

Original Research Article

<https://doi.org/10.20546/ijcmas.2017.612.106>

Effect of Different Extenders on the Quality of Mongrel Dog Semen Preserved at 5°C on the Basis of Hypo-Osmotic Sperm Swelling Test (HOSST)

Amy Zorinkimi*, F.A. Ahmed and K. Lalrintluanga

Department of Animal Reproduction, Gynaecology and Obstetrics, College of Veterinary Sciences and Animal Husbandry, Central Agricultural University, Selesih, Aizawl, Mizoram – 796014, India

*Corresponding author

ABSTRACT

Keywords

Extenders, Semen quality, Chilled temperature, HOSST.

Article Info

Accepted:
10 October 2017
Available Online:
10 December 2017

Semen extenders are diluent added to semen to preserve its fertilizing ability and also act as buffers that protects the sperm cells from their own toxic byproducts, cold shock and osmotic shock during the chilling and as a source of nutrition. The study was undertaken to compare the effect of 3 different extenders viz., BTS, CDE and TRIS on preserved mongrel dog semen at chilled temperature upto 72 hours. A total of 21 ejaculates were utilized to study the effects of these 3 extenders on semen quality on the basis of hypo-osmotic sperm swelling test. The overall HOSST-reacted sperm was found to be significantly ($P<0.01$) higher in CDE and TRIS extenders than in BTS extender.

Introduction

Dogs were the first species of animals in which semen was collected, artificial insemination performed and off springs obtained successfully by Spallanzani in 1776 and even though no other domesticated animal has had such a long history of close relationship with humans as the dog (Wayne and Vila, 2001), still very little work has been done till date for the evaluation and preservation of dog's semen to fully exploit the genetic potential of the male animals. It was only in the year 1954 that the first successful A.I. using chilled dog semen was reported by Harrop and that the use of frozen-thawed semen was reported by Seager

in 1969. Preserved frozen semen is inseminated routinely in cattle, sheep and goats and much research has also been carried out in these species.

However, there is limited use of frozen dog semen, owing mainly to fertility when compared with fresh or chilled semen and also, to some extent, owing to the regulations controlling the use of artificial insemination.

The use of frozen semen has, therefore, been limited to inter-continental transfer of genetic material and long-term storage of spermatozoa (Srivastava and Mathur, 2011).

Chilled extended semen has been used more increasingly than frozen ones for artificial insemination in canine. Different types of semen extenders have been evaluated for their capability to keep chilled dog semen motile over time (Bouchard *et al.*, 1990; Province *et al.*, 1984). Extenders for chilled semen usually contain egg yolk (EY) that has an important role in protecting sperm cells from cold-shock (Beccaglia *et al.*, 2008). It is impossible to have fertilization with a physically inactive membrane as some physiological processes during fertilization (capacitation, acrosomal reaction, fusion of sperm and ovum) demand an active membrane (Jeyendran *et al.*, 1984). However, fertilization of oocyte will not occur if the sperm membrane is biochemically inactive, even if it remains structurally intact (Dobranic *et al.*, 2005). The HOS test is therefore a better indicator of fertilization potential than supravital staining (Tamuli and Watson, 1992).

Non-descript or no definable type or breed of dogs are said to be mongrel dogs. In this work, mongrel dogs found in Mizoram are used for the experiment.

Materials and Methods

A total of 21 semen ejaculates obtained from three mongrel dogs were used to study the effect of three extenders on quality of semen during preservation for upto 72 hours. Each ejaculates was split into three equal parts using split sample technique and kept in three different vials and extended (1:5) with Beltsville Thawing Solution (BTS), Citrate Dextrose Egg yolk extender (CDE) and Tris-buffered egg yolk extender (TRIS). The extended semen was preserved at 5°C in a refrigerator for upto 72 hours. The preserved semen was examined for progressive motility, live sperm, intact acrosome and HOSST-reaction at 0, 24, 48 and 72 hours of preservation.

