Anim. Reprod., v.2, n.2, p.139-144, April/June, 2005

The hypoosmotic swelling test in fresh goat spermatozoa

J.F. Fonseca[1,3], C.A.A. Torres[2], V.V. Maffili[2], A.M. Borges[2], A.D.F. Santos[2], M. T. Rodrigues[2], R.F.M. Oliveira[2]

[1]Embrapa Caprinos, CP D10, CEP 62011-970, Sobral, CE, Brasil
[2]Departamento de Zootecnia, Universidade Federal de Viçosa, Av. P. H. Rolfs, s/n, CEP 36571-000, Viçosa, MG, Brasil.

Abstract

The hypoosmotic swelling test (HOST) has been proved to be a good tool for evaluating the membrane integrity of spermatozoa of various domestic animals including cattle, horses, and swine. However, the best approach for using this technique in goat semen has not been tested. The present study aimed to establish the best hypoosmotic solution (HS) for testing membrane integrity in fresh goat semen. Sodium citrate and fructose based solutions (S) with the following osmolarities (mOsm/l) were used: 50 (S1), 75 (S2), 100 (S3), 125 (S4), 150 (S5), 175 (S6), 200 (S7), 250 (S8), 290 (S9) and 300 mOsm/l (S10). Twenty-eight semen samples were collected from seven mature bucks (four collections per buck) at 48 hour intervals. After macroscopic evaluation, 10µl of semen was immediately added to 2ml of each solution and incubated for one hour at 37ºC. Sequentially, 20µl of semen diluted in HS were evaluated with oil immersion using a phase-contrast microscope. A total of 200 spermatozoa were counted in at least five different fields, and sperm tails were classified as non-coiled, coiled, and strongly coiled. The respective percentages of spermatozoa with coiled tails (coiled plus strongly coiled) in the ten solutions listed above were 34.1, 38.8, 45.3, 51.5, 46.8, 42.8, 38.2, 29.0, 19.4 and 23.1%. Percentages of strongly coiled spermatozoa were: 6.8, 10.6, 21.5, 25.3, 24.3, 21.5, 19.3, 12.4, 6.4, and 7.9 for the ten solutions, respectively. According to total coiling, S4 was superior to S1, S7, S8, S9, and S10 ($P<0.05$). According to strong coiling, S4 was superior to S1, S2, S8, S9, and S10 ($P<0.05$). Results suggest that the 125 mOsm/l solution would be best for use in HOST in fresh goat spermatozoa.

Keywords: hypoosmotic swelling test, sperm, tail, goat

Introduction

The small ruminant industry has experienced a great expansion during the last two decades. In tropical and subtropical areas of Brazil, goats have proved to be a good option for production of milk, meat, and leather. As breeds expand, assisted reproductive technologies are necessary to assess and improve the efficiency of reproduction. In male goats, semen analyses is based upon methods developed in other domestic species. Thus, it becomes necessary to test and adapt techniques to the peculiarities of goat semen in the case of the hypoosmotic swelling test (HOST) that is used to verify sperm membrane integrity.

The importance of the membrane in maintaining both biochemical and structural integrity of the spermatozoon is well known (Cabrita *et al*., 1999). When exposed to hypoosmotic solutions, biochemically-active spermatozoa increase their volume in order to establish equilibrium between the fluid compartment within the spermatozoa and the extracellular environment. Swelling causes changes in both cell size and shape that can be evaluated using a phase contrast microscope (Cabrita *et al*., 1999). This swelling process culminates in promoting a spherical expansion of the cell membrane covering the tail, thus forcing the flagellum to coil inside the membrane. Coiling of the tail begins at the distal end of the tail and proceeds toward the midpiece and head as the osmotic pressure of the suspending media is lowered (Jeyendran *et al*., 1984). There are references for the use of HOST in cattle (Correa and Zavos, 1994; Revell and Mrode, 1994), swine (Vasquez *et al*., 1997; Zou and Yang, 2000), horses (Neild *et al*., 1999), and humans (Jeyendran *et al*., 1984). The best osmolarity for a hypoosmotic solution has already been established for these species. Although Fonseca *et al*. (2001) reported the ability of different concentrations of fructose-citrate solutions to cause swelling of spermatozoa from frozen-thawed semen of bucks, there is no reference for use of this assay in fresh caprine semen.

