International Journal of Multidisciplinary Approach and Studies

ISSN NO:: 2348 – 537X

Effect of Different Extenders on the Quality of Boar Semen During Preservation At 18^0C

Lalrintluanga. K*, Deka. B.C., Nath. K.C.***, L. Hmar****, Bhuyan. D****, & Biswas. R.K.*****,**

Department of Animal Reproduction, Gynaecology and Obstetrics
College of Veterinary Science, Assam Agricultural University, Khanapara, Guwahati.

ABSTRACT

A total of 24 semen ejaculates obtained from seven Large White Yorkshire (LWY) boars were used to study the effect of different extenders on the quality of boar semen during preservation at 18^0C. The semen were extended using Beltsville Thawing Solution (BTS), Androhep, Fructose Egg Yolk (FEY) and GPSE. (Glucose Potassium Sodium tartrate Sodium citrate edate) extenders. At about 15-20 minutes after collection, each ejaculate was split into 4 parts and was diluted at the rate of 1:12 with the four extenders. The diluted semen was then filled in glass/plastic vials (20 ml) with cap. For each extender per ejaculate one vial was preserved at 18^0C in BOD incubator for 72 hours. The preserved semen vials were turned upside down once daily. The average sperm motility was 51.66 ± 1.80, 53.25 ± 2.09, 48.04 ± 2.12 and 42.96 ± 2.02 % respectively, in BTS, Androhep, GPSE and FEY extenders at72 hrs of preservation. While live percentage, intact acrosome and HOSST reacted sperm were 72.71 ± 1.11, 70.08 ± 1.12 and 39.38± 1.87 % respectively in BTS; 73.42 ± 1.15, 70.21 ± 1.31 and 42.08 ± 2.31 % respectively in Androhep, 70.25 ± 1.21, 68.38 ± 0.98 and 40.21 ± 2.23 % in GPSE and 69.17 ± 1.10, , 67.88 ± 1.17 and 37.58 ± 2.25 % respectively in FEY extender after 72 hours of preservation at 18^0C. The percentage of progressively motile sperm differed significantly between extenders ($P<0.05$) and between preservation periods ($P<0.01$). The percentage of progressively motile sperm in BTS and Androhep extenders was significantly ($P<0.05$) higher than that in FEY extender but it did not differ significantly from that in GPSE extender. The live sperm per cent differed significantly between extenders ($P<0.05$) and between preservation periods ($P<0.01$) but not due to interaction. Critical difference test revealed that the per cent of live sperm in Androhep and BTS was higher than in GPSE and FEY extenders. The incidence of intact acrosome did not vary significantly between extenders but varied significantly ($P<0.01$) between preservation periods. The mean HOSST reacted sperm did not differ significantly between extenders. The study may be concluded that both Androhep and BTS extenders were equally good and were better than GPSE and FEY extenders in term of progressive motility, live percentage, acrosomal integrity and hypo-osmotic sperm swelling test (HOSST) for preservation of boar semen.

Key words: Boar, extender, HOSST, Preservation, semen.

Liquid storage of boar semen is superior to that of frozen semen with respect to easier processing procedure, yielding higher artificial insemination doses per ejaculate because of higher cell survival, resulting in both high fecundity rates and bigger litter size. The fertility is maintained even with low number of spermatozoa in the inseminate with more than one

million sperm cells per breeding unit. However, the fertility of the semen is gradually lost during extended period of preservation. Large number of boar semen extenders have been tried and claimed to be good but there exists high variability among different extenders in terms of viability and fertilizing capacity of spermatozoa. Therefore, it is essential to find out the suitable extender for preservation of boar semen in liquid state. Present study has been carried out to assess the viability and fertilizing ability of spermatozoa during in vitro storage of boar semen at 18^0C using different extenders.

MATERIALS AND METHODS

A total of 24 semen ejaculates obtained from seven Large White Yorkshire (LWY) boars maintained at Regional Pig Breeding Farm, Govt. of Mizoram, Selesih, were collected by gloved hand technique with the help of dummy sow as a mount. The collected semen was strained through filter gauze into a graduated collecting beaker of 500 ml capacity to remove the gel portion. The extenders used were Beltsville Thaw Solution (BTS), Androhep, Fructose Egg Yolk (FEY) and GPSE. (Glucose Potassium Sodium tartrate Sodium citrate edate).

