar 5, collections 4 and 6, in Boar 2, collection 5, and in Boar 9, collections 2 and 3. The pattern of presentation of this primary abnormality was irregular. Abnormal heads observed above the 5% level were regarded as a sign of poor semen quality in Boar 1, collections 4 and 6, in Boar 2, collections 4 and 5, and in Boar 8, collection 1. The appearance of this primary abnormality did not seem to be due to the cold stress. The percentage of coiled tails was elevated in Boar 9 at collection 1.

In order to evaluate statistically the effect of cold stress in the morphology of the semen, all the primary abnormalities were pooled for each collection. The statistical analysis showed that when the semen collections of Group 1 and Group 2 were compared, there was not a significant difference among the different collections (Table 8).

Table 9 shows that no difference in the percentage of primary abnormalities between the cold stress and non-

Table 7. Morphological study: Percentage counts per 100 sperm cells per sample of each animal

Boar	Group[a]	Collection[b]	Proximal droplet	Abnormal heads	Coil tail	Secondary abnormalities[c]	Pooled primary abnormalities group mean
1	1	1	0	4	1	2	13
		2	2	2	1	3	
		3	6	2	0	6	
		4	10	15	3	1	
		5	9	5	0	0	
		6	10	15	3	7	
3		1	1	2	1	4	
		2	3	1	2	13	
		3	1	1	1	10	
		4	0	3	5	6	
		5	9	3	3	8	
		6	2	5	5	48	
5		1	5	3	1	2	
		2	1	1	5	2	
		3	20	4	2	7	
		4	1	5	0	1	
		5	18	7	2	4	
		6	14	4	2	7	

[a] 3/group.

[b] Pre-cold stress collection 1 and 2 - $\bar{X}$ = 5 primary abnormalities, post-cold collections, 4, 5 and 6 $\bar{X}$ = 14.7 primary abnormalities.

[c] All the secondary abnormalities were pooled.

Table 7 (Continued)

Boar[a]	Group	Collection[b]	Proximal droplet	Abnormal heads	Coil tail	Secondary abnormalities[c]	Pooled primary abnormalities group mean
2	2	1	3	1	0	4	7
		2	4	6	1	1	
		3	2	3	2	1	
		4	11	9	0	2	
		5	14	9	1	15	
		6	5	3	0	0	
4		1	1	2	0	2	
		2	0	1	0	6	
		3	2	3	0	0	
		4	0	5	0	0	
		5	2	6	2	1	
		6	5	7	0	22	
6		1	0	0	0	25	
		2	11	4	2	3	
		3	1	5	0	1	
		4	1	3	0	3	
		5	0	1	0	3	
		6	2	2	0	10	
7	3[d]	1	6	7	0	5	12
		2	6	3	3	9	
		3	4	3	2	11	
		4	6	1	0	33	
		5	2	3	0	10	
		6	1	2	1	5	

[d]Control group.

Table 7 (Continued)

Boar[a]	Group	Collection[b]	Proximal droplet	Abnormal heads	Coil tail	Secondary abnormalities[c]	Pooled primary abnormalities group mean
8		1	8	13	0	5	
		2	7	4	0	2	
		3	5	2	0	4	
		4	7	3	0	2	
		5	1	4	3	3	
		6	1	4	2	4	
9		1	10	2	13	33	
		2	17	2	0	49	
		3	17	3	0	28	
		4	10	1	2	23	
		5	11	2	3	23	
		6	11	1	0	6	

Table 8. Analysis of variance of morphological characteristics of semen among collections[a]

Source of variance	Degrees of freedom	Sum of squares	Mean square	F-value
Model	35	2658.75	75.96	-
Group[a]	1	283.56		2.39
Animal (Group)	4	475.22		-
Collection	5	598.25		2.32
Group - Collection	5	272.47		1.06
Animal - Collection (Group)	20	1029.44		-
Corrected Total	35	2658.75		

[a]Groups 1 and 2.

Table 9. Analysis of variance of morphological characteristics of semen among groups[a,b]

Source of variance	Degrees of freedom	Sum of squares	Mean square	F-value
Model	23	2204.22	95.83	2.65*
Group	2	313.44	156.72	4.38*
Animal (Group)	6	747.55	124.59	3.45*
Collection	5	130.00	26.00	0.72
Group - Collection	10	1013.00	101.32	2.81
Error	30	1083.11	36.10	-
Corrected Total	53	3287.33	-	

[a]S.D. = 6.0.

[b]Groups 1 and 2 vs. Group 3.

*P<.05.

cold stress groups was found (Table 9). This table also shows that there were individual variations that accounted for differences between Groups 1 and 2, rather than the effect of treatments.

E. Evaluation of the Testicular Samples

The effects of cold stress upon the germinal epithelium were studied, using three histological characteristics as indicators of damage viz., 1) number of elongated spermatids per seminiferous tubule cross-section (Figure 13), 2) the presence of giant cells (multinucleated cells) in the tubular cross-section (Figure 10), and 3) histopathological studies of the germinal epithelium in these cross-sections.

1. Number of elongated spermatids

The average numbers of elongated spermatids per cross-section in each boar are listed in Table 10.

Table 10 shows that the effect of cold stress upon the number of spermatids per cross section was not homogeneous; on the contrary, it seemed that the effect had considerable individual variability.

