EFFECT OF DIFFERENT CRYOPROTECTANTS ON SPERM PLASMA MEMBRANE INTEGRITY OF OSMANABADI AND SIROHI BUCK SEMEN

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ABSTRACT

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Semen was collected from six Osmanabadi and six Sirohi bucks weekly for six weeks by Artificial Vagina method. Semen was diluted using Tris Egg yolk Glycerol (TYG), Tris Egg yolk Ethylene Glycol (TYE) and Tris -Dimethyl sulphoxide (DMSO) dilutors (TYD) and evaluated for sperm plasma membrane integrity by HOS test at pre (5°C) and post freezing (-196°C at 24 and 72 hours) stage. At pre freezing (5°C) stage in Osmanabadi buck semen there was no significant difference (P>0.05) across different dilutors with respect to HOS positive sperm percentage but in Sirohi bucks significantly higher sperm plasma membrane integrity was observed in TYD and TYG than TYE dilutor. At 24 hours post freezing stage in Osmanabadi buck semen significantly higher plasma membrane integrity was noted in TYD than TYG and TYE dilutors. In Sirohi buck semen significantly higher plasma membrane integrity was noted in TYD than TYE dilutor, TYG being intermediate. At 72 hrs post freezing stage in Osmanabadi bucks semen there was no significant difference (p>0.05) across different dilutors with respect to HOS positive sperm percentage. In Sirohi bucks semen significantly, higher (P<0.05) percentage of plasma membrane integrity was observed in TYD than TYE dilutors. It was concluded that in both the breeds higher plasma membrane integrity was observed in TYD dilutor which indicates that DMSO was more effective cryoprotectant than ethylene glycol and glycerol in preserving sperm membrane integrity during cryopreservation of Osmanabadi and Sirohi bucks semen.

Key words: Osmanabadi semen, Sirohi semen, Cryoprotectants, Plasma membrane integrity, Buck frozen semen

INTRODUCTION

Satisfactory conception rates have been achieved in goats using washed or unwashed frozen-thawed semen extended in egg yolk diluents. In recent years, more attention has been given for evaluating sperm membrane integrity as it is not only important for sperm metabolism, but a correct change in the properties of the membrane is required for successful union of the male and female gametes, i.e. for sperm capacitation, acrosome reaction, and binding of spermatozoon to the egg surface. Thus, the integrity and functional activity of the sperm membrane is of fundamental importance in the fertilization process, and assessment of membrane function may be a useful indicator of the fertilizing ability of spermatozoa. Hypo-osmotic swelling test is one of the procedures used in the evaluation of functional integrity of plasma membrane of spermatozoa (Jeyendran *et al.*, 1984). HOS test is used as a method complementing the routine sperm analysis (Nie and Wenzel, 2001). As in other species of domestic animals (Correa *et al.*, 1997; Neild *et al.*, 1999), goat spermatozoa had a similar

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pattern of swelling when exposed to a hypoosmotic medium.

Tail coiling begins at distal end of the tail and proceeds towards the mid-pice and head as the osmotic pressure of the suspending media is lowered (Jeyendran *et al.*, 1984). With this views in mind the present study was designed to evaluate the effect of dilutors on membrane integrity of Osmanabadi and Sirohi buck semen at pre-freeze (5°C) and post freeze stage (24 & 72 hrs) by using Hypo-osmotic swelling test.

MATERIALS AND METHODS

Semen from six Osmanabadi and six Sirohi bucks was collected aseptically and hygienically by artificial vagina method at weekly interval for over six weeks period. Total 36 ejaculates were collected for evaluation. Collected semen was diluted (160 to 180 million sperm /ml) in three different dilutors like Tris Egg yolk Glycerol (TYG) (Davis *et al.*, 1963), Tris Egg yolk Ethylene Glycol (TYE) (Rohilla *et al.*, 2005) and Tris -Dimethyl sulphoxide (DMSO) (TYD). This diluted semen was kept at 5°C for four hours for equilibration. The equilibrated semen was frozen using standard protocol of semen freezing in French medium (0.5ml) straws. The straws were thawed in water bath at 37°C for 30 seconds. Semen samples were evaluated for sperm plasma membrane integrity by Hypo osmotic swelling test (HOST) at the time of dilution, pre-freezing (5°C) and post freezing (24 and 72 hours) stage.

Two hypo-osmotic solutions were prepared as 2.7% aqueous solution of fructose (1.351 gm/50 ml distilled water) and 1.47% aqueous solution of sodium citrate (0.735 gm/50 ml distilled water). Equal volumes of both solutions (0.5 ml each) were mixed and kept in an incubator at 37°C for 10 minutes. 50 µl of semen was added in the above hypo-osmotic solution and incubated at 37°C for 30 minutes, 10 µl of this mixture was observed under 10 X objective with covered glass slide to determine the number of spermatozoa showing swollen head and coiled tail indicating sperms with intact plasma membrane (HOS positive sperm).Total hundred spermatozoa were counted to determine the percentage of HOS positive spermatozoa.

