EFFECT OF SCROTAL INSULATION ON PHYSICAL AND MORPHOLOGICAL SEMEN FEATURES IN YOUNG CATTLE

[Efeito da insulação scrotal sobre as características físicas e morfológicas do sêmen de touros jovens]

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ABSTRACT - The aim of this study was to evaluate the physical and morphological characteristics of semen from young cattle subjected to experimental testicular degeneration by scrotal insulation method. There were used three animals of the Holstein breed with an average of 12 months of age undergoing breeding soundness examination weekly for a period of 10 weeks. Semen was collected from animals by the method of electroejaculation and evaluated for physical characteristics of semen, sperm morphology and thermo-resistance test. Scrotal insulation for 120 hours increased scrotal temperature by 3.4 °C. The rectilinear progressive sperm motility and sperm vigor decreased between 7 to 28 days post-insulation ($p<0.05$) and sperm incidence with abnormal head increased between 21 to 35 days post-insulation. There was a decrease ($p<0.05$) in sperm motility during thermo-resistance test, for the periods post-insulation, indicating low sperm viability. The testicular degeneration induced by insulation bags may be temporary and even animals during puberty and adolescence have the ability for restructuring of the seminiferous epithelium and restoration of normal sperm production, although there is need for more recovery time.

Keywords: cattle, scrotal insulation, semen, testicular degeneration

RESUMO: O objetivo deste estudo foi avaliar as características físicas e morfológicas do sêmen de bovinos jovens submetidos à degeneração testicular experimental pelo método de insulação escrotal. Foram utilizados três animais da raça Holandesa, com uma média de 12 meses de idade, submetidos a exame andrológico semanalmente por um período de 10 semanas. O sêmen foi coletado pelo método de eletroejaculação e os animais avaliados quanto às características físicas e morfológicas do sêmen e teste de termorresistência. A insulação escrotal por 120 horas aumentou a temperatura escrotal em 3,4 °C. A motilidade espermática progressiva retilínea e vigor espermático diminuiram entre 7 a 28 dias pós-insulação ($p <0,05$) e a incidência de espermatozóides com a cabeça anormal aumentou entre 21 a 35 dias pós-insulação. Houve uma diminuição ($p<0,05$) na motilidade espermática durante o teste de termorresistência, nos períodos pós-insulação, indicando baixa viabilidade espermática. A degeneração testicular induzida por sacos de isolamento pode ser temporária e até mesmo animais durante a puberdade e adolescência têm a capacidade de reestruturação do epitélio seminífero e restauração da produção espermática normal, embora haja necessidade de maior tempo de recuperação.

Palavras-chave: bovinos, degeneração testicular, insulação scrotal, sêmen

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INTRODUCTION

Spermatogenesis is under the physiological control of neuroendocrine system and undergoes direct influence of the Scrotal/testicular thermoregulation (Byers and Glover, 1984).

In mammals, normal spermatogenesis depends on ideal maintenance of scrotal temperature, which in bulls should be kept between 2-6 °C below of body temperature that are produced fertile sperm (Gabaldi and Wolf, 2002; Barros et al., 2011; Brito et al., 2012).

Elevation of room temperature causes testicular degeneration by interfering with thermoregulatory mechanism and has been referenced as the main cause of subfertility in cattle breeding, especially those living in unfavorable environment (Fonseca, 1976; Blanchard et al. 1996; Nascimento and Santos, 1997; Barros et al., 2011). Elevation of the testicular temperature increases metabolism and oxygen demand, but testicular blood flow is limited (Brito et al., 2012).

The testicular degeneration involves degenerative changes in the epithelium of seminiferous tubules, which lose their ability to produce physiologically normal spermatozoa (Wildeus and Entwistle, 1986; Bernardi, 1990; Vogler et al. 1993), or even affect the total sperm production (Ross and Entwistle, 1979). In general, the causes of testicular degeneration are related to etiological processes more generalized and, in many cases it is not possible to recognize the primary cause (Nascimento and Santos, 1997), however, a recognized cause of testicular degeneration is the thermal injury.