In Group 1 as well as in Group 2 (cold-stressed), there were two boars (Boars 1 and 4), in which there was practically no elongated spermatids discernible in the seminiferous

Table 10. Study of the number of elongated spermatids per seminiferous tubule cross-section (50 tubule cross-sections were counted)[a]

Boar	Group	Number of elongated spermatids per seminiferous tubule cross-section[b]
1	1	0.60
3		32.88
5		29.58
		$\overline{X}^{c}$ = 21.00
2	2	31.32
4		1.26
6		29.58
		$\overline{X}$ = 20.72
7	3	38.68
8		37.24
9		30.70
		$\overline{X}$ = 35.40

[a]Std. error = 8.28

[b]50 cross sections were counted and the mean obtained.

[c]Average number of elongated spermatids per cross section in each group.

tubules. In the remaining animals of these two groups, the numbers of elongated spermatids were similar.

In spite of the damage observed in these two boars, when the cold stressed groups were compared to the control (Group 3), no difference was found. The comparison of the two groups that were cold stressed did not reveal any difference.

2. Giant cells

The percentage of tubules in which multinucleated giant cells were observed is shown in Figure 8.

In this study, the number of giant cells per cross-section was not evaluated, only their presence or absence was noted. However, it was observed that in many instances, there was more than one giant cell per cross-section.

The mean percentage of tubules with giant cells was 46.66% in Group 1. Group 2 showed a mean percentage of 60.66%. At comparison between Groups 1 and 2, there was no significant ($P<0.05$) difference. Nevertheless, when these two groups were compared to Group 3, a significant difference ($P<0.05$) was observed (Table 11).

The presence of giant cells in seminiferous tubule is highly indicative of damage to the spermatogenic cells in the seminiferous epithelium.

Figure 8. Graphic illustration of the seminiferous tubules (%) in which giant cells were detected

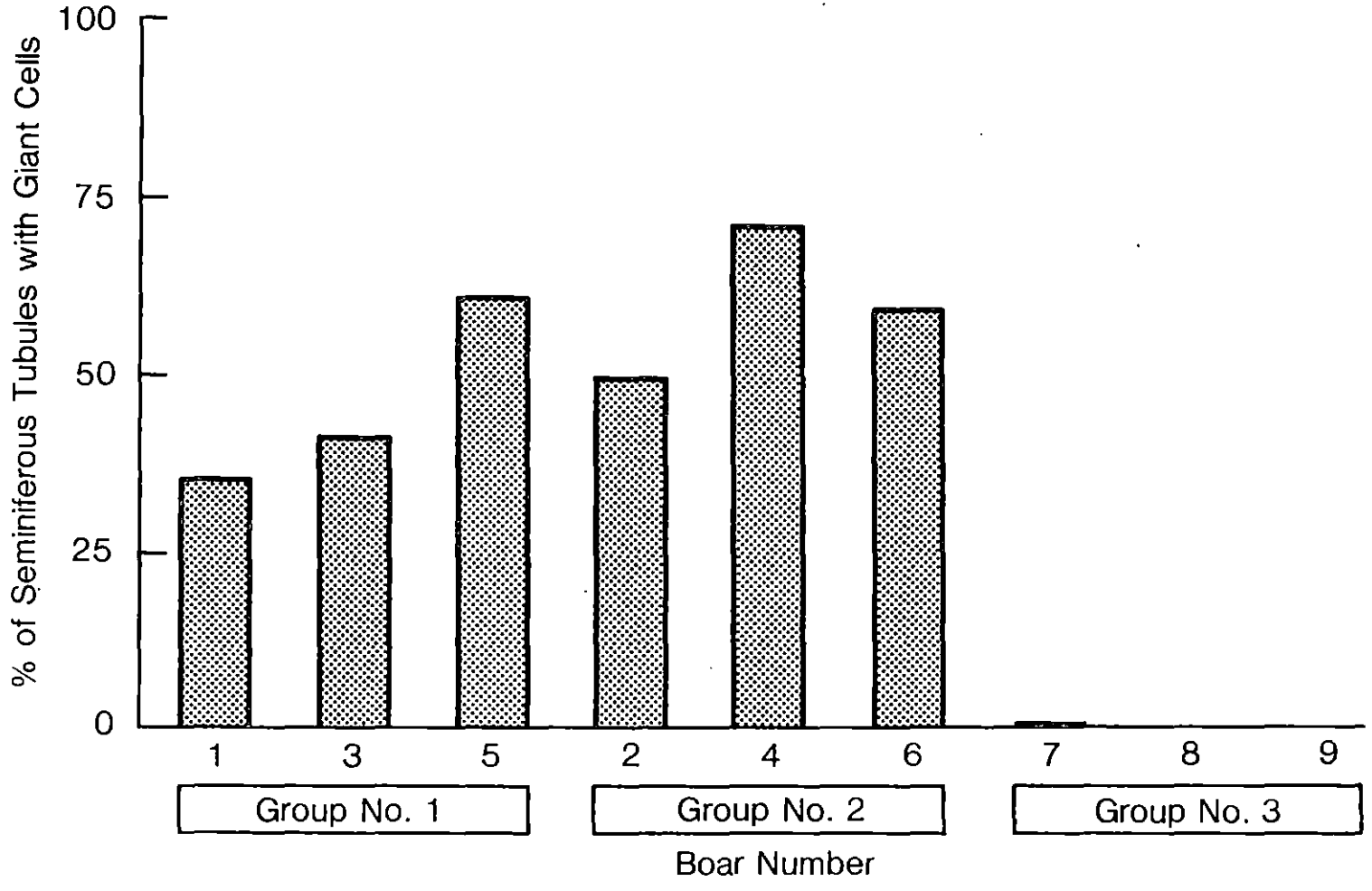

% of Seminiferous Tubules with Giant Cells
100
75
50
25
0
1
3
5
2
4
6
7
8
9
Group No. 1
Group No. 2
Group No. 3
Boar Number

Table 11. Analysis of variance of the percentage of seminiferous tubules with giant cells (50 cross-sections) [a,b]

Source of variance	Degrees of freedom	Sum of squares	Mean square	F-value
Group	2	6054.22	3027.11	29.61*
Error	6	613.33	102.22	
Corrected Total	8	6667.55		

[a] Contrast Group 1, Group 2 vs. Group 3 - $P<0.05$.