Table 1. Composition of extenders:

	BTS	Androhep	GPSE	FEY
Glucose – D (g)	3.715	2.600	3.5	-
Fructose 8.8 %	-	-	-	80 ml
EggYolk	-	-	-	20ml
Tri-sodium citrate (g)	0.600	0.800	0.3	-
E.D.T.A. di-sodium salt (g)	0.125	0.240	0.2	-
Sodium Hydrogen carbonate (g)	0.125	0.120	-	-
Potassium chloride (g)	0.075	-	-	-
HEPES (g)	-	0.900	-	-
BSA (g)	-	0.250	-	-
Potassium sodium tartrate(g)	-	-	1.0	-
Gentamicin sulphate (µg/ml)	150	150	150	150
Distilled water upto	100 ml	100 ml	100ml	-
pH	7.2	6.8	6.8	6.8

All the constituents of the extenders except egg yolk in FEY extender were mixed and kept overnight at 5^0C in a refrigerator. Just before collection of semen the extenders were warmed to 37^0C and the egg yolk was added in case of Fructose Egg Yolk extender. At about 15-20 minutes after collection, each ejaculate was split into 4 parts and was diluted at the rate of 1:12 with the four extenders. The diluted semen was then filled in glass/plastic vials (20

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ml) with cap. For each extender per ejaculate one vial was preserved at 18^{0}C in BOD incubator for 72 hours. The preserved semen vials were turned upside down once daily.

Semen was evaluated for sperm motility, live sperm count, intact acrosome, hypo-osmotic sperm swelling test (HOSST) at 0 (immediately after dilution), 24, 48 and 72 hours of preservation.

Sperm motility: A drop of diluted semen was placed on a glass slide prewarmed to 37^{0}C using a prewarmed stage of Sperm Class Analyzer (SCA). A cover slip was then placed over the drop of semen and the sperm motility was estimated using the Computer Assisted Sperm Analysis (CASA) system (Sperm Class Analyzer, Microptic SL. Barcelona). The sperm motility was recorded from 0 to 100 based on the percentage of progressively motile spermatozoa.

Live sperm count: Live sperm count was determined using eosin-nigrosin staining technique (Beatty, 1957). Two hundred sperms were counted in each smear using a manual counter in CASA (Sperm Class Analyzer) to determine the percentage of live sperm.

Intact acrosome: The incidence of intact acrosome was studied using Giemsa staining technique of Watson (1975). A total of two hundred spermatozoa were studied in each smear using a manual counter in CASA (Sperm Class Analyzer) to determine the percentage of intact acrosome.

Hypo-osmotic sperm swelling test (HOSST) : Hypo-osmotic sperm swelling test was done as per Jeyendran *et al.* (1984) with slight modification. The Hypo-osmotic solution having an osmotic strength of 100 mOsm was used for this study.

Statistical analysis of the data was done using Systat version-12 Soft ware Inc. San Jose, USA.

RESULTS AND DISCUSSION

The mean values of sperm motility, live sperm count, intact acrosome and HOSST reacted sperm in BTS, Androhep, GPSE and FEY extenders at different hours of preservation at 18° C are presented in Tables 2.

Sperm motility in BTS extender recorded in the present study was comparable to the reports of earlier workers (Khan *et al.,* 2006, Naskar *et al.,* 2006$_a$ and Pande *et al.,* 2007). However, the value in BTS extender in the present study was higher than the report of Aalbers *et al.* (1983) and Cerovsky and Vinter (1985). This might be due to the difference in preservation temperatures and/or, due to breed difference. The value in this study was lower than the report of Kantharaj and Athman (2007) at 15°C in Large White Yorkshire. This difference in the same breed of pigs might be due to the variations in preservation temperature, difference in dilution rates, pH of extenders, age of boars and processing of semen. Higher sperm motility percentage at 18°C was reported by Tyngkan (2009) in Hampshire boars. This might be due to the difference in breed of boars. The percentage of progressively motile sperm in GPSE extender in the present study was lower than that reported by earlier workers (Tamuli, 1982; Bhuyan, 1989; Bujarbaruah, 1989; and Pande *et al.,* 2007). This might be due to difference in preservation temperature, age and breed of boars. The sperm motility in FEY extender in Hampshire boar semen reported by Lalrintluanga (1994) was higher and that reported by Tyngkan (2009) was lower than the values found in the present study. This might

