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CORRELATIONS BETWEEN HYPOOSMOTIC SWELLING TEST AND THE CLASSICAL EVALUATION OF GOAT SEMEN

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ABSTRACT

We evaluated the correlation between the assessment of the functional integrity of the sperm membrane by hypoosmotic swelling test using solutions with different osmolarities, and the conventional assessments of fresh semen in goats. A total of 24 ejaculates from three goats was obtained by artificial vagina and immediately submitted to the classical evaluation. Samples were divided into five aliquots and subjected to hypoosmotic test using distilled water (0 mOsm/L), and sodium citrate and fructose solutions at different osmolarities (50; 100; 150 and 200 mOsm/L). The 100 mOsm/L solution showed the highest percentage of reacted sperm (34.8%), but distilled water was the one with the lowest values (20.8%). No significant correlations were detected between the reacted sperm verified by the hypoosmotic swelling test and other semen characteristics (P> 0.05). Nevertheless, we recommend the carry out of the hypoosmotic test by using a 100 mOsm/L sodium citrate and fructose solution to assess the functional integrity of the sperm membrane in caprine species.

KEYWORDS: caprine; hyposmotic swelling test; sperm.

CORRELAÇÕES ENTRE O TESTE HIPOSMÓTICO E A AVALIAÇÃO CLÁSSICA DO SÊMEN DE CAPRINOS

RESUMO

Avaliou-se a correlação entre a avaliação da integridade funcional de membrana espermática por meio do teste hiposmótico, utilizando-se soluções com diferentes osmolaridades, e as avaliações convencionais do sêmen fresco de caprinos. Foram utilizados 24 ejaculados de três caprinos machos, obtidos por vagina artificial, os quais foram imediatamente submetidos à avaliação clássica. Em seguida, as amostras foram divididas em cinco alíquotas e submetidas ao teste hiposmótico, utilizando-se água destilada (0 mOsm/L) e soluções de citrato de sódio e frutose de diferentes osmolaridades (50; 100; 150 e 200 mOsm/L). A solução de 100 mOsm/L obteve a maior média de espermatozoides reagidos (34,8%) ao teste hiposmótico (P < 0,05); por outro lado, a água destilada foi a que apresentou os menores valores (20,8%). Não foram detectadas correlações significativas entre os espermatozoides reagidos ao teste hiposmótico e as demais características seminais (P > 0,05). Apesar disso, recomenda-se que o teste hiposmótico seja realizado utilizando-se uma solução à base de citrato de sódio e frutose apresentando 100 mOsm/L para a avaliação da integridade funcional da membrana espermática na espécie caprina.

PALAVRAS-CHAVE: caprino; espermatozoide; teste hiposmótico.

INTRODUCTION

The tests used in semen analysis routine consist, primarily, in the evaluation of concentration, morphology and sperm motility, recommended by the Brazilian College of Animal Reproduction (CBRA, 1998). However, there is evidence that these parameters alone are not sufficient to estimate the fertility potential of an ejaculate. As the plasma membrane is involved in metabolic exchanges with the environment, the study of its functionality is essential, given the strong influence of its biochemical activity in the processes of sperm capacitation and fertilization. Besides, when it is carried out with the traditional parameters of semen evaluation, it results in more accurate determination of fertility rates (LEBOUEF et al., 2006).

JEYENDRAN et al. (1984) proposed the use of the hypoosmotic test (HOST), because it was considered a simple and accessible method, capable of detecting intense alterations in sperm functionality in samples that would not be discarded if only the results of sperm motility and morphology were considered. This test is characterized by the influx of fluids into the sperm cells under hypoosmotic conditions, until balance between the compartments is reached, being indicative that the water transport through the membrane is occurring normally (INAMASSU et al. 1999).

Despite the relative simplicity of the HOST, several points can be worked out to make it a test of high reliability. Researchers can discuss aspects such as which solute to use, ideal osmolarity of the solution, and the number of cells to be counted (MELO & HENRY, 1999). Studies with canine (BUENO et al. 2001), equine (MELO & HENRY, 1999), bovine (SIQUEIRA et al., 2006) and buffalo semen (LODHI et al. 2008) revealed the existence of correlation between the HOST and other seminal parameters, demonstrating that this test could be used as a fertility predictive test. FONSECA et *al.* (2005) tested solutions from 50 to 300 mOsm / L in an attempt to identify the most appropriate osmolarity to be used in the evaluation of goat's fresh semen. However, the authors evaluated the integrity of the membrane alone and did not establish a correlation between the results obtained with other parameters of sperm quality.

Therefore, this study aimed to evaluate the correlation between the results of membrane integrity, found through the HOST, using solutions with different osmolarities, and conventional tests of goat's fresh semen.

MATERIAL AND METHODS

The experiment was conducted in a property in the city of Natal, Rio Grande do Norte State, located at the geographical coordinates 5º11' South Latitude and 37º West Longitude, with an average elevation of 16 m. The semen processing was performed in the Laboratory of Animal Germplasm Conservation of the Federal Rural University of the Semi-arid (UFERSA).

