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Research Article

Preservation of cross-bred boar semen*

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ABSTRACT

Twenty four semen samples having more than 75 per cent progressively motile spermatozoa were obtained from six cross-bred boars (Large White yorkshire X Indigenous). The part of neat semen was diluted at the rate of 1:2 with each of four dilutors viz. Glucose Potassium sodium tartrate Sodium citrate dihydrate Edate (GPSE-1), Kiev dilutor, Beltsville Liquid (BL-1) and Beltsville Thawing Solution (BTS) and preserved at 15°C. The progressive motility of spermatozoa in the preserved semen was assessed at 0, 24, 48 and 72 hours after preservation to compare the efficacy of the dilutors. GPSE-1 dilutor maintained better sperm motility than other dilutors at each hour of preservation. The percent motile spermatozoa in GPSE-1 dilutor was 84.81 ± 0.17, 75.71 ± 0.27, 70.0 ± 0.33 and 59.9 ± 0.64 at 0 hour, 24 hour, 48 hour and 72 hour of preservation, respectively. Only GPSE-1 and BTS dilutors maintained over 50 percent motile spermatozoa upto 72 hours after preservation. The EDTA, Potassium sodium tartrate and Sodium citrate dihydrate in GPSE-1 dilutor worked synergistically and maintained sperm motility for longtime.

Key words: Cross-bred boar, Semen, Preservation.

INTRODUCTION

For the maximum utilization of quality semen from males of superior genotype, preservation of semen for long period *in vitro* without lowering the inherent fertilizing ability of the spermatozoa is of utmost importance. Scant literature on the preservation of cross-bred boar semen is available in India. Hence, the present investigation was undertaken to compare the efficiency of certain diluents for preservation of cross-bred boar semen at 15°C.

MATERIALS AND METHODS

Six cross-bred boars (Large White Yorkshire X Indigenous) aged 10 to 18 months maintained at the All India Coordinated Research Project on Pigs, Jabalpur were used for the study. All the boars were kept under identical management. Semen was collected by the "Gloved Hand Method". Twenty four semen samples, four from each boar, having more than 75 per cent progressively motile spermatozoa were used in the study. Immediately after collection the part of neat semen was diluted at the rate of 1 : 2 with each of the four dilutors viz. Glucose Potassium sodium tartrate Sodium citrate dihydrate Edate (GPSE-1), Kiev dilutor, Beltsville Liquid (BL-1) and Beltsville Thawing Solution (BTS) in sterile glass ampoules. The diluted semen samples were preserved at 15°C. The efficacy of dilutors was compared by assessing the progressive motility of spermatozoa in the preserved semen just after dilution at 0 hour, 24, 48 and 72 hours of preservation.

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Table 1. Composition of dilutors

Contents	GPSE -1	KIEV	BL-1	BTS
Glucose	3.5 gm	6.00 gm	2.90 gm	3.70 gm
Potassium sodium tartrate	1.0 gm	-	-	-
Sodium citrate dihydrate	0.3 gm	0.375 gm	1.00gm	0.60 gm
Sodium salt of EDTA	0.2 gm	0.375 gm	-	0.125 gm
Sodium bicarbonate	-	0.120 gm	0.20 gm	0.125gm
Potassium chloride	-	-	0.03 gm	0.075 gm
Distilled water	100ml	100ml	100ml	100ml

After autoclaving at 15 Ib pressure and 121°C temperature for 15 minutes the dilutors were fortified with streptopenicillin at the dose rate of 1000 ug/ml.

RESULTS AND DISCUSSION

The average percent motile sperm at different hours of preservation in different dilutors are presented in Table 2. The percentage of motile spermatozoa varied significantly ($P<0.05$) among diluents and hours of preservation. There was a significant ($P<0.05$) interaction due to dilutors X hours of preservation (Table 3). In the present study the sperm motility was best maintained by GPSE-1 dilutor followed by BTS, Kiev and BL-1 dilutors. Several workers also reported GPSE as a best dilutor in comparison to Kiev and BL-1 dilutors (Tamuli and Rajkonwar, 1987 and Rao *et al,* 1992). The percent sperm motility in GPSE-1 dilutor in the present study is in close agreement with the findings in Landrace boars (Tamuli *et al.,* 1986) and native boars (Rao *et al.,* 1991).

Table 2. Effect of dilution on sperm motility of cross-bred boar (mean ± SE)

Hours of Preservation	Dilutors			
	GPSE-1 %	Kiev %	BL-1 %	BTS %
0 hour	84.81 ±0.17"	80.08 ±0.16^{b}	79.54 ± 0.24^{C}	83.86 ±0.16^{b}
24 hours	75.71 ±0.27"	70.35 ± 0.26^{C}	70.68 ±0.19°	73.47 ±0.36^{b}
48 hour	70.0±0.33^{a}	52.27 ± 0.43^{C}	55.01 ± 0.40^{C}	67.60 ± 0.46^{b}
72 hours	59.9 ± 0.64^{a}	45.41 ± 0.69^{C}	38.22 ± 0.58^{d}	55.29 ±0.57^{b}

Means bearing same superscript within a time interval do not differ significantly ($P<0.05$)

Table 3. Critical difference test for preservation of semen

Dilutors	Means
GPSE - 1	65.29^{a}
Kiev	58.96^{C}
BL-1	57.34^{d}
BTS	63.10^{b}

Means bearing same superscript do not differ significantly ($P < 0.05$)

