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Effect of liquid nitrogen levels during storage of frozen semen of Tharparkar bulls

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In a herd a sire is always valued more than the cow, as the cow can hardly produce a dozen of progeny in her entire span of reproductive life, while a sire has the potential of producing several thousands of progeny. With the increasing trend of artificial insemination (AI) in the field, demand of good quality semen is increasing simultaneously with the result of increasing importance of elite bulls day by day. Spermatozoa of the bull can be stored at liquid nitrogen temperature (–196º C) for indefinite periods and after thawing retain relatively high rates of fertility (Graham *et al*. 1984). Sherman (1990) reported that there is no further loss in quality of frozen semen which is continuously stored at –196ºC under the surface of liquid nitrogen. Quintin *et al*. (1997) found that maintaining optimum liquid nitrogen level in the container is very much essential during prolonged storage and transportation of semen. Lack of optimum liquid nitrogen even for few hours in the container may result in complete destruction of sperm bank (Hafez 1993). The information on the level of liquid nitrogen in Tharparkar breed of cattle is meagre, hence, an attempt was made to study the effects of different levels of liquid nitrogen on various aspects of post thaw seminal characteristics.

Semen ejaculates (48) from 6 bulls (8 ejaculates from each bull) of 4 to 5 years of age maintained at Central Semen Station (CSS) Anjora, Durg (Chhattishgarh) during 2010–2011, were collected from April to July. All the bulls were maintained in identical feeding and management regimes according to minimum standard protocol (MSP) of Government of India. Bulls were maintained under single pens housing system and standard feeding schedule was planned to ensure intake of 2–3 kg dry matter/100kg body weight using the feedstuffs, concentrate: 0.4% of the body weight (2.0–3kg), green fodder: 40 to 60% of the balance dry matter (20–30 kg), roughages: remaining 40–60% (7–9kg), mineral mixture: 1% of concentrate (25–30g). Semen from experimental bulls was collected once a week, in morning hours between 7.00 and 8.30 AM before feeding by using artificial vagina (40 cm long and 6.5 cm in diameter) maintained at 42–45°C in incubator. A male partner of the same species was used as a dummy for semen collection. Two false mounts were provided to each bull before collection. Immediately after collection, the semen was kept at 37°C in a water bath placed inside the passbox. Semen was diluted in tris diluent and freezing was carried out after equilibration under standard conditions (Graham *et al.* 1985).

Post thaw progressive motility was assessed 24 h after freezing. Frozen semen samples were thawed in a water bath maintained at 37°C for 30 sec and thawed straws were wiped with tissue paper to remove the water. Laboratory seal end was cut with the scissors and semen straw was evacuated in a sterile sugar tube by cutting factory seal end. A small drop of semen was placed on clean glass slide and cover slip was placed over the semen drop. Progressive motility was determined on a subjective scale of 0–100% with nearest 5% intervals at 200× magnification of a phase contrast microscope fitted with the stage warmer (37°C).

The hypo osmotic swelling test was carried out as per the procedure described by Jeyendran *et al.* (1984) whereas cervical mucus penetration test was performed as described by Kremer (1965). The data were analyzed statistically using standard procedure as per Snedecor and Cochran (1994). Effect of different level of liquid nitrogen on PTM (%), HOST (%) and CMPT (mm/min) in Tharparkar bulls is presented in Table 1.

The post thaw motility was 52.22±1.09 (range 45– 60%), 49.44±0.80 (range 45–55%), 44.44±0.80 (range 40–50%) and 29.72±0.95 (range 25 – 40%) at full, 3/4th, 1/2, and 1/4th of the straw level of liquid nitrogen respectively, in Tharparkar bulls. The overall per cent post thaw motility was

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significantly higher (P<0.01) when the level of liquid nitrogen was full, 3/4th, and 1/2 of the level of straws as compared to 1/4th level of straws in Tharparkar bulls (Table 1).

Similar effect of level of liquid nitrogen was observed in Sahiwal and Red Sindhi bull (Pathak 2008). Hafez (1993) reported that insufficient liquid nitrogen in the container even for few hours may result in complete destruction of stored semen. However, Das *et al.* (2002) reported that frozen semen straws can be stored in 2 litre liquid nitrogen container with acceptable post-thaw motility for 10 days without filling liquid nitrogen and showed a gradual decrease in percent post-thaw motility after 10 days of storage. In present study at 1/4th level of straws a significant decline in motility may be due to decline in various post thaw cyto-morphological characteristics.

The overall post thaw per cent HOST positive sperm at full , 3/4th, 1/2 and 1/4th liquid nitrogen level of straws in Tharparkar bulls was 54.39±2.54 (range 30 - 72), 51.89±2.61 (range 28 - 71), 48.50±2.47 (range 25 - 65) and 40.11±2.21 (range 20 - 50), respectively (Table 1).

