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Effect of different extenders on cryopreservation of Hampshire and crossbred boar semen

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ABSTRACT

The comparative efficacy of extenders, viz. LEYG (lactose egg yolk glycerol), BTSLEYG (Beltsvilli thawing solution lactose egg yolk glycerol), KEYG (Kiev egg yolk glycerol) and BTSEYG (Beltsvilli thawing solution egg yolk glycerol) were evaluated for cryopreservation of boar semen. Sperm rich fraction of ejaculates (12 each from 3 Hampshire and 3 crossbred boars) were collected by gloved-hand method using dummy sow. Ejaculates having more than 75% initial motility were taken for the study. Each ejaculate was divided in to 4 aliquots and processed with 4 different extenders using 3 h holding time and 1 h equilibration period and frozen in liquid nitrogen using 0.5 ml french medium straws. Each sample was evaluated just after collection and 24 h post-thawing for initial motility, live sperm count, acrosomal integrity and HOST reacted spermatozoa. Results revealed that out of 4 extenders, BTSLEYG was superior than LEYG, KEYG and BTSEYG in respect of post-thawing sperm motility (51.38±0.87 vs 46.79±1.15, 32.50±0.92, 42.71±0.89%), live sperm count (56.83±0.74 vs 55.37±0.96, 45.75±1.34, 54.83±0.81%), acrosomal integrity (56.83±0.74 vs 55.08±0.96, 43.37±0.70, 54.83±0.81%) and hypo-osmotic-reacted spermatozoa (43.79±0.83 vs 39.00±0.91, 36.37±1.01, 36.41±0.71%) respectively. Therefore, it may be concluded that BTSLEYG can be successfully used for cryopreservation of boar spermatozoa using 3% glycerol concentration with 3 hours holding and 1 h equilibration period.

Key words: Boar, Cryopreservation, Extenders, Semen, Spermatozoa

Boar semen differs in several respects from the semen of other domestic animals as it is produced in large volumes and is extremely sensitive to sudden cooling immediately following collection. The goal of developing more effective freezing extenders to both stabilize the sperm membranes and extend the functional life span of thawed sperm has still not been achieved (Roca *et al*. 2006). Studies were made to evolve suitable extenders and methods for freezing of boar semen with varying success. However, there are still many weaknesses in the sperm cryopreservation process that should be improved before frozen thawed boar semen can be applied with the same efficiency of liquid semen in routine AI programmes in commercial pig farms (Mercadoa *et al*. 2009). One important aspect is the standardization of freezing extenders. In India, not much work has been done for freezing of boar semen. Therefore, the present study was aimed to find out suitable extender for cryopreservation of boar semen with optimum motility and membrane integrity.

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MATERIALS AND METHODS

Semen collection and processing: Sperm-rich fraction of the ejaculates (72) were collected from 3 Hampshire (HS) and 3 crossbred (Hampshire × Khasi Local with 75% of exotic inheritance), maintained at the institute. The boars were in use for breeding purpose and the age varied from 15 to 36 months. All the boars were clinically examined for sexual as well as for general health and were selected on the basis of clinical normality. Semen was collected twice weekly by gloved hand technique using dummy sow, in a sterilized plastic bottle inside an insulated thermos flask. The gel portion of semen was removed at the time of collection by using gauze inside the Butchner funnel. Immediately after collection, semen was brought to the laboratory in a thermos flask (at 35°C) for further processing. Each ejaculate was divided in to 4 aliquots and initial dilution was made by using holding fraction of different extenders (Table 1) and was kept in BOD incubator for 3 h at 24 °C. After 3 h of holding time,

Table 1. Composition of different extenders used for cryopreservation of boar semen

Extender	Holding fraction	Freezing fraction	
		Fraction-I	Fraction-II
LEYG	8.8% Lactose solution	8.8% lactose (80 ml) egg yolk (20 ml)	8.8% lactose (74 ml) egg yolk (20 ml) glycerol (6 ml)
BTSLEYG	BTS Diluent	11% lactose (80 ml) egg yolk (20 ml)	11% lactose (74 ml) egg yolk (20 ml) glycerol (6 ml)
KEYG	Kiev Diluent	Kiev diluent (80 ml) egg yolk (20 ml)	Kiev diluent (74 ml) egg yolk (20 ml) glycerol (6 ml)
BTSEYG	BTS Diluent	BTS (80 ml) egg yolk (20 ml)	BTS diluent (74 ml) egg yolk (20 ml) glycerol (6 ml)