All the four dilutors maintained over 50 per cent motile spermatozoa upto 48 hours of preservation, however, only GPSE-1 and BTS dilutors could maintain this motility up to 72 hours of storage. It was observed that decrease in motile sperm percentage during first 24 hours of preservation was nearly similar in all diluents. Thereafter, sperm motility declined at slow rate in GPSE-1 and BTS-diluted semen, whereas at a faster rate in kiev and BL-1 diluted semen and both could not maintain the minimum 50 percent sperm motility beyond 48 hours of preservation. This is in accordance with the findings of Tamuli *et al.* (1986), however, Pursel *et al.* (1974) and Bhuyan *et al.* (1992) reported comparatively higher sperm motility in semen diluted with BL-1 extender at 72 hours of preservation. The per cent motility in Kiev-diluted semen corroborated with the findings of Vijaykumaran and Iyer (1980). In the present study, BTS-diluted semen showed better sperm motility in comparison to Kiev dilutor which supported the findings of Tackacs *et al.* (1991) and Machaty *et al.* (1992). The synergistic action of EDTA, potassium sodium tartrate and sodium citrate dihydrate maintained highest percentage of motile spermatozoa in GPSE-1 diluted semen upto 72 hours. The EDTA either depresses the enzymatic activity of proteases (Plisko, 1966) or prevents the decrease of aldolase enzyme action (Kurilo, 1968). EDTA is a chelating agent which in combination with tartrate prevents the formation of lysolecithin and other highly toxic compounds that destroy fertilizing capacity of boar spermatozoa (Pursel, 1979). BL-1 dilutor lacks EDTA in its composition and it may be a factor that it showed poor sperm motility in later hours of storage. The crystalline tartrate is a highly reducing substance, and it protects spermatozoa from lipid peroxide formation and prevents cold shock damage and sperm cell agglutination (Tamuli and Rajkonwar, 1987). The lack of potassium sodium tartrate may be a cause of lower sperm motility in semen diluted with kiev and BL-1 dilutors. Glucose, besides providing energy, along with potassium sodium tartrate also provides optimum motility, osmolarity, buffering capacity and obviates the harmful effects of dilution and thus supports the viability and motility of spermatozoa. However, the availability of nutrients alone is not adequate to support livability of sperm without an added buffer to the diluents (Murthy and Rao, 1975) and therefore GPSE. Kiev, BL-1 and BTS dilutors were enriched with sodium citrate dihydrate to provide buffering activity.

It is evident from the present study that GPSE-1 dilutor is best for preservation of boar semen at 15°C followed by BTS, Kiev and BL-1 dilutors and the difference in motile sperm percentage in various dilutors is due to their chemical composition.

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REFERENCES

Bhuyan, D., Borgohain, B.N., Ahmed, K. and Deka, B.C. (1992). Effect of KIEV, GPSE-1 and BL-1 extenders on the semen quality of crossbred boar. Indian J. Anim. Reprod.**13**: 41-43.

Kurilo, J.V.G (1968). Activity of aldolase in fresh and stored boar semen. Svinarstvo Kygiv, 7: 41-51, 147-148. (Cited by Rao *el* al., 1991).

Machaty, Z., Takacs, T. and Gathy, I. (1992). Fertilizing capacity of boar semen diluted with Beltsville TS (BTS) and modified Kiev (MK) extenders in relation to storage time and number of spermatozoa per insemination dose. Anim. Reprod. Sci., **29** : 289-295.

Murthy, P.R. and Rao, A.R. (1975). Preservation of boar semen. Indian. Vet. J., **52**: 415-420.

Plisko, N.T. (1966). Effect of synthetic diluents on carbohydrate metabolism and physiology of boar spermatozoa and activity of antibiotics. Zh. Zhivot Vet., (3): 58, 260. (Anim. Breed. Abstr., **36**: 641).

Pursel, V.G. (1979). Advances in preservation of swine spermatozoa. *Belltsville Symposia in Agriculture Research and Animal Reproduction.* Allanheld Osumum and Co. New Jersey, pp. 148 (Cited by Tamuli and Rajkonwar, 1987).

Pursel, V.G, Johnson, L.A. and Schulman, L.L. (1974). Acrosome morphology of boar spermatozoa during *in vitro* aging. J. Anim. Sc, **38**: 113.

Rao, B.V., Chetty. A.V., Ramachandraiah, S.V. and Sreekumaran, P.K. (1991). Preservation of native boar semen. Indian J. Anim. Reprod., **12**: 148-150.

Rao, M.M., Ramachandraiah, S.V, Cherry, A.V. and Sriraman. P.K. (1992). Studies on cross-bred boar semen characteristics and preservation. Indian J.Anim. Reprod., **13**: 141-142.

Takacs, T., Machaty, Z. and Gathy, I. (1991). Fertilizing capacity of boar semen plotted against the storage and changes in the sperm cell counts using Beltsville Thawing Solution or the Modified Kiew diluent. Magyar Allator vosok Lapja.. **46**: 224-228. Internet.

Tamuli, M.K. and Rajkonwar, C.K. (1987). Innovation of boar semen dilutors. Indian Vet. J., **64**: 782-784.

Tamuli, M.K., Rajkonwar, C.K. and Goswami, B.K. (1986). Preservation of boar semen, Indian J. Anim. Reprod., **7**: 70-72.

Vijaykumaran, V. and Iyer, C.P.N. (1980). Extenders for preservation of boar semen. Kerala J. Vet. Sci. **11**:215.