BOVINE SEMEN THAWED AT 28ºC OR 37ºC CAN BE USED FOR AI TILL 90 MIN POST-THAW

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ABSTRACT

The post-thaw keeping quality of frozen bull semen at 15 min interval upto 150 min post-thaw was assessed following thawing under tap water (28ºC) or ideal (37ºC) temperature. Under both thawing temperatures, upto 120 min post-thaw, the progressive motility (>50%), sperm viability (>60%), sperm abnormalities (<7%) and acrosome integrity (>75%) were within the acceptable limlits of frozen bovine semen production, whereas, >40% HOST-positive sperms were maintained upto 90 min post-thaw. Between groups, there was no difference (p>0.05) in the post-thaw outcome of seminal parameters. Thus, based upon the results of seminal parameters of frozen thawed bull semen, it can be suggested that semen straws thawed under tap water (28ºC) or ideal (37ºC) temperature can be used for artificial insemination till 90 min post-thaw in situations where carrying of liquid nitrogen cylinder is not possible.

Keywords: Bull, Frozen, Semen, Temperature, Thawing

INTRODUCTION

For a successful artificial insemintaion (AI) in dairy animals, the procedure of thawing of frozen semen and the subsequent handling of semen should be appropriate. Mostly, the cryopreserved bovine semen is thawed by plunging straws in 37ºC water bath for 30-60 seconds. However, an inappropriate semen handling technique results in temperature variation leading to poor post-thaw viability (Brown *et al.*, 1991). In hilly terrains, carrying semen straws stored in liquid nitrogen containers to farmer's premises and hence following ideal semen thawing procedures before AI is very difficult. On the contrary, in hilly terrains, usually followed practice of thawing of semen in tap water and carrying frozen thawed semen for a period of time before AI may affect the semen quality. Therefore, the present study was planned to assess the keeping quality, at periodic intervals, of bull semen thawed under tap water (28ºC) or ideal (37ºC) temperature.

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MATERIALS AND METHODS

Twenty-two semen straws, each, from two healthy Holstein Friesian bulls with proven fertility were used in the present study. One half of the straws thawed in water bath at 28ºC (T_1) and the others thawed at 37ºC (T_2) for 60 sec were maintained at that temperature till subsequent evaluations. The parameters like progressive motility of sperms (at 400X), sperm viability (eosin-nigrosin stained semen smear at 400X), sperm abnormalities, acrosome integrity (Giemsa stain smear) and Hypo-osmotic swelling test (HOST; Jeyendran *et al.*, 1984) were evaluated at 15 min interval till 150 min post-thaw, with six repetetions of each. The results were analysed statistically by Student's t-test using SPSS17.0 software.

RESULTS AND DISCUSSION

Immediately following thawing under tap water (28ºC) or ideal (37ºC) temperature, the recorded progressive motility of sperms was >60% which was maintained at 50% till 120 min post-thaw (Table 1). The minimum acceptable post-thaw motility of frozen bull

semen is 50% as per the minimum standard protocol for bovine frozen semen (DAHD, 2014).

The live sperm count which was around 75-80% immediately following thawing, under both thawing temperatures, was maintained within acceptable limit of 60% till 120 min post-thaw (Table 1). The sperm abnormalities, especially curled and bend tail abnormalities, increased gradually during post-thaw period, however, these were maintained within aceptable limit of 7-9% till 150 min post-thaw (Table 1).

About 90-92% spermatozoa had intact acrosomes immediately after thawing under both temperatures, followed by continuous decline in acrosome integrity to ~75% by 150 min post-thaw (Table 1). The minimum standard protocol for the production of bovine frozen semen requires ≥65% sperms with intact acrosomes (DAHD, 2014). In a previous pre- and post-thaw thermal insult study, it was suggested that acrosomal integrity of cryopreserved bovine sperms was more sensitive than sperm motility (DeJarnette *et al.*, 2000).