In scrotal insulation, the cremaster muscle, pampiniform plexus and dartos tunic lose their ability to accomplish thermoregulation, allowing exposure of the seminiferous tubules at high temperatures, hyperthermia and a degenerative process occurring in the testes (Bernardi, 1990; Blachard et al. 1996; Camp, 1997; Rahman et al., 2011), with damage to the spermatogenic process (Fonseca, 1976; Hafez and Hafez, 2004).

Since the first half of this century has already produced work to the knowledge of the problems, arising from the increase of testicular temperature on the quality of semen produced. Lagerlof (1938) when experimentally induced the testicular temperature rise recorded an increase of sperm changes, which became a constant concern of researchers, especially after the expansion of the technique of artificial insemination. The increase in scrotal temperature, natural or induced by scrotal insulation interferes with testicular thermoregulation, predisposing to degeneration of the gonadal germinal epithelium. This temperature rise has consequences for sperm quality and subsequently to fertilization and embryo survival by interfering directly in fertility (Mieusset et al. 1992).

The aim of this study was to evaluate the physical and morphological characteristics of semen from young cattle subjected to experimental testicular degeneration by scrotal insulation method.

MATERIALS AND METHODS

There were used three Holstein breed with an average of 12 months of age. The animals were kept in paddocks containing *Brachiaria decumbens*, mineral salt and water ad libitum. Before starting the experiment, the animals were submitted to clinical evaluations and only those who were clinically healthy composed the experimental group.

The experiment lasted 10 weeks, during which the animals were subjected to one semen collection per week, totalizing 10 sessions of samples by animal, divided into three phases: the pre-insulation phase (control samples), corresponded to the first two weeks of the experiment (days -14 and -7), which was made the breeding soundness examination of bulls. The scrotal insulation phase with duration of 120 hours (day 0) corresponded to the period in which the animals remained with a freezer bag in the scrotum and the post-insulation phase corresponded to the period after withdrawal of thermal bag, with semen evaluation weekly for 7 weeks.

To promote the insulation, the scrotum was involved with cotton and plastic layers in the following order: cotton (layer of 0.5 cm thickness), plastic bag, cotton and another plastic bag. These bags were placed with adhesive tape in the spermatic cord region, being careful not to damage the testicular circulation. During the insulation, the thermal variation of the scrotum was monitored every 24 hours after beginning of the scrotal insulation, with the aid of a digital thermometer and the measurements were made on the back of the scrotum, inside the insulation bag, as recommended by Fonseca (1976).

Throughout the trial period, during breeding soundness examination, after restraint in appropriate trunk, the animals were subjected to clinical examination of the reproductive organs, evaluating the scrotum, testis, epididymis, spermatic cord, foreskin, penis, seminal vesicles

and ampules. Then the values for scrotal circumference (SC) and testicular consistency were recorded. Semen was collected by electroejaculation method, and analyzed the physical aspects of the ejaculate (appearance, sperm motility and sperm vigor). With the aid of a microscope in a magnification of 100x, analyzing the progressive motility (0-100%) and the spermatic vigor (0-5) according to the procedures recommended by the Brazilian College of Animal Reproduction (1998).

Thereafter, for thermo-resistance test, the remaining volume of each ejaculate was diluted in a 1:1 ratio, amid citrate-egg yolk without glycerol (sodium citrate 2.94%, 1% fructose, 100,000 UI/mL streptomycin, and egg yolk 20%) (Mies Filho, 1982). The samples were placed in test tubes, kept at 37 °C in water bath for evaluation of individual motility and spermatic vigor (every 30 minutes during 3 hours) (Vogler et al. 1991).