be due to difference in breed, dilution rate, pH of extender, processing of semen, and /or preservation temperature. The percentage of progressively motile sperm differed significantly between extenders (P<0.05) and between preservation periods (P<0.01). The significant difference in

progressively motile sperm between extenders was in agreement with that of earlier workers (Cheng, 1988; Dirkseng, 1991; Lalrintluanga, 1994; Naskar, 2006a; Pande *et al.,* 2007 and Tyngkan, 2009). Critical difference test revealed that the percentage of progressively motile sperm in BTS and Androhep extenders was significantly (P<0.05) higher than that in FEY extender but it did not differ significantly from that in GPSE extender. However, it was observed in the present study that 50 per cent progressive motile sperm was maintained in all the extenders upto 48 hours of preservation at 18^0 C. The significant (P<0.05) decline in sperm motility along with the increase in preservation period observed in the study was in accordance with the reports of earlier workers (Das *et al.*, 2005a; Khan *et al.,* 2006; Naskar, 2006a; Kantharaj and Athman, 2007; Pande *et al.,* 2007 and Tyngkan, 2009). The decrease in sperm motility during preservation probably occurred owing to sperm cell senescence and exhaustion of metabolites in the preservation media (Tamuli, 1993). The higher decline rate of sperm motility in FEY extender might be due to lack of buffer in the extender leading to rapid reduction of pH due to the presence of high fructose (8.8 per cent) and thus producing more lactic acid whereas in other three extenders the sugar was glucose and its concentration varied from 2.6 to 3.7 per cent. The interaction between extender and preservation period was not significant for sperm motility. This indicated that the effect of extenders and the preservation periods on sperm motility was independent.

The mean live sperm per cent at 72 hours of preservation was comparable to the finding of Tyngkan (2009) in Hampshire boar semen preserved at 18°C, but the present value in BTS extender was higher than that reported in Hampshire boar semen preserved at 22^0C (Das *et al.,* 2005c) and 18^0 C (Khan *et al.,* 2006). The higher live sperm count in the present study might be due to the difference in breed and /or preservation temperature. The mean live sperm count in GPSE in this study at 48 hours of preservation was comparable with that of 75.38 per cent (Bhuyan, 1989) in crossbred boar semen. The value at 72 hours of preservation in GPSE was comparable to that of Tamuli (1982). The present value was higher than that of Bujarbaruah (1989) and lower than that of Talilepzuk (1998) in Hampshire boar semen preserved at 15°C. Live sperm percentage in FEY extender in this study was higher than that in Hampshire boar semen preserved for 48 and 72 hours (Lalrintluanga, 1994; Tyngkan, 2009). The differences mentioned might be due to breed difference, preservation temperature, pH of the extender, dilution rate and processing of semen. The live sperm per cent differed significantly between extenders (P<0.05) and between preservation periods (P<0.01) but not due to interaction. Critical difference test revealed that the per cent of live sperm in Androhep and BTS was higher than in GPSE and FEY extenders. The high live sperm count in Androhep might be due to the content of HEPES, a zwitterionic organic buffer, which was known to capture heavy metal and control pH (Crabo *et al.,* 1972).

