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A STUDY OF THE EFFECTS OF ELEVATED TEMPERATURE OF SCROTUM BY INSULATION IN DIFFERENT BREEDS OF BULLS*

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Değişik ırk boğalarda skrotal insulasyonla ısı artışının spermatozitler üzerindeki etkisi

Özet: *Bu çalışmada özel bir torba uygulamakla skrotum ısısı beden ısısına yakın bir duruma getirilmiş ve kısa süreli skotal insulasyonların sperma karakteristikleri üzerine etkisi ve aynı zamanda İsveç Kırmızısı, yarım kan Montafon ve yerli boğalarda farklılıkları incelenmiştir.*

Kısa süreli insulasyonlarda volum, motilite ve konsantrasyonda değişiklik olmamış, diğer taraftan uzun süreli insulasyonlaraa motilite ve konsantrasyonda belirli düşmeler görülmüştür.

Uzayan insulasyon süreleri sperma karakteristiklerinde oozukluğun artmasına, düzelmenin gecikmesine neden olmuş, düzelmenin şekillenebilmesi için uzun bir zamanın geçmesi gerekmiştir.

İsveç Kırmızısı boğalarda sperma karakteristikleri üzerine termal etkinin, yerli ve yarım kan boğalarımızdan daha fazla olduğu aynı süreli insulasyonlar uygulamak suretiyle karşılaştırmalı çalışmada ortaya konmuştur.

Anomalik baş ve proksimal protoplazmik damlacıklarda artma yerli ve yarım kan boğalarımıza göre oldukça fazla bulunmuştur.

Düzelme bütün boğalarda tam olarak şekillenmekle beraber yerli ve yarım kanlarda İşveç Kırmızısı boğalardan daha çabuk oluşmuştur.

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Zusammenfassung: *Bei diesen Untersuchungen konnten wir durch Anwendung einer besonderen Hülle eine bis zur Körpertemperatur erhöhte Scrotaltemperatur erreichen. Unsere Absicht war, aie Auswirkungen einer kurzzeitigen Scrotalisoation auf die Spermaqualitat verschiedener Bullen zu untersuchen una Unterschiede zwischen den einzelnen Rassen festzustellen (Einhemische, Montafonkreuzungtiere und Schwedische Rotbunte).*

Es traten keine Veranderungen auf bezüglich Volumen Beweglichkcit und Konzentiration des Spermas nanch kurzzeitiger Isolierung des Scrotums, Jedoch konnten wir erhebliche weminderung der Beweglihkeit und der Konzentration als Folge von Langezeitisolation festellen.

Die Verlangerung der Isolationzeit verursachet unerwünschte Veranderungen der Spermaqualit, und die Etholungsphase beanspruchte eine längere zeit.

In ahnlichen Arbeiten mit gleichen Isolationzeitraumen zeiten wir, dass die Spermaqualitat der Schwed. Rotb. durch thermische Einflüsse starker beeintrachtigt wurde als die von einheiminischen und Kreuzungssbullen.

Die Zunahme anomaler Köpfe und Proximaler Protoplasmatropfen war bei den Schwed. Rotb. grösser als bei den anderen Versuchsrassen.

Die Wiederherstellung der ursprünglichen Spermaqualitat wurde bei allen Tieren erreicht, aber der benötigte Zeitraum war bei einheimischen und Kreuzungsbullen kürzer.

Summary: *In this rearch by applying a special bag we have been able to perform a higg scrotal temperature as near as the body temperature and our intention was to determine th. effect of short period scrotal insulations on semen characteristics of different bulls and also to deter mine whether there are differences in response between Native, Montafon crossbred and Swedish Red Breed bulls.*

No changes have occurred in respect to volume, motility and concentration attributable to the short period insulations; however obvious decreases have been obsered in the motility and the concentration as a result of long period insulations.

The prolongation of the insulation periods have caused some undesired changes in the spermatozoal characteristics and the recovery has needed a longer period of time.

