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## Effect of Different Freezing Rates and Thawing Methods on the Quality of Frozen Boar Semen

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**Abstract** A total of 30 ejaculates from three Hampshire crossbred boars were used to study the effect of freezing rates and thawing methods on quality of frozen boar semen. Semen was frozen using three freezing rates-30<sup>0</sup>c/min, 50<sup>0</sup>c/min and 70<sup>0</sup>c/min in a programmable biological semen freezer. The semen characteristics viz., sperm motility, live sperm, HOST reacted spermatozoa and intact acrosome were studied after thawing using three different thawing methods viz., 37<sup>0</sup>c for 30 sec, 50<sup>0</sup>c for 12 sec and 70<sup>0</sup>c for 10 sec. Out of three different freezing rates and thawing methods freezing rate 30<sup>0</sup>c/min with thawing method 70<sup>0</sup>c for 10 sec resulted better semen quality.

**Keywords** *Boar Semen; Freezing Rate; Thawing Method*

### 1. Introduction

Pig farming has been occupying a vital position in uplifting the rural economy by providing self-employment and supplementary income. The scarcity of superior boar and high cost for rearing them by the small and marginal farmers necessitated the use of artificial insemination (AI). At present liquid semen has been used in AI of pig. Since the first successful AI with frozen boar semen in 1971 (Crabo and Einarsson), several studies have been made (Bwanga, 1991; Wagner and Thibier, 2000; Baishya, 2013) on freezing and thawing procedure of boar spermatozoa, but the use of frozen thawed semen has remain very low. Preservation of semen for longer period in-vitro without lowering the inherent fertilizing ability of sperm is essential for success of AI and the current status of boar semen cryopreservation is still considered poor to fair. Frozen thawed boar sperm quality is influenced by many factors such as freezing and thawing protocols, composition of diluents used in the processes, susceptibility to cryoinjury of the spermatozoa etc (Johnson, et al., 2000). The present experiment was carried out to study the effect of different freezing rate and thawing methods on the quality of frozen boar semen.

## 2. Materials and Methods

A total of 30 ejaculates were collected by simple fist method, once in a week, from three Hampshire cross bred boars maintained at AICRP on pig, Assam Agricultural University, Khanapara, Guwahati, Assam in a thermo flask. Gel free semen sample was brought to the laboratory in the thermo flask at 35°C and evaluated for its initial quality. The suitable semen sample was kept in BOD incubator at 24°C in a conical flask for 3 hours. After 3 hours of holding time sample was centrifuged at 1500 rpm for 10 minutes. The supernatant fluid was discarded and the centrifugate was extended using Lactose egg yolk glycerol extender (Park and Pursel, 1985). The partially extended semen was cooled gradually at 5°C at the rate of 1°C per 3 minutes and finally extended at 5°C with glycerolated extender (1: 1.5). The extended semen was then equilibrated for 1 hour. French medium straw (0.5ml) were used for filling the extended semen. Freezing was done with programmable freezing machine (IMV Technologies, XRP 60-S, Cryo Diffusion 49, Rue de Verdun, 27690 Lery, France) using three different freezing rates viz., Freezing rate- I (30°C/min), Freezing rate- II (50°C/min) and Freezing rate- III (70°C/min). After 16 hours of storage in liquid nitrogen, the frozen semen was thawed in warm water using three different thawing methods viz., Thawing method- I (37°C for 30 sec), Thawing method- II (50°C for 12 sec) Thawing method-III (70°C for 10 sec). The semen sample was evaluated for sperm motility, live sperm count, HOST reacted sperm and intact acrosome after thawing.