The incidence of intact acrosome at 72 hours of preservation at 18^0C in BTS extender (70.08 ± 1.12) in the present study was slightly lower than the report of 75.39 per cent by Tyngkan (2009) but somewhat higher than the report of 64.32 per cent by Khan *et al. (*2006) in Hampshire boar semen preserved at 18^0C and 53.42 and 51.62 per cent by Das *et al. (*2005_c) in Hampshire and crossbred boar semen respectively preserved at 22°C. The difference might

be due to difference in temperature and/or due to breed or dilution rates. The percentage of intact acrosome in GPSE diluent in the present finding (68.38 ± 0.98 %) at 72 hours of preservation was in agreement with the observations of Tamuli (1982) and Talilepzuk (1998). The present finding at 48 hours of preservation in GPSE (74.42 ± 1.12 %) was in agreement with the findings of Bhuyan (1989) in Hampshire and crossbred boar semen. The present mean value in FEY extender (74.04 ± 1.09 %) at 48 hours of preservation at 18^0C was in agreement with that at 5°C but lower than that at 15^0C (Lalrintluanga, 1994). However, at 72 hours of preservation, Tyngkan (2009) reported much lower incidence of intact acrosome (44.61 %) in Hampshire boar. This difference might be due to difference in dilution rate, pH of extender or breed of boar.

The incidence of intact across some did not vary significantly between extenders but varied significantly (P<0.01) between preservation periods. On the other hand, Dirkseng (1991) reported significantly higher percentage of intact acrosome in Androhep and BW-25 extenders than in Kiev and BTS diluents during preservation for 144 hours at 15°C. On critical difference test, it was observed that the mean intact acrosome decreased significantly (P<0.01) as the preservation period increased from 0 through 72 hours. Similar observations were also made by earlier workers (Tamuli, 1982; Bhuyan, 1989; Lalrintluanga, 1994; Talilepzuk, 1998; Das *et al.,* 2005c; Khan *et al.,* 2006 and Tyngkan, 2009). The mean percentage of intact acrosome did not differ significantly due to interaction of extender and preservation period which showed that the main effects were independent.

The mean HOSST reacted sperm at 0 hour of preservation were slightly higher than the report of 55.33 per cent in Hampshire and 52. 45 per cent in crossbred (Das *et al.,* 2005_d) and 55.44 per cent (Das *et al.,* 2006_b) in the incubation temperature of 37°C for 60 minutes and 57.50 per cent in Hampshire in the incubation temperature of 37°C for 30 minutes in 150 mOsm/litre (Khan *et al.,* 2006). The difference in percentage of HOSST reacted sperm might be due to the difference in breed (Das *et al.,* 2006_b), incubation period and difference in osmolarity of hypo-osmotic solution as the present study was done in Yorkshire boar semen with the incubation temperature of 37°C for 60 minutes in 100 mOsm/litre of hypo-osmotic solution. The difference in response to HOSST between breeds might be due to difference in physical and bio-chemical properties of plasma membranes of the spermatozoa of various breeds resulting in differences in the degree to which electrolytes and non-electrolytes penetrate their membranes (Guraya, 1987). During the HOSS test, the bio-chemically active spermatozoa, when exposed to hypo- osmotic stress will undergo swelling due to the influence of water and subsequently increase in volume to establish equilibrium between the fluid compartment within the spermatozoon and the extracellular environment (Drevius and Erikson, 1966; Jeyendran *et al.,* 1984). The optimal hypo-osmotic medium should exert an osmotic stress large enough to cause an observable increase in volume, but small enough to prevent lyses of the sperm membranes (Jeyendran, 1984; Zavos, 1990). But the optimal osmolarity of hypo-osmotic solution for HOSS test varied in different species of animals. The optimal osmolarity of hypo-osmotic solution giving the maximum HOSST reacted sperm was reported to be 150 mOsm/litre for neat bull semen (Revell and Mrode, 1994) and 100 mOsm/litre for frozen thawed bull semen (Correa and Zavos, 1994; Revell and Mrode, 1994; Singh *et al.,* 2004), 100 mOsm/litre in buffalo bull semen (Pant, 2002), 125 mOsm/litre in fresh goat semen (Fonseca *et al.,* 2005), and 100 mOsm/litre in boar semen (Vazquez *et al.,* 1997; and Samardzija *et al.,* 2008).