We selected three adult male goats (~2 years of age) to be used in the experiment. The animals underwent complete clinical evaluation and andrologic assessment. The animals were kept at pasture, having as main forage support native vegetation, *Caatinga*, with free access to water and mineral supplementation. Two days before semen collection, we separated the males from females and kept them in a paddock covered with ceramic tiles. The entire experiment was conducted during the dry season.

We collected 24 ejaculates from three goats (eight ejaculates per animal) by means of an artificial vagina and with the aid of an estrogen female as mannequin. We collected the semen in graduated tubes and immediately transferred them for evaluation, according to the norms of the CBRA (1998). We observed the color, appearance and volume of fresh semen. We subjectively evaluated the microscopic criteria as mass motility (0-5) and progressive motility (%) using a light microscope with 100x magnification. We assessed the percentage of viable spermatozoa by means of a semen smear stained with Bromophenol Blue, where 200 cells were counted at a magnification of 400X (DERIVAUX, 1980). We determined the percentage of morphologically normal sperm by counting 200 cells in a semen smear stained with Rose Bengal in optical microscopy at 1000x magnification. In this same smear, we also determined acrosome integrity of sperm cells, establishing the proportion of sperm with intact and non-intact acrosome.

Aiming at evaluating the functional integrity of the plasma membrane, we carried out HOST. For this, we prepared a solution based on sodium citrate (50%) and fructose (50%) at 200 mOsm/L, according to the method of REVELL & MRODE (1994). As proposed by CORREA & ZAVOS (1994), we prepared serial dilutions in distilled water forming solutions of four different osmolarities: 50; 100; 150 and 200mOsm /L

We divided the semen samples into five aliquots of 10 L, adding one of the them to the tube containing 1 mL of distilled water (0 mOsm/L) and the other ones to tubes containing 1 mL of the basic

solution of sodium citrate and fructose (50; 100; 150 and 200 mOsm/L). The tubes were incubated in a water bath and after 40 minutes at 37°C, we evaluated an aliquot of semen from each tube under contrast phase microscopy (400X), and counted 200 cells. The spermatozoa showing a bent and swollen tail were considered to have a functional plasma membrane (FONSECA et al., 2005). The result was expressed as a percentage, after the difference between the percentage of HOST-reactive spermatozoa and the percentage of spermatozoa presenting tail pathologies, during the examination of sperm morphology (MELO & HENRY, 1999).

For statistical analysis of the characteristics evaluated, we employed the Statistical Analysis System (SAS 6.10, SAS Institute Inc., Cary, NC, USA). The consistency of the data and descriptive analysis (means and standard deviation) of features of interest to the study were carried out through the use of PROC MEANS, at 5% probability ($P < 0.05$). Variables were tested with Pearson's correlation.

RESULTS

The fresh semen of goats showed yellowish color, milky aspect and the total volume of ejaculates was 1.1 ± 0.1 mL. The mass motility was 3.85 ± 0.16 and individual progressive motility, 96.35 ± 0.9%. A total of 94.05 ± 1.26% of viable sperm, 76.7 ± 1.47% of sperm with normal morphology, and 99.68 ± 0.22% of sperm with intact acrosome were observed.

Regarding the functional integrity of the membrane, the solution with 100 mOsm/L presented the highest percentage ($P < 0.05$) of HOST-reactive sperm (Table 1). However, none of the solutions was significantly correlated with the results of any of the semen traditional analysis (Table 2).

Table 1. Mean (± standard deviation) of the percentage of goat's sperm reactive to hypoosmotic solutions of different osmolarities (0; 50; 100; 150 and 200 mOsm/L, n = 24 ejaculates)

	Osmolarity of the solutions (mOsm/L)				
	0	50	100	150	200
Mean	20.60[e]	30[c]	34.80[a]	33.25[b]	25.45[d]
Standard deviation	1.93	3.49	3.6	3.62	2.64

[a,b,c,d,e] different lower case letters in the same row differ among each other ($P < .05$)

Table 2. Pearson correlation coefficients (r) among hypoosmotic solutions of different osmolarities and the semen characteristics evaluated in goat's fresh semen (n = 24 ejaculates)

	Osmolarity of the solutions (mOsm/L)				
Semen characteristics	0	50	100	150	200
Individual motility	0.18	0.34	0.27	0.18	0.21
Mass motility	-0.24	-0.32	-0.38	-0.31	-0.18
Sperm viability	-0.09	-0.03	-0.14	-0.16	-0.08
Normal Morphology	-0.20	-0.11	-0.14	-0.06	-0.10
Acrossome integrity	0.14	-0.25	-0.13	-0.20	-0.21

* There were no significant correlations among the parameters evaluated ($P > .05$).