[b] SD = 10.11

* $P<.05$.

3. Histopathological status of the germinal epithelium

The result of the study of the effect of cold stress upon the germinal epithelium and the type of damage are illustrated in Figure 9.

The histopathological status was classified according to the integrity and degree of damage that the germinal epithelium showed. The degree of damage was classified in three types, the criteria used have been mentioned in Material and Methods.

Type I damage (Figure 10) was a slight alteration underwent by the germinal epithelium, which was observed mainly in 4 boars (Boars 2, 3, 5 and 6); it was also detected in boars in the control Group 3 (Boars 7, 8 and

Figure 9. Graphic illustration of the effect of cold stress upon the germinal epithelium

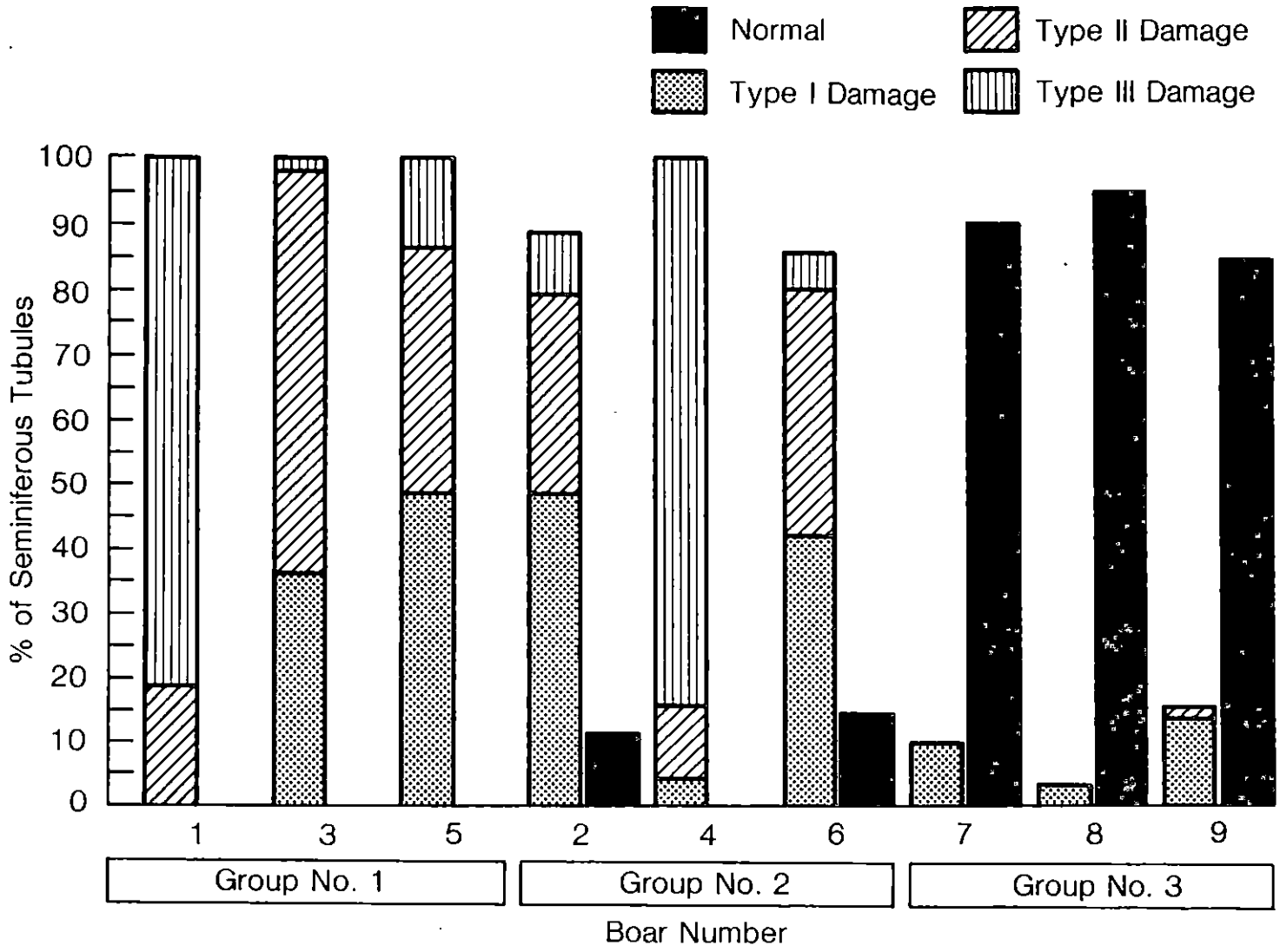
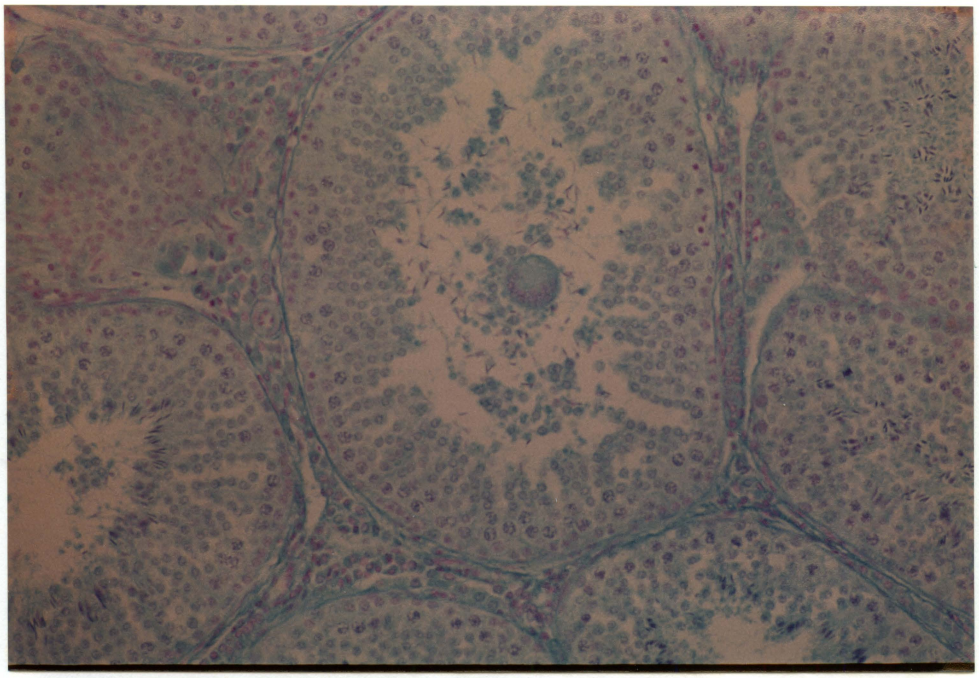

