Short Communication

LDL SUPPLEMENTATION ON VITAL CHARACTERISTICS OF CRYOPRESERVED CANINE SEMEN

S. Rajput[1], N. Arunmozhi*[2], S. Rangasamy[3], T.A. Kannan[4], S.Rajalakshmi[5] and P. Sridevi[6]

Department of Veterinary Gynaecology and Obstetrics
Madras Veterinary College
Tamil Nadu Veterinary and Animal Sciences University
Chennai, TamilNadu, India

ABSTRACT

The present study was aimed at studying the effect of Low-density lipoprotein (LDL) on vital characteristics of canine spermatozoa when compared to semen extended with egg yolk (EY). Sixteen dogs aged between 2-6 years with body condition score of 4-5 on a scale of 0-9 were selected. Semen was collected by digital manipulation and was subjected to pre freeze evaluation. After pre freeze evaluation semen was diluted and cryopreserved by conventional method. Post-thaw evaluation at 24 h revealed highly significant differences in mean percentages of progressive motility, viability, plasma membrane integrity, acrosomal integrity and mitochondrial membrane potential between the semen extended with 20 per cent EY and 8 per cent LDL. However, no significant difference was observed in the mean percentage of sperms showing DNA integrity between the semen extended with 20 per cent EY and 8 per cent LDL at 24 hours post-thaw. This might be due to the detrimental effects of EY on spermatozoa during the process of cryopreservation. It was concluded that LDL has beneficial effects and minimizes the damages caused to spermatozoa during cryopreservation.

Keywords: Canine semen, Cryopreservation, LDL, Vital characteristics

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Canine breeding has gained more importance during the recent years. The demand for cryopreservation of semen from valuable studs is increasing. However, cryopreservation of semen causes remarkable decrease in quality of semen due to osmotic stress (Chatterjee and Gagnon, 2001). Egg yolk (EY) is the compound most commonly used in semen extenders for protection against cold shock and disruption during freezing and thawing of spermatozoa. Low Density Lipoprotein (LDL), a major component of egg yolk has been reported to have a cryoprotective effect. Substances other than LDL also are reported to be present in egg yolk which contribute towards the diminished motility and inhibition of sperm cell respiration.

*Corresponding author, email: drarunmozhivet@gmail.com
[1]Postgraduate student
[2]Assistant Professor
[3]Assistant Professor
[4]Professor and Head, Education Cell
[5]Assistant Professor, Department of Veterinary Microbiology
[6]Professor and Head (Retd.)

These arguments concerning the presence of cryoprotective antagonists in egg yolk has reinforced interest in the use of LDL extracted from egg yolk in the extender rather than complete egg yolk (Bencharif *et al*., 2008). With this background, the present study was carried out to compare the morphological and functional parameters in frozen thawed canine semen extended with EY and LDL.

The present study was conducted in 16 male dogs of different breeds aged between 2-6 years with a body condition score of 4-5 on a scale of 0-9 and body weight between 15-50 kg. The ejaculate from each dog was collected by digital manipulation and the sperm rich fraction was subjected to physical evaluation including volume, colour, consistency, Gross / initial motility, individual/progressive motility, sperm concentration, viability and sperm abnormalities. Following which the sperm rich fraction was diluted in the ratio of 1:1 or 1:2 depending on the sperm concentration with TRIS-glucose bound extender containing 20 per cent egg yolk in trial 1 and with TRIS-glucose bound extender containing 8 per cent low-density lipoprotein (LDL) in trial 2. LDL was extracted as per the procedure described by Moussa *et al.*, (2002). Then cryopreservation was done as per conventional method. The effect of LDL supplementation on progressive motility, viability, plasma membrane integrity, acrosome membrane integrity, DNA integrity and mitochondrial membrane potential at 24 hours post-thaw were evaluated and compared with Tris-egg yolk extender.

Percentage of progressive motile spermatozoa was assessed by the procedure described by Rota *et al*. (1995). The percentage of live spermatozoa was calculated by the method described by Johnston *et al*. (2001). Eosin – Nigrosin staining method was adopted for estimation of live and dead spermatozoa percentage in the semen sample. Hypo-osmotic sperm swelling (HOS) test is a semi-quantitative test involving submersion of spermatozoa into hypo-osmotic solution (Jeyendran *et al*., 1984). Integrity of sperm DNA was assessed by using Acridine orange staining as reported by Chohan *et al*., (2004). Acrosomal integrity of sperm cells was evaluated by using Giemsa stain. Mitochondrial membrane potential of the sperm cells was assessed by using a cationic carbocyanine dye called JC-1 (Selvaraju *et al*., 2008).

The accumulated data was analyzed by unpaired t-test as per Snedecor and Cochran (1994) using SPSS computer program (Version 20.0; SPSS Co., Chicago, IL, USA). The mean value of $P<0.05$ was considered statistically significant and $P<0.01$ was considered highly significant and $P>0.05$ was considered non-significant.

The semen samples diluted with EY and LDL extender were subjected to cryopreservation and evaluated for progressive motility, viability, plasma membrane integrity, acrosomