EFFECT OF LIQUID NITROGEN LEVELS DURING STORAGE ON QUALITY OF FROZEN SEMEN OF GIR BULLS*

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ABSTRACT

A total of 50 semen ejaculates collected from 5 Gir bulls were frozen and stored in 3 liter container to study the effect of different levels of liquid nitrogen on various post thaw seminal attributes. Overall mean values of post thaw motility, hypo osmotic swelling reactive sperm and cervical mucus penetration were 54.33 ± 1.18, 52.33 ±1.37, 47.00 ± 1.18, 28.67 ± 1.14%, 55.73 ± 2.08, 53.93 ± 1.70, 48.93 ± 2.18, 38.07 ± 1.79%, and 29.13 ± 0.96, 28.33 ± 0.83, 26.53 ± 1.06 and 19.87 ± 0.72 mm while liquid nitrogen level was full, 3/4th, half and 1/4th of frozen semen straw, respectively. The overall percent post thaw motility, percent HOS positive sperm and the mean distance traveled by vanguard spermatozoa (CMPT) 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. Overall mean values for post thaw motility, hypo osmotic swelling test and cervical mucus penetration test were best observed during the full level of liquid nitrogen and dropped significantly when level of liquid nitrogen reached to 1/4th level of semen straws in 3 liter LN_2 container.

Key words: CMPT, HOST, PTM, Seminal attributes, Gir bulls.

INTRODUCTION

Semen analysis is a valuable diagnostic tool to assess the fertility status of the male (Lodhi *et al.*, 2008). Spermatozoa of the bull can be stored at liquid nitrogen temperature (-196°C) for indefinite periods and after thawing retain relatively high rates of fertility (Graham *et al.* 1984). Lack of optimum liquid nitrogen even for few hrs in the container may result in complete destruction of sperm bank (Hafez, 1993). The information on the effect of level of liquid nitrogen on frozen semen in Gir 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.

MATERIALS AND METHODS

The study was conducted on five Gir bulls of 4 to 5 years age maintained at Central Semen Station (CSS) Anjora, Durg (Chhattishgarh) during 2010-2011. All the bulls were maintained in identical feeding and management regimen according to minimum standard protocol (MSP) of Government of India. Bulls were maintained under single pens housing system and

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standard feeding schedule was planned to ensure intake of 2-3 kg dry matter/ 100 kg body weight using the feedstuffs, concentrate: 0.4% of the body weight (2-3 kg), green fodder: 40 to 60 % of the balance dry matter (20-30 kg), roughages: remaining 40-60% (7-9 kg), mineral mixture: 1% of concentrate (25-30 g). Semen from experimental bulls was collected once a week, in morning hours between 7.00 and 8.30 A.M. before feeding by using Artificial Vagina. 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. A total of 50 semen ejaculates from five bulls (10 ejaculates from each bull) were collected from April to July. Semen was diluted in tris diluent and freezing was carried out after 4 hours of equilibration under standard conditions (Graham *et al.,* 1985).

Frozen semen straws were stored in a 3 liter liquid nitrogen container. These straws were evaluated for post thaw motility, CMPT and HOST when the level of liquid nitrogen was full and 24 hours after the level of liquid nitrogen reached to 3/4th, 1/2 and 1/4th of the level of straws. Frozen semen samples were thawed in a water bath maintained at 37°C for 30 seconds and the content was evacuated in a sterile sugar tube by cutting seal. Progressive motility was determined on a subjective scale of 0-100% with nearest 5% intervals at 200x 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 was analyzed statistically using standard procedure of ANOVA as per Snedecor and Cochran (1994).

RESULTS AND DISCUSSION

The findings on effect of different level of liquid nitrogen on PTM (%), HOST (%) and CMPT (mm) of Gir bulls sperms are presented in Table.