The intactness of sperm cell memebrane as revealed by HOST outcome is a good model for predicting the potential fertility of bull semen and was compared with sperm motility measurements and fertility trials (Padrik *et al.*, 2012). In the present study, immediately after thawing, 62-68% sperms had HOST-positive reaction under both thawing protocols (Table 1). The percentage of HOST positive sperms reached 42-47% within 90 min after thawing and 20-28% by 150 min after thawing (Table 1). The minimum cut off limit for HOST-reactive sperms is 40% as per the minimum standard protocol for the production of bovine frozen semen (DAHD, 2014).

Based upon the results of present study regarding seminal parameters during post-thaw period, it was found that acceptable limits for the bovine frozen semen were maintained till 90 min post-thaw and the outcome was similar ($p>0.05$) following semen thawing under tap water (28ºC) or ideal (37ºC) temperature. In a previous field trial conducted to determine the effect of thawing of semen at 35ºC compared with ambient temperature (14±3ºC) revealed the absence of difference in fertility between the two thawing temperatures (Vishwanath, 1998). Thus, carrying the LN_2 containers to hilly terrains can be avoided in situations where the inseminator can perform the AI within 90 min post-thaw. However, the fertility studies of

Table 1. Post-thaw semen parameters of frozen bull semen thawed at at 28ºC (T_1) and 37ºC (T_2) for 60 sec and maintained at that temperature till end of evaluations. '0' min refers to start of thawing

Time (min)	Seminal Parameters (%)									
	Progressive Motility		Viability		Sperm abnormality		Acrosome integrity		HOST	
	T_1	T_2	T_1	T_2	T_1	T_2	T_1	T_2	T_1	T_2
0	61.7±2.6	61.7±2.6	77.3±2.2	77.8±2.2	3.3±0.8	3.3±1.5	92.2±2.0	90.5±1.4	65.5±3.2	66.0±2.8
15	59.2±2.0	59.2±2.0	77.2±2.5	77.0±1.9	5.0±1.6	3.8±1.3	91.0±1.6	91.3±1.6	64.3±3.7	63.5±3.3
30	58.3±2.6	59.2±2.0	75.8±2.3	77.3±2.0	5.0±1.7	4.5±1.1	90.8±2.4	90.3±2.1	60.0±3.7	61.0±2.2
45	57.5±4.2	59.2±2.0	74.8±1.9	75.5±2.1	5.8±1.9	4.7±1.0	88.7±2.3	89.3±2.1	56.7±2.5	54.8±2.5
60	57.5±4.2	59.2±2.0	72.3±1.9	73.5±2.1	7.0±1.6	5.5±1.6	86.3±2.5	87.3±2.7	52.7±2.7	50.7±2.5
75	54.2±2.0	55.8±3.8	70.2±3.0	72.3±3.3	6.7±1.5	5.8±1.2	83.7±1.0	86.0±3.0	48.0±3.0	49.3±2.7
90	51.7±2.6	53.3±2.6	67.8±1.9	69.5±2.4	6.5±1.9	6.5±1.1	80.7±2.0	82.0±3.8	43.7±1.0*	45.7±2.3*
105	50.8±2.0	50.0±3.2	66.2±3.2	67.0±2.0	8.2±1.2	6.2±2.6	76.8±1.5	78.5±2.3	36.5±3.2	37.8±3.1
120	50.8±2.0*	50.0±3.2*	63.0±2.3*	64.5±2.1*	7.5±2.1	7.0±1.7	75.0±3.1	76.3±2.9	32.8±5.9	33.3±3.8
135	45.8±3.8	45.8±2.0	58.7±2.3	59.5±2.7	8.7±1.9	7.8±2.2	74.0±2.9	76.3±2.0	26.8±4.5	29.2±2.6
150	43.3±2.6	44.2±2.0	55.7±3.1	57.0±2.0	8.5±1.4*	7.8±1.6*	74.3±3.0*	75.5±2.7*	22.8±2.8	26.0±2.8

$p>0.05$, between treatments for all the parameters at any given point of time; *Cut-off limits for each test

frozen thawed semen (at 28ºC and 35ºC) during post-thaw period are essential for the final recommendation for field practice.

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