Normal
Type I Damage
Type II Damage
Type III Damage
% of Seminiferous Tubules
100
90
80
70
60
50
40
30
20
10
0
1
3
5
2
4
6
7
8
9
Group No. 1
Group No. 2
Group No. 3
Boar Number

Figure 10. Photomicrograph of seminiferous tubule displaying damage type I. Multinucleated giant cell in the lumen



9), but in a small percentage of tubules.

Type II damage (Figure 11) could be characterized as a mild type of dearrangement of the architecture of the seminiferous tubule. It was spread in all boars in the cold stress groups. In Boar 4, this type of damage occurred only in a small percentage of tubules. It was observed in one boar (Boar 9) in the control group. However, the percentage of its occurrence was negligible.

The severe type of damage, which was typified as almost complete destruction of the seminiferous tubule epithelium was denominated Type III (Figure 12). This kind of damage was observed mainly as the primary type in two boars (Boars 1 and 4). It was also detected in all boars of Groups 1 and 2 in different proportions. This type of damage was not seen in the control group (Group 3).

The presence of normal seminiferous tubules (Figure 13), which characterized the control group (Group 3), was only observed in two boars in Group 2 (Boars 2 and 6).

When the percentage of normal tubules in the control group was compared to the ones in the cold stress groups, a significant difference ($P<0.05$) was found (Table 12).

In seminiferous tubules that experienced Type III and Type II damage, there was a vacuolization of the germinal epithelium. This vacuolization was also indicative of

Figure 11. Photomicrograph of seminiferous tubules displaying two types of damages (the seminiferous tubule of the right hand corner shows damage type II, and damage type III is shown by the seminiferous tubule in the center of the picture. Several giant cells are observed in the lumen of these tubules)

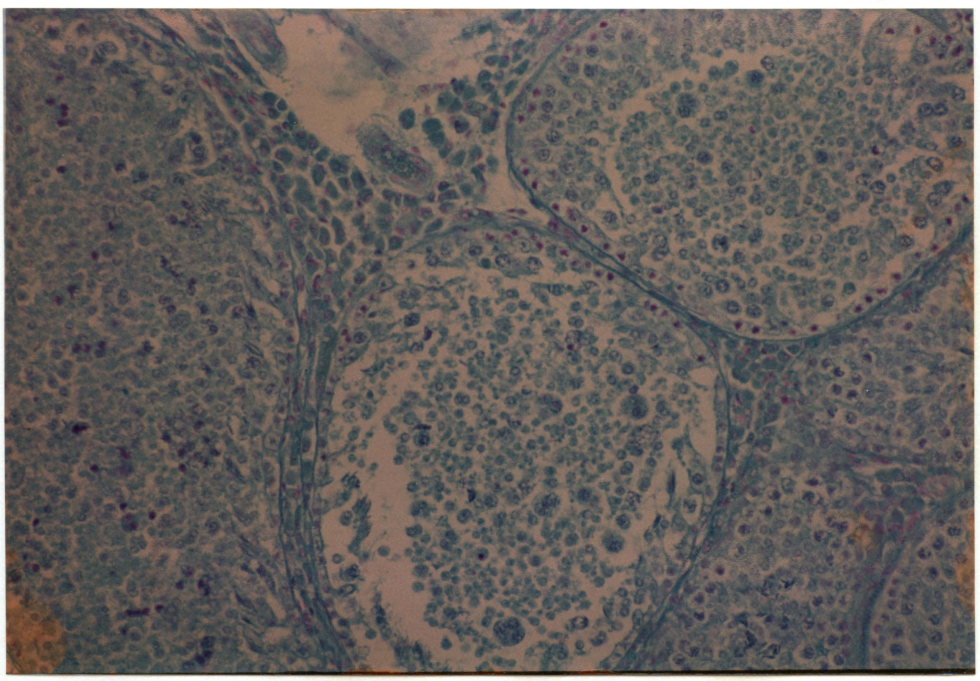


Figure 12. Photomicrograph of seminiferous tubules showing damage type III (note that there is complete destruction of the seminiferous epithelium with hypertrophy of the interstitial cells, no elongated spermatids are observed)

