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Efficiency of thawing cum controller unit against normal water bath for determining the cattle sperm quality parameter test

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Abstract
Artificial insemination (AI) is the first generation reproductive biotechnology that has made a biggest contribution to the genetic improvement, particularly in dairy cattle. Frozen semen in straws has become the universally accepted unit of storage and transfer of bovine genetics to cattle procedures which depends on preserve the functional activity of spermatozoa (viability and fertilizing ability) [1]. High viability and motility of spermatozoa are important factors for successful artificial insemination (AI) because a significant correlation between post-thawing sperm viability and subsequent conception rate has been reported [2]. Many techniques are there in India to thaw frozen semen straws before insemination which not only affecting the motility of sperm it also affect the sperm morphology ex Acrosome intactness. For this a study was conducted in which 15 bulls and buffaloes frozen semen straws are thawed and tested in different commercially available portable thawing kit in compare to normal water bath and results are found to be satisfactory. There was non-significant difference in between sperm quality parameters thawed in all the three thawing unit and thawing cum controller can be used in the farms where the artificial inseminations processes are carried out.

Keywords: Artificial insemination, thawing cum controller unit, Normal water bath, Post Thaw motility, Percent intact Acrosome, HOS test.

1. Introduction
Artificial insemination is a reproduction technique used to introduce semen into a female vagina, cervix, uterus or fallopian tubes without sexual intercourse. This method is predominantly used in animal breeding. AI is cheaper and closer to natural reproduction compared with other methods of assisted reproduction, including in vitro fertilization. AI helps to pass on desirable characteristics of a male more quickly [3]. As AI plays such a critical role in the reproduction and genetic improvement of farm animals, it is essential that studies continue to improve methods commonly used. There has been significant research conducted on temperature variances and their effects on thawed semen. These studies have concluded that temperature variances can cause significant mortality rates of spermatozoa. Thawed semen may be exposed to temperature variances in transfer from the thaw bath to the female, thus alternative procedures to reduce this variation could increase the conception rates in cattle[4]. Thawing of frozen semen brings back the frozen spermatozoa to life and to the physiologically active state. Therefore, it is essential to carry out thawing carefully at an optimal temperature for sufficient time to minimize the loss of semen quality during thawing procedure. Worldwide, different workers conducted trials to determine the optimal thawing temperature and duration, and to know the adequate thawing rate that may result in the highest percentage of viable spermatozoa in different species after thawing process [5, 6]. Evaluation of the quality of frozen semen has been based on a variety of methods, including routine semen analysis (motility, morphology and Acrosome integrity) [7], zona free hamster ova test and hypo-osmotic swelling test [8]. The plasma membrane is of crucial importance to freeze-thaw survival of spermatozoa and regarded as the primary site of freezing injury [9]. Hypo-osmotic swelling test (HOST) has recently been shown to be useful in detecting subtle changes in the sperm membranes of rams, boars, stallions, dogs and bulls [10-13].

As the post thaw motility is one of the most important processes in artificial insemination so the aim of the present study was to the comparative effect of different thawing unit on Motility HOS test and Percent Intact Acrosome of frozen cattle and buffalo semen.

2. Materials methods

Frozen semen from 15 different bulls breed and buffalo breed were used. Cattle semen Packaged in 0.25 ml French straws were provided by the laboratory of the Department Quality Control of Frozen Semen assessment lab BAIF Development and Research Foundation (Uruli Kanchan Pune). Only semen with motility and concentration values higher than 70 % and 600 x 106/ml were used in this study. Semen was extended with egg yolk extender at a rate to obtain 20 x106 spermatozoa/straw. Extended semen was cooled to 5 °C in 45 minutes, and after glycerolisation it was subjected to equilibration for2 hours. Semen in straws was frozen at -110 °C for 7 minutes and stored in liquid nitrogen. Semen of 15 different breed bulls and buffaloes of was used in this study.

2.1 Thawing procedures

The straws were thawed by placing them into different portable thawing units (Mayuresh Thawing cum controller unit Horizontal and Vertical, Mayuresh Industries Kolhapur Maharashtra) and conventional water bath for 30 seconds at 37 degree Celsius. Four mini frozen semen straws from each bull and buffaloes were submitted to different thawing procedures in three different thawing units.

2.2 Assessment of frozen-thawed semen

Post thaw motility: Post-thaw sperm motility was assessed subjectively using a phase- contrast microscope (x 400) with a warm slide (38 °C).

Sperm membrane integrity: Sperm membrane integrity was assessed with minor modifications [15]. The semen was submitted to HOS test. Add 0.1 ml of the specimen containing the spermatozoa with 1 ml of hypo-osmotic solution sodium chloride (NaCl). The solution is then incubated at 37°c for one hour. Similarly 0.1 ml of semen is incubated in 1.0 ml in Distilled water for one hour. A single drop from both solutions is placed on the clean glass slide and covered with a cover glass and observed under phase-contrast microscope at x 400 magnifications. A total of 200 sperm are counted in at least 5 different fields and reactive and nonreactive sperms are counted (Both in Distilled water and NaCl).

