



Relationship between hypo-osmotic swelling response of spermatozoa with post-thaw motility in crossbred bulls

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The success of cryopreservation of semen and artificial insemination greatly depends on quality of semen used for insemination. The frozen-thawed semen is evaluated by quality control tests and among them the post-thaw motility (PTM) examination is the most important one. Motility is an essential indicator of healthy spermatozoa, but it alone is not enough to evaluate the fertilizing ability of the semen. Fertilizing ability deteriorates in spermatozoa before it loses its motility. Hypo-osmotic swelling test (HOST) is one of the quality control tests to evaluate the functional integrity of sperm plasma membrane (Correa and Zavos 1994), which is of fundamental importance in fertilization process. Studies on correlation between PMT and HOST were carried out for buffalo bulls (Dhawan *et al.* 2002, Chaudhari *et al.* 2007, Lodhi *et al.* 2008), exotic cow bulls (Kumar and Singh 2007) and indigenous cow bulls (Lodhi *et al.* 2008, Chaudhari *et al.* 2009). However, the information on correlation between PMT and HOST is scanty in crossbred cow bulls. Hence the study was attempted to know the correlation and report the same in crossbred bulls.

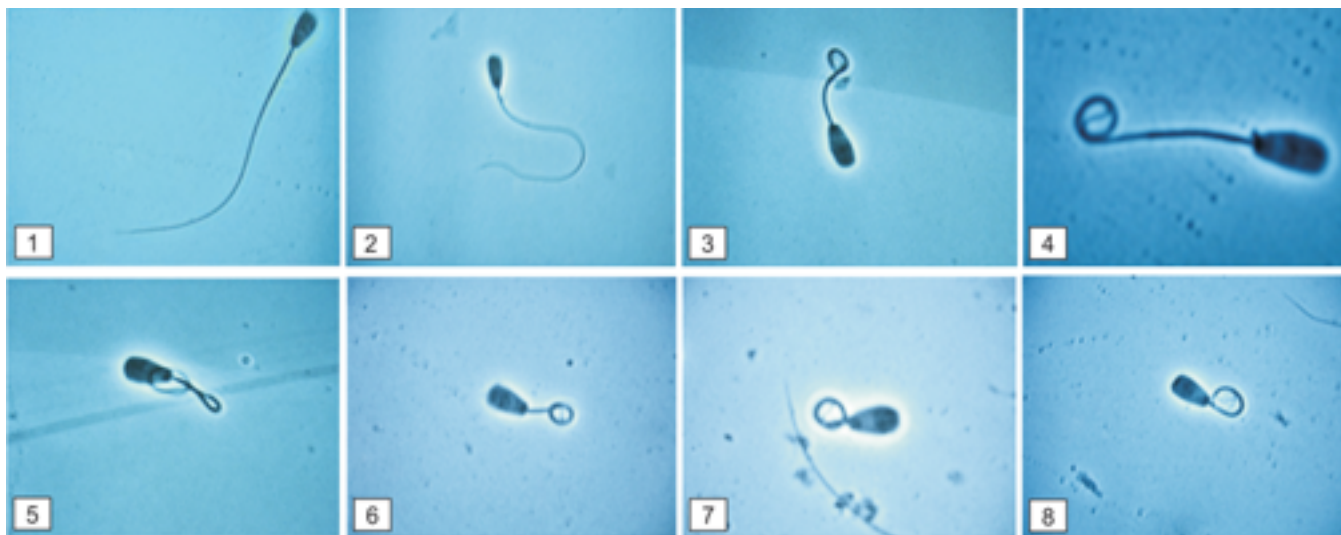
The present study was undertaken on crossbred bulls to find out the relationship between HOST and PTM in frozen - thawed semen. Records of 153 semen samples were studied which were prepared during February 2005 to September 2006 at Pashu Samvardhan Kendra, Dudhsagar Research and Development Association, Jagudan. The semen were collected by artificial-vagina method and diluted with Tris-egg yolk-citric acid-fructose-glycerol extender. Diluted semen was packed in 0.25 ml French Mini straws and was equilibrated for 4 h at 4°C in cold handling unit. Thereafter,