An aliquot of each ejaculate was stored in 1 mL of saline-buffered formalin solution (Hancock, 1956) and subsequently analyzed, adopting the criteria established for Blom (1973), classifying the abnormal sperm in major, minor and total defects. We emphasize that in the present study, all the defects present in spermatic cells were recorded, regardless of the number or classification as major or minor defect, unlike current methods, which records only one sperm abnormality by spermatozoon (most apical) (CBRA, 1998) since the purpose of this study was to evaluate the scrotal thermal stress effect on spermatogenesis.

For data evaluation, we used the statistical software SAEG (UFV, 2007). For all data (testis size, physical and morphological sperm evaluations, additional tests) were performed the analysis descriptive statistics (average and standard deviations). For quantitative data, were performed the variance analysis and when significance was observed for the F test, was performed test comparison of averages by DMS with 5% of error probability.

RESULTS

In Table 1 are demonstrated the climate averages during the experimental period.

After scrotal insulation, the three animals showed good health and no injuries were found for internal and external genitalia. The testes showed no changes in the mobility, symmetry and inguinal position. The epididymides were within the normal standards required for bulls. The spermatic cords had elongated aspect, under the cremaster muscle relaxation and increase in diameter due to vasodilatation of pampiniform plexus, indicating natural mechanism of thermo-regulatory of organs. The glands were considered normal in size and consistency.

The SC was negatively affected, presenting reduced from the seventh day after scrotal insulation (Table 2).

Table 1: Averages and standard deviations of daily maximum and minimum temperature and relative air humidity during the experimental period of scrotal insulation in young cattle.

WEEKS	MAXT (°C)	MINT (°C)	DAYT (°C)	RH (%)
1	26.13±4.18	18.07±1.88	20.74±2.88	81.14±8.07
2	27.97±2.27	16.33±3.56	21.01±2.10	75.57±6.32
3	29.20±1.56	19.21±1.42	22.81±1.39	79.14±4.63
4	27.34±2.09	19.29±1.69	22.36±1.74	80.28±7.89
5	31.49±1.81	18.61±1.88	24.37±1.12	69.14±6.62
6	26.64±3.93	20.19±0.63	23.07±1.48	87.00±7.39
7	25.47±3.28	19.17±0.74	21.78±1.05	87.14±9.26
8	29.61±2.50	20.26±0.57	23.66±0.72	82.86±3.02
9	28.64±2.14	19.60±1.66	22.87±1.12	82.28±4.53
10	26.50±2.91	19.81±2.02	22.41±1.80	87.00±6.19
AVERAGE	27.89±1.85	19.05±1.67	22.51±1.12	81.15±5.66

*ANOVA (p>0.05); MAXT: Maximum room temperature; MINT: Minimum room temperature; DAYT: daily average temperature; RH: relative humidity.

The scrotal insulation caused a reduction in the individual motility and spermatic vigor. (Table 2) from the 7th day after withdrawal of the bag and remain in decline until the 4 week post-insulation when then began to rise, both motility and spermatic vigor (48.38% and score 2.66, respectively), but in the last evaluation (8 week) post-insulation averages still had not returned to the values recorded in the pre-insulation phase (75% and score 3, respectively, for motility and vigor).

After removal of the insulation bag, the physiological thermoregulation of the testes was restored and returned to its tense-elastic consistency (35 days post-insulation), reflecting in the next

session of semen collection, where the animals showed spermatozoon in the ejaculate. However, testicular consistency restored did not indicate the correct functioning of the gonads, as verified by the high incidence of sperm abnormalities of testicular origin in ejaculates (Table 3).

Table 2: Weekly variation in spermiogram of three young Holstein breed submitted to scrotal insulation on days -7 to 0.