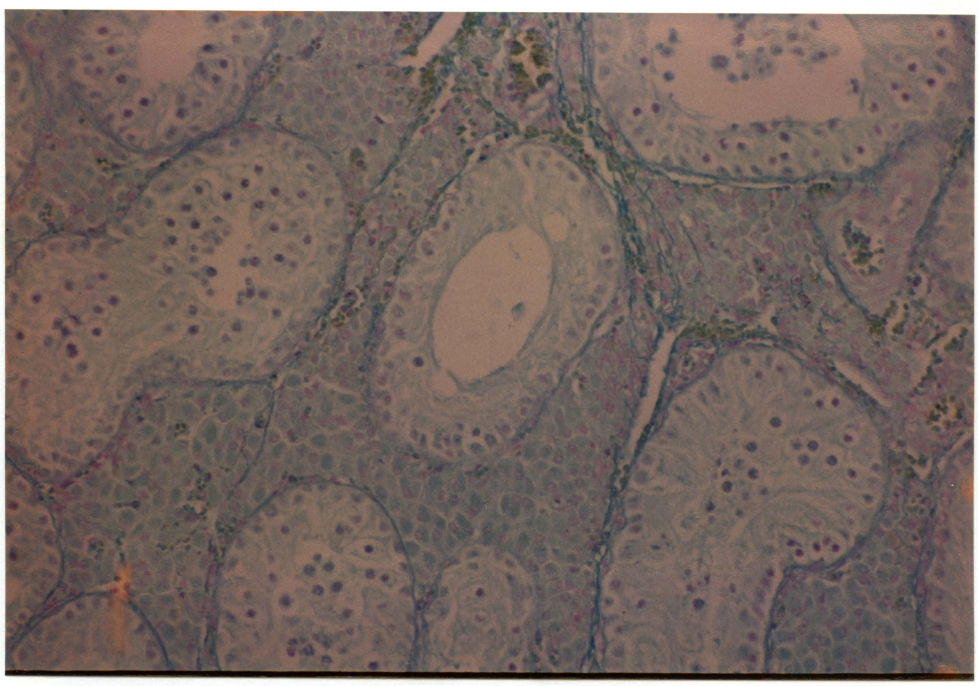


Figure 13. Photomicrograph of normal seminiferous tubules (note the integrity of the epithelium)

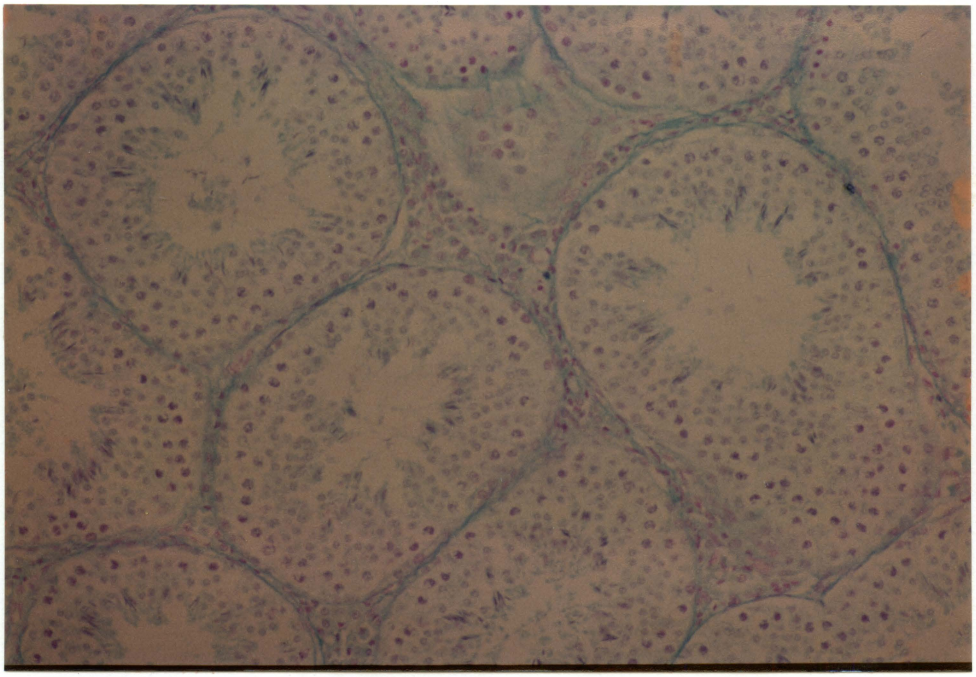


Table 12. Analysis of variance of the percentages of normal seminiferous tubules (50 cross-sections)[a,b]

Source of variance	Degrees of freedom	Sum of squares	Mean square	F-value
Group	2	14790.22	7395.11	237.70*
Error	6	186.66	31.11	
Corrected Total	8	14976.88		

[a]Contrast Group 1, Group 2 vs. Group 3 - P<.05. Contrast Group 1 vs. Group 3 - P<.05.

