EVALUATION OF SPERM PLASMA MEMBRANE INTEGRITY OF FROZEN-THAWED MALABARI BUCK SEMEN

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ABSTRACT

The present study was aimed to establish the best hypo-osmotic solution for evaluating membrane integrity in frozen-thawed Malabari buck semen. Sodium citrate and fructose based solutions with different osmolarities (50, 75, 100, 125, 150, 175 and 200 mOsm/L) were compared in 54 frozen-thawed semen samples. Based on the observations, 50 mOsm/L was found to be the most suitable hypo-osmotic solution for HOST.

Key words: Plasma membrane integrity, HOST, Frozen thawed semen, Malabari buck.

INTRODUCTION

Semen cryopreservation and artificial insemination with frozen thawed buck semen has been proved to be the most potent technique to ameliorate genetic value in native goats (Sundararaman and Edwin, 2008). Cryopreservation is known to cause damage to plasma membrane integrity of the spermatozoa (Azerêdo *et al.,* 2001). Plasma membrane integrity (PMI) is essential for survival of sperm in the female reproductive tract and to maintain the fertilizing ability by acting as a selective barrier between components of the intracellular and extra-cellular environment (Gwathmey *et al.*, 2006).

Although, many modern techniques like molecular probes, oocyte penetration test are used to determine the status of plasma membrane integrity, hypo-osmotic swelling test (HOST) is considered as best procedure since it is simple, rapid, inexpensive and can be performed at field level (Gupta and Singh, 2019). HOST is based on the semi-permeability of the intact plasma membrane, which allows the sperm to swell under hypo-osmotic conditions (Jeyendran *et al.,* 1984). Hypo-osmotic concentration to be used in HOST varies among species (Fonseca *et al.,* 2005). Hence, the present study was aimed to determine the suitable osmolarity of the hypo-osmotic solution for frozen-thawed Malabari buck spermatozoa.

MATERIALS AND METHODS

Frozen semen straws from 54 Malabari bucks were obtained from Kerala Livestock Development Board, Frozen Semen Station, Dhoni, Palakkad, Kerala.

Stock solution for hypo-osmotic swelling test was prepared by dissolving trisodium citrate (9.8 g) and fructose (18 g) in double glass distilled water (1000 ml) to yield hypo-osmotic solution with 200 mOsm/L. Seven different osmolarities (mOsm/L) were prepared by serial dilutions of stock solution using double distilled water as proposed by Correa and Zavos (1994).

Required osmolarity (mOsm/L)	Volume of Stock solution (ml)	Volume of distilled water added (ml)
50	50	150
75	75	125
100	100	100
125	125	75
150	150	50
175	175	25
200	200	0

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Thawing was performed by immersing the frozen semen straw in a water bath at 37^0 C for 30 sec, wiped individually and emptied to eppendorf tubes kept at 37^0 C in a water bath. Frozen thawed semen (0.1 ml) was added to each of 1 ml of hypo-osmotic medium with different osmolarities (50, 75, 100, 125, 150, 175 and 200 mOsm/L). After incubating it for 30 minutes at 37^0 C, a drop of solution containing semen was placed on a clean, dry glass slide and covered with a cover slip. At least 200 spermatozoa on each slide were examined (magnification 1000x) under phase contrast microscope and the percentage of spermatozoa reacted to hypo-osmotic solutions with different osmolarities were recorded and stratified into four groups namely, Group A: no tail coiling, Group B: tail coiling at distal end, Group C: mild tail coiling and Group D: strong tail coiling, according to the different intensity of tail coiling of hypo-osmotically affected spermatozoa as described previously by Mordel *et al.* (1993) with slight modifications. Statistical analyses of the data were carried out as per standard technique (Snedecor and Cochran, 1989).

RESULTS AND DISCUSSION

The results of the present study are summarized in Table 1. Based on different intensity of tail coiling, maximum percentage of tail coiling was observed in Group C and minimum percentage of tail coiling was observed in Group B in all osmolarities of hypo-osmotic solution. There is no significance difference in the mean percentage of spermatozoa with coiling at distal end between different osmolarities of hypo-osmotic solution.

The mean percentage of mild tail coiling with hypo-osmotic solution did not differ significantly (P e" 0.05) between 50 and 70 mOsm/L; 75 and 100 mOsm/L; 150 and 175 mOsm/L; and 175 and 200 mOsm/L. At 125 mOsm/L, tail coiling percentage showed significant difference with hypo-osmotic solution of other osmolarities. Strong coiling of tail of spermatozoa did not differ significantly (P e" 0.05) with 150 and 175 mOsm/L and 175 and 200 mOsm/L.