PERIOD	WEEKS	DAYS	SC	MOT	VIG
Pre-insulation	1st	-14	25,16^{NS}	75.00^{a}	3.00^{a}
	2nd	-7	25.16 ^{NS}	75.00^{a}	3.00^{a}
Insulation	3rd	0	26.83 ^{NS}	70.00^{a}	3.33^{a}
Post-insulation	4th	7	26.50 ^{NS}	10.00^{c}	3.00^{ab}
	5th	14	26.16 ^{NS}	10.00^{c}	1.33^{c}
	6th	21	25.93 ^{NS}	3.33^{c}	0.66^{c}
	7th	28	25.83 ^{NS}	*	*
	8th	35	26.16 ^{NS}	48.38^{b}	2.66^{ab}
	9th	42	26.40 ^{NS}	41.66^{b}	1.66^{bc}
	10th	49	26.66 ^{NS}	43.33^{b}	2.5^{ab}

SC: Scrotal circumference; MOT: progressive motility; VIG: Spermatic vigor. NS: non-significant to 5%. a - c: different letters in the same column, there is a difference ($p < 0.05$) by LSD tests. *Azoospermia.

Table 3: Weekly variation in spermiogram of three young Holstein breed submitted to scrotal insulation on days -7 to 0.

	SPERMATIC CHANGES (%)								
DAYS	ACR	HD	IP	TD	PD	SSD	PSD	TSD	TNC
-14	5.35^{cde}	11.69^{c}	2.98^{ef}	6.69^{NS}	27.70^{NS}	12.54^{cd}	40.26^{d}	54.43^{d}	54.77^{ab}
-7	5.58^{de}	20.00^{c}	4.75^{ef}	7.75^{NS}	13.91^{NS}	13.58^{cd}	38.41^{d}	52.00^{d}	59.66^{a}
0	3.25^{e}	17.00^{c}	2.5^{f}	7.58^{NS}	34.50^{NS}	12.5^{d}	52.33^{cd}	64.83^{cd}	46.00^{abc}
7	8.72^{cde}	84.03^{b}	40.6^{a}	22.44^{NS}	16.95^{NS}	14.46^{bcd}	161.34^{a}	175.81^{a}	5.48^{de}
14	24.75^{b}	148.50^{a}	22.75^{b}	15.33^{NS}	11.91^{NS}	29.25^{a}	194.00^{a}	223.25^{a}	2.75^{e}
21	40.33^{a}	135.00^{a}	27.16^{b}	8.41^{NS}	10.58^{NS}	21.41^{b}	200.08^{a}	221.50^{a}	2.58^{e}
28	*	*	*	*	*	*	*	*	*
35	16.16^{bc}	41.50^{c}	15.33^{c}	11.83^{NS}	38.91^{NS}	20.50^{bc}	103.25^{b}	123.75^{b}	20.16^{de}
42	16.00^{bc}	35.58^{c}	9.00^{de}	10.16^{NS}	17.25^{NS}	14.91^{bcd}	73.08^{bcd}	88.00^{bcd}	31.41^{bcd}
49	14.00^{cb}	32.66^{c}	12.16^{cd}	12.08^{NS}	27.25^{NS}	13.33^{cd}	84.83^{bc}	98.16^{bc}	29.91^{cd}

ACR: Acrosome defects, HD: head defects, IP: intermediate piece defects; TD: tail defects; PD: protoplasmic droplet; SSD: secondary sperm defects; PSD: primary sperm defects; TSD total sperm defects; TNC: total of normal cells. NS: non-significant to 5%. a - c: different letters within the column, there are differences ($p < 0.05$). *Azoospermia.

In this study, it were observed an increase of sperm defects during the experimental period ($p<0.05$). The defects that had a higher frequency were head with abnormal coloration, narrow head at the base, abnormal head, abaxial midpiece insertion and acrosome defects.

With respect to sperm abnormalities, there was an increase of protoplasmatic droplet; immediately after removal of the insulation bag, with the prevalence proximal protoplasmic droplet; to the detriment of distal protoplasmic droplet.

