EFFECT OF DIFFERENT EXTENDERS ON HYPO-OSMOTIC SWELLING OF DOG SEMEN PRESERVED AT 5° C

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ABSTRACT

The objective of the present study was to evaluate the preservability of different extenders for dog semen on the basis of hypo-osmotic swelling test (HOST). Semen samples from three Labrador Retriever (LR) dogs were collected by digital manipulation method at weekly intervals. The first and second fractions of the ejaculates were collected combinedly in a single collection cup. The semen samples were then extended @ 1:4 in Tris-Egg Yolk- Citric Acid-Glucose (TEYCAG), Tris-Egg Yolk- Citric Acid-Fructose (TEYCAF) and Egg Yolk-Citrate-Glycine-Glucose (EYCGG) extenders by split sample technique and preserved at 5° C. The preserved semen was evaluated for HOST-reacted sperm at 24, 48, 72, 96 and 120 hours of preservation. The mean incidence of HOST- reacted sperm was 93.44 ± 1.92, 92.20 ± 1.72 and 85.65 ± 3.01; 87.99 ± 4.35, 85.56 ± 3.82 and 75.34 ± 6.86; 85.03 ± 4.23, 82.25 ± 4.32 and 68.88 ± 7.46; 82.20 ± 4.42, 78.75 ± 5.17 and 59.33 ± 5.10; and 79.51 ± 4.25, 70.00 ± 3.63 and 52.50 ± 3.71 per cent in TEYCAG, TEYCAF and EYCGG extenders at 24, 48, 72, 96 and 120 hours of preservation respectively. Analysis of variance revealed that the mean incidence of HOST-reacted sperm differed significantly ($P<0.01$) between extenders and between preservation periods. But the interaction between extender and preservation period was not significant. The mean percentage of HOST-reacted sperm was significantly ($P<0.05$) higher in TEYCAG and TEYCAF extenders from that in EYCGG extender irrespective of hour of preservation. The incidence of HOST-reacted spermduring 48 to 120 hours of preservation decreased significantly ($P<0.05$) from that at 24 hours of preservation irrespective of extender. It was significantly ($P<0.05$) lower at 96 and 120 hours as compared to that at 48 hours, and at 120 hours than at 72 hours of preservation irrespective of extender. Per cent HOST-reacted spermatozoa did not differ significantly between TEYCAG and TEYCAF extenders irrespective of preservation period which might suggest that the two extenders were equally efficacious in providing functional integrity of the plasma membrane of LR dog spermatozoa during preservation.

Key words: Extenders, HOST-test, Dog semen, Preservation

INTRODUCTION

Hypo-Osmotic Swelling Test (HOST) is performed to determine the functional integrity of the plasma membrane of spermatozoa in a simplified way. Movement of sperm in the female genitalia depends partly on sperm tail activity. Intact state of sperm plasma membrane is indicative of the fertilizing potential of spermatozoa both *in vivo* and *in vitro*. It can also be used to examine the capacity of spermatozoa for preservation in addition to the standard semen analysis procedures. Hypo-osmotic swelling test for canine sperm was reported by England and Plummer (1993) and Kumi-Diaka (1993)after incubating the canine semen in 60-mOsm fructose solution for 45 min at 37°C. Pinto and Kozink (2008) reported that there was no significant difference in percentage of HOST-reacted canine sperm between 1 and 60 minutes of incubation in 100 mmol sucrose solution.

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But since there was no report on hypo-osmotic swelling of LR dog sperm preserved in different extenders under refrigeration condition, the same was carried out in the present work so as to find an extender that could maintain the integrity of canine sperm plasma membrane required for successful artificial insemination (A.I.).