Collection of semen

Collection of semen from dogs was done by digital manipulation following the method described by Christensen (1984) with slight modifications. Semen was collected from individual dogs without using an oestrous bitch as a teaser, in a glass graduated tube. With the gloved hand (using a non-latex glove), initial friction movements are performed and the penile sheath is gently pulled back behind the bulbusglandis. As soon as penile erection starts, a constant pressure is maintained caudal to the bulbus with the fingers encircling a penis like a ring at this level. Erection and eventually ejaculation is achieved. Ejaculation started in dogs within a minute of erection of the penis. The first fraction of the ejaculate was clear and watery with few or no spermatozoa. The second fraction was opaque, viscous and milky white and is sperm-rich. In the present study, the first and second fractions of the ejaculate were collected in the same tube and is used for the test acrosome and HOSST-reaction at 0, 24, 48 and 72 hours of preservation.

Preparation of extenders

The extenders were prepared before use. Aliquot buffers used in all the three extenders were prepared beforehand separately and kept in a refrigerator at 5° C. Egg yolk in CDE and TRIS and antibiotics in each of the extenders were added just before use.

Composition of the extenders used

Beltsville Thawing Solution (BTS)

Composition:

- a) Glucose- D 3.715 g
- b) EDTA disodium salt 0.6g
- c) Sodium Hydrogen Carbonate 0.125 g
- d) Potassium chloride 0.125 g

- e) Distilled water ad 100 ml
- f) Penicillin 1,00,000 I.U
- g) Streptomycin 100 mg

pH was adjusted to 6.8 using 5% citric acid or N/10 NaOH solution, using a pH meter.

Citrate dextrose egg-yolk extender (CDE)

Composition:

- a) Sodium citrate 1.45 g
- b) Dextrose 1.25 g
- c) Egg yolk 20 ml
- d) Distilled water ad 100 ml
- e) Penicillin 1,00,000 I.U
- f) Streptomycin 100 mg

pH was adjusted to 6.8 using 5% citric acid or N/10 NaOH solution, using a pH meter.

Tris-buffered egg-yolk extender (TRIS)

Composition:

- a) Tris 2.4 g
- b) Citric acid monohydrate 1.3 g
- c) Fructose 1.0 g
- d) Egg yolk 20 ml
- e) Distilled water ad 100 ml
- f) Penicillin 1,00,000 I.U
- g) Streptomycin 100 mg

pH was adjusted to 6.8 using 5% citric acid or N/10 NaOH solution, using a pH meter.

Composition of hypo-osmotic solution (100 mOsm/L osmolality)

- Trisodium Citrate 0.49 g
- Fructose 0.99 g
- Double glass distilled water ad 100 ml

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.

Results and Discussion

The mean percentage of HOSST-reacted sperm was 91.95 ± 3.57 , 92.80 ± 3.51 and 92.28 ± 4.35 per cent at 0 hour; 87.77 ± 4.57 , 89.93 ± 3.89 and 90.15 ± 4.24 per cent at 24 hours; 80.68 ± 7.30 , 85.52 ± 3.37 and 86.66 ± 3.92 per cent at 48 hours; and 66.51 ± 24.26 , 81.66 ± 3.61 and 83.37 ± 3.97 per cent at 72 hours of preservation in BTS, CDE and TRIS extenders respectively.

Analysis of variance revealed that the mean HOSST-reacted sperm differed significantly ($P < 0.01$) between extenders and between preservation periods.

The interaction between extender and preservation period also differed significantly ($P < 0.01$) in respect of HOSST-reacted sperm.

It was observed that the overall mean percentage of HOSST-reacted sperm differs significantly ($P < 0.05$) between BTS and CDE, but the mean percentage of HOSST-reacted sperm did not differ significantly ($P < 0.05$) between CDE and TRIS extenders.