There was a reduction in percentages of normal cells in the ejaculates from 7 days and increased at 35 days post-insulation (Table 3). Although there was an improvement in the percentage of normal cells until 49 days post-insulation, the values were still below the initial (pre-insulation). If these animals had a longer follow-up, perhaps the normal cells values would return to that recorded in the pre-insulation period.

DISCUSSION

During insulation, the scrotal temperature varied from 34.6 ºC to 39.9 ºC, being close to (35.1 to 36.6 ºC) those reported by Barth and Bowman (1994) in taurine animals subjected to scrotal insulation. In this study, only the third day post-insulation reached the average temperature of 38 °C, indicating that the technique used by several cotton layers, was not very efficient in raising the temperature within 48 hours.

In situations of high ambient temperature, body temperature is maintained by peripheral vasodilatation, sweating and shortness of breath, allowing the body heat loss. However, the mechanisms of thermoregulation are limited and if not sufficient, resulting in hyperthermia causing

thermic stress. Heat stress, hypoxia and oxidative stress resulted in apoptosis of sperm. In that regard, it has been suggested that testicular hyperthermia, in particular scrotal insulation, may adversely affect spermatogenic cells (Rahman et al., 2011). In this study, during the insulation, the average temperature of the environment and physiological animal was 22.51 °C (Table 1) and 37 °C, respectively, which did not affect the increase in scrotal temperature caused by thermal bag.

Throughout the experimental period (pre, during and post-insulation), the daily average temperature of 22.51 ± 1.12 ºC and relative humidity of 81.15 ± 5.66% were within the comfort range (22.34 to 23.04 °C) for the Holstein breed, according to standards described by Armstrong (1994), showing that environmental factors did not exert negative effect on the characteristics of homeostasis of animals (Table 1). These observations were also found in the gonads by the tense-elastic consistency (physiologically normal) and average scrotal temperature of 34.9 and 34.8 °C in the first and second weeks pre-scrotal insulation, and an average body temperature of 38.8 °C presented throughout the experimental period.

After placing the thermic bag, the scrotal temperature rose gradually reaching a temperature of 38.2 °C only after 48 hours remaining until the moment of its withdrawal. This caused the change of testicular consistency where all the bulls began to show slight sagging testicular, remained so for a 30 days after after withdrawal of the bag. The rise of 3-4 °C with the use of the scholarship was also reported by Wildeus and Entwistle (1983), who observed an increase in scrotal temperature immediately after preparation of the insulation bag, with an average difference of 4.05 °C temperature between bulls and insulated control, however, being higher to those achieved and recorded by Barth and Bowman (1994), probably because of the time (6 days vs. 48 hours) use of the bag and the number of cotton layers (4 vs. 3) that were greater in number and time, respectively when compared to this study.

Although there was no statistical difference in SC ($p > 0.05$), we found an increase of approximately 1.7 cm in SC animals at day 0 and decreased to 1 cm from the 7th until the 28th day post-insulation. These observations corroborate those recorded by Collins et al. (1997) who observed no difference in SC between adult goats and the control group. However, reduction in the average values of SC coincides with the period in which the animals showed testicular consistency slightly flaccid (28 days after beginning of the insulation) and the reduction of the physical aspects of the semen, resulting in an absence of spermatozoa in the 5 week after beginning of the insulation. This probably occurred due to the low extra-gonadal reserve, has also been observed in bulls insulated by Wildeus and Entwistle (1983). However, in this study, this feature may be due to young age of the animals that were used in the adolescence phase or after puberty, which is not observed in the structure of the seminiferous epithelium, with few layers of germ cells and large epithelial losses, so that the spermatogenic process is still inefficient to establish extra-gonadal reserve.

Arteaga et al. (2005) observed that animals submitted to scrotal insulation for four days had higher sperm motility than insulated animals for eight days (66.4 vs. 41.5%, respectively). These authors found that sperm motility decreased below pre-insulation levels in both groups on day 7 after removal of the insulation and returned to pre-insulation on day 38 only in the insulated group for four days. Pezzini et al. (2006) using Curraleiro and Holstein breed, observed, post-insulation for 48 hours, a reduction in sperm motility from the fifth and ninth days, respectively. These results differ from other studies that report the occurrence of a change in sperm motility from the 12 day post-insulation (Vogler et al. 1993; Barth and Bowman, 1994; Brito et al. 2003).