DISCUSSION

When the hypoosmotic test was first described for the evaluation of human semen, JEYENDRAN et al. (1984) tested solutions with varying osmolarities from 50 to 300 mOsm/L, obtaining the best results of sperm reaction with the 150 mOsm/L solution. The same authors also tested different solutes (sodium citrate, sucrose, melitose, fructose and sodium chloride) and associations between them, and found that the best results were obtained with the association of sodium citrate (50%) and fructose (50%). In the present study, we verified the superiority ($p < 0.05$) of the solution of sodium citrate and fructose at 100 mOsm /L to detect goat HOST-reactive sperm compared to the other tested solutions. Similarly, MARTINS et al. (2006) obtained values of 33.35% goat sperm reactive to the test using the solution of 100 mOsm/L. FONSECA et al. (2005) also tested sodium citrate and fructose solutions with osmolarity ranging from 50 to 300 mOsm/L, and found that the highest percentage

(51.5%) of spermatozoa reactive to the test was found in solution at 125 mOsm/L, which was not used in this study, but whose osmolarity is close to the 100 mOsm/L solution.

Overall, we verified a low response of goat's semen to the hypoosmotic test, given that even the highest values of reactive spermatozoa were around 33.35%. These results are lower than those observed by SALGUEIRO et al. (2003), who found an average of over 50% of reactive spermatozoa in the same species. As this study was conducted in the rural area of Mossoró, RN, which is a region of extreme daily temperature around 35-39 °C, it is possible that the low values are associated with the influence of high ambient temperatures on semen quality by changing the epididymal maturation process, compromising the structure of the plasma membrane of the sperm (HUANG et al., 2000), thereby affecting the response of spermatozoa to the hypoosmotic test.

We verified in this study that the lowest values (20.6%) of sperm reactive to the test were obtained from the use of distilled water as hypoosmotic solution at 0 mOsm1/L. In contrast, studies with equine semen found higher results of reacted sperm when distilled water was used as hypoosmotic solution compared to other solutes (DELL'AQUA et al., 2002; MELO et al., 2003). Distilled water was superior to other solutions in assessing the membrane functional integrity of both equine and canine spermatozoa (QUINTELA et al. 2010). However, differences in the composition of the plasma membrane could make goat sperm present a lower response to this environment, when compared to other species.

In this study, no correlation was found between sperm reactive to the hypoosmotic test and the other semen characteristics of goats. Similarly, SANTOS et *al.* (2006) found no correlation between sperm motility and HOST values in fresh semen of the same species. Furthermore, ENGLAND & PLUMMER (1993) demonstrated no correlation between the percentage of spermatozoa reactive to the hypoosmotic test and other semen parameters in dogs, which corroborates the data from this study.

On the other hand, NUR et al. (2005) demonstrated that there is a positive, moderate and highly significant correlation ($r = 52.3\%$, $P < 0.001$) between the results of the hypoosmotic test and the sperm motility in goats. This correlation has also been described in studies with bovine semen (SIQUEIRA et al., 2007). In addition, high positive correlations between motility and HOST were found in fresh semen of equines (MELO & HENRY, 1999), dogs (KUMI-DIAKA, 1993; RODRÍGUEZ-GIL et al., 1994; BUENO et al., 2001), buffaloes and bulls (CORREA & ZAVOS, 1994; LODHI et al., 2008; ZÚCCARI et al., 2009). Also, the response to HOST has also been positively correlated with motility as well as with viability and normal morphology of spermatozoa of humans (JEYENDRAN et *al.* 1984), equines (MANTOVANI et al., 2002; MELO et al., 2005), buffaloes and bulls (LODHI et al., 2008).

The existence of correlations between the hypoosmotic test and the evaluation of other semen characteristics in different species is indeed controversial. Correlation coefficients as low as those described in this study were also found by other authors for semen of goats (MARTINS et al., 2006), equines (NEILD et al., 1999; SNOECK et al., 2007) and dogs (INAMASSU et al.,1999). According to these authors, this fact was due to the specificity of the HOST, because the edema of sperm cells is indicative of integrity of the sperm membrane, while motility depends not only on the transport of substances passing through the membranes but also on many other biochemical functions, such as metabolism and sperm microtubule action of the fibers of the spermatozoa tail.

Regardless of whether there is or not correlation with other semen characteristics, we emphasize that the hypoosmotic test provides important information about the integrity of the plasma membrane of sperm cells (JEYENDRAN et *al.,* 1984). The use of different specific tests that evaluate important structural or functional aspects of sperm cell allows us to improve the selection of breeders and of means and protocols of seminal conservation. Thus, the results of such tests should be added and viewed as complementary tests and not as exclusive.

CONCLUSIONS

Although any particular correlations between the results of the hypoosmotic test and the other evaluations of goat semen could be determined, it is recommended that such test is carried out using a solution based on sodium citrate and fructose at 100 mOsm/L to evaluate functional integrity of sperm membrane in this species.

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