RESISTANCE OF RABBIT SPERMATOZOA TO AMBIENT STRESS AND TO 24-HOUR REFRIGERATION AT 5° C

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ABSTRACT

The effect of temperature upon seminal characteristics of New Zealand White rabbits was studied, through comparison of the quality of freshly collected semen with that of semen kept 24 hours at 5 °C, considering two different periods (seasons). A saline diluting solution was used, being made up of trizma, glucose, EDTA (ethylene diamine tetra-acetic) and citric acid (pH 6.8 - 7.0). The experimental design was a randomized blocks and the variable studied were motility, strength, spermatozoa concentration and pathologies. During the hot period, values of motility and strength were lower (71.9% and 3.0 *vs.* 85.6% and 3.4) as compared to cool period. The occurrence of spermatozoa pathologies increased in the hot period (33.9% *vs.* 25.4%), especially, the presence of protoplasmic droplets (16.2% vs. 11.3%). Results were similar between freshly collected semen and refrigerated one, except the higher ($p < 0.05$) occurrence rate of protoplasmic droplets in fresh semen (15.0% vs. 11.4%).

Key words: New Zealand White, semen, quality, temperature, pathology.

INTRODUCTION

Seminal characteristics vary among individuals and between ejaculates of the same individual. The differences encountered must be considered relative (UBILLA & REBOLLAR, 1995). BATTAGLINI *et al*. (1992) evaluated the qualitative and quantitative characteristics of rabbit semen, studied the existing correlations and estimated the repeatability of these events. Low repeatability of measurements was obtained, particularly, the number of spermatozoa per ejaculate (0.20) and motility (0.12). Semen volume and spermatozoa concentration vary, approximately, between 0.1 and 1.5 ml, and 1×10^6 and 1×10^9/ml, respectively. According to the latest authors, the low volume of ejaculate and low concentration of spermatozoa, in addition to poor resistance of the cells during long period storage, limit the use of artificial insemination in the rabbit.

Differently of other species, rabbit semen doesn't resist to dilution, due to sensitiveness to hypertonic solutions, and to cryoprotector agents containing hydroxyl groups (CASTELLINI *et al.*, 1992), as glycerol (LÓPEZ & ALVARIÑO, 1998). Freezing is not yet a

practical means for maintaining viability of rabbit spermatozoa. Semen storage for short periods, normally less than 48 hours, at temperatures between 5 and 25 °C, is practice commonly adopted (ALVARIÑO *et al.*, 1996).

In Brazil, data about semen preservation in rabbits and andrological evaluation are still very few. The environment in which animals are kept are subject to seasonal variations, where hot and wet climate is predominant. The hypothesis of the present trial is that thermal stress may affect the quality of fresh and refrigerated semen The aim of this experiment was to draw the andrological profile of rabbit bucks, and assess the qualitative characteristics of either freshly collected semen or kept at 5 °C for 24 hours, during a hole year period.

MATERIALS AND METHODS

The andrological survey of 17 New Zealand White rabbit bucks was carried out, during the period of June 2001 to September 2002. The mean of maximal temperature during the hot period (October till April) was 29.5 °C and the mean minimal was 17.5 °C. During the cool period (May till September) the mean maximal was 27.4 °C while the mean of minimal temperature was 12.7 °C.

To assess the effects of ambient stress, temperature and time of storing on semen quality, semen was collected with artificial vagina, and basically, the same routine was conducted for both fresh and refrigerated semen. The first step was dilution with saline solution (1 part of semen and 4 parts of solution). The diluting solution was made up of trizma, glucose, EDTA (ethylene diamine tetra-acetic) and citric acid (pH 6.8 - 7.0). After that, spermatozoa motility and strength were analyzed, as parameters of fresh semen quality. Concentration and morphological analyses of spermatozoa were also performed. In this case, the aliquot was fixed with a solution of formol 1% and the dilution factor brought up to 50 (1 part of the semen solution with 9 parts of formol). The targeted spermatozoa pathologies were those of the head (separated head and abnormalities of acrosomal and plasmatic membranes), pathologies of the intermediary segment (protoplasmic droplets and edema) and those of the tail (insertion and conformation).

The andrological evaluation of the bucks was made by assessing these variables during the whole year. The effects of storage were obtained through the comparison of the characteristics of the two sub-samples taken from the first dilution. A sub-sample was analyzed immediately, while the other was refrigerated at 5 °C for 24 hours, returned to physiological temperature by incubating 5 minutes in water-bath at 37 °C, and then analyzed.