Changes in the progressive motility and vigor are related to changes in spermatogenesis and disturbances in the epididymis, where sperm acquire their motility (Hafez and Hafez, 2004). In this study, it is likely that the effect of temperature increase occurred primarily in the epididymis, interfering in the process of maturation of sperm cells. It is noteworthy that during puberty, the epididymis did not show normal due to be hormone-dependent (testosterone), resulting in production of ejaculates with low and irregular sperm concentrations, reaching normal standards only after sexual maturity.

The sperm concentration was not measured because of the sampling method used, since the eletroejaculator tends to disguise the true concentration of the ejaculate (Guimarães, 1993). However, the ejaculates were with watery aspect from 7 days post-insulation, and maintained this characteristic until 35 days post-insulation, when it started to produce opalescent or milky aspect until the end of the experiment.

There were observed variation in type and percentage of morphologically abnormal spermatozoa post-insulation produced (Table 3), which can be explained by the fact that the appearance of various abnormalities follows a chronological order (Vogler et al. 1993).

Furthermore, after scrotal insult, the abnormality type is linked to the spermatogenesis stage throughout the insulation life. Arteaga et al. (2005) noticed that eight days of scrotal insulation caused major effects on sperm quality compared to four days, suggesting that the scrotal insulation not only has a deleterious effect on sperm quality but also that the severity of defects is related to length insulating.

Regarding sperm defects, according to Walters et al. (2006), the increase in the concentration of sperm abnormalities besides interfering negatively in the ejaculate quality may affect the fertilizing ability of sperm or embryo survival. According to Takahashi (2012) and Saadi et al. (2013), elevated testicular temperature induced by impaired scrotal/testicular thermoregulation is believed to be a common cause of abnormal spermatogenesis and impaired sperm function in domestic farm animals and in humans, with outcomes ranging from subclinical infertility to sterility. This principle has been widely utilized in research studies to induce various sperm abnormalities, including pyriform sperm.

Pezzini et al. (2006) observed increase of normal head from the 5 day post-insulation, with a peak incidence at 23th day for Curraleiro (9%), and on 30th day for Holstein breed (13%). Fonseca and Chow (1995) found that the abnormalities observed among the first who came forward were proximal droplets, normal heads and folded and coiled tails, indicating the beginning of degenerative disease. Head anomalies were also the main sperm changes recorded by Fonseca (1976). However, Wildeus and Entwiltle (1983) who observed a higher frequency of changes in tail, protoplasmic droplets and acrosome recorded different results.
Folded and coiled tails are acquired defects probably in the epididymal transit, because of cell exposure to secretions epididymal sperm abnormal (Barth and Bowman, 1994). However, in this experiment there was no difference ($p>0.05$) in the occurrence of changes in tail recorded in the pre and post-insulation periods. Different result recorded by Pezzini et al. (2006) where the incidence of tail folded strongly differed from pre-treatment at 16th days post-insulation.

In the pre-insulation period (-14) animals already had high incidence of proximal protoplasmatic droplet (Table 3), is common during adolescence, due to not epididymal maturation (Guimarães, 1993). Wildeus and Entwistle (1983) and Blanchard et al. (1996) observed a high frequency of this abnormality in bulls and stallions insulated, respectively. These observations are due to high temperature have an effect on the normal production of testosterone thus impairing the epididymal function (androgen dependent) (Amann, 1987; Barth and Oko, 1989; Gabaldi et al., 1999), thereby preventing sperm maturation and loss of protoplasmic droplet (Hafez and Hafez, 2004).