Sperm morphology: **Percent Intact Acrosome by Staining Technique (Giemsa method)**

Equal drops of 0.2% trypan blue and frozen semen are mixed with tip and smeared. A thin smear of semen is made on a clean grease free slide. After drying, the slide is immersed in ethanol absolute (No more than 1% of water) for 10 minutes at 37 °c and fixed. Than the slide is immersed in freshly prepared Giemsa stain overnight and then washed in running tap water and dried. From stock Giemsa stain solution, 3ml is taken and mixed with 2ml of Sorenson's phosphate buffer (ph-7.0) and 45ml-distilled water. Fresh stain should be prepared every time for staining the smear. Examine the smear under the oil immersion objective of the microscope and count total of 100 sperms recording number of sperms with intact, altered and completely lost acrosome. Calculate the % of acrosome alteration in the form of loose, detached, ruffled, knobbed, vacuolated acrosome.

2.3 Statistical analysis

The mean semen parameters obtained from 15 bulls and 15 buffaloes were subjected to an analysis of variance (ANOVA, SPSS 10.0) by General Linear Model procedure using the factor bull and the factor group (thawing protocol).

3. Results and discussion

The comparative study of Mayuresh Thawing kit (vertical and horizontal) with conventional water bath was conducted in Central Research Station BAIF Development and Research Foundation Uruli Kanchan. All the main Quality control parameter test are performed like Post thaw motility test, Percent Intact Acrosome test and HOS test for both the species of 15 (Cattle and Buffaloes) are done. Results of the average and the standard error of the for the entire test for Cattle (Post thaw motility, Acrosome test and HOS test of Cattle in Horizontal thawing kit, conventional water bath and vertical thawing kit) and buffalo (Post thaw motility of Cattle, Acrosome test and HOS test in Horizontal thawing kit, conventional water bath and vertical thawing kit) are tabulated above. Sperm quality parameters like post thaw motility, percent intact acrosome and HOS test, total morphological defects spermatozoa are given in Table 1 and 2 shows the values quality parameters value for each thawing unit (Mayuresh Thawing cum controller unit Horizontal and Vertical) and conventional water bath for 30 seconds at 37 degree Celsius) for each bull and buffalo semen sample.

The Mean ± SEM of post thaw motility of cattle semen and buffalo semen thawed in each thawing unit (Mayuresh Thawing cum controller unit Horizontal and Vertical) and normal water bath is estimated to be (53.6±1.58, 54.0±1.77, 54.0±1.11). Percent intact acrosome of Mean ± SEM value of cattle and buffalo semen thawed in each thawing unit (Mayuresh Thawing cum controller unit Horizontal and Vertical) and conventional water bath are (53.6±1.58, 54.0±1.77, 54.0±1.11). Like this the Mean ± SEM of HOS reacted sperm percentage of cattle semen thawed in each thawing unit (Mayuresh Thawing cum controller unit Horizontal and Vertical) and conventional water bath are (53.6±1.58, 54.0±1.77, 54.0±1.11) respectively.All the semen parameters results were statistically nonsignificant in all the three thawing units among bulls. (Table 3) shows the significant values for motile spermatozoa in all the three thawing units P =0.590) Percent intact acrosome (P =0.207), other HOST reacted sperm percentage (P = 0.401). Thawing procedures which plays the most significant role in artificial insemination but in villages peoples doesn't take it seriously so they use to thaw frozen semen in inappropriate manner like in pockets, under arms or in rubbing in palms etc all these are not a good process to thaw the frozen semen straws as the thawing doesn't done properly by all this manners. As thawing of frozen semen should be properly thawed for a good result for artificial insemination process so it affects the percentage of conceiving rate.

And conception rates can also be vary based on semen quality, temperatures and other environmental conditions. This study evaluated the efficiency of convenient water bath with (Mayuresh thawing cum controller horizontal and vertical unit) and all the three thawing unit are having more or less the same value for the entire three sperm quality parameter test.

Table 1: Effect of Different Thawing Units on Various Post-Thaw Sperm Quality Parameters of Cattle Semen

Sperm Quality of Parameters	Thawing units		
	Normal water bath	Vertical thawing unit	Horizontal thawing unit
Post Thaw motility	54.0 ± 1.77	54.0 ± 1.11	53.6 ± 1.58
Percent Intact Acrosome	71.4 ± 0.89	70.1 ± 0.97	70.8 ± 1.01
HOST reacted sperm percentage	63.4 ± 1.44	64.3 ± 1.19	64.4 ± 1.45

Table 2: Effect of Different Thawing Methods on Various Post-Thaw Sperm Quality Parameters of Buffalo Semen

Sperm Quality of Parameters	Thawing units		
	Normal water bath	Vertical thawing unit	Horizontal thawing unit
Post Thaw motility	56.3 ± 0.59	57.3 ± 0.67	55.2 ± 0.81
Percent Intact Acrosome	70.9 ± 0.86	69.8 ± 0.78	72.0 ± 0.85
HOST reacted sperm percentage	66.8 ± 1.51	63.1 ± 1.08	66.2 ± 0.94

Table 3: Interpretation of results of the three thawing units in compare with (P<0.05) significant value

Sperm quality parameters	No. samples observed (N)	Significant value (P<0.05) for all the three thawing unit
Post thaw motility	30	0.590
Percent Intact Acrosome	30	0.207
HOST reactive percentage	30	0.401

4. Conclusion

Thawing procedure is just as important as the freezing procedure in terms of its impact on the survival of spermatozoa [15]. Inseminators use to carry frozen-thawed semen straw either in pocket which was not a good practice and it's also effect the motility and sperm abnormalities. After performing the Quality control tests by both the Mayuresh Thawing-Cum-Controller units both Horizontal and Vertical in comparison to normal water bath the results are found to be satisfactory. And Mayuresh Thawing-Cum-Controller units both Horizontal and Vertical can be used in Artificial Insemination for thawing purpose in fields.

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