The figures recorded in the present study during 24 to 72 hours of preservation were in agreement with that reported by Rota *et al.*, (1995) in the pool semen of German Shepherds, Bernese mountain, Flat-coated Retriever, Labrador Retriever, Rottweiler, Hovawart and crossbred dogs when preserved at 4°C for the same duration using egg-yolk-tris extender, Das (2012) in Labrador Retriever dogs Using TEYCAF and TEYCAF extenders.

However, the present values were found to be much higher than that observed by Varela Junior *et al.*, (2009) in the semen of Cocker Spaniel and German Shepherd dogs preserved in Tris-glucose plus 20 percent egg yolk at 5°C during the corresponding period.

The over-all mean percentage of HOSST-reacted sperm in BTS differs significantly ($P < 0.05$) from CDE, but there was no significant difference between CDE and TRIS.

In the present study, there is significant ($P < 0.01$) difference between extenders, between preservation periods and due to interactions.

This indicated that the main effects were not independent. Therefore, CDE and TRIS extenders are considered to be better choice for preservation of mongrel dog semen at chilled temperature (5°C) on the basis of HOSST than that of BTS.

References

- Beccaglia, M., Anastasi, P., Chigioni, S. and Luvoni, G. C. (2008). Tris-Lecithin extender supplemented with antioxidant catalase for chilling of canine semen. 6th *Int. Symp. Can. and Fel. Reprod.*
- Bouchard, G. F., Morris, J. K., Sikes, J. D., Youngquist, R. S. (1990). Effects of storage temperature, cooling rates, and two different semen extenders on canine spermatozoal motility. *Theriogenology*; 34: 147-157
- Das, A. (2012). Seminal attributes and preservation of dog semen at liquid state. MVSc Thesis, College of Veterinary Sciences and Animal Husbandry, Assam Agricultural University.
- Dobranic, T., Samardzija, M., Cergolj, M. and Prvanovic, N. (2005). Determination of membrane integrity of canine spermatozoa. *Vet. Arhiv*; 75(1): 23-30.
- 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 sperm membrane and its relationship to other semen characteristics. *J. Reprod. Fert.* 70: 219-28.
- Ponglowhapan, S., Essen-Gustavsson, B. and Linde Frosberg, C. (2004). Influence of glucose and fructose in the extender during long-term storage of chilled canine semen. *Theriogenology*. 62: 1498-1517.
- Province, C. A., Amann, R. P., Pickett, B.W. and Squires, E. L. (1984). Extenders for preservation of canine and equine spermatozoa at 5°C . *Theriogenology*. 22: 409-415.
- Rota, A., Strom, B., Linde-Forsberg, C. (1995). Effects of seminal plasma and three extenders on canine semen stored at 4°C . *Theriogenology*; 44:885-900.
- Srivastava, A. K. and Mathur, A.K. (2011). Recent advances in the area of canine semen preservation. *Ind J. Can Pract*; 3(1): 21-28.
- Tamuli, M. K., Watson, P. F. (1992). Effect of temperature of incubation on the development of resistance to cold stress and hypo osmotic stress in boar spermatozoa incubated for up to 24 hours. *Proc. 12th Int. Cong. Anim. Reprod*; pp. 1484-1486.
- Varela Junior, A.S., Corcini, E.D., Ulgium, R.R., Alvarenga, M.V.F., Bianchi, I., Correa, M.N., Lucia Jr., T. and Deschamps, J.E. (2009). Effect of low density lipoprotein on the quality of cryopreserved dog semen. *Anim. Reprod. Sci.* 115: 323-327.
- Wayne, R. K. and Vila, C. (2001): Phylogeny and Origin of the domestic dog. *In: The Genetics of the Dog*. CABI Publishing. pp. 1-3.

How to cite this article:

Amy Zorinkimi, F.A. Ahmed and Lalrintluanga, K. 2017. Effect of Different Extenders on the Quality of Mongrel Dog Semen Preserved at 5°C on the Basis of Hypo-Osmotic Sperm Swelling Test (HOSST). *Int.J.Curr.Microbiol.App.Sci*. 6(12): 961-964.
doi: <https://doi.org/10.20546/ijcmas.2017.612.106>