Similar results were observed by Gabaldi et al. (1999) who reported increased proximal protoplasmic droplet between the seventh and tenth day of the post-insulation period, suggesting epididymis function impaired and the emergence of this morphological change. However, Pezzini et al. (2006) observed the increase of proximal protoplasmic droplet only from 23 days post-insulation suggesting that the occurrence of this defect in the ejaculate may be a result of changes still in the spermatids development process or spermatocytes. These authors noted differences between breeds, with values of proximal protoplasmic droplet ranging from 3.5% in Curraleiro to 13% in the Holstein breed (sexually mature animals with an average age of 6 years). Already Collins et al. (1997) found no difference in the percentages of proximal protoplasmic droplets from goats of groups submitted and not submitted to scrotal insulation.

From the 7 day post-insulation, there was a decrease in protoplasmic droplet percentage and an increase in abnormalities of head and abnormal sperm cells (table 3) related to high temperatures induced in the testes. These results are consistent with those found by Vogler et al. (1991) and Santos and Simplicio (2000) who found increased of head abnormalities in sperm of cattle and goats submitted to scrotal insulation. However, Blanchard et al. (1996) observed the appearance of abnormalities in the head only 17 of the 35 days post-insulation in stallions.
Barth and Bowman (1994) found that the normal cells percentages reached lowest values 14 days post-insulation returning to pretreatment values 35 days post-insulation. Horn et al. (1999) found 38% decrease in the normal cells percentage at 23 days post-insulation and the initial values were again obtained from day 44 post-insulation. The variation in the normal cells percentage post-insulation recorded by these authors in relation to this experiment can be explained by differences in age of animals used. While Barth and Bowman (1994) and Horn et al. (1999) worked with animals of 24 months of age, in this study it was used animals with 12 months, which were more predisposed to testicular degeneration by not having the complete structure of the seminiferous epithelium as well as epididymal maturity.

In addition to the morphology changes of sperm at 28 days, post-insulation all animals had ejaculates

with low sperm concentration and large number of atypical cells (stem cells, giant, epithelial, pyocytes, and leukocytes). Blanchard et al. (1996) found that some spermatogenic cells rounded (spermatocytes and round spermatids) appeared in the ejaculate approximately in 15-30 days post-insulation.

From 35 days post-insulation, the animals initiate the recovery of sperm motility and vigor, which coincided with the start of improvement in major and total defects (Table 2 and 3). Santos and Simplicio (2000) reported restoration of spermatic group on average 8 weeks and a half and 10 weeks after removal of the insulation bag for Moxotó and MOPA, respectively. According to Setchell (1998) generally the spermatic morphology returns to the values obtained in the pre-treatment with approximately 6 weeks after insulation, which was not seen in this study. However, according to the author, even after cell morphology returned to normal, this sperm has a low fertilizing capacity and there is an increased in embryonic death incidence.

In the thermo-resistance test performed in this experiment in post-insulation periods, there was no decrease in the individual motility ($p > 0.05$) during the three hours of incubation in the test execution. However, we can see the difference in initial individual motility in different post-insulation collects. Low values of sperm motility between days 7-28 post-insulation, occurred because the semen showed with high rates of sperm alterations. Vogler et al. (1991) noted that for the non-frozen sperm the initial spermatic motility when compared to the final, did not differ between the days -6, -3, pre-insulation (period 1) and days 3, 6 and 9 post-insulating (period 2.) However, recorded difference between the periods 1, -2 and period 3 (days 12, 15, 18 and 21 post-insulation).

CONCLUSIONS

The data evaluated in this study suggest that the experimental testicular degeneration in young Holstein breed significantly affect spermatogenesis, demonstrating that in pre-puberty or adolescence, induced testicular degeneration occurs temporarily, but the experimental post-insulation period was not enough for complete restoration of spermatogenesis occurred and there is need for more time for recovery, indicating that young animals are more susceptible to degeneration than adult animals or mature sexually.

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