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Effect of certain additives on the quality of boar semen during preservation at 15°C and 5°C

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Abstract

The study was aimed to evaluate the effect of four additives viz. $KMnO_4$, Vitamin E, Butylatedhydroxytoluene and trehalose on quality of boar semen during preservation at 15°C and 5°C up to 96 hours. 24 ejaculates were utilized for the study. Sperm motility was significantly higher with BHT irrespective of preservation temperature and period. However, the mean sperm motility was significantly higher ($P<0.05$) in semen preserved at 15°C than 5°C. Live sperm was significantly ($P<0.05$) lower in Trehalose than $KMNO_4$, Vitamin E and BHT. The mean percentage of live intact acrosome irrespective of temperature and preservation period was significantly higher ($P<0.05$) with BHT than others additives. Semen in Modena extender with BHT had significantly higher ($P<0.05$) percentage of HOST-reacted sperm at different preservation periods irrespective of different temperature. In conclusion, Butylatedhydroxyltoluene (BHT) was found to be superior to $KMNO_4$, Vitamin E and Trehalose for preservation of Hampshire boar semen.

Keywords: Hampshire, semen, additives, preservation, quality

Pig is considered as the most important livestock in North East Region, India, contributes 28% of total country's pig population (Khan *et al.* 2011). Inferior local pigs necessitate the up-gradation with superior germ plasm. Hence, in order to enhance the production potentiality of indigenous pig population of this region, crossbreeding is the only tool with elite exotic breeds through Artificial Insemination (AI). The main idea behind semen preservation is to extend the usefulness of superior germ plasm for the purpose of maximizing number of doses of semen obtainable from a given ejaculate, without

reducing fertility and by extending the fertile life of the doses to facilitate their effectiveness use in breeding (Purdy *et al.* 2008). Several commercial semen extenders have been introduced with the objectives of reducing the metabolic activity during preservation which can be obtained by semen dilution into an appropriate medium and by lowering the temperature (Gadea, 2003). Swine semen cooled at 5°C would be a cheaper alternative to keep with increasing the use of AI. Another benefit of preservation at 5°C is that bacterial growth is reduced, which would improve the quality of semen (Althous and Lu, 2005). However, Reactive oxygen species (ROS) is responsible for sperm dysfunction due to lipid peroxidation of membrane during preservation. To check the level of ROS and promote sperm survival and motility during preservation several additives have been used successfully in supplementation with extender (Roca *et al.* 2005). Keeping in view the facts cited above the study has been planned.

Materials and Methods

Semen samples were collected from four Hampshire boars by simple fist technique. A total of 24 ejaculates were used to evaluate the effect four additives viz. $KMnO_4$, Vitamin E, Butylatedhydroxyltoluene (BTS) and Trehalose in Modena extender on quality of semen during preservation at 15°C and 5°C up to 96 hours. For preservation, each ejaculate was extended (1:4) with Modena (Weitze, 1991) extender. The extended semen was split into four parts, each part was added with $KMnO_4$ (6.25µM), Vitamin-E (100µM), Butylatedhydroxyltoluene (100µM) and trehalose (2mM) and preserved into 2 mlof micro centrifuge tube at 15°C and 5°C in a BOD incubator up to 96 hours. Prior to evaluation semen was thawed at 37°C for two minutes and gently shaken for homogenization. The evaluation of sperm motility, live sperm, live intact acrosome and HOST reacted sperm at 0 hour (immediately after extension), 24, 48, 72 and 96 hours of preservation.

The data was analyzed statistically by using software SPSS version 17.0.

Results and Discussion

In the present study different additives were utilized to study their effect on quality of boar semen during preservation. The results obtained are presented in Table1.

