Indian Journal of Animal Sciences **84** (2): 170–172, February 2014/Short communication

Effect of post-thaw thermal resistance test on motility, membrane integrity and migration capacity of sperm in Gir bulls

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Received: 5 July 2013; Accepted: 29 September 2013

Key words: Frozen semen, Gir bulls, Incubation duration

The quality of frozen-thawed semen is one of the most influential factors affecting conception (Saacke 1984). The small number of frozen-thawed spermatozoa in each insemination dose must be of very high quality to ensure acceptable pregnancy rates (Brinsko and Varner 1993). 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 cryo-preserved spermatozoa (Januskauskas and Zilinskas 2002). The percentage of progressively motile spermatozoa declines significantly at different intervals of post-thaw incubation (John *et al*. 1979). The variation in temperature during freezing and thawing of semen inevitably reduces the proportion of motile and live spermatozoa and causes ultra-structural, biochemical and functional damage (Maurya and Tuli 2003). Sperm penetration is affected by penetration time, semen quality and properties of cervical mucus. The information on the effects of post-thaw thermal resistance test on motility, membrane integrity and migration capability of sperm in Gir breeds of bulls are however meager, hence, an attempt was made to study the effect of post-thaw incubation period on sperm motion characteristics, intactness of sperm cell membrane and sperm penetration distance of frozen Gir bull semen at room temperature.

Semen ejaculates (50) from 5 Gir bulls (10 ejaculates from each bull), 4 to 5 years age maintained at Central Semen Station Anjora, Durg (Chhattishgarh), were collected during 2010–2011. Bulls were maintained under single pens housing system and standard feeding schedule 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% (7–9 kg), mineral mixture: 1% of cattle feed (25–30g). Semen was collected once a week, between 7.00 and 8.30 AM before feeding by using artificial vagina (40 cm long and 6.5 cm in dia). 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).

The stored semen straws were thawed at 37°C for 30 sec, taken into a small sugar tube and incubated in the water-bath at 37°C for 0 h (control), 15 min, 30 min, 45 min and 60 min and then per cent post-thaw motility (forward 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 at 37°C), hypo-osmotic swelling test (Jeyendran *et al.* 1984) and cervical mucus penetration test (Kremer 1965) were carried out. The data were analyzed statistically using standard procedure as per Snedecor and Cochran (1994).

Effect of post-thaw incubation duration on PTM (%), HOST (%) and CMPT (mm) in Gir bulls is presented in Table 1. The overall post-thaw motility at 0 h, 15 min was significantly higher ($P<0.01$) than 30 min, 45 min and 60 min in Gir bulls. Similar to our findings, Pathak (2008) also observed significantly higher ($P<0.01$) post-thaw motility at 0 h, 5 min and 15 min than 30 min and 60 min of post-thaw incubation in Sahiwal bulls as well as in Red Sindhi bulls. Taraphder *et al*. (2001) reported a significant reduction in motility after 1 h of incubation. The reduction in motility after 15 min post-thaw incubation is due to increased oxidative metabolism, increased toxicity of the medium due to peroxide formation, with the increase in the number of dead spermatozoa. Dhami *et al*. (1994) also found lower motility i.e. 20.90% after 1 h of post-thaw incubation. Similarly Dhami and Sahni (1995) reported the values as

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Table 1. Effect of post-thaw incubation duration on PTM (%), HOST (%) and CMPT (mm) in Gir bulls

Bull No.	Post-thaw incubation duration														
	Post-thaw motility (PTM %)					Hypo-osmotic swelling test (HOST %)					Cervical mucus penetration test (CMPT mm)				
	0 h	15 min	30 min	45 min	60 min	0 h	15 min	30 min	45 min	60 min	0 h	15 min	30 min	45 min	60 min
892	53.33 ± 1.67	53.33 ± 1.67	46.67 ± 1.67	43.33 ± 1.67	35 ± 2.87	49.67 ± 2.03	47.67 ± 2.85	37 ± 1.53	27.33 ± 3.28	23 ± 1.15	28 ± 1.15	26.67 ± 1.76	18 ± 1.53	12.33 ± 1.85	8.33 ± 0.88
BP-943	55 ± 2.89	53.33 ± 3.33	48.33 ± 1.67	41.67 ± 1.67	31.67 ± 1.67	56 ± 2.31	52.67 ± 1.45	44.33 ± 2.73	38.33 ± 2.6	28.33 ± 2.02	29.33 ± 3.18	28.33 ± 0.88	19.67 ± 0.88	13.67 ± 0.88	8.33 ± 1.2
MP-120	50 ± 2.89	48.33 ± 1.67	41.67 ± 1.67	38.33 ± 1.67	31.67 ± 1.67	47 ± 3.61	47 ± 5	39.33 ± 1.2	24.33 ± 2.33	23 ± 1.73	24 ± 4.04	23.33 ± 2.19	16.33 ± 0.33	11.67 ± 1.33	6 ± 1.53
BP-902	55 ± 2.89	53.33 ± 3.33	50 ± 2.89	46.67 ± 1.67	41.67 ± 1.67	54.33 ± 4.81	54.67 ± 3.18	43.33 ± 7.62	38 ± 7.81	20.67 ± 1.45	29 ± 3.05	24 ± 2.52	17.33 ± 3.18	13.33 ± 1.45	9.33 ± 1.45
BP- 934	58.33 ± 1.67	56.67 ± 1.67	50 ± 0	46.67 ± 1.67	38.33 ± 1.67	55.67 ± 2.18	53.33 ± 2.03	49.33 ± 1.45	41 ± 1.15	23 ± 4.04	29.67 ± 2.19	30.33 ± 1.76	21.33 ± 2.03	13.33 ± 1.2	9.33 ± 0.88
Overall	54.33 ± 1.18[a]	53 ± 1.17[a]	47.33 ± 1.08[b]	43.33 ± 1.05[c]	35.67 ± 1.27[d]	52.53 ± 1.53[a]	51.07 ± 1.45[a]	42.67 ± 1.82[b]	33.8 ± 2.36[c]	23.6 ± 1.10[d]	28 ± 1.23[a]	26.53 ± 1.00[a]	18.53 ± 0.85[b]	12.87 ± 0.55[c]	8.27 ± 0.56[d]