The per cent HOST positive sperm was found significantly lower (P<0.01) when the level of liquid nitrogen remained 1/4th level of straws as compared to full, 3/4th, and 1/2 levels in overall values in Tharparkar bulls. Similarly Pathak (2008) also found significantly lower (P<0.01) per cent HOST positive sperm when the level of liquid nitrogen remained 1/4th level of straws as compared to full, 3/4th, and 1/2 levels in Sahiwal bulls while there was no significant decline observed in per cent HOST positive sperms from full to 1/4th level of liquid nitrogen in Red Sindhi bull. The decline in per cent HOST positive sperms at 1/4th level indicated that the alteration in the temperature during storage may result in disruption of chemical as well as functional integrity of plasma membrane.

The mean sperm penetration distance travelled (mm/min) by vanguard spermatozoa at full, 3/4th, 1/2 and 1/4th liquid nitrogen level of straws in Tharparkar bulls was 29.39±1.16 (range 22 - 40), 28.39±1.20 (range 20 - 40), 27.61±1.22 (range 20 - 39) and 22.94±1.47 (range 13 - 35), respectively (Table 1). The mean distance traveled by vanguard spermatozoa at 1/4th level was significantly (P<0.01) lower than full, 3/4th and 1/2 liquid nitrogen level in Tharparkar bulls. The mean sperm penetration distance in full, 3/4th level of liquid nitrogen was much higher than those reported by Shrivastava and Kumar (2006) in cattle and buffalo semen. A significant lower value of sperm penetration distance travelled at 1/4th level may be due to decrease in post-thaw motility and damage to spermatozoa due to temperature variations.

The post thaw motility, hypo osmotic swelling and cervical mucus penetration followed similar pattern under different levels of liquid nitrogen with highest values in full whereas lowest in 1/4th level of liquid nitrogen on various post-thaw seminal characteristics.

Based on lower values of PTM, HOST and CMPT in 1/

Table 1. Effect of different level of liquid nitrogen on PTM (%), HOST (%) and CMPT (mm/min) in Tharparkar bulls

Bull No.	Post thaw motility (PTM%)				Hypo osmotic swelling test (HOST%)				Cervical mucus penetration test (CMPT mm/min)			
					Level of liquid nitrogen							
	Full	3/4th	Half	1/4th	Full	3/4th	Half	1/4th	Full	3/4th	Half	1/4th
7032	55.00±2.89	51.67±1.67	46.67±1.67	31.67±1.67	54.00±5.57	52.00±5.69	48.33±5.24	39.00±5.69	29.33±1.45	28.67±1.20	27.33±1.45	26.00±2.08
7110	51.67±1.67	48.33±1.67	43.33±1.67	28.33±1.67	59.33±3.48	57.33±3.18	53.33±3.18	46.33±2.73	27.67±3.18	26.00±3.46	25.33±2.91	17.67±1.76
6990	48.33±1.67	46.67±1.67	41.67±1.67	26.67±1.67	49.67±7.06	46.67±7.26	44.00±7.37	35.67±6.89	33.33±4.81	32.67±4.67	31.33±5.36	26.33±6.77
6957	55.00±2.89	51.67±1.67	46.67±1.67	31.67±1.67	50.33±11.05	47.67±10.74	44.67±10.99	36.33±8.76	30.00±3.06	28.67±2.73	28.33±3.38	23.67±3.18
7043	50.00±0.00	48.33±1.67	43.33±1.67	28.33±1.67	54.67±3.76	52.33±3.48	48.67±3.18	42.00±3.79	30.00±2.00	30.00±2.00	28.33±2.40	24.00±2.65
7097	53.33±4.41	50.00±2.89	45.00±2.89	31.67±1.67	58.33±7.80	55.33±8.95	52.00±7.23	41.33±5.55	26.00±2.08	24.33±2.33	25.00±2.31	20.00±3.21
Overall	52.22 ±1.09a**	49.44 ±0.80a**	44.44 ±0.80a**	29.72 ±0.95b**	54.39 ±2.54a**	51.89 ±2.61a**	48.50 ±2.47a**	40.11 ±2.21b**	29.39 ±1.16a**	28.39 ±1.20a**	27.61 ±1.22a**	22.94 ±1.47b**

** P≤0.01; Means bearing different superscript (a, b) in row differ significantly.

4^{th} LN_2 level in containers as compared to full, $3/4^{th}$ and ½, it was concluded that in the post thaw motility, hypo osmotic swelling and cervical mucus penetration of sperms were decreased on progressive decline in the level of liquid nitrogen in containers. Therefore to ensure better post thaw motility, hypo osmotic swelling and cervical mucus penetration of semen straws the level of liquid nitrogen should not fall below ½ of the level of straw while storage of Tharparkar bull semen.

SUMMARY

The experiment was conducted to study the effects of different levels of liquid nitrogen on various aspects of post-thaw seminal characteristics. Semen ejaculates (48) from Tharparkar bulls (6) of 4 cosy ears of age were collected. Post-thaw progressive motility was assessed 24 h freezing. HOST PMT and CPMT of spream decreased on progressive decline in the level of N_2, and it was observed that level of N_2, should not fall below ½ of the level of show while storage of semen of Tharparkar bull.

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