In comparable work, by applving the same insulation periods we have shown that the thermal influnce on the spermatozoal characteristics was found to be more effective in Swedish Red Breea bulls than in our Native and crossbred bulls.

The increase in the abnormal heads and proximal protoplasmic droplets were greater in Swedish Red Breed than in our Native and crossbred bulls.

Recovery has occurred to some extent in all bulls but was more rapid and more nearly complete in Native and crossbred bulls when compared with Swedish Red Breed bulls.

Introduction

The testicle is one of the organs in the body, most sensitive to external influences. The scrotum is a structure which one of its functions is to regulate the environmental tmperature of the testicle. This is maintained by the cremaster muscle by which of its mechanism the testicular temperature is kept at an optimal level for spermatogenesis (11).

It has long been known that undescended mammalian testes have no spermatozao and for the most part are devoided of all germinal cells, the Sertoli reticulum is retained almost without exeption and sometimes spermatogonia are present in small numbers (8).

When a temperature, that is slightly higher than the normal, is subjected directly on the scrotum, the local thermo regulation fails and the testicular temperature rises so that it causes a testicular degeneration. Lagerlöf (6) was the first carrying out some experiments wiht scrotal insulation designed so as to produce the testicular degenetion, artificially in the bull. He showed that by the insulation of the scrotum any desired degree of degeneration of spermatogenic epithelium could be induced and the character and extent of the denera-tion or regeneration could be traced by means of the periodic semen examinations.

Later, the same type of experiments were performed by some other investigators on bulls (1,4,5) and on rams (3,8,10).

These investigations in bulls have shown that the insulation of scrotum which lasts 4 to 5 days, does not entail any material diminution of the number of spermatozoa but, on the other hand,a considerable increase in the number of pathological spermatozoa. After the insulation which has caused a degeneration has been removed for after a period of 4 to 6 weeks the semen picture was again normal. In the case of more prolonged disturbance, 11 to 15 days, in the temperature regulating function of the scrotum, very considerable changes occur in the semen picture. The number of spermatozoa

rapidly decreased and the number of underdeveloped, pathological spermatozoa increased. The spermiogenesis ceased completely and quickly and during a period of about 4 months practically no spermatozao were developed.

Skinner and Louw (12) tried to determine the critical duration of high ambient temperatures required in affecting the spermatogenesis adversely in Bos Indicus and Bos Taurus.

Lagerlöf (7) has shown that in semen from bulls with good fertility, there are normally about 2–5 % of spermatozoa which have proximal protoplasmic droplets. Higher percentage in such droplets mostly indicates a disturbance in spermiogenesis and often considered as the first symptom.

Material and Methods

2 Swedish Red Breed (SRB) one used twice, one crossbred Montafon used twice and two Native Breeds a total of 5 bulls have been used in our 7 experiments. The bulls had undergone through clinical examinations after their arrival in the clinic. A series of semen semples examined prior to the onset of the experiments confirmed normal semen pictures.

During the experimental period the bulls were kept at a temperature of between 17°C and 24°C.

At each semen collection, a sterilized rubber artificial vagina was used. The temperature of the artificial vagina was adjusted between 45° C–65°C just before collection. The rubber cone and the graded collecting tubes were prewarmed to 36°C in an incubator. These were attached to the rubber artificial vagina just before collection with an insulated covering in order to maintain the temperature. Two samples were taken at each collection.

The volume of the semen was recorded from the graded collecting tubes to the nearest 0.1 ml, after that the semen examined for density. The wave motion and motility were determined subjectively through a microscope on a slide warming stage. The spermatozal concentration was measured by actual counting with a heamositometer which is generally accepted as the most reliable method.

Morphological examination of the spermatozoa was carried out according to the methods described by Lagerlöf (6).