*Antibiotic (gentamycin) @ 150 µg/ml of extender was added in each diluent.

semen was centrifuged, supernatant was discarded and freezing fraction-I of the different extender was added to make the volume 50% of the final volume. Temperature was reduced gradually from 24°C to 5°C within 1½ h by keeping the samples in BOD incubator. Then freezing fraction-II (glycerol fraction) of different extender was added at 5°C.

Filling and sealing of straws: French-mini straws with 4 different colors of PVA powder (polyvinyl alcohol powder) were taken for 4 different extenders. All the equipments related to the filling and sealing of straws were kept in cold handling cabinet at 5°C. After 30 min of equilibration, the straws were filled with the semen and the open end was sealed with PVA powder. At the end of 1 h of equilibration period, the straws were placed on precooled (5°C) towel and were dried by rolling between the two folds of towel.

Semen freezing: The straws were arranged horizontally 5 cm above the liquid nitrogen level in a thermocole box and exposed for 10–12 min to liquid nitrogen vapors. Immediately after vapour freezing, the straws were transferred into the goblet containing liquid nitrogen and transferred to liquid nitrogen container for freezing and storage. Each sample was evaluated for initial motility, live sperm count, acrosomal integrity and HOST test before freezing (fresh semen) and post thawing. The data were analyzed using analysis of variance (ANOVA) and critical difference test as per Snedecor and Cochran (1967).

RESULTS AND DISCUSSION

Semen characteristics were assessed both in fresh and post-thawed semen and the results were presented in Table 2.

Sperm motility: The mean initial motility of spermatozoa in Hampshire and Crossbred boars in the present investigation were in close proximity with the report of Naskar *et al.* (2006) in Hampshire and crossbred boars but higher than the findings of Bhuyan *et al.* (1991). These variations might be attributed to differences in breed, age, season and method of examination of sperm motility. In the present study, no variation was found in the initial motility either between the

Table 2. Characteristics of spermatozoa in fresh and cryopreserved semen of Hampshire and crossbred boars

Sperm characteristics	Breed	Undiluted fresh semen	Post-thawing			
			LEYG	BTSLEYG	KEYG	BTSEYG
Motility (%)	HS	88.00±0.72	48.17^{a}±1.11	51.08^{a}±1.22	33.33^{b}±1.18	42.00^{c}±1.15
	CB	86.25±0.81	45.42^{a}±1.99	51.67^{b}±1.28	31.67^{c}±0.42	43.42^{a}±1.38
	Overall	87.12±0.54	46.79^{a}±1.15	51.38^{b}±0.87	32.50^{c}±0.92	42.71^{d}±0.89
Live sperm count (%)	HS	90.25^{A}±0.47	55.75±1.32	56.91±1.05	49.00^{A}±2.08	55.66±1.20
	CB	88.16^{B}±0.45	55.00±1.40	56.75±1.08	42.50^{B}±1.13	54.00±1.07
	Overall	89.20±0.34	55.37^{a}±0.96	56.83^{a}±0.74	45.75^{b}±1.34	54.83^{ac}±0.81
Acrosomal integrity (%)	HS	90.42^{A}±0.56	55.75^{a}±1.32	56.91^{a}±1.04	44.25^{b}±0.85	55.66^{a}±1.20
	CB	88.00^{B}±0.47	54.41^{a}±1.47	56.75^{a}±1.08	42.50^{b}±1.13	54.00^{a}±1.07
	Overall	89.23±0.39	55.08^{a}±0.98	56.83^{a}±0.74	43.37^{b}±0.70	54.83^{a}±0.81
HOST reacted spermatozoa (%)	HS	60.14^{A}±1.03	38.50^{a}±1.33	44.66b±0.81	36.83a±1.79	37.83a±1.02
	CB	56.47^{B}±0.85	39.50^{ac}±1.28	42.90^{a}±1.45	35.91^{c}±0.98	35.00^{bc}±0.84
	Overall	58.30±0.70	39.00^{a}±0.91	43.79^{b}±0.83	36.37^{c}±1.01	36.41^{c}±0.71

genetic groups.