[b]SD = 5.5.

*P<.05.

alterations in the tubular epithelium.

Another finding in the evaluation of the testicular samples was the marked "hypertrophy" of the Leydig cells in the boars that were subjected to cold stress when compared with the ones in the control group (Figure 10). This "hypertrophy" may have been relative rather than actual, since a reduction in the size of the seminiferous tubules made the Leydig cells more evident. The actual number of Leydig cells interspersed between the seminiferous tubules was not counted.

V. DISCUSSION

A. Testicular Temperature

Exposure of boars to low environmental temperatures (-15 ± 3°C) caused a significant ($P<.05$) increase in testicular temperatures. During cold stress, the mean testicular temperature was 40.7°C, while the mean testicular temperatures were 36.2°C and 36.6°C, respectively, for pre-cold and post-cold stress periods. There was no significant difference ($P<0.05$) in testicular temperature between pre-cold and post-cold stress periods. This indicates that the rise in testicular temperature was related to cold stress. This is also evidenced by the abrupt change in testicular temperature that occurred within 24 hours of change in environmental temperatures (Figures 3, 4 and 5).

Rectal temperatures were also recorded for the three treatment periods. The mean rectal temperature did not differ significantly ($P<0.05$) from one period to another.

The mean testicular temperatures of boars exposed to room temperatures was 2.6°C lower than the mean rectal temperature. Though this temperature gradient is similar to that reported by Stone (1981), the mean testicular temperature (36.2°C) in this study was 0.6°C higher than reported by Stone (1981). This higher value would be the result of slightly elevated testicular temperatures for two days

following surgery. Stone (1981) did not record testicular temperatures until two days postsurgery.

During the period of cold stress, the scrotal surface underwent severe and prolonged contracture (Figure 7). It is hypothesized that the scrotal contracture moved the testes closer to the body wall which had a higher temperature and also altered the thermoregulatory mechanism of the testis.

Scrotal contraction caused a reduction in surface exposure of the testicular artery (Shaffik, 1977), thus, reducing the heat exchange between the testicular artery and testicular veins, and increasing the amount of heat transported into the testis by the testicular artery. Furthermore, heat dissipation through the scrotal skin is reduced by a decrease in scrotal surface and vasoconstriction of the blood vessels in the scrotal skin during cold stimulus. The testis can also gain heat from the body when being in close contact with it. Kellaway et al. (1971) observed that when the testicles in bull calves were pushed close to the body wall with elastrator bands in order to produce pseudochryptorchidism, the temperature of these testicles was 3°C higher than the temperature of testicles remaining in the scrotum. In this study, the gain of heat by the testis was probably the result of both altered testicular thermoregulatory mechanism and closeness of the testis.

Measurements of blood flow to the testes and in the pampiniform plexus as well as temperature gradients in the blood, testis and scrotal skin were not within the scope of this study. These measurements would further elucidate the mechanisms involved in elevated testicular temperatures during cold stress.

Due to the lack of reports on studies of effects of low temperatures upon testicular temperature in the literature, it is difficult to interpret certain values recorded in this experiment. For instance, Boar 3 of Group 1 showed very high testicular temperature during the cold stress period, reaching 43°C on one day. This temperature may reflect mild inflammatory response in the testicle or a faulty reading by the recorder. Another unexpected testicular temperature (35.6°C) occurred during cold stress of Boar 1. This may have been due to a faulty reading or excessive cooling of the testicle when the boar lay on the cold concrete floor.

B. Semen Quality

In this study, sperm cell motility and morphology were used to evaluate semen quality. However, there were no breeding trials for assessment of fertility.

The evaluation of samples collected in the post-cold

stress period showed a significant reduction ($P<0.05$) in sperm motility when compared to the semen collected in the pre-cold stress period. These findings are not in agreement with those reported by Swierstra (1970a). He did not observe any decrease in sperm motility of boars exposed to low environmental temperatures (-15°C to -20°C). However, in rats exposed to low ambient temperatures (-5°C) and hypoxia for seven days, sperm motility was reduced. Sperm motility altogether ceased when the exposure time was prolonged to 21 days. Moreover, it has been shown that either high ambient temperatures or elevated testicular temperatures can cause a reduction in sperm motility (VanDemark and Free 1970, McNitt and First 1970, Cameron and Blackshaw 1980, Stone 1981/1982).

Sperm motility of the post-cold stress collections was slightly lower in boars of Group 1 than in boars of Group 2, but this was not significantly different ($P<0.05$). This difference could be due to breed or individual animal variation or some minor effect from the thermistor implantation. Though sperm motility in the pre-cold collections from the three groups was similar, a significant difference ($P<0.05$) in pooled sperm motility between cold-stressed groups and control was detected. Also, the effect of experimental procedure (Groups 1 and 2) on motility was not significant ($P<0.05$). Therefore, this

decrease in sperm motility seems related to cold stress.

The study of morphological characteristics of the sperm cells from boars that were cold-stressed revealed that the number of primary abnormalities in post-cold stress collections was not significantly ($P<0.05$) higher than in the pre-cold stress ones. Nonetheless, in post-cold stress collections from Boars 1, 2 and 5, the percentages of abnormal heads were higher than levels regarded as normal (Hurtgen et al., 1977), but this pattern was not consistent for all cold-stressed boars.