## 3. Results and Discussion

The result of different semen characteristics observed in the present study revealed that the better semen quality was observed in the freezing rate of 30°C/min with the thawing method of 70°C for 10 sec (Table 1). Statistical analysis revealed that the mean sperm motility, live sperm count and intact acrosome did not differ significantly between freezing rates, between thawing methods and due to freezing x thawing interaction while the mean HOST reacted spermatozoa differed

**Table 1:** Percentage (Mean± SE)\*Of Sperm Motility, Live Sperm, HOST Reacted Sperm and Intact Acrosome in Boar Semen after Freezing in Lactose Egg Yolk Glycerol Extender with Different Freezing Rates and Thawing Methods

	Motility (%)			Live Sperm (%)			HOST Reacted Sperm (%)			Intact acrosome (%)		
	TM-I	TM-II	TH-III	TM-I	TM-II	TH-III	TM-I	TM-II	TH-III	TM-I	TM-II	TH-III
FR-I	45.30 ±0.88	44.40 ±1.92	47.20 ±2.51	52.30 ±1.47	51.30 ±1.58	54.80 ±1.81	50.00 ±2.01	47.90 ±1.97	52.40 ±1.42	54.90 ±1.08	53.20 ±1.24	51.20 ±1.64
FR-II	42.30 ±2.12	44.00 ±1.99	43.40 ±2.09	53.60 ±1.50	53.30 ±0.72	52.00 ±1.11	49.40 ±1.75	46.90 ±1.97	51.10 ±0.91	52.10 ±1.57	54.00 ±1.16	51.80 ±1.91
FR-III	43.30 ±2.56	44.30 ±2.39	47.10 ±2.88	53.30 ±1.47	52.40 ±1.63	56.50 ±0.72	50.10 ±1.03	45.90 ±1.18	47.20 ±2.51	50.90 ±1.36	51.70 ±1.20	53.80 ±0.94

\*Nos of observation=10, FR means Freezing Rate, TM means Thawing Method

significantly ( $P < 0.01$ ) between thawing methods but did not differ significantly between freezing rates and due to freezing x thawing interaction. The significantly ( $P < 0.01$ ) higher post thaw HOST reacted spermatozoa observed in freezing rate-I + thawing method –III in the present study was lower than the values reported by Eriksson and Martinez (2000) and Hernandez et al. (2007). The different semen qualities recorded in the present study did not differ significantly between freezing rates. It might be due to narrow range of freezing rate to demonstrate any significant effect on sperm survival which was in agreement with that of Eriksson and Martinez (2000). Result obtained in the present study led to the conclusion that out of the three freezing rates freezing rate of 30°C/min was superior as apparently higher sperm quality was obtained. It was reported that the optimum freezing rate using 0.5 ml straws was 30°C/min with 3% glycerol (Fiser and Fairfull, 1990) and for 0.25 ml straws was 50°C/min with 1.5% glycerol (Woelders and Den Besten, 1993). Watson (1995) reported that boar spermatozoa tolerated a range of freezing rate and Martinez and Wallgren (2011) reported that the

optimum freezing rate for boar spermatozoa was in the range of 30 - 50<sup>0</sup>c/min. The post thaw sperm motility, live sperm, HOST reacted spermatozoa increased along with the increase in thawing rate from 37<sup>0</sup>c for 30 sec to 70<sup>0</sup>c for 10 sec. This was in agreement with that of Fisher et al. (1993) and Hernandez et al. (2007). Hernandez et al. (2007) reported that fast thawing method of 70<sup>0</sup>c for 8 sec (1800<sup>0</sup>c/min) improved post thaw sperm quality than the thawing method 37<sup>0</sup>c for 20 sec (1200<sup>0</sup>c/min). However both temperature and timing for fast thawing method (70<sup>0</sup>c for 10 sec) is very crucial to be applicable to commercial situation, since slightly longer period in the water bath at 70<sup>0</sup>c would raise the temperature inside the straw to non-physiological limit causing irreversible damage to sperm.

#### 4. Conclusion

It can be concluded from the present study that freezing rate 30<sup>0</sup>c/min was found to be better than the freezing rates 50<sup>0</sup>c/min and 70<sup>0</sup>c/min. The thawing method 70<sup>0</sup>c for 10 sec was found to be better than the thawing methods 50<sup>0</sup>c for 12 sec and 37<sup>0</sup>c for 30 sec.

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