Sperm motility

The highest mean percentage of sperm motility was 87.92±0.79, 76.73±2.90, 67.08±2.39, 57.92±2.96 and 52.00±3.88 at 0, 24, 48, 72 and 96 hours of preservation

Table 1: Mean sperm motility of Modena extended Hampshire boar semen containing different additives at 15°C and 5°C

Preservation period	Sperm motility							
	15°C				5°C			
	$KMNO_4$	Vita-E	BHT	Trehalose	$KMNO_4$	Vita-E	BHT	Trehalose
0 h	87.33±0.78	87.92±0.79	87.92±0.73	86.67±1.23	87.33±0.78	87.92±0.79	87.92±0.73	86.67±1.23
24 h	76.67±2.44	72.50±1.90	76.73±2.90	66.25±2.76	55.17±2.88	64.58±3.17	71.67±2.27	54.58±3.82
48 h	67.08±2.39	60.42±2.21	66.25±3.77	45.83±3.45	52.33±2.23	51.25±2.47	59.33±3.25	35.83±3.48
72 h	55.00±3.59	52.91±2.59	57.92±2.96	40.00±3.97	48.25±2.97	46.25±2.83	56.25±3.29	25.42±2.57
96 h	52.00±3.88	45.42±4.03	51.17±4.98	26.25±4.40	42.50±3.76	39.17±3.44	42.08±3.05	20.00±2.57

Table 2: Mean live sperm of Modena extended Hampshire boar semen containing different additives at 15°C and 5°C

Preservation period	Live sperm							
	15°C				5°C			
	$KMNO_4$	Vita-E	BHT	Trehalose	$KMNO_4$	Vita-E	BHT	Trehalose
0 h	84.33±0.80	75.79±1.68	81.29±1.89	77.96±2.00	84..33±0.80	75.79±1.68	81.29±1.89	77.96±2.00
24 h	68.08±3.85	73.47±3.37	76.68±1.85	71.01±3.62	66.30±3.00	65.44±2.59	73.42±1.90	60.63±3.76
48 h	65.50±2.88	68.23±3.78	73.17±2.39	63.00±3.22	62.11±2.72	61.24±2.84	65.43±3.51	55.29±4.08
72 h	64.02±2.71	67.01±2.68	70.17±2.41	61.08±2.98	61.13±3.13	57.83±3.27	60.04±3.19	53.16±3.32
96 h	62.15±4.70	57.98±4.25	65.33±4.36	54.58±4.02	56.18±3.29	55.50±4.33	59.68±4.36	51.83±4.45

at 15°C with Vitamin E, BHT, $KMNO_4$, BHT and $KMNO_4$ respectively (Table 1). The corresponding values in semen preserved at 5°C were 87.92±0.79, 71.67±2.27, 59.33±3.25, 56.25±3.29 and 42.50±3.76 with Vitamin E, BHT, BHT, BHT and $KMNO_4$ respectively.

Analysis of variance revealed that sperm motility differed significantly ($P<0.01$) among additives, between preservation temperatures and among preservation periods. Results of critical difference test indicated that overall percentage of sperm motility was significantly higher with BHT followed by $KMNO_4$, Vitamin E and Trehalose irrespective of preservation temperature and period. However, the mean sperm motility was significantly higher ($P<0.05$) in semen preserved at 15°C than 5°C. There was no available literature on using $KMNO_4$ as an additive for preservation of semen but beneficial effect of very low concentration of K^+on $KMNO_4$ in cell biology had been confirmed by many workers (Abuladze *et al.* 2009).

In the present study, sperm motility in Modena extender was comparable to the reports of early workers (Funahashi and Sano, 2005; Kadirvel *et al.* 2005). However, the present findings were higher than the reports of Khan et al. (2006).