freezing of semen was carried out in programmable bio-freezer up to -140°C and immediately the straws were transferred into the liquid nitrogen. On next day, the straws were thawed at 37°C for 30 sec and PTM was examined under phase contrast microscope. Proportion of progressively motile spermatozoa was expressed in percentage. Samples with 50% (N, 110) and 60% (N, 43) PTM were chosen and subjected to HOST. It was performed by mixing 1 ml of hypo osmotic solution (0.367 g sodium citrate in 50 ml and 0.675 g fructose in 50 ml of double distilled water) with 0.1 ml of frozen thawed (37°C for 30 sec) semen and incubated at 37°C for 1 h. After incubation slides were prepared and examined under phase-contrast microscope for counting curled-tailed spermatozoa. Sperms with various types of curling patterns (Type B, C and D) were considered as reacted sperms, while sperms with no swelling/curling were considered as non-reacted sperms (Type A) as per Arangasamy and Singh (2002). A total of 200 spermatozoa were counted in different fields and total number of curled-tailed spermatozoa was calculated (Type B, C, D). The data were subjected to the 2 sample t test (Snedecor and Cochran 1989).

In the present investigation, the HOS reacted sperm tail percentage were 54.76 ± 0.93 and 63.40 ± 1.21 for sample with 50% (N,110) and 60% (N,43) PTM, respectively. Highly significant ($P < 0.01$) difference was observed between 2 groups. Similarly, highly significant correlation between pre-freeze HOST values and PTM was reported by Sivaramlingam *et al.* (2002). They suggested the possibility of using this test for screening of ejaculates to be used for freezing. Arangasamy and Singh (2002) reported the greater % of tail swelling in test samples compared to control which might be because of increased motility and livability of spermatozoa after treatment with certain additives like PGF₂ alpha, BHT and oxytocin. Chaudhari *et al.* (2007) also reported the highly significant difference among the 2 groups with 50 and 60% PTM in Mehsana buffalo bulls. Again, Chaudhari *et al.* (2009) reported similar findings in Kankrej

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Figs 1–8. 1–2. Type A sperms: no swelling, no membrane reaction. 3–4. Type B sperms: swelling of tip of tail. 5–6. Type C sperms: swelling of mid piece or different type of hair pin like swelling pattern. 7–8. Type D sperms: complete tail swelling.

bulls. Kumar and Singh (2007) also observed significant correlation between HOST reactive spermatozoa with progressively motile spermatozoa (r , 0.86, $P < 0.01$). Similarly, Lodhi *et al.* (2008) reported highly significant correlation between HOS test score and progressive motility (%) in Nili-Ravi buffalo and Sahiwal cow bull semen. This test depends up on the positive correlation between coiling/bending of the flagellum/tail in response to lowered osmotic pressure and high fertilizing ability of the semen (Van der Ven *et al.* 1986). In hypo-osmotic solution, the response of individual sperm varied and different types of coiling patterns were reported by Prasad *et al.* (1999), Arangasamy and Singh (2002) and Mandal *et al.* (2006). In the present study, sperms with no swelling / curling were considered as non-reacted sperms and shown in Figs 1–2. Whereas, sperms with various types of curling patterns were considered as reacted sperms and shown in Figs 3–8. In almost all samples examined in the present study, some of the HOS reacted spermatozoa showed non-progressive motility. This is in accordance with Revell and Mrode (1994), Pant *et al.* (2002) and Chaudhari *et al.* (2007, 2009).

SUMMARY

The relationship between hypo-osmotic swelling test with post-thaw motility in crossbred bulls was studied. The samples with 50% (110) and 60% (43) PTM were examined for HOST. Sperms with various types of curling patterns were considered as reacted and sperms with no swelling/curling were considered as non-reacted. With higher motility the HOS reacted sperms were increased in count. Positive correlation between the % of post thaw motility of spermatozoa and the spermatozoa that reacted to the hypo-osmotic swelling test was observed. In addition to post-thaw motility, hypo-osmotic swelling test can be used as a regular

base test to judge the best quality cryo-preserved semen for artificial insemination programme. Further studies are required to find out the relationship of these two tests with the field fertility.

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