MOTILITY OF FROZEN THAWED SEMEN AFTER THERMAL RESISTANCE TEST

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ABSTRACT

The data used in this study is an outcome of visit of Central Monitoring Unit (CMU) for evaluation of Semen Station of Kerala and Chitely Dairy, Maharashtra. To assess the post-thaw motility of frozen semen, frozen straws were taken out randomly from the stored semen and were thawed at 37° C for 15 seconds and motility was recorded. The same samples were subjected to thermal incubation test in water bath at 37° C and progressive motility (%) was recorded at 30 and 60 minutesafter incubation. It was observed that the mean post-thaw motility in exotic breeds (HF and Jersey) was 52.40 per cent followed by 50.85 per cent in crossbreds (HF,Jersey, and other crosses).The per cent mean value observed in Indigenous breeds (Tharparkar and Gir) was 45.0 per cent and the difference in post thaw motility was non significant ($p<0.05$) among breed groups. The overall mean post-thaw motility was 51.02 per cent.Further these thawed samples were incubated at 37° C for 60 minutesand there was consistent reduction in the motility at 30 and 60 minutes. This reduction varied from 6.29 per cent(pure exotic) to 11.25 per cent(indigenous) semen after 30 minutes of incubation and the differences were significant ($p<0.05$) among breed groups. However, at 60 minutes breed difference in the per cent drop of motility on incubation was non-significant. It was observed that there was no significant difference in the post-thaw motility and reduction in motility after incubation of frozen semen among the evaluated semen stations. Among the semen production stations, the average post- thaw motility of frozen semen varied from 52.70 to 49.70 per cent and motility after 60 minutes of incubation varied from 36.62 to 40.63 per cent, respectively. The results indicated that the reported semen stations are following the Minimum Standard Protocol (MSP) for freezing and thawing and maintaining their semen quality as per the standard guidelines of MSP.

Key words: Percent motility, Frozen thawed semen, Crossbred bull, Incubation test

INTRODUCTION

Post-thaw sperm motility is the most commonly used parameter for evaluation of frozen thawed semen. There is a considerable variation among breeds and individual bulls in retaining fertilizing capacity after freezing and thawing. This capacity is further assessed by subjecting spermatozoa to incubation test, which can be conducted easily in field as well as farm conditions. The data used in the present study is an outcome of visit of members of Central Monitoring Unit for evaluation of Semen Station of Kerala and Chitely Dairy, Maharashtra, to find out any variation in frozen semen quality among the breeds, breed combinations and location of frozen semen production centres etc.

MATERIALS AND METHODS

The data used in this study are the recording of observations of Central Monitoring Unit team of Govt. of India for evaluation of Semen Stations at Kerala and Chitley Dairy, Maharashtra. To record the post-thaw

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motility, the frozen semen doses were taken out from the semen storage containers at random and were thawed and subjected to incubation test. The frozen semen straws were from four categories of breeding bulls, i.e., pure exotic (HF, Jersey), crossbreds bulls (HF crosses,Jersey crosses), Indigenous (Tharparkar, Gir) and Murrahbuffalo bulls.

Thawing was done at 37°Cfor 30 seconds in a water bath and thawed samples were incubated at 37°Cfor 60 minutes. Per cent progressive motility of thawed semen was observed under phase contrast microscope with thermal stage immediately after thawing and then at every 30 minutes interval.The obtained data were grouped into 4 categories, viz., pure exotic (HF, Jersey), crossbreds bulls (HF crosses, Jersey crosses, others), Indigenous (Tharparkar,Gir) and Murrah buffalo bulls. Data were subjected to statistical analysis by performing ANOVA. The homogeneity among means was tested by Duncan's Multiple Range Test(Snedecor and Cochran 1994). Depending upon exotic components, the crossbred bulls were further categorized into 3 groups, viz.,HF crossbred, Jersey Crossbred and Sunandini crossbred and data were analyzed separately.

RESULTS AND DISCUSSION

The overall mean post-thaw percent motility just after thawing (0 h) was (52.02±0.57). In pure exotic bulls, the mean post-thaw per cent motility was 52.42.In crossbred bulls it was 50.85 and the value in Indigenous breeds (Tharparkar,Gir) was 45.00, which was slightly lower than the other breeds, although there was no significant difference in the post-thaw motility in all the four categories of semen in this study. Present values of post-thaw motility were similar to those reported in Frieswal (HF crossbred) bulls (Mandal *et al.* 2013); however, higher values in crossbred Jersey and HF bulls have been reported by Nagendrakumar and Kathiresan (2011).Like present findings, similar values of post-thaw motility in Murrah buffalo bulls' semen have been reported by Tiwari *et al.* (2011).There existed significant differences in sperm concentration, initial motility and post-thaw motility of spermatozoa among different breeds, species and bulls of crossbred categories having different breeds' combinations and their levels of exotic inheritance (Singh *et al.*,2012). The per centinitial and post-thaw motility differed significantly among various crossbred categories and their ranking order was observed as HF X H > J X H > BS X H.

On subjecting to incubation test, samples showed a consistent reduction in the motility on incubation at 37°C after 30 and 60 minutes. The reduction in per cent motility after 30 minutes of incubation varied significantly ($P<0.05$) from 6.29 in pure exotic animals to 11.25 indigenous bulls. After 60 minutes of incubation, the decline in per cent motility ranged from 11.94 to 16.25; however,the difference was non-significant ($P>0.05$) among the different breeds used in the present study. Maintaining frozen thawed sperm at 37°C for 1-2 hours after thawing partially mimics the exposure of sperm to female reproductive tract just after insemination (Kastelic, 2013). Thus sustenance of sperm motility on incubation test for an hour is an indirect assessment of bull fertility and present study showed 12 to 16 per cent reduction in motility, which was quite acceptable.

When the data were analyzed for different semen stations, it was observed that the overall per cent post-thaw motility ranged from 52.70 to 49.50. However, the values were slightly higher at Mattupatty and ChitaleSemen Stations. The reduction in post-thaw motility was slightly more in Kulathpuza and minimum in Chitale after 30 and 60 minutes of post-thaw incubation. Retention of sperm motility with extending lifespan of spermatozoa has been a major area of interest to circumvent the infertility problems in domestic animals.

The post-thaw motility data were also analyzed considering the type of crossbred used for semen freezing. The mean per cent motility varied from 50.28 to 50.79 at 0h, 42.5 to 43.16 after 30 minutes and 38.06 to 41.88 after 60 minutes of incubationin HFcrossbreds, Jersey crossbreds and Sunandini bulls, respectively. Although the sperm per cent motility declined almost linearly over the time of incubation, however, there was no significant difference among

the crossbred categories in motility at 0h (at thawing), 30 minutes and 60 minutes after incubation at 37°C. Shortened longevity of spermatozoa might play an important role on failed fertilization particularly when delivery of sperm in female reproductive tract is accomplished well before ovulation. Ability of sperm to maintain motility in *in-vitro* conditions simulating the uterine environment might give better indication than the present test; nonetheless, present test is relatively simple and inexpensive assay for quality assessment of frozen semen. The results obtained in the present study indicated that all the observed bull stations are performing well and maintaining the optimum semen quality in frozen samples.

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