Live sperm

The overall mean percentage of live sperm was significantly ($P<0.05$) lower in Trehalose than $KMNO_4$, Vitamin E and BHT, but no significant difference was observed among the later three additives (Table 2). The mean live sperm was significantly higher ($P<0.05$) in semen preserved at 15°C than 5°C. The mean percentage of live sperm in Modena extender in the current study was higher than the reports of others (Khan *et al.* 2006; Lalrintluanga *et al.* 2009)

Live intact acrosome

The highest percentage of live intact acrosome at 0, 24, 48, 72 and 96 hours of preservation at 15°C was 83.17±0.68, 71.22±2.60, 65.00±2.40, 62.71±2.61 and 52.46±3.95 with $KMNO_4$, BHT, BHT, BHT and BHT respectively (Table-3). The corresponding values at 5°C were 83.17±0.68, 66.83±2.40, 55.48±3.33, 53.61±3.58 and 49.58±4.24 with $KMNO_4$, BHT, BHT, BHT and BHT respectively.The overall mean percentage of live intact acrosome irrespective of temperature and preservation period was significantly higher ($P<0.05$) with BHT than others. The mean live intact acrosome in this study was higher than the report of Boonsorn *et al.* (2010).

Table 3: Mean live intact sperm of Modena extended Hampshire boar semen containing different additives at 15°C and 5°C

Preservation period	Live intact sperm							
	15°C				5°C			
	$KMNO_4$	Vita-E	BHT	Trehalose	$KMNO_4$	Vita-E	BHT	Trehalose
0 h	83.17±0.68	76.13±1.83	78.13±1.89	76.46±1.91	83.17±0.48	75.13±1.73	76.13±1.49	75.46±1.81
24 h	68.55±3.53	63.47±4.16	71.22±2.60	61.58±3.57	60.51±3.30	55.90±2.58	66.83±2.40	51.25±3.79
48 h	60.08±3.15	59.08±3.52	65.00±2.40	59.56±3.41	51.41±3.51	49.46±3.20	55.48±3.33	43.13±3.90
72 h	53.73±3.36	53.43±3.12	62.71±2.61	46.33±3.21	49.63±3.02	47.88±3.39	53.61±3.58	39.49±3.32
96 h	45.67±4.74	43.25±4.14	52.46±3.95	38.58±4.21	43.17±3.29	42.89±3.10	49.58±4.24	37.83±3.94

Table 4: Mean HOST-reacted sperm of Modena extended Hampshire boar semen containing different additives at 15°C and 5°C

Preservation period	HOST- reacted sperm							
	15°C				5°C			
	$KMNO_4$	Vita-E	BHT	Trehalose	$KMNO_4$	Vita-E	BHT	Trehalose
0 h	60.50±2.44	61.63±2.21	68.42±2.59	57.92±2.34	59.55±2.44	60.63±2.11	66.42±1.59	56.92±2.74
24 h	53.75±2.21	52.38±2.91	58.92±3.05	49.63±2.94	46.54±2.03	47.54±2.64	56.46±3.03	46.42±2.25
48 h	51.42±3.05	49.92±2.46	55.54±2.50	48.75±2.38	43.42±2.57	46.00±2.26	51.75±2.83	44.83±2.13
72 h	45.58±3.39	47.00±2.55	47.88±2.86	43.63±2.80	41.50±2.98	45.33±2.52	46.75±3.21	42.33±3.75
96 h	41.42±2.79	42.50±2.70	46.25±2.71	41.92±2.64	36.71±2.59	40.58±3.26	42.67±3.31	40.38±3.24

HOST-reacted sperm

Semen in Modena extender with BHT had significantly higher ($P<0.05$) percentage of HOST-reacted sperm at different preservation periods irrespective of different temperature (Table 4). In the present study, the percentage of HOST-reacted sperm was in close agreement with the findings of Ziaullah *et al.* (2012). However, these findings were higher than that reported by others (Correa *et al.* 2006; Zou and Yang 2000).

Conclusion

The present study concluded that the Butylatedhydroxyltoluene (BHT) was found to be superior to $KMNO_4$, Vitamin E and Trehalose for preservation of Hampshire boar semen. This advanced technology could be used to maintain optimum fertility under field condition with superior germplasm.

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