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SHORT COMMUNICATION

Standardization of hypo-osmotic solutions to study the functional integrity of post thaw bull spermatozoa

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ABSTRACT

Functional integrity of sperm plasma membrane was studied in post thaw bull semen using Hypo-osmotic swelling test (HOST). Frozen semen after thawing was subjected to hypo-osmotic swelling test (HOST) in hypo-osmotic solutions of osmolarity 75, 100 and 150 mOsmol/L. The overall average tail curling with 75, 100 and 150 mOsmol/L were recorded 17.55±8.28, 46.46±11.97 and 34.27±7.97%, respectively. Maximum tail curling was observed with 100 mOsmol/L solution which was significantly ($P < 0.05$) higher than the 75 and 150 mOsmol/L solution.

Key words : HOST, functional integrity, post thaw semen, curled tail spermatozoa, bull

Semen quality assessed through physical parameters such as sperm count, motility, concentration and morphology is of low predictive value to fertility (Kumi-Diaka, 1993). Moreover, post thaw motility assessment alone is not the absolute technique to evaluate the fertilizing ability of spermatozoa (Glass and Ericsson, 1979), hence, more reliable tests are needed for the same. Hypo-osmotic swelling test evaluates the functional integrity of sperm plasma membrane (Correa and Zavos, 1994) which is of fundamental importance in fertilization process. Scanty reports are available on thawed bull semen exposed to HOST.

Therefore, this investigation was carried out to compare the functional integrity of post thaw bull sperm exposed to three different hypo osmotic solutions viz., 75, 100 and 150 mOsmol/L. Frozen semen (medium French straws) produced from four healthy bulls (three cross bred and one pure bred Holstein Fresian) under thrice a week collection schedule at dairy farm, P.A.U., Ludhiana, was used for the purpose. Hypo osmotic swelling test was performed as per the method of Correa and Zavos (1994) by mixing one ml of hypo osmotic solution with 0.1 ml of frozen thawed (37°C for 30 sec) semen and incubating it at 37°C for one hr. Hypo osmotic solutions of osmolarity 75 and 100 mOsmol/L were prepared by serial dilution of 150 mOsmol/L solution, which was prepared by dissolving 7.35 g of sodium citrate and 13.15 g of Fructose in one litre of double distilled water. After incubation, slides were prepared and examined under phase contrast microscope (400X) for curled tailed spermatozoa. A total of 200 spermatozoa were counted in different field and total number of curled tailed spermatozoa was calculated. Similarly, 0.1 ml of semen was incubated in normal saline and number of curled tailed spermatozoa was found. This number in normal saline was deducted from the number in a hypo osmotic solution. The resultant figure was taken as the sperm tail curling as an effect of HOST. Data were analyzed using randomized block design and critical difference test.

The sperm tail curling for each bull exposed to 75, 100 and 150 mOsmol/L solutions varied from 36.42±7.27 to 38.92±7.93%, from 45.92±10.52 to 48.17±12.65% and from 33.42±5.99 to 35.17±8.83%, with overall average of 37.55±8.28, 46.46±11.97 and 34.27±7.97%, respectively. Significant ($P<0.05$) difference in tail curling was observed between three solutions, maximum curling being with 100 mOsmol/L solution (Table 1).

Correa and Zavos (1994), however, reported 60 and 48 per cent tail curling in bull semen subjected to 100 and 150 mOsmol/L solutions, respectively. Jeyendran *et al.* (1984) also found higher values of sperm tail curling (60.1%) in human semen exposed to 150 mOsmol/L solutions, respectively. Jeyendran *et al.* (1984) also found higher values of sperm tail curling (60.1%) in human semen exposed to 150 mOsmol/L solution. Only live and biochemically active spermatozoa show typical tail curling when exposed to hypo-osmotic medium (Bredderman and Foote, 1969). The optimal Hypo-osmotic medium should exert enough osmotic stress to cause an

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Table 1. Average sperm tail curling (% Mean ±SEM) in post thaw semen exposed to HOST using three hypo-osmotic solutions

Bull No.	n	Hypo-osmotic solutions		
		75 mOsmol/L	100 mOsmol/L	150 mOsmol/L
HHS 973	6	37.83±8.70	45.92±11.14	34.75±7.55
1F 804	6	37.00±8.58	45.92±10.52	35.17±8.83
1F 1013	6	36.42±7.27	45.83±12.77	33.75±8.42
AHF 113	6	38.92±7.93	48.17±12.65	33.42±5.99
Overall average	24	37.55±8.28[a]	46.46±11.97[b]	34.27±7.89[c]
CD 5%			1.8514	

Overall means with different superscripts differ significantly ($P < 0.05$)

observable increase in volume but small enough to prevent lysis of plasma membrane (Jeyendran *et al.*, 1984). Maximum tail curling with 100 mOsmol/L may be due to its optimal osmotic pressure offered to the spermatozoa.

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