The objective of this study was to establish the most suitable hypoosmotic solution for testing membrane integrity of fresh goat semen.

[3]Corresponding author: jeferson@cnpc.embrapa.br
Received: May 17, 2005
Accepted: July 8, 2005

Materials and Methods

Location

This study was carried out in March and April (first half of local breeding season) at the Goat Section of the Department of Animal Science of the Federal University of Viçosa (UFV), in Viçosa, Zona da Mata of Minas Gerais, Brazil, situated at 20º45' S latitude and 42º51' WG longitude. The local average altitude is 692.73 m above sea level with a dry winter and humid summer climates. The average annual temperature is 20.9º C and annual rainfall is 1203 mm^3. The local breeding season extends from March to June.

Animals

Eight mature bucks, with documented good fertility (used in both artificial insemination and natural mounting programs), three Saanen and five Alpine breeds, were used. Animals were kept in individual pens and fed corn silage and a concentrate diet twice a day according to requirements. Water and mineral salt were available *ad libitum*.

Hypoosmotic solution preparation

A 300 mOsm/l trisodium citrate and fructose based solution was made according to the method of Revell and Mrode (1994). As proposed by Correa and Zavos (1994), serial dilutions were carried out in triple-distilled water yielding solutions of 10 different osmolarities (mOsm/l): 50 (S1), 75 (S2), 100 (S3), 125 (S4), 150 (S5), 175 (S6), 200 (S7), 250 (S8), 290 (S9), and 300 (S10). Solutions were aliquoted into 3 ml polyethylene tubes (2ml/tube) and then stored at -20ºC until use.

Semen collection, incubation and evaluation

Semen was collected every 48 hours between 8:00 and 10:00 a.m. by means of an artificial vagina and using an estrogenized female (1ml of estradiol benzoate at 48 hour interval subcutaneous) as a sucubus. Four collections were made for each male. After collection, semen samples were immediately evaluated for volume, mass movement, and progressive motility. A volume of 10µl was gently mixed in each of the 2ml hypoosmotic solutions previously described and incubated for one hour in a water bath at 37ºC. After incubation, 20µl of the solutions containing semen were placed on a microscope slide, covered with a cover glass and evaluated using a phase-contrast microscope at 1000 x magnification. A total of 200 spermatozoa were counted in at least five different fields. Spermatozoa were classified as swelled (coiled) according to description used by Revell and Mrode (1994). Besides total coiling, a strong coiling was the description given when the tail became very coiled. One Saanen buck was eliminated from the experiment due to excessive seminal pathology, mainly in the sperm tail which could have confounded swelling readings due to extensive coiling.

Statistical analyses

Data, presented as the mean ± SD, were analyzed by a two-way analysis of variance (Neild *et al.*, 1999). Friedman's two-way analysis of variance by ranks was applied to evaluate differences among hypoosmotic solutions, semen samples, and bucks (BioEstat 2.0; Ayres *et al*., 2000). Statistical significance was set at $P < 0.05$.

Results

Spermatozoa, suffering from different grades of coiling as well as the percentages of strongly coiled and total coiling after exposure to different concentrations of hypoosmotic solutions, are presented in Fig. 1. According to these data (Fig.2), goat spermatozoa appeared to suffer increasing coiling from the 50 mOsm/l solution and reached a maximum value with the 125 mOsm/l solution after which coiling began to decrease reaching the minimum score with the 290 mOsm/l solution. This tendency was noted both in the strong and total coiling endpoints. According to total coiling, S4 was superior to S1, S7, S8, S9, and S10 ($P<0.05$). When considering strong coiling, S4 was superior to S1, S2, S8, S9, and S10 ($P<0.05$).

There was no interaction among solutions, collections, and bucks ($P>0.05$). Data from S3, S4, and S5, that had a similar increased percentage of coiling, were grouped to assess differences among collections or bucks. The percentage of total coiling increased significantly ($P<0.05$) from the first to fourth semen collection (Fig. 3) and differed ($P<0.05$) among bucks (Fig. 4).