After thawing, a significantly higher (P<0.05) overall sperm motility was recorded in BTSLEYG than in LEYG, KEYG and BTSEYG respectively. Post-thaw sperm motility observed in BTSLEYG extender in the present study was in close proximation with Flores *et al.* (2008) in LEYG extender. However, lower and higher post thaw sperm motility than the present finding were reported by Saravia *et al.* (2007).

Live sperm count: The mean % of live spermatozoa recorded in the present study in Hampshire and crossbred boars was close to the values reported by Khan *et al.* (2011) in Hampshire boars. In the present investigation, a significantly (P<0.01) higher live sperm count was reported in Hampshire than in crossbred boars. This might be due to variation between the genetic groups.

Post-thaw live sperm count was significantly ((P<0.01) lower in KEYG than in LEYG, BTESLEYG and BTSEYG. This was similar to the findings of Saravia *et al.* (2007) and Flores *et al.* (2008). Higher post-thaw sperm viability in BTSLEYG extender might be due to presence of potassium chloride in the holding fraction (BTS) which was absent in Kiev diluent. (Jhonson *et al.* 2000) reported that BTS maintain intracellular concentration of K^+ inside the cell and minimal addition of milimolar concentration of K^+ may helpful in maintaining the motility of spermatozoa. Low pH of Lactose egg yolk glycerol (pH 6.2; Westendorf *et al*. 1975) was also one of the factors for increased post-thaw sperm viability in BTSLEYG extender.

Acrosomal integrity of spermatozoa: The mean intact acrosome percentage was 90.42±0.56% in Hampshire 88.0±0.47% in crossbred boars. The present findings were in close proximity with the findings of Khan *et al.* (2006) in Hampshire boars. The mean-intact acrosome % in the present study was significantly (P<0.01) higher in Hampshire than in crossbred boars. This might be variation between the genetic groups.

Acrosomal integrity after freezing and thawing was also significantly (P<0.01) lower in KEYG than in LEYG, BTSLEYG and BTSEYG extenders. Post-thawing % of intact acrosome recorded in the present study was close to the findings of Saravia *et al*. (2007) and Flores *et al*. (2008). The lower acrosomal integrity in KEYG extender may due to difference in the composition of the extenders. Paquignon (1985) reported that the % of spermatozoa with normal acrosome was higher with lactose extender than with glucose containing extenders. This might be due to difference in the composition, pH and osmolarity of hoding and freezing fraction of the extenders (Pursel *et al*. 1972).

Hypo-osmotic swollen spermatozoa: The mean hypo-osmotic swollen sperm percentage observed in the present investigation in Hampshire and crossbred boars was in agreement with the finding reported by Khan *et al.* (2006) in Hampshire boars. A significantly (P<0.01) higher mean intact acrosome obtained in the Hampshire boars in the present study than the crossbred boars might be due to genetic variations among the groups.

After thawing, there was a significantly (P<0.01) higher hypo-osmotic swollen spermatozoa percentage in BTSLEYG than in LEYG, KEYG and BTSEYG extenders, respectively. Our findings are in close agreement with Wolders *et al.* (2005) who also reported 41.2% plasma membrane integrity when frozen in BTSEYG extender.

Sperm motility and acrosomal integrity was affected by the osmotic pressure of the diluent and glucose favoured sperm motility while lactose reduces acrosome damage (Martin–Rillo *et al*. 1980). In this study, semen was extended with 8.8% lactose solution in LEYG, with BTS diluent in BTSLEYG and BTSEYG and with Kiev diluent in KEYG extender and held at 24 °C for 3 h. Low pH of 8.8% lactose solution in LEYG extender, absence of buffer and low osmolarity in holding fraction of LEYG extender might have had the adverse effect during cooling and freezing on motility and membrane integrity of spermatozoa.

It is concluded from our study that out of the 4 extenders (LEYG, BTSLEYG, KEYG and BTSEYG), BTSLEGY (belts ville thawing solution lactose egg yolk glycerol) was superior in post-thawing motility, live sperm count, acrosomal integrity and hypo-osmotic reacted spermatozoa when frozen with 3% final glycerol concentration for 1 h equilibration period.

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