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Assessment of Sperm Viability in Extended Boar Semen during Long Term Storage at 15˚C

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ABSTRACT

A total of 24 ejaculates comprising 6 ejaculates from each of four trained healthy crossbred Hampshire boars of one to two years of age were selected for the present study. Semen was collected by Simple fist method to study the effect of Modena extender on quality of semen during preservation at 15˚C up to 120 hours. The results revealed that the sperm motility, live intact acrosome, HOST-reacted spermatozoa, sperm membrane protein, sperm cholesterol level decreased significantly ($P<0.01$) with increase in hour of preservation. The highest conception rate (93.33%) was observed at immediately after extension and lowest conception (40%) was observed at 120 hours of preservation. In conclusion, the boar spermatozoa undergo several changes including diminished motility, viability and alterations in membrane permeability during long term storage at 15°C with Modena extender.

Keywords: Boar, semen, liquid preservation, Modena extender, sperm viability

In the swine industry, more than 99% of artificial inseminations (AIs) performed around the world today with extended liquid semen (Wagner and Thibier, 2000) whether on the same day or stored at 15°–20°C for 1–5 days before AI (Johnson *et al.,* 2000). Success of A.I. depends on preservation of semen for longer period of time without reducing the fertility. Boar sperm is more sensitive to storage at cool temperatures than the sperms of other domestic species. Fertility of boar spermatozoa decreases with increase in storage time during liquid preservation. During storage, the boar spermatozoa undergo several changes including diminished motility, viability and alterations in membrane permeability. The aim of this study was to assess the effect of time of storage and the dynamic changes in sperm cell characteristics in normospermic boar semen stored in long-term extender, in order to determine the susceptibility to damage of particular structures of spermatozoa during cooling and storage at 15 °C for 120 hours of post collection.

MATERIALS AND METHODS

A total of 24 ejaculates, six from each four trained healthy crossbred Hampshire boars (Hampshire × Indigenous) of one to two years of age maintained at ICAR-All India Coordinated Research Project (AICRP) on Pig, Assam Agricultural University, Guwahati, Assam, India were selected for the present study. The semen was collected twice weekly by Simple fist method (Tamuli, 1994). Immediately after collection, the semen samples were evaluated for volume, concentration and initial sperm motility. The ejaculates having initial sperm motility 70 per cent or more were used for the present study. The semen samples after primary evaluation were extended with Modena extender (Glucose 2.75 g, Sodium citrate 0.69 g, Sodium

bicarbonate 0.1 g, EDTA sodium salt 0.23 g, Tris- buffer 0.56 g, Citric acid 0.29 g, Triple glass distilled water up to 100 ml, Strepto- penicillin 180 mg/100 ml; at pH 6.9) at 1:3 ratio (Moretti, 1981). After that the sample was kept in a BOD incubator at 22˚C for four hours of holding. The preserved semen prior to evaluation was brought to 37˚C for two minutes and gently shaken in between palms for homogenization. The preserved semen sample on all occasions was evaluated for sperm motility (Blom, 1950), live intact acrosome (Watson, 1975), hypo-osmotic swollen test, HOST (Jeyendran *et al.,* 1984), sperm membrane protein (Srivastava *et al.*, 2013a) and total sperm cholesterol (Srivastava *et al.*, 2013b) at 0 hour (immediately after holding), 24, 48, 72, 96 and 120 hours of preservation at 15˚C.

The insemination was done at 2nd and 3rd day of observed oestrus with 50 ml of diluted semen (conc. 1000 × 10^6 sperm per dose) by using intrauterine insemination device (Deep golden pig™ catheter, IMV Technologies, L'Aigle, France) which was inserted through the vagina into the cervix. The inner tube extended 200 mm beyond the tip of the outer catheter lying in the uterine body or the posterior uterine horn. The sows not returning to estrus within 24 days after the first insemination, pregnancy was determined by ultrasongraphy (Aloka, SUPER EYE, SSD-500, Tokyo, Japan) at day 45 (AI = day 0). The statistical analysis of data was done using SAS Enterprise Guide 4.2 version.