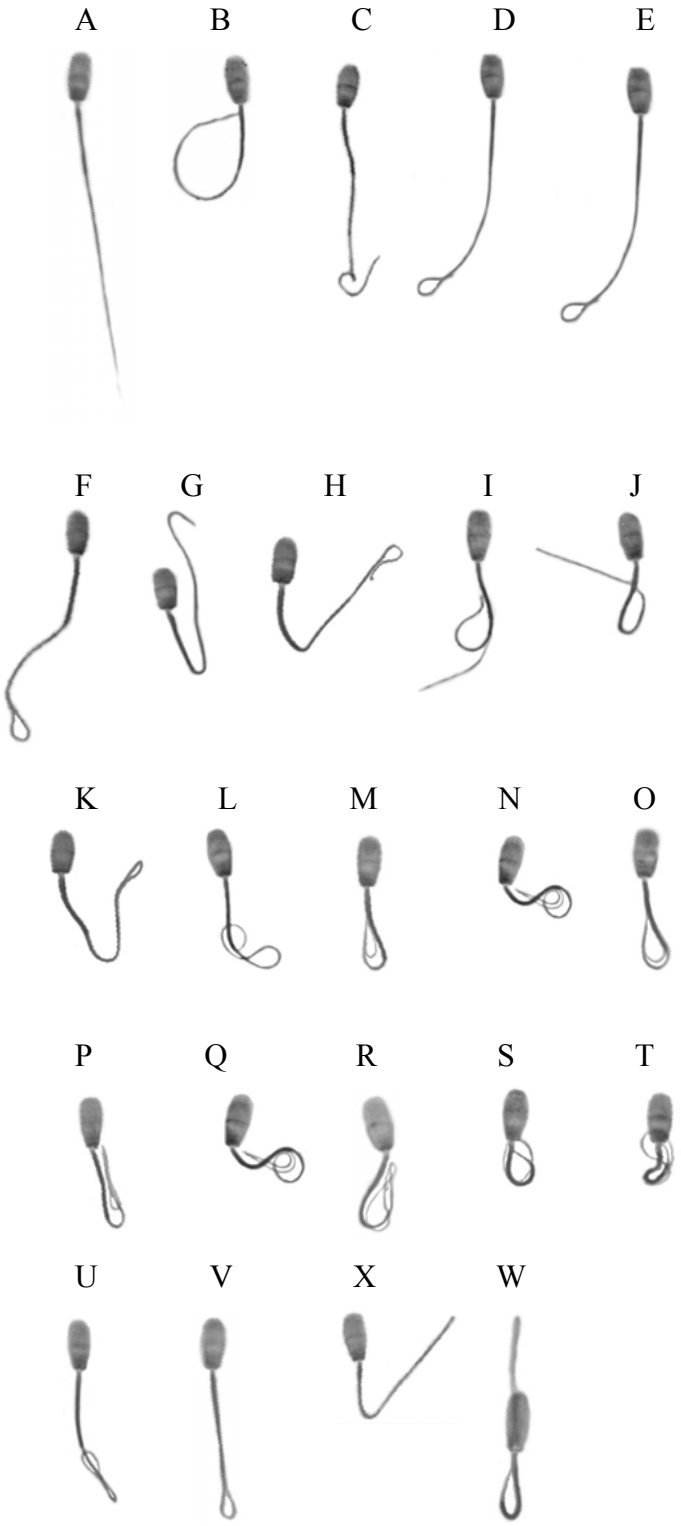

Figure 1. Goat spermatozoa suffering from different grades of coiling (phase contrast microscope, 1000x magnification): (A) not coiled, (B-J) coiled spermatozoa and (K-W) strongly coiled spermatozoa. Note that none spermatozoa have entrapped cytoplasmic droplet.