The semen smears were stained with Carbol fuchsin (William's method) for examination of pathological spermatozoa heads. A thousand of spermatozoa were counted in order to determine the incidence of different types of pathological heads. To determine the percentage of live spermtozoa a smear was made on a warm stage (37. 5°C) using Eosin-Nigrosin, simultaneously to the measurement of initial motility. Five hundred spermatozoa were counted.

A wet preparation was prepared by mixing the semen with a buffered formol-saline solution, a drop of which was placed on a slde to be examined under a phase-contrast microscope for proximal protoplasmic droplets. Two hundred spertozoa were counted.

For the scrotal insulation an insulating bag, a double layer plastic bag with a light insulating material between the layers was used. The bag was placed carefully over the scrotum until both testicle were completely enclosed. Care was taken not to disturb the circulation. The mouth of the bag was then carefully sealed to the scrotal neck using adhesive tape to prevent heat loss from inside the bag.

The durations of the testicular insulations were as shown in table I.

The temperature inside the bag was taken by using a thermometer which could measure the temperature without causing any heat loss from inside the bag. Rectal temperature was taken simultaneously. The temperature of scrotal skin varied between 31 8°–36.0° C. The semen collection were made regularly.

Results

The experiments have shown that degeneration of seminal epithelium can proceed very rapidly and if the degenerative process has not gone too far, a regeneration can also be accomplished in a relatively short period of time. If the effect is of a more serious nature, the spermiogenesis may be arrested for several months and regeneration can not be seen for the following 3-4 months in exotic breeds and 2–3 months in Native Breeds. The regeneration always takes a longer time than degeneration.

The volumes of the ejaculates did not differ significantly during the experimental procedures. Each ejaculate ranged between 2–5 m.l

The sperm concentration at the control collections before insulation were approximately 800.000 to 1.900.000 spermatozoa/ mm^3.

No and Breed	Duration of the insulation (days)	Total hours	Frequency of application of the insulation bag	Percentage of the Primary Abnormalities		Percentage of the prox. proto. droplets		
				Before İnsulation	10–12 days after İnsulation	Normal	Before Degeneration	Before Regenation
I SRB	4 days	48	Each Second days	14.6 %	62.8 %	1.5 %	49.5 %	26.0 %
II SRB	3 days	36	Each Second days	6.2 %	27.0 %	0.5 %	7.5 %	3.5 %
III SRB	6 days	144	Continuously	5.8 %	94.0 %	1.0 %	27.5 %	27.5 %
IV Crossbred. mont.	4 days	48	Each Second days	7.4 %	22.8 %	0.5 %	3.5 %	3.5 %
V Crossbred. mont.	3 days	36	Each Second days	5.2 %	14.0 %	1.5 %	2.0 %	2.0 %
VI Native	6 days	144	Continuously	9.4 %	32.2 %	0.5 %	3.0 %	6.0 %
VII Native	10 days	240	Continuously	2.0 %	53.4 %	1.0 %	27.0 %	27.0 %

10 to 15 days after the first application of insulation, the sperm concentration decreased to 100.000 spermatozoa / mm^3 in some severe cases.

The sperm motility was affected by the long term insulations. It fell sharply from normal (70–85 %) to 20–30 %.

Following the 9th. day, the percentage of the pathological spermatozoa heads began to rise above normal. The majority of these were pear-shaped and narrow at tha base.

In experiments III and VI, the duration of testicular insulation continued 6 days. In the third experiment SBR (101 Masselberg) was used as the experimental bull. On the 7 th. day after the first application of insulation the percentage of pear-shaped spermatozoa was 10.8 % underdeveloped ones 28.1 % and the free heads 11.2 % and the total percentage of the primary abnormalities was 67.4 %. On the 10 th. day it was 94 % and the majority was underdeveloped ones (87.0 %).

In experiment VI Native breed (Sarı) was used and the testicular degeneration occurred some how later and it was milder as compared to SRB bull. On the 16 th. day after the first application of insulation the persentage of the pear-shaped spermatozoa was 4.8 %, narrowness at the base 4.2 %, free sperm heads 7.4 % the total percentage of primary abnormalities was 22.2 % and reached to 31.2 % on the 18 th. day (Table I).