RESULTS AND DISCUSSION

The mean percentage of motile spermatozoa at 0, 24, 48, 72, 96 and 120 hours of preservation were presented in the Table 1. It was recorded that the percentage of sperm motility decreased significantly (P<0.01) with increase in hour of preservation. This is in agreement with the observation of earlier workers (Estienne *et al.*, 2007; Kadirvel *et al.*, 2016). Significant decrease in the sperm motility with increase in hour of preservation could be due to progressive decline in nutrient content in extender with increased periods of preservation and the loss of adenosine triphosphate (ATP) and cyclic AMP, as well as calcium uptake are characteristics of decreased motility (Kadirvel *et al.*, 2016). It was also observed in the present study that the interactions between the extender and preservation periods in case of sperm motility was significant which were corroborated with the findings of the earlier workers (Lalrintluanga, 1994; Khan *et al.*, 2006; Tyngkan, 2009; Kadirvel *et al.*, 2016).

The incidences of live intact acrosome at 0, 24, 48, 72, 96 and 120 hours of preservation were presented in the Table 1. The mean live intact acrosome was significantly (P<0.01) decreased with the increase in hour of preservation in the Modena extender. This is in agreement with the observation of earlier workers (Lalrintluanga, 1994; Kommisrud *et al.*, 2002). Gradual increase in the proportion of acrosomal damage with increase in hour of preservation could be due to peroxidation effect (Pursel, 1979). It was observed that there was increase in phospholipids and cholesterol in the seminal plasma on storage and high concentrations of these plasmatic components (free radicals) caused destructive changes in sperm membrane (Dimitrov *et al.,* 2009).

The mean percentage of HOST-reacted spermatozoa at 0, 24, 48, 72, 96 and 120 hours of preservation were presented in the Table 1. In the present study, there was a significant reduction in the percentage of HOST reacted spermatozoa with advancement of storage days in the Modena extender which could be due to the fact that there was a decrease in membrane fluidity and ATP content during storage. Dilution and cooling render the boar sperm membrane more permeable (Ortman and Rodriguez-Martinez, 1994). Cold-induced reorganization of membrane particles, although partially reversible, could influence membrane function in a number of ways. These include an increase of permeability (leakage of cations and enzymes), a reduction in enzyme activity

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Table 1: Sperm motility (mean ± SE) in hampshire boars in modena extender at different hours of preservation periods at 15˚C

Observations	Preservation periods (hour)						Effect
	0	24	48	72	96	120	
Sperm motility (%)	85.42^{a} ± **0.85**	75.21^{b} ± **0.97**	70.83^{c} ± **1.03**	66.04^{d} ± **0.90**	60.21^{e} ± **0.93**	51.04^{f} ± **0.90**	P<0.01
Live intact acrosome (%)	95.32^{a} ± **0.28**	92.45^{b} ± **0.35**	89.14^{c} ± **0.43**	85.68^{d} ± **0.59**	80.83^{e} ± **0.77**	76.09^{f} ± **0.94**	P<0.01
HOST-reacted spermatozoa (%)	73.53^{a} ± **0.79**	69.39^{b} ± **0.78**	65.43^{c} ± **0.73**	60.06^{d} ± **0.72**	54.16^{e} ± **0.72**	46.71^{f} ± **0.65**	P<0.01
SMP(mg/10^{9} spermatozoa)	13.68^{a} ± **0.18**	10.11^{b} ± **0.16**	9.13^{c} ± **0.18**	7.88^{d} ± **0.14**	6.77^{e} ± **0.16**	6.06^{f} ± **0.21**	P<0.01
Cholesterol(mg/10^{8} spermatozoa)	31.84^{a} ± **0.23**	29.75^{b} ± **0.22**	27.08^{c} ± **0.16**	23.28^{d} ± **0.15**	19.13^{e} ± **0.22**	16.22^{f} ± **0.21**	P<0.01
Conception rate (%) n=15	93.33(14)	80.00 (12)	73.33 (11)	66.66 (10)	46.66 (7)	40.00 (6)	

Means bearing different superscripts in a row differed significantly (P<0.01)

and diffusion controlled membrane processes, and changes in lateral motion in channels (De Leeuw *et al.*, 1990).