The mean percentage of spermatozoa not reacting to hypo-osmotic solution was lower in 50 mOsm/L (35.85 ± 1.79) when compared to other osmolarities and higher in hypo-osmotic solution with 200 mOsm/L, when compared to other osmolarities (73.63 ± 1.58).

The mean percentage of total (sum of mean percentage of spermatozoa showing coiling at distal end, mild coiling and strong coiling) spermatozoa reacted to hypo-osmotic solution was significantly higher ($P < 0.05$) at 50 mOsm/L (64.15 ± 1.79) compared to hypo-osmotic solution with other osmolarities.

The ability of spermatozoa to swell when subjected to hypo-osmotic conditions implies intact membrane function, i.e. the ability of the membrane to allow passage of water in order to establish an equilibrium between the fluid compartment within the spermatozoon and the external surroundings (Jeyendran *et al.,* 1984).

The reaction of spermatozoa to hypo-osmotic conditions may be due to movement of biochemical and physical substances across the membrane of spermatozoa. Karger *et al.* (2016) reported that even due to osmotic stress during cryopreservation process, osmotic gradient is generated across the sperm cell membrane and spermatozoa with intact membranes swell resulting in the curling of the tails due to a water influx. Neild *et al.* (2000) suggested that high percentage of tail coiling of spermatozoa is an indication of functional spermatozoa with a functional intact membrane.

Based on the present study, the 50 mOsm/L solution was found to be most suitable osmolarity of hypo-osmotic solution for HOST in frozen-thawed Malabari buck spermatozoa. On contrary, Ranjan *et al.* (2009) reported that 75 mOsm/L was the most adequate for use in HOST for frozen-thawed Sirohi and Jamunapari buck spermatozoa. The difference in the results may be due to the difference in the breed of goats used.

In the present study with 50 mOsm/L hypo-osmotic solution, the mean percentage of tail coiling was 64.15 %, which was higher than the results obtained (ranged from 44 to 54%) by the previous authors with 100 mOsm/L in Malabari frozen-thawed semen (Sundararaman *et al.*, 2016). This indicated that 50 mOsm/L was superior to 100 mOsm/L to evaluate the plasma membrane integrity of Malabari buck frozen-thawed spermatozoa.

Fonseca *et al.* (2005) reported that the percentage of tail coiling was maximum at 125 mOsm/L of hypo-osmotic solution in fresh goat semen. However, the present study was done with frozen-thawed semen sample. Karger *et al.* (2016) reported that there is a significant difference in the mean percentage of spermatozoa reacted to the HOS test between fresh and frozen-thawed semen.

In conclusion, hypo-osmotic solution with 50 mOsm/L appeared to be most suitable to evaluate the plasma membrane integrity of Malabari buck frozen-thawed spermatozoa. Since the HOS test is quick, simple and inexpensive, it can be deployed for routine semen evaluation procedure even in field conditions.

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Table 1. Different types of tail coiling percentage of frozen-thawed Malabari buck spermatozoa with different osmolarities of hypo-osmoti solutions (Mean ± SE)

Intensity of tail coiling (GROUP)	Different osmolarities of hypo-osmotic solution						
	50 mOsm/L	75 mOsm/L	100 mOsm/L	125 mOsm/L	150 mOsm/L	175 mOsm/L	200 mOsm/L
Group A	35.85 ± 1.79^{a}	43.07 ± 1.95^{ab}	47.7 ± 2.01^{b}	55.74 ± 1.69^{c}	63.15 ± 1.53^{d}	67.89 ± 1.65^{de}	73.63 ± 1.58^{e}
Group B	9.22 ± 0.38^{a}	9.15 ± 0.41^{a}	10.19 ± 0.46^{a}	10.56 ± 0.43^{a}	10.59 ± 0.41^{a}	10.56 ± 0.5^{a}	9.7 ± 0.56^{a}
Group C	37.15 ± 0.95^{a}	33.52 ± 1.13^{ab}	30.81 ± 1.19^{b}	26.07 ± 1^{c}	21.52 ± 0.9^{d}	18.44 ± 0.97^{de}	14.93 ± 0.95^{e}
Group D	17.78 ± 0.93^{a}	14.26 ± 0.81^{b}	11.3 ± 0.7^{c}	7.63 ± 0.5^{d}	4.74 ± 0.37^{e}	3.11 ± 0.31^{ef}	1.74 ± 0.21^{f}
TOTAL	64.15 ± 1.79^{a}	56.93 ± 1.95^{ab}	52.3 ± 2.01^{b}	44.26 ± 1.69^{c}	36.85 ± 1.53^{d}	32.11 ± 1.65^{de}	26.37 ± 1.58^{e}

Within rows, means without a common superscript differ significantly ($p < 0.05$)

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