In experiment I and IV the testicular insulation was kept for 4 days, i.e. the application was performed each second day that means 48 hours totally.

After the insulation the percentage of the proximal protoplasmic droplets was 49.5 % and on the 17th day the total percentage of the primary abnormalities reached to 62. 8 %, in experiment I (SRB, 134 Masselberg).

After the same scrotal insulation period, on the 18th day the percentage of the primary abnormalities was only 24.8 %, and there found no significance in proximal protoplasmic droplets, in experiment IV (Crossbred Montafon).

In experiments II and V the duration of scrotal insulation were 3 days, i.e. this application was each second day, totally 36 hours.

In experiment II (SRB 101 Masselberg) 17 days after the scrotal insulation the percentage of the pathological spermatozoa was above

the normal (27.0 %), but in experiment V (Native breed) it was only 14 % and remained in the normal range. While there was a slight change in the proximal protoplasmic droplets in the second experiment, there was no change in experiment V.

In the last experiment a Native bul was used and the scrotal insulation was applied longer than in the other experiments, in order to determine the difference between the exotic purebred bulls and the Native ones. The insulation lasted 10 days. After the first application of insulation the total percentage of primary abnormalities was 53.4 % on the 17 th day, and the proximal protoplasmic droplets was 27.0 % on the 14 th day. The results we obtained from purebred bulls were higher in comparison to this last one.

The percentage of the proximal protoplasmic droplets increased before the sharp fall in sperm concentration and at the beginning of the regeneration phase, the percentage of these droplets began to rise again, that is the increase in the proximal protoplasmic droplets could be seen in short insulations and occurred in two periods: at the beginnings of degeneration and regeneration. Because of the sharp fall in sperm concentration, the increase in number of the proximal protoplasmic droplets could not be seen in long period insulations.

Discussion

During recent years emphasis has been placed on the improvement of animal production in hot countries of the world. In most cases this was tried to achieve by the importation of animals grown in the temperate zone. Dairy cattle introduced in this way, however have frequently failed to thrive. The direct effect of climate on the animals has been an important factor in failure. As we are in the subtropical zone, we decided to deterimine the effect of heat by means of scrotal insulation on different breeds of bulls and also to determine whether there are difference in responses in purebred SRB, native and crossbred Montafon bulls.

No changes have occurred in respect to volume, motility and concentration attributable to the short period insulations (2). Obvious decrease have been observed, however in the motility and concentration as a result of long period insulations. These results are similar to those reported by Lagerlöf (6), Koefoed-Johnsen (5) and Gustafsson (3) Skinner-Louw (12), but the initial motility was less affected in the Native than in SRB bulls.

The prolongation of the insulation periods has caused undesired changes in the spermatozoal characteristics and it has taken a much longer time for regeneration than degeneration. This is in agreement with the findings of Lagerlöf (6).

The increase in spermatozoal abnormalities was singnificantly greater in SRB than in Native and crossbred Montafon bulls. The first type of spermatozoal morphological abnormality appeard was pear-shaped spermatozoa. However large number of bent and coiled tails appeared in several bulls. These results are similar to those reported by Casady et al (2), Glover (3) and Skinner-Louw (12).

Approximately at the end of one week, after the first application of insulation, the percentage of the proximal protolasmic droplets increased, but fell down to the normal limits afterwards, because of the sharp fall in sperm concentration. The percentage of proximal protoplasmic droplets began to rise again during the regeneration period, and gradually fell to the normal percentage. Lagerlöf (6) has found similar results.

The data collected in this study on semen quality indicated that the Native and Montafon crossbred bulls were more resistant to scrotal insulation than the SRB purebred bulls.

Finally, it would appear that even short-term exposure to heat stress may be an important factor in bovine fertility under practical condions. This emphasizes the need for the protection of bulls, especially the exotic ones, from severe heat stress.

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