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

35.85% after 1 h. Reduction in motility could be due to increased oxidative metabolism, increased toxicity of the medium due to peroxide formation with the increase in number of dead spermatozoa (John *et al*. 1979).

The overall per cent HOS positive sperm (Table 1) was significantly higher (P<0.01) at 0 h (control) and 15 min than 30 min, 45 min and 60 min post-thaw incubation in Gir bulls. However, Pathak (2008) found that the overall per cent HOS positive sperm was significantly higher (P<0.01) at 0 h (control) than 5 min, 15 min, 30 min and 60 min post-thaw incubation in Sahiwal bulls and Red Sindhi bulls.The per cent HOS positive sperm at 0 h of post-thaw incubation was much higher than those reported by 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 per cent HOS positive sperm at 30 min, 45 min and 60 min post-thaw incubation showed that membrane permeability got inversely affected after long holding duration.

The overall SPD (mm) at 0 h and 15 min (Table 1) was significantly higher (P<0.01) than 30 min, 45 min and 60 min in Gir bulls. Our observations for mean sperm penetration distance at 0 h of post-thaw incubation were higher than those reported by Shrivastava and Kumar (2006) in cattle and buffalo semen. Pathak (2008) found that the overall SPD (mm) at 0 h was significantly higher (P<0.01) than 5 min, 15 min, 30 min and 60 min in Sahiwal bulls and in Red Sindhi bulls. A significant lower values of sperm penetration distance travelled at 30 min, 45 min and 60 min post-thaw incubation may be due to change in spermatozoa motility and damage to spermatozoa as evidenced in this study.

Decline in membrane integrity and sperm penetration distance could be due to leakage of intercellular enzyme and inhibition of respiration as a result of plasma membrane damage on cryo-preservation (White 1993). Bull sperms were found to be more susceptible to peroxidation, due to higher ratio of unsaturated to saturated fatty acid (Halliwell and Gutterridge 1984) which results in irreversible membrane damage while incubation.

Post-thaw motility, per cent hypo-osmotic swelling of sperms and cervical mucus penetration ability of spermatozoa remain unaffected up to 15 min post-thaw incubation while there is significant decline at 30, 45 and 60 min which indicated that increasing post-thaw incubation duration affects post-thaw motility, cell membrane permeability and cervical mucus penetration ability of spermatozoa which may affect fertility.

SUMMARY

Incubation test assesses the livability of spermatozoa in female reproductive tract and thereby the longevity of the sperms. Indirectly, it assesses the freezing injuries occurred to sperms during the deep freezing process. 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 sec and taken into a small sugar tube

and incubated in the water-bath at 37°C for 0 h, 15 min, 30 min, 45 min and 60 h and per cent post-thaw motility (PTM%), hypo-osmotic swelling test (HOST%) and cervical mucus penetration test (CMPT – mm) were carried out. The PTM declined from 54.33±1.18% at 0 h to 53.00±1.17% – 15 min, 47.33±1.08% - 30 min, 43.33±1.05% - 45 min and 35.67±1.27% at 60 min. HOST reduced from 52.53±1.53% at 0 h to 51.07±1.45%- 15 min, 42.67±1.82%- 30 min, 33.80±2.36%- 45 min and 23.60±1.10% at 60 min. Similarly, CMPT reduced from 28.00±1.23 mm at 0 h to 8.27±0.56 mm at 60 min. The overall post-thaw motility, HOST and CMPT at 0 h and 15 min were significantly higher than 30 min, 45 min and 60 min in Gir bulls. Post-thaw motility, hypo-osmotic swelling test and cervical mucus penetration test remain unaffected up to 15 min post-thaw incubation while there is significant decline in at 30, 45 and 60 min, which indicated that increasing post-thaw incubation duration affects post-thaw motility, 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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