EFFECT OF POST THAW INCUBATION ON QUALITY OF FROZEN THARPARKAR BULL SEMEN*

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Received : 01.08.2013 Accepted : 31.12.2013

ABSTRACT

The period spent after thawing and before insemination may affect the quality and fertility of post thaw semen. The frozen semen was thawed at 37°C for 30 seconds and taken into a small sugar tube and incubated in the water bath at 37°C for 0, 15, 30, 45 and 60 min and post thaw motility (PTM %), hypo osmotic swelling test (HOST %) and cervical mucus penetration test (CMPT – mm/60 min) were carried out. The mean (range) of PTM observed at respective intervals was 52.22 ± 1.09 (45-60), 51.39 ± 0.97 (45-60), 46.94 ± 0.59 (45-50), 40.28 ± 0.85 (35-45) and 32.22 ± 0.92 (25-40) %. The corresponding values of HOST were 54.39 ± 2.54 (30-71), 51.28 ± 1.79 (40-65), 44.77 ± 1.87 (32-58), 34.17 ± 1.49 (23-43) and 24.50 ± 1.10 (16-32) % and CMPT 29.39 ± 1.16 (22-40), 27.78 ± 0.92 (23-36), 23.11 ± 0.81 (19-31), 16.39 ± 0.16 (12-24) and 10.28 ± 0.54 (7-15) mm/60min. The overall post thaw motility at 0, 15 and 30 min was found to be significantly higher ($P<0.01$) than 45 and 60 min in Tharparkar bulls, however overall HOST and CMPT were significantly higher ($P<0.01$) at 0 and 15 min than at 30, 45 and 60 min. Post thaw motility remained unaffected up to 30 min post thaw incubation while there was significant decline in HOS reactive sperm and cervical mucus penetration at 30, 45 and 60 min which indicated that motility may remain good but increasing post thaw incubation duration affects cell membrane permeability and cervical mucus penetration ability of spermatozoa which may affect fertility.

Key words: CMPT, HOST, Incubation period, PTM, Tharparkar bull semen

INTRODUCTION

Fertility is a measure of reproductive success. In males, it can be defined as the ability of a bull to produce semen that will result in a successful pregnancy. The main disadvantage of post breeding bull fertility evaluations is that they assess the fertility of any given bull, after the bull has been bred to his female counterparts. In case of an infertile bull, damage has been done before fertility results become available. Hence, prediction of fertility prior to breeding rather than post breeding could largely increase reproductive efficacy (Rodriguez-Martinez, 2003). Frozen semen is most frequently damaged during handling after thawing and prior to the insemination. The duration between thawing and insemination also have influence on fertilizing ability of frozen semen when it is exposed to 37°C temperature for longer duration after thawing (Singh and Pant, 1999). For this reason, proper assessment of the post-thaw quality of spermatozoa is of highest interest for AI industry, since it can provide insights upon the fertilizing capacity of the cryopreserved spermatozoa. The information on the effect of duration of post thaw incubation in exotic and crossbred bulls are available in plenty but, studies in Tharparkar breed

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of cattle is however meager, hence, an attempt was made to study the post thaw incubation test (thermo resistance test) on frozen Tharparkar bull semen.

MATERIALS AND METHODS

The study was conducted on six Tharparkar bulls of 4 to 5 years age maintained at Central Semen Station (CSS) Anjora, Durg (C.G.) 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 standard feeding schedule is planned to ensure intake of 2-3 kg dry matter/100kg body weight using the feedstuffs, concentrate: 0.4% of the body weight (2-3kg), green fodder: 40 to 60 % of the balance dry matter (20-30 kg), roughages: remaining 40-60% (7-9kg), mineral mixture: 1% of cattle feed (25-30g). Semen from experimental bulls was collected once a week, in morning hours 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. A total of 48 semen ejaculates from six bulls (8 ejaculates from each bull) were collected from April to July. 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 4 hours of equilibration under standard conditions (Graham *et al.,* 1985).

The stored semen straws were thawed at 37°C for 30 second, taken into a small sugar tube and incubated in the water bath at 37°C for 0 (control), 15, 30, 45 and 60 min and evaluation of post thaw motility, hypo osmotic swelling test (Jeyendran *et al.*, 1984) and cervical mucus penetration test (Kremer, 1965) was carried out after each interval.