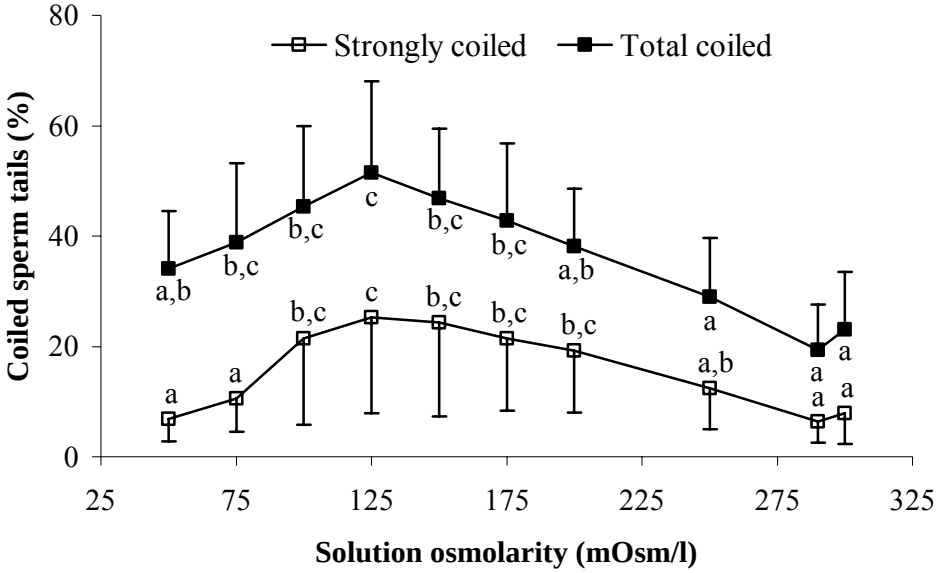

Figure 2. Effect of different concentrations of hypoosmotic solutions (mOsm/l) on the coiling of fresh goat spermatozoa (mean ± SD). Means with different superscript differed significantly among solutions (Friedman; P<0.05).

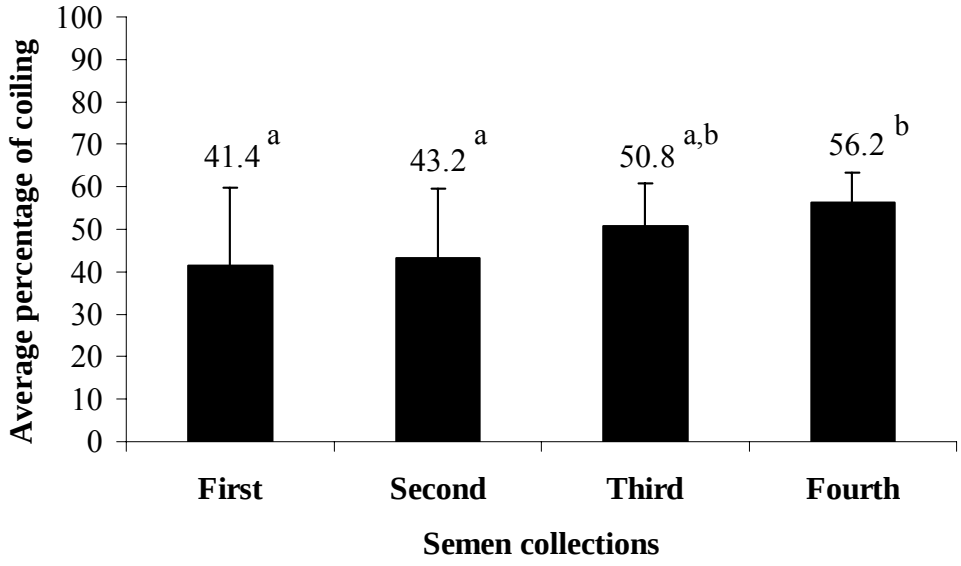

Figure 3. Average percentage of total coiling in fresh goat spermatozoa (200 cells counted per sample) exposed to hypoosmotic sodium citrate / fructose based solutions (grouped 100, 125 and 150 mOsm/ml) in four subsequent semen samples. Means with different superscript differed significantly (Friedman; P<0.05).