Analysis of variance was performed using SAS. For spermatozoa concentration and pathologies, means were compared by the test of Student. For motility and strength, nonparametric analyses were performed, with the means compared by the test of Wilcoxon.

RESULTS AND DISCUSSION

Concentration of spermatozoa as well as the number of inseminating doses, was not affected by periods (hot or cool), as shown in Table 1. Production of inseminating doses is obtained multiplying concentration by motility, and reflects the concentration of viable cells capable of reaching oocyte in the oviduct. Although motility decreased in the hot period (Table 2), this didn't affect the number of viable cells required per dose (6.10^6 cells/0.5 ml).

Table 1: Concentration and production of inseminating doses of rabbit semen during hot (October till April) and cool period (May till September), respectively

Period	Spermatozoa concentration (10^6 sptz/ml)	Inseminating doses production
Hot	143.2[a]	15.0[a]
Cool	127.0[a]	15.0[a]
CV (%)	26.2	36.4

[a] Means with the same superscript in the same column are equal, by the test of Student ($p > 0.05$)

Table 2: Motility and strength of rabbit spermatozoa during hot (October till April) and cool period (May till September), respectively

Period	Motility	Strength
Hot	71.9[b]	3.0[b]
Cool	85.6[a]	3.4[a]

[ab] Means with different superscripts within a column are different by the test of Wilcoxon ($p< 0.05$)

Apart from being included in total spermatozoa pathologies, acrosomal membrane abnormalities and protoplasmic droplets were also computed separately due to their impact on oocyte fertilization and because of the high frequency observed. Within the hot period, increased the occurrence of protoplasmic droplets as well as the total pathologies (Table 3).

Table 3: Occurrence of seminal pathologies in the rabbit in function of period (hot and cool)

Period	Total pathologies (%)	Acrosomal pathology (%)	Protoplasmic droplets (%)
Hot	33.9[b]	4.4[a]	16.2[b]
Cool	25.4[a]	5.1[a]	11.3[a]
CV (%)	18.0	25.5	39.5

[ab] Means with different superscripts within a column are different by the Student test (p<0.05)

There was no effect of refrigeration upon motility and strength (Table 4). The occurrence of seminal pathologies remained within acceptable limits (Table 5). Trials on this issue normally refer to inseminating capacity of semen, and reveal no significant alterations on fertility and prolificacy rates by storing in such conditions (EGEA & ROY, 1992; ALVARIÑO *et al*., 1996; LOPEZ & ALVARIÑO, 1998).

Table 4: Motility and strength of rabbit spermatozoa, in either freshly collected semen or refrigerated at 5 °C for 24 hours

Status	Motility (%)	Strength (score 0-5)
Fresh	73.2[a]	3.7[a]
Refrigerated	66.4[a]	3.5[a]

[a] Means with the same superscript in the same column are equal, by the Wilcoxon test (p.0.05)

A reduction in the occurrence of protoplasmic droplets was registered with refrigerated semen (Table 5). This fact might be explained by the period of maintenance of semen in iso-osmotic medium, which might allow that the spermatozoa, through beating of their tails, release the protoplasmic droplets, especially the distal ones.

Table 5: Occurrence of spermatozoa pathologies in the rabbit, either in freshly collected semen or refrigerated at 5 °C for 24 hours

Status	Total pathologies (%)	Acrosomal pathology (%)	Protoplasmic droplets (%)
Fresh	25.4[a]	6.2[a]	15.0[b]
Refrigerated	28.2[a]	6.6[a]	11.4[a]
CV (%)	14.3	22.8	31.0

[ab] Means with different superscripts within a column are different by the Student test (p<0.05)

CONCLUSION

Andrological evaluation of rabbit bucks during the whole year, revealed the deleterious effect of ambient stress, caused by thermal discomfort. This effect was detected through semen analysis. However, the accuracy of these analyses is not guaranteed, once the coefficients of variation were high.

The results obtained demonstrated the viability of refrigerated semen, based on comparison with results of routine analyses and on minimal acceptable standards. Further prospective studies are needed, especially, the evaluation of refrigeration conditions, such as refrigeration curve and utilization of biomarkers, that reflect better

the sub-clinical damages caused to the cells as result of stress due to cold shock, especially during transition period.

This initial study helped define the methodological needs for our further experiments, e.g. the utilization of more sensible and accurate analyses. The evaluation of production animals is the way to knowing the biological material available and to determine related technical needs.

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