MATERIALS AND METHODS

Six ejaculates from each of three adult Labrador Retriever dogs were collected by digital manipulation method and first and second fractions of the ejaculates were collected in a single collection cup. The semen samples were then extended @ 1:4 in Tris-Egg Yolk-Citric Acid-Glucose (TEYCAG) (Vestergen *et al.*, 2005), Tris-Egg Yolk- Citric Acid-Fructose (TEYCAF) (Foote, 1970), and Egg Yolk-Citrate-Glycine-Glucose (EYCGG) (Foote and Leonard, 1964) extenders by split sample technique. The extended semen samples preserved at 5°C for 24, 48, 72, 96 and 120 hours were subjected to hypo-osmotic solution at room temperature. The functional integrity of the sperm membrane was studied by using a Hypo-Osmotic solution as per the method described by Jeyendran *et al.* (1984).

Composition of Hypo-osmotic Solution (150 mOsm /L osmolality)

Sodium Citrate Dihydrate	0.735 g
Fructose	1.351 g
Double glass distilled water ad	100 ml

Preserved semen was incubated in the hypo-osmotic solution for 1 minute. A total of 200 spermatozoa were examined in different fields at a magnification of 400X using a phase contrast microscope for determining the status of sperm swelling. Statistical analysis of the data was done following Snedecor and Cochran (1994).

RESULTS AND DISCUSSION

The mean values of HOST-reacted sperm preserved for 24, 48, 72, 96 and 120 hours in the extenders are presented in Table.

The figures recorded in the present study in TEYCAG and TEYCAF extenders during 24 to 96 hours of preservation were in agreement with that reported by Rota *et al.*(1995) for sperm intact membrane in the pooled semen of different dogs when preserved at 4°C for the same duration using egg-yolk- tris extender. The present finding in TEYCAG extender at 24, 48, 72 and 96 hours of preservation was found to be much higher than that observed by for sperm membrane integrity Varela Junior *et al.* (2009) in the semen of Cocker Spaniel and German Shepherd dogs preserved in Tris glucose plus 20 per cent egg yolk at 5°C during the corresponding period. The discrepancies in findings might be attributed to the variations in breed and age of the dogs, seasons, preservation temperature, quality of extender components and method of evaluation.

In the present study it was observed that there was significant ($P<0.01$) difference in mean HOST-reacted sperm between extenders and between preservation periods. However, the interaction between extender and preservation period was not statistically significant. This indicated that the main effects were independent. In the present study the percentage of HOST-reacted sperm did not differ significantly between TEYCAG, TEYCAF and EYCGG extenders at all hours of preservation. Although non-significant, the percentage of HOST-reacted sperm was higher in TEYCAG than in TEYCAF extender at 120 hours of preservation. Per cent HOST-reacted spermatozoa did not differ significantly between TEYCAG and TEYCAF extenders irrespective of preservation period. This might suggest that the two extenders were equally efficacious in providing functional integrity of the plasma membrane of spermatozoa during preservation. However, the percentage of HOST-reacted sperm was somewhat higher in TEYCAG than in TEYCAF extender during 24 to 96 hours of preservation. The mean percentage of HOST-reacted sperm was significantly ($P<0.05$) lower in EYCGG extender as compared to that in TEYCAG and TEYCAF extenders irrespective of hour of preservation. This might indicate that the percentage of spermatozoa with an intact membrane

decreased in EYCGG extender consequential to its impaired protection of spermatozoan membrane. Differential action of the extenders in shielding sperm membrane in dog semen exposed to hypo-osmotic solution has been documented. Rota *et al.* (1995) reported that integrity of plasma membrane of spermatozoa was better maintained in Egg yolk-Tris extender than in Egg yolk-milk and Egg yolk-cream extender when dog semen preserved at 4°C was subjected to hypo-osmotic swelling test.