RESULT AND DISCUSSION

The sperm plasma membrane integrity percentage at different freezing stages in different dilutors of Osmanabadi and Sirohi bucks are shown in table 1.

At pre-freezing (5°C) stage there was no significant difference ($P>0.05$) between the two breeds of bucks with respect to HOS sperm percentage in TYG and TYD dilutors but significantly higher HOS sperm per cent was noted in TYE dilutor for Osmanabadi than Sirohi bucks. In Osmanabadi bucks there was no significant difference ($P>0.05$) across different dilutors with respect to HOS sperm percentage. In Sirohi bucks there was significant difference between TYD and TYE as well as TYG and TYE dilutors. Significantly higher plasma membrane integrity observed in Sirohi bucks was in TYD and TYG than TYE dilutor. Salvador *et al.* (2006) in Murciano-Granadina bucks observed 21 to 31 % HOS positive sperm percentage in semen stored at 5°C, which was much lower than present observations. It was opined that washing of semen might be affecting the plasma membrane integrity. Fernandez-Santos *et al.* (2006) observed in Red Deer epididymal spermatozoa at pre freeze stage the HOS positive sperm percentage in dilutor containing 6% glycerol was 80-85 % which was higher than present findings. This may be due to difference in the species and source of spermatozoa.

It is also evident that at 24 hours post freezing stage there was no significant difference ($P>0.05$) between the two breeds of bucks but there was a significant difference ($P<0.05$) across different dilutors with respect to HOS positive sperm percentage. The results further indicated that in Osmanabadi bucks semen there was a significant difference between TYG and TYD as well as TYD and TYE dilutors. Significantly higher plasma membrane integrity was noted in TYD than TYG and TYE dilutors. In Sirohi bucks semen significantly higher plasma membrane

integrity observed was in TYD than TYE dilutor. There was no significant difference between TYG and TYD as well as TYG and TYE dilutors with respect to sperm plasma membrane integrity. The literature pertaining to post freezing plasma membrane integrity of bucks semen was limited and hence comparison was also made with other species. Arangasamy *et al.* (2005) in Murrah buffalo bulls recorded 66.4 ± 0.65% HOST values for post thawed semen which was in close agreement with present observations. Low plasma membrane integrity (less than 50 percent) was observed by Fernandez-Santos *et al.* (2006) in Red Deer epididymal spermatozoa and Gang Zhang *et al.* (2007) in bucks from China.

At 72 hours post freezing stage there was a significant difference (P<0.05) between the two breeds of bucks with respect to HOS positive sperm percentage in TYD and TYE dilutors. Plasma membrane integrity was significantly higher in Osmanabadi than Sirohi bucks in TYE dilutor. There was a significant difference (P<0.05) across different dilutors with respect to HOS positive sperm percentage only in Sirohi buck semen.

Significantly, higher percentage of plasma membrane integrity observed was in TYD than TYE dilutors. In both the breeds higher plasma membrane integrity observed was in TYD dilutor which indicates that DMSO was more effective cryoprotectant than ethylene glycol and glycerol in preserving sperm membrane integrity on frozen storage of Osmanabadi and Sirohi bucks semen. Azerêdo *et al.* (2001) reported that in buck spermatozoa plasma membrane integrity was reduced in Tris solution, before and after freezing which was also observed in present study. The plasma membrane integrity was reduced in both Osmanabadi and Sirohi bucks semen on cryopreservation.

It can be concluded that DMSO was abetter option as a cryoprotectant for preserving Osmanbadi and Sirohi buck semen.

TABLE 1 PLASMA MEMBRANE INTEGRITY PERCENTAGE OF SPERMATOZOA AT DIFFERENT FREEZING STAGES IN DIFFERENT DILUTORS OF OSMANABADI AND SIROHI BUCKS

Breed/freezing stage	Pre-freezing			24hours post freezing			72 hours post freeing		
	TYG	TYD	TYE	TYG	TYD	TYE	TYG	TYD	TYE
Osmanabadi	72.50^{ab} ±1.03	74.22^{a} ±1.03	72.05^{ab} ±2.54	68.83^{cde} ±1.95	72.66^{ab} ±1.78	68.72^{cde} ±1.46	67.27^{de} ±1.16	70.16^{bcd} ±1.42	67.83^{de} ±1.23
Sirohi	71.00^{bc} ±1.15	72.11^{ab} ±1.15	67.83^{de} ±0.62	68.83^{cde} ±0.59	70.11^{bcd} ±0.84	66.50^{ef} ±0.76	66.33^{ef} ±0.72	67.50^{de} ±1.14	63.66^{f} ±1.10

Means with common superscript do not differ significantly. C.V.=3.921, F-value- 6.036* Significant at 5% level, C.D (0.05) = 3.120

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