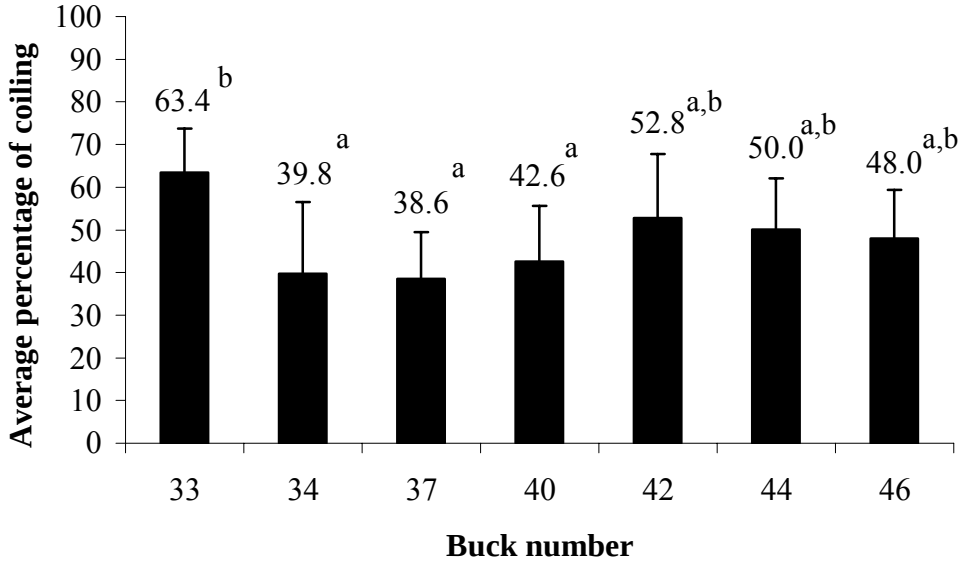

Figure 4: Average percentage of total coiling in fresh goat spermatozoa (200 cells counted per sample) exposed to hypoosmotic sodium citrate / fructose based solutions (grouped 100, 125 and 150 mOsm/ml) for Alpine (33 to 42) and Saanen (44 and 46) bucks. Means with different superscripts differed significantly (Friedman; $P<0.05$).

Discussion

As shown in other domestic species of animals (Correa *et al*., 1997; Neild *et al*., 1999), goat spermatozoa had a similar pattern of swelling when exposed to a hypoosmotic medium. It is proposed that under such conditions, biochemically-active sperm cells, with intact membranes, absorb water and swell increasing in volume to establish equilibrium between the fluid compartment within the spermatozoa and the extracellular medium. It culminates into a spherical expansion of the cell membrane covering the tail, thus forcing the flagellum to coil inside the membrane. Tail coiling begins at the distal end of the tail and proceeds towards the midpiece and head as the osmotic pressure of the suspending media is lowered (Jeyendran *et al*., 1984). In this study, "total coiling" referred to the percentage of sperm cells evaluated that suffered coiling, and the term "strong coiling" referred to the percentage of sperm cells that suffered coiling with more intensity. Considering differences in the coiling intensity, it could be speculated that the membrane integrity can present some degree of variation, being more evident under some hypoosmotic solutions. It is important to differentiate loops and bends produced by exposing normal spermatozoa to a hypotonic solution (coiling) from cytoplasmic droplets in principal piece. Coiling means that spermatozoa are viable while cytoplasmic droplets are a pathological condition (Barth and Oko, 1989).

As proposed by Jeyendran *et al*. (1984), the optimal hypoosmotic solution should exert an osmotic stress large enough to cause an observable increase in volume but small enough to prevent lyses of the sperm membrane. In the present study, this was found between 100 to 200 mOsm/l. This result was similar to the 150 mOsm/l found in cattle (Revel and Mrode, 1994), 150 mOsm/l in humans (Jeyendran *et al*., 1984), but different when compared to 50 to 150 mOsm/l found for swine (Vazquez *et al*., 1997) and 25 to 100 mOsm/l for equine spermatozoa (Neild *et al*., 1999). This justifies the need for finding the best hypoosmotic concentration to be used in HOST according to the species being evaluated.