The mean levels of sperm membrane protein (SMP) at 0, 24, 48, 72, 96 and 120 hours of preservation were presented in the Table 1. The sperm membrane protein levels decreased significantly (P< 0.01) while preservation period increased in Modena extender. The diminished sperm membrane proteins might be due to sub lethal damage which was occurred during preservation leading to loss of sperm surface proteins (Lessard *et al.,* 2000), segregation of membrane proteins (De Leeuw *et al.,* 1990), inactivation of membrane- bound enzymes and decreased lateral protein diffusion within the membrane (Watson, 1995).

The mean level of sperm cholesterol at 0, 24, 48, 72, 96 and 120 hours of preservation were presented in the Table1. In the present study, it was observed that the sperm cholesterol levels decreased significantly (P< 0.01) while preservation period increased in Modena. The present finding was in close agreement with the observation reported by Cerolini *et al.* (2001) and Maldjian *et al.* (2005). Membrane cholesterol has stabilizing effect on spermatozoa membrane; hence any change in its content is expected to induce reorganization or destabilization of membrane as reported by Srivastava *et al.* (2013a). There was increased in phospholipids and cholesterol in the seminal plasma of Hampshire boar with increased in preservation hours which was preserved at 15°C from 0 to 72 hours as reported by Chutia *et al.* (2010). The alteration in the cholesterol level might be due to damage to their membrane during cooling.

The overall conception rates were presented in the Table 1. The maximum conception was found at 0 hour of preservation with Modena extender in where A.I. was done immediately after extension of semen samples. Chanapiwat *et al.* (2014) found 88.9% of conception rate by using Modena extender at 24 hours of preservation at liquid state which was higher than the present finding. Chutia *et al.* (2016) found 71.4% of conception rate by using BTS extender at 48 hours of preservation at liquid state which was lower than the present finding. It was observed that the conception rate was decreased while preservation periods increased which might be due to progressive decline of sperm motility, viability and sperm membrane permeability as well as the nutrient content in the extender with increased periods of preservation (Kadirvel *et al.*, 2016).

CONCLUSION

In conclusion, the present study revealed that the boar spermatozoa undergo several changes including diminished motility, viability and alterations in membrane permeability while increasing the time of storage at 15°C with Modena extender. The conception rate was decreased while preservation period was increased at 15°C with Modena extender. For better fertility and conception, Modena extender can be used for preservation of boar semen at 15°C from 0 to 72 hours.

REFERENCES

Blom, E. 1950. A simple rapid staining method for the differentiation between live and dead sperm cells by means of Eosin and Nigrosin. *Nord. Vet. Med.,* **2:** 58.

Cerolini, S., Maldjian, A., Pizzi, F. and Gliozzi, T.M. 2001. Changes in sperm quality and lipid composition during cryopreservation of boar semen. *Reproduction,* **121**: 395–401.

Chanapiwat, P., Olanratmanee, E., Kaeoket, K. and Tummaruk, P. 2014. Conception rate and litter size in multiparous sows after intrauterine insemination using frozen-thawed boar semen in a commercial swine herd in Thailand. *J. Vet. Med. Sci.* **76**(10): 1347–1351.

Chutia, T. 2010. Preservation of boar semen at liquid state. *M.V.Sc. Thesis*, Assam Agricultural University, Guwahati-22.

Chutia, T., Lalrintluanga, K., Ahmed. F.A. and Lego, A. 2016. Use of 'wooden dummy sow' for training of boar for semen collection and the semen quality of trained boar. *Indian Journal of Animal Reproduction,* **38** (2):6-8.

De Leeuw, F.E., Chen, H.C., Colenbrander, B. and Verkleij, A.J. 1990. Cold-induced ultrastructural changes in bull and boar sperm plasma membranes. *Cryobiology*, **27**: 171-183.

Dimitrov, S., Atanasov, V., Dichlyanova, E. and Petroval, R. 2009. Comparision of three commercial diluents for short term storage of boar semen. *Trakia J. Sci.,* **7**(1): 58-62.

Estienne, M. J., Harper, A. F. and Day, J. L. 2007. Characteristics of sperm motility in boar semen diluted in different extenders and stored for 7 days at 18°C. *Reprod. Biol.*, **7**(3): 221-231.