The percentages of primary abnormalities in semen from cold-stressed boars were not statistically different ($P<0.05$) from the percentages of primary abnormalities in semen of boars kept at room temperatures. However, there was a significant difference ($P<0.05$) between the cold-stressed boars; that was due mainly to individual effect rather than treatment (see Table 9).

Reports of the effect of low environmental temperatures on sperm cell morphology are scant or incomplete. Swierstra (1970a), in his study of the effect of cold stress on boar semen characteristics, did not include a morphological study of the semen. Riar et al. (1979) reported an increase of abnormal spermatozoa in rats after cold stress, but neither did he mention the kinds of abnormalities nor

their percentages.

It has been shown that an increase in testicular temperature caused by either high environmental temperatures or direct heating of the testes produces an increase in primary abnormalities, including abnormal heads, coiled tails and proximal droplets (McNitt and First 1970, Wettemann et al. 1976, Cameron and Blackshaw 1980). Therefore, it is difficult to explain the fact that despite increased testicular temperatures and damage to the germinal epithelium, the number of primary abnormalities was not significantly altered in all the post-cold stress collections. One consideration is the selective phagocytosis of damaged cells by the rete testis (Goyal 1982).

The presence of moderate spermatozoa numbers in semen samples from boars with evidence of seminiferous tubule damage is probably due to the infrequency of collection and the reduced volume collected by electroejaculation. Thus, a few viable tubules could produce a relatively normal sample. It is also very likely that the time interval from cold stress and testicular tissue collection was insufficient to see the maximum effect on spermatozoa numbers and morphology. It has been estimated that the spermatogenic process requires 34 days and epididymal transport 9-12 days (Swierstra 1968). The last semen sample was collected 15

days after removal of this boar from cold stress conditions and may not have timely reflected the degree of testicular damage.

C. Histopathological Status of the Germinal Epithelium

Histological studies of testicular tissue obtained 15 days after cold stress revealed different degrees of damage in seminiferous epithelium. The damage to the germinal epithelium ranged from slight damage to the epithelium (Type I), which was characterized by areas of missing cells, slight vacuolization of the epithelium and an increase of cell debris in the lumen, to a complete destruction of the germinal epithelium (Type III). However, the degree of damage displayed in the testicular tissue by the different boars was not uniform. Boars 1 and 4 showed greater percentages of severe damaged tubules (82% and 85%, respectively). The remaining cold-stressed boars had higher percentages of slight and mild damaged seminiferous tubules (Type I and Type II). This probably reflects individual susceptibility to cold stress.

Comparison of the histological status of testicular tissue from cold-stressed boars (Groups 1 and 2) and non-cold stressed (Group 3) showed a marked difference. The germinal epithelium of the latter animals did not have

detectable damage (Figure 9).

In this study it was observed that there was little change in the number of elongated spermatids in testes of cold-stressed boars as compared to controls. Elongated spermatids seem not to be affected as much as the round spermatids (VanDemark and Free 1970). Multinucleated giant cells were discernible in a high percentage of seminiferous tubules of cold-stressed boars. These giant cells are believed to be formed by fusion of damaged round spermatids (VanDemark and Free 1970). Therefore, it was concluded that a significant damage to the round spermatids and consequently to the spermiogenesis process had occurred in cold-stressed boars.

VI. CONCLUSIONS AND SUMMARY

This study showed that exposure to low environmental temperature can result in an increase in testicular temperature. This seemed to occur as a result of scrotal contraction that altered scrotal and testicular location, hindering the thermoregulatory mechanisms of the testis. The elevation in testicular temperature during cold stress (-15°C $\pm$ 3°C) resulted in an inversion of the rectal-testis temperature gradient from +2.6°C to -2.1°C and subsequently, damage to the germinal epithelium. This damage was not uniform but varied from severe to mild in individual boars. The presence of many damaged seminiferous tubules indicates the detrimental effect of cold stress upon the seminal epithelium. Furthermore, high incidence of giant cells in the seminiferous tubules and a reduction in the size of the seminiferous tubule were indicative of damages to testicular tissue during cold stress.

Sperm motility was also adversely affected by cold stress. There was a significant decrease ($P<0.05$) in spermatozoa motility in semen collections from cold-stressed boars.

Morphological abnormalities in the spermatozoa were not consistently observed at significant values in cold-stressed boars, despite the elevated testicular temperatures,

altered tubular architecture and reduced sperm motility.

The findings of this study require more in-depth study in order to elucidate the mechanisms involved in the elevation of testicular temperature when exposed to low environmental temperature. Also, there is a need to determine the tolerance limits of duration of cold stress and the relative ambient temperature that produces testicular damage. Subsequent studies should include breeding trials as a measure of fertility under normal physiological conditions. Finally, there is need to determine the duration of infertility associated with this type of testicular insult.

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IX. APPENDIX

Table 13. Motility (%) study

Boar	Group	Collection[a]	Percentage life (%)	Scoring
1	1	1	85	4
		2	85	4
		3	70	3
		4	65	3
		5	50	2
		6	65	3
3	1	1	90	4
		2	95	5
		3	65	3
		4	70	3
		5	60	3
		6	50	2
5	1	1	75	4
		2	90	4
		3	65	3
		4	70	3
		5	60	3
		6	80	4
2	1	1	75	4
		2	80	4
		3	90	4
		4	60	3
		5	70	3
		6	60	3
4	1	1	95	5
		2	80	4
		3	80	4
		4	90	4
		5	85	4
		6	85	4

[a]Pre-cold stress collections: Groups 1 and 2, post-cold stress collections: Groups 4, 5 and 6.