The incidence of HOST-reacted sperm during 48 to 120 hours of preservation decreased significantly ($P<0.05$) from that at 24 hours of preservation irrespective of extender. This suggested that the integrity of plasma membrane and functional intactness of spermatozoa decreased with increase in preservation period. Rota *et al.* (1995) also recorded that the percentage of swollen canine sperm *i.e.*, with an intact membrane decreased over time when dog semen preserved in Egg yolk-Tris, Egg yolk-milk and Egg yolk- cream extender at 4°C underwent hypo-osmotic swelling test. It was revealed in the present investigation that although the HOST-reacted spermatozoa differed significantly between preservation periods, it did not differ significantly between each successive hour of observation during preservation at 5°C irrespective of extender. The percentage of HOST-reacted sperm was significantly ($P<0.05$) lower at 96 and 120 hours of storage as compared to that at 48 hours of preservation and it was also significantly ($P<0.05$) lower at 120 hours of preservation than that at 72 hours of storage. This might imply that the decline in percentage of HOST-reacted sperm during preservation was not abrupt but rather gradual and the reaction of sperm plasma membrane to hypo-osmotic solution diminished gradually with prolongation of preservation period.

It could be concluded that Labrador Retrieveer dog semen extended in TEYCAG and TEYCAF extenders were equally efficacious in providing functional integrity of the plasma membrane of dog spermatozoa during preservation that was necessary for use of successful AI.

Table: Incidence (%) of HOST-reacted Labrador Retriever dog spermatozoa (mean ± S.E.) in different extenders during different hours of preservation at 5° C

Extender	Preservation period (hour)					
	24	48	72	96	120	Overall
TEYCAG	93.44± 1.92	87.99± 4.35	85.03± 4.23	82.20± 4.42	79.51± 4.25	85.63^{a}± 1.87
TEYCAF	92.20± 1.72	85.56± 3.82	82.25± 4.32	78.75± 5.17	70.00± 3.63	81.75^{a}± 2.12
EYCGG	85.65± 3.01	75.34± 6.86	68.88± 7.46	59.33± 5.10	52.50± 3.71	68.34^{b}± 3.15
OVERALL	90.43^{a}± 1.49	82.97^{bc}± 3.11	78.72^{cd}± 3.46	73.43^{de}± 3.61	67.34^{e}± 3.43	

^{a,b,c,d,e}Means having at least one letter superscript in common do not differ significantly within column and within row.

REFERENCES

England, G.C. and Plummer, G.C. (1993). Hypo-osmotic swelling of dogspermatozoa. *J. Reprod. Fertil. Suppl.*, **47:** 261–270.

Foote, R.H. and Leonard, E.P. (1964). The influences of pH, osmotic pressure, glycine, and glycerol on the survival of dog sperm in buffered yolk extenders. *Cornell Vet.,* **54:** 78-89.

Jeyendran, R.S., Van-der-Ven, H.H., Perez-Pelaez, M., Crabo, B.G. and Zaneveld, L.J.(1984). Development of an assay to assess the functional integrity of the human spermmembrane and its relationship to other semen characteristics. *J. Reprod. Fert.,* **70:** 219-28.

Kumi-Diaka, J. (1993). Subjecting the caninespermatozoa to the hypoosmotic swelling test. *Theriogenology*, **39:** 1279–1289.

Pinto, C.R.F. and Kozink, D.M. (2008). Simplified hypoosmotic swelling test (HOST) of fresh and frozen-thawed canine spermatozoa. *Anim. Reprod. Sci.*, **104:** 450-455.

Rota, A., Strom, B. and Linde-Frosberg, C. (1995). Effects of Seminal Plasma and Three Extenders on canine semen stored at 4°C. Swedish Univ. Agric. Sci., Uppsala, pp. 885-900.

Snedecor, G.W. and Cochran, W.G. (1994). Statistical Methods, 8th Edn, Iowa State University Press, Ames, Iowa.

Varela Junior, A.S., Corcini, C.D., Ulgium, R.R., Alvarenga, M.V.F., Bianchi, I., Correa, M.N., Lucia Jr., T. and Deschamps, J.C. (2009). Effect of low density lipoprotein on the quality of cryopreserved dog semen. *Anim. Reprod. Sci.*, **115**: 323-327.

Verstegen, J.P., Onclin, K. and Iguer-Ouada, M. (2005). Long-term motility and fertility conservation of chilled canine semen using egg yolk added Tris-glucose extender: In vitro and in vivo studies. *Theriogenology*, **64**: 720-733.