International Journal of Multidisciplinary Approach and Studies ISSN NO:: 2348 – 537X

The mean HOSST reacted sperm did not differ significantly between extenders. The lower percentage of HOSST reacted sperm in FEY might be due to the absence of EDTA in FEY extender whereas it was present in other three extenders. EDTA prevented initiation of capacitation and acrosome reaction (Johnson *et al.,* 2000) thereby it could be associated with preventing the physical and biochemical damage of the plasma membrane. The mean percentage of HOSST reacted sperm differed significantly between preservation periods (P<0.01). On critical difference test it was observed that HOSST reacted sperm percentages decreased significantly (P<0.05) with each increase in preservation period. This could be due to progressive decrease in biochemical activity of spermatozoa with increase in preservation period. The significant decrease in HOSST reacted sperm along with the increase in preservation period in the present study was in agreement with Das *et al.* (2006_b) in crossbred boar semen.

The study may be concluded that both Androhep and BTS extenders were equally good and were better than GPSE and FEY extenders in term of progressive motility, live percentage, acrosomal integrity and hypo-osmotic sperm swelling test (HOSST) for preservation of boar semen.

Table-1. Mean ± S.E. Of Progressively Motile Sperm, Live Sperm, Intact Acrosome And Hosst Of Boar Spermatozoa Preserved In Different Extenders At 18^0c

Extenders	Preservation period				
	0 hr	24 hr	48 hr	72 hr	**Overall**
Progressively motile sperm (%)					
BTS	77.16 ± 1.56	67.75 ± 1.60	59.12 ± 1.91	51.66 ± 1.80	64.00^{a}± 5.51
Androhep	76.58 ± 1.47	68.38 ± 1.84	59.58 ± 1.93	53.25 ± 2.09	64.60^{a}± 5.21
GPSE	76.58 ± 1.47	65.33 ± 1.83	55.88 ± 2.31	48.04 ± 2.12	61.45^{ab}±6.15
FEY	76.71 ± 1.39	64.92 ± 1.64	51.83 ± 2.31	42.96 ± 2.02	59.10^{b}± 7.40
Overall	76.91^{a}± 0.74	66.59^{b}± 0.86	56.60^{c}± 1.09	48.98^{d}± 1.07	
Live sperm (%)					
BTS	92.63 ± 0.55	86.08 ± 0.70	79.21± 0.93	72.71 ± 1.11	82.66^{a}± 4.30
Androhep	92.63 ± 0.53	86.21 ± 0.67	79.79 ± 1.03	73.42 ± 1.15	83.01^{a} ± 4.13
GPSE	92.25 ± 0.55	85.21 ± 0.78	77.88 ± 1.12	70.25 ± 1.21	81.40^{b} ± 4.73
FEY	92.29 ± 0.64	84.83 ± 0.69	77.04 ± 0.93	69.17 ± 1.10	80.83^{b} ± 4.98
Overall	92.40^{a} ± 0.28	85.60^{b} ± 0.35	78.50^{c}± 0.51	71.40^{d} ± 0.59	
Intact acrosome (%)					
BTS	88.29 ± 0.90	81.96 ± 1.10	75.75 ± 1.14	70.08 ± 1.12	79.02 ± 1.07
Androhep	88.42 ± 0.95	82.83 ± 1.08	76.54 ± 1.09	70.21 ± 1.31	79.47 ± 1.10
GPSE	87.67 ± 1.12	81.75 ± 1.16	74.42 ± 1.12	68.38 ± 0.98	78.05 ± 1.09
FEY	88.54 ± 0.88	81.96 ± 1.21	74.04 ± 1.09	67.88 ± 1.17	78.10 ± 1.09
Overall	88.24^{a}± 0.48	82.10^{b}± 0.56	75.23^{c}± 0.55	69.10^{d}± 0.57	
HOSST reacted sperm (%)					
BTS	64.75± 4.34	53.33 ± 1.90	45.83±1.87	39.38± 1.87	50.82± 1.92
Androhep	61.58 ± 2.36	55.04 ± 2.03	48.41±2.14	42.08 ± 2.31	51.76 ± 2.14
GPSE	60.46 ± 2.05	53.17 ± 2.05	46.21±2.23	40.21 ± 2.23	50.01 ± 2.08
FEY	60.04 ± 1.90	52.37 ± 1.91	43.75±2.14	37.58 ± 2.25	48.40 ± 1.94
Overall	61.71^{a}±1.14	53.48^{b}± 0.97	46.05^{c}±1.04	39.81^{d} ± 1.08	

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