The overall percent post thaw motility was significantly higher (P<0.01) when the level of liquid nitrogen was full and 3/4th of the level of straws as compared to ½ and 1/4th level of straws (Table 1). Pathak (2008) observed that the overall percent post thaw motility was significantly higher (P<0.05) 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 Sahiwal and Red Sindhi bulls. 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 refilling liquid nitrogen and showed a gradual decrease in percent post thaw motility after 10 days of storage. In present study at ½ and 1/4th level of straws a significant decline in motility may be due to decline in various post thaw cyto-morphological characteristics.

The percent 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 Gir bulls. Similarly Pathak (2008) also found significantly lower (P<0.01) percent 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 percent HOST positive sperms from full to 1/4th level of liquid nitrogen in Red Sindhi bull. The decline in percent HOST positive sperms at 1/4th level indicate that the alteration in the temperature during storage may result in disruption of chemical as well as functional integrity of plasma membrane.

The mean distance traveled by vanguard spermatozoa at 1/4th level was also found to be significantly (P<0.01) lower than full, 3/4th and 1/2 levels (Table 1). 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 values 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.

Based on lower values of PTM, HOST and CMPT in 1/4th LN_2 level in containers as compared to full, 3/4th and ½, it was concluded that the post thaw motility, hypo osmotic swelling and cervical mucus penetration of sperms 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 Gir bull semen.

Table : EFFECT OF DIFFERENT LEVEL OF LIQUID NITROGEN ON PTM (%), HOST (%) AND CMPT (MM) IN GIR BULLS

Bull No.	Level of Liquid Nitrogen in 3 liter Container											
	Post Thaw Motility (PTM %)				Hypo Osmotic Swelling Test (HOST %)				Cervical Mucus Penetration Test (CMPT mm)			
	Full	3/4th	Half	1/4th	Full	3/4th	Half	1/4th	Full	3/4th	Half	1/4th
892	53.33 ± 1.67	51.67 ± 1.67	48.33 ± 1.67	33.33 ± 1.67	55.67 ± 3.52	55.33 ± 1.45	53.00 ± 1.53	41.33 ± 3.28	30.00 ± 2.00	29.00 ± 1.73	27.67 ± 1.76	19.00 ± 0.58
BP-943	55.00 ± 2.88	53.33 ± 1.67	46.67 ± 1.67	28.33 ± 1.67	52.33 ± 4.33	51.00 ± 2.51	44.33 ± 2.96	38.00 ± 1.53	28.67 ± 1.45	28.33 ± 2.02	29.33 ± 0.88	22.00 ± 0.58
MP-120	50.00 ± 2.88	46.67 ± 4.40	45.00 ± 2.89	25.00 ± 2.87	48.00 ± 6.00	47.00 ± 5.03	39.67 ± 7.42	34.00 ± 7.37	25.67 ± 3.84	24.67 ± 2.02	20.67 ± 2.96	16.67 ± 2.03
BP-902	55.00 ± 2.88	53.33 ± 3.33	46.67 ± 4.41	28.33 ± 3.33	60.33 ± 3.38	56.67 ± 3.18	51.67 ± 2.02	37.76 ± 1.76	30.33 ± 1.20	30.00 ± 1.15	28.00 ± 1.00	19.67 ± 1.45
BP-934	58.33 ± 1.66	56.67 ± 1.67	48.33 ± 3.33	28.33 ± 1.67	62.33 ± 2.40	59.67 ± 2.91	56.00 ± 2.51	39.33 ± 5.23	31.00 ± 1.15	29.67 ± 1.45	27.00 ± 1.52	22.00 ± 1.15
Overall	54.33 ± 1.18^{a}	52.33 ± 1.37^{a}	47.00 ± 1.18^{b}	28.67 ± 1.14^{c}	55.73 ± 2.08^{a}	53.93 ± 1.70^{ab}	48.93 ± 2.18^{b}	38.07 ± 1.79^{c}	29.13 ± 0.96^{a}	28.33 ± 0.83^{a}	26.53 ± 1.06^{a}	19.87 ± 0.72^{b}

Means of a trait bearing different superscript (a, b, c, d) in a row differ significantly (P<0.01)

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