The data was analyzed statistically using standard ANOVA procedure as per Snedecor and Cochran (1994).

RESULTS AND DISCUSSION

The mean post thaw motility (PTM) at 0, 15, 30, 45 and 60 min post thaw incubation was 52.22 ± 1.09 (45-60), 51.39 ± 0.97 (45-60), 46.94 ± 0.59 (45-50), 40.28 ± 0.85 (35-45) and 32.22 ± 0.92 (25-40) %, respectively. The overall post thaw motility at 0, 15 and 30 min was found to be significantly higher ($P<0.01$) than 45 min and 60 min. The findings are in close agreement with findings on Sahiwal bulls and Red Sindhi bulls at 0 and 15 min and higher than 30 and 60 min (Pathak, 2008). Dhami *et al.* (1994) also found lower motility *i.e.* 20.90 % after 60 min of post thaw incubation in Murrah buffalo under tropical climate. Similar findings on post thaw incubation motility of cattle and buffalo spermatozoa have also been reported by other workers (Tuli *et al.,* 1985; Sahni and Mohan, 1988) whereas Pramanik (1996) observed higher values of motility of frozen semen after 0, 1 and 2 hrs of incubation. Reduction in motility could be due to increased oxidative metabolism and increased toxicity of the medium due to peroxide formation with the increase in number of dead spermatozoa (John *et al.*, 1979).

The mean percent hypo osmotic swelling test (HOST) positive sperms at 0, 15, 30, 45 and 60 min post thaw incubation were 54.39 ± 2.54 (30-71), 51.28 ± 1.79 (40-65), 44.77 ± 1.87 (32-58), 34.17 ± 1.49 (23-43) and 24.50 ± 1.10 (16-32) % in Tharparkar bulls. The overall percent HOST positive sperm was significantly higher ($P<0.01$) at 0 and 15, than 30, 45 and 60 min post thaw incubation. The present observation is in close agreement with finding on Sahiwal and Red Sindhi bulls (Pathak, 2008). The percent HOS positive sperm at 0 hr of post thaw incubation was much higher than those reported by Sivaramlingam (1994), Prasad *et al.* (1999) and Rasul *et al.* (2000), while Pramanik (1996) reported 57.48 % HOS positive sperm at 0 hr incubation. A significant lower value of percent HOS positive sperm at 30 and 60 min post thaw incubation shows that membrane permeability affected inversely after long holding duration.

The overall cervical mucus penetration test (CMPT) by spermatozoa at 0, 15, 30, 45 and 60 min of post thaw incubation was 29.39 ± 1.16 (22-40), 27.78 ± 0.92 (23-36), 23.11 ± 0.81 (19-31), 16.39 ± 0.76 (12-24) and 10.28 ± 0.54 (7-15) mm/60 min, respectively. The overall CMPT (mm) at 0 and 15 min, was significantly higher ($P<0.01$) than, 30, 45 and 60 min in Tharparkar bulls. Present observations are in close agreement with finding on Sahiwal and Red Sindhi bulls (Pathak, 2008). The observations in our study for mean sperm penetration distance at 0 hr of post thaw incubation was much higher than those reported by Dev *et al.* (1996), Shrivastava and Kumar (2006) in cattle and buffalo semen. A significant lower values of sperm penetration distance travelled at 30, 45 and 60 min post thaw incubation may be due to change in spermatozoa motility and damage to spermatozoa as evidenced in this study.

Post thaw motility remained unaffected up to 30 min post thaw incubation while there was significant decline in hypo osmotic swelling test and cervical mucus penetration test at 30, 45 and 60 min which indicated that motility may remain good but increasing post thaw incubation duration affects cell membrane permeability and cervical mucus penetration ability of spermatozoa which may affect fertility.

ACKNOWLEDGEMENTS

Authors are highly thankful to Dean, College of Veterinary Science and Animal Husbandry, Anjora, Durg and Director Veterinary Services (C.G.) for permitting to carry out the research project and providing necessary facilities.

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