Table 13 (Continued)

Boar	Group	Collection[a]	Percentage life (%)	Scoring
6	2	1	85	4
		2	80	4
		3	65	3
		4	70	3
		5	90	4
		6	80	4
7	3	1	90	4
		2	80	4
		3	80	4
		4	90	4
		5	95	5
		6	90	4
8	3	1	90	4
		2	95	5
		3	85	4
		4	95	5
		5	85	4
		6	95	5
9	3	1	50	2
		2	70	3
		3	60	3
		4	75	4
		5	85	4
		6	85	4

Table 14. Record of daily rectal temperature at different environmental temperatures

Boar	Group	Rectal temperature	Environmental temperature (°C)
1	1	38.3	23±3°C
		38.8	
		38.8	
		38.3	
		37.8	
		38.3	
		39.0	
		38.8	
		38.5	0±3°C
		38.8	
		38.0	-15±3°C
		38.8	
		38.8	
		38.8	
		39.1	
		39.1	
		38.8	
		38.5	
		38.7	
		38.8	
		38.7	23±3°C
		38.7	
		38.6	
		38.4	
		38.5	
		38.5	
		38.6	
		39.4	
2	2	39.4	23±3°C
		39.1	
		38.6	
		38.8	
		39.0	
		38.5	
		38.5	
		38.5	

Table 14 (Continued)

Boar	Group	Rectal temperature	Environmental temperature (°C)
2	2	38.2	0±3°C
		38.3	
		38.5	
		38.5	
		38.5	
		38.4	
		38.3	
		38.8	
		38.8	
		38.7	
		39.1	
		39.1	
		38.3	
		38.4	23±3°C
		38.6	
		38.5	
		38.4	
		38.6	
		38.5	
		38.5	
3	1	39.0	23±3°C
		38.7	
		38.5	
		38.8	
		38.1	
		38.1	
		38.7	
		38.4	
		38.7	0±3°C
		38.5	

Table 14 (Continued)

Boar	Group	Rectal temperature	Environmental temperature (°C)
3	1	38.7	-15±3°C
		38.8	
		38.7	
		38.7	
		38.7	
		38.8	
		39.0	
		38.2	
		38.6	
		38.5	
		38.7	23±3°C
		38.6	
		38.5	
		38.4	
		38.3	
		38.3	
		38.3	
		38.0	
		38.3	
		38.3	
		38.5	
		38.5	
4	2	38.8	23±3°C
		38.8	
		38.7	
		38.8	
		38.7	
		38.3	
		37.2	
		38.3	
		37.2	0±3°C
		38.8	

Table 14 (Continued)

Boar	Group	Rectal temperature	Environmental temperature (°C)
4	2	38.4	-15±3°C
		37.2	
		37.3	
		37.5	
		37.5	
		37.2	
		37.7	
		37.7	
		38.8	
		38.3	
		38.3	23±3°C
		38.4	
		38.6	
		38.2	
		38.6	
		38.4	
		38.4	
5	1	38.8	23±3°C
		38.3	
		38.8	
		38.3	
		38.3	
		38.3	
		38.3	
		38.3	
		38.3	0±3°C
		37.1	
		38.0	-15±3°C
		38.8	
		38.7	
		38.7	
		38.6	
		38.6	
		38.2	
		38.4	
		38.5	
		38.5	

Table 14 (Continued)

Boar	Group	Rectal temperature	Environmental temperature (°C)
5	1	38.4	23±3°C
		38.6	
		38.5	
		38.4	
		38.5	
		38.6	
		38.5	
6	2	38.8	23±3°C
		38.8	
		38.9	
		39.0	
		39.4	
		38.5	
		38.6	
		38.8	
		38.7	0±3°C
		38.8	
		38.8	-15±3°C
		38.7	
		38.7	
		38.7	
		38.3	
		38.5	
		38.5	
		38.9	
		39.1	
		38.9	
		38.8	23±3°C
		38.4	
		38.6	
		38.3	
		38.6	
		38.4	
		38.5	

Table 14 (Continued)

Boar	Group	Rectal temperature	Environmental temperature (°C)
7	3	39.0	23±3°C
		38.8	
		38.7	
		38.3	
		38.4	
		38.3	
		38.8	
		38.8	
		38.6	
		38.5	
		38.8	
		38.8	
		38.6	
		38.7	
		38.3	
		38.7	
		38.6	
		38.2	
		38.4	
		38.7	
		38.6	
		38.5	
		38.4	
		38.7	
		38.3	
		38.5	
		38.5	
8	3	39.1	
		38.7	
		38.5	
		38.6	
		38.4	
		38.8	
		38.5	
		39.0	
		38.4	
		38.6	
		38.4	

Table 14 (Continued)

Boar	Group	Rectal temperature	Environmental temperature (°C)
8 (Continued)	3	38.4	
		38.7	
		38.9	
		38.5	
		38.7	
		38.6	
		38.5	
		38.4	
		38.4	
		38.5	
		38.3	
		38.2	
		38.4	
		38.6	
		38.7	
		38.5	
9	3	39.2	
		38.6	
		38.4	
		38.9	
		38.8	
		38.9	
		38.6	
		38.5	
		38.4	
		38.7	
		38.6	
		38.4	
		38.2	
		38.6	
		38.4	
		38.1	
		38.3	
		38.4	
		38.6	
		38.7	
		38.2	
		38.3	
		38.7	
		38.4	
		38.3	
		38.7	
		38.7	