Combining data of both strong and total coiling, the 125 mOsm/l solution yielded the best results. Moreover, at 125 mOsm/l, sperm cells appeared to undergo strongest coiling. This indicates that this solution caused a more evident swelling and subsequent coiling and should be chosen to be used in HOST with goat fresh spermatozoa. It is known that the percentage of coiling varies according to bulls (Prasad *et al*., 1999), bucks, or season (Kale *et al*., 2000) and it is strongly correlated to mass movement, progressive motility, live sperm count, total intact acrosome, sperm concentration (Prasad *et al*., 1999), and fertility (Jeyendran *et al*., 1984). In the present study, some of these findings were confirmed in that coiling varied according to bucks and semen samples. Under the conditions of this study, HOST proved to be a suitable technique for testing membrane status of goat spermatozoa. It could be a valuable and practical tool for accurate assessment of

the individual sperm cell rather than the population as a whole. The concentration of 125 mOsm/l appeared to be the most adequate for use in HOST for goat fresh spermatozoa, and it could aid the routine analyses of goat semen.

Acknowledgments

Authors want to thank the National Council for Scientific and Technological Development (CNPq, Ministry of Science and Technology, Brazil) and Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG) for financial support.

References

Ayres M, Ayres Jr M, Ayres DL, Santos AS. 2000. *BioEstat 2.0: aplicações estatísticas nas áreas de ciências biológicas e médicas*. Belém: Editora Sociedade Civil Mamirauá, MCT – CNPq. pp.45-47.

Barth AD, Oko RJ. 1989. *Abnormal morphology of bovine spermatozoa*. Ames: Iowa State University Press. pp.285

Cabrita E, Alvarez R, Anel E, Herráez MP. 1999. The hypoosmotic swelling test performed with Counter: a method to assay functional integrity of sperm membrane in rainbow trout. *Anim Reprod Sci*, 55:279-287.

Correa JR, Zavos PM. 1994. The hypoosmotic swelling test: its employment as an assay to evaluate the functional integrity of the frozen-thawed bovine sperm membrane. *Theriogenology*, 42:351-360.

Correa JR, Heersche Jr, G, Zavos PM. 1997. Sperm membrane functional integrity and response to frozen-thawed bovine spermatozoa during the hypoosmotic swelling test incubation at varying temperatures. *Theriogenology*, 47:715-721.

Fonseca JF, Torres CAA, Santos ADF, Rovay H, Borges AM, Barbosa LP, Maffili VV, Fraga DBM. 2001. The hypoosmotic swelling test in goat spermatozoa. *Rev Bras Reprod Anim*, 25:436-438. (abstract).

Jeyendran RS, Van Der Ven HH, Perez-Pelaez M, Crabo BG, Zaneveld LJD. 1984. Development of an assay to assess the functional integrity of the human sperm membrane and its relationship to other semen characteristics. *J Reprod Fertil*, 70:219-228.

Kale MM, Manik RS, Tomer OS. 2000. In-vitro assessment of crossbred buck fertility. *Indian J Anim Sci*, 70:25-29.

Neild D, Chaves G, Flores M, Mora N, Beconi M, Agüero A. 1999. Hypoosmotic test in equine spermatozoa. *Theriogenology*, 51:721-727.

Prasad JK, Kumar S, Mohan G, Shanker U, Agarwal SK. 1999. Hypo-osmotic swelling tests (HOST) and its response in fresh and freeze-thawed semen. *Indian J Anim Sci*, 69:766-769.

Revell SG, Mrode RA. 1994. An osmotic resistance test for bovine semen. *Anim Reprod Sci*, 36:77-86.

Vasquez JM, Martinez EA, Martinez P, Garcia-Artiga C, Roca J. 1997. Hypoosmotic swelling of boar spermatozoa compared to other methods for analyzing the sperm membrane. *Theriogenology*, 47:913-922.

Zou C-X, Yang Z-M. 2000. Evaluation on sperm quality of freshly ejaculated boar semen during in vitro storage under different temperatures. *Theriogenology*, 53:1477-1488.