Jeyendran, R.S., Van der Ven, H.H., Perez-Pelaez, M., Carbo, B.G. and Zaneveld, L.J.D. 1984. Development of an assay to assess the functional integrity of the human sperm membrane and its relationship to other semen characteristics. *Journal of Reproduction and Fertility.* **70**: 219-228.

Johnson, L. A., Weitze, K. F., Fiser, P. and Maxwell, W. M. C. 2000. Storage of boar semen. *Animal Reproduction Science,* **62**: 143-172.

Kadirvel, G., Ponraj, P., Thulasiraman, S., Andonissamy, J., Naskar, S., Das, A., Hasin, D. and Baishya, S. 2016. Efficacy of different extenders on sperm characteristics and fertility in crossbred pigs of north-easten India. *Veterrinarski archive,* **86**(4): 515-528.

Khan, M. H., Naskar, S., Das A. and Bordoloi, R. K. 2006. Comparative efficacy of different diluents on liquid preservation of boar semen. *Indian J. Anim. Sci.*, **76** (10): 780-783.

Kommisrud, E., Paulenz, H., Sehested, E. and Grevle, E. 2002. Influence of boar and semen parameters on motility and acrosome integrity in liquid boar semen stored for five days. *Acta vet. Scand.*, **43**: 49-55.

Lalrintluanga, K. 1994. Preservation of boar semen in liquid state. *M.V.Sc. Thesis*, Assam Agricultural University, Khanapara, Guwahati-22.

Lessard, C., Parent, S., Leclerc, P., Bailey, J.L. and Sullivan, R. 2000. Cryopreservation alters the level of the bull sperm surface protein P25b. *J. Androl.*, **21**: 700 –7.

Maldjian, A., Pizzi, F., Gliozzi, T., Cerolini, S., Penny, P and Noble, R. 2005. Changes in sperm quality and lipid composition during cryopreservation of boar semen. *Theriogenology*, **63** : 411–421.

Moretti, J. 1981. Artificial insemination of swine: fertility using several liquid semen diluents. 8th International Pig Veterinary Society Congress, Ghent, Belgium, 293 pp.

Ortman, K. and Rodriguez-Martinez, H. 1994. Membrane damage during dilution, cooling and freezing-thawing of boar spermatozoa packaged in plastic bags. *Zentralbl Veterinarmed A.,* **41**(1):37-47.

Pursel, V. G. 1979. Effect of cold shock on Boar sperm treated with Butylated Hydroxytoluene. *Biol. Reprod.*, **21**: 319-324.

Srivastava, N., Jerome.A., Srivastava, S.K., Ghosh, S.K. and Amit, K. 2013a. Bovine seminal PDC-109

protein: An review of biochemical and functional properties. *Anim. Reprod. Sci.,* **138**: 1-13.

Srivastava, N., Srivastava, S.K., Ghosh, S.K., Amit, K., Perumal, P. and Jerome, A. 2013b. Acrosome membrane integrity and cryocapacitation are related to cholesterol content of bull spermatozoa. *Asian Pacific Journal of Reproduction,* **2**(2): 126-131.

Tamuli, M.K. and Watson, P.F. 1994. Use of a simple staining technique to distinguish acrosomal changes in the live sperm sub-population. *Anim. Reprod. Sci.*, **35**: 247-250.

Tynkan, L. 2009. Effect of holding, removal of seminal plasma and extender on preservation of boar semen at 18°C. *M.V.Sc. Thesis*, Assam Agricultural University, Khanapara, Guwahati-22.

Wagner, H. G. and Thibier, M. 2000. World statistics for artificial insemination in small ruminants and swine. In: Proceedings of the 14th International Congress of Animal Reproduction, Stockholm, **2** (15): 3.

Watson, P. F. 1975. Use of Giemsa stain to detect change in acrosome of frozen Ram spermatozoa. *Vet. Rec.,* **97**(1)**:** 12.

Watson, P.F. 1995. Recent developments and concepts in the cryopreservation of spermatozoa and assessment of their post-thawing function. *Reprod. Fertil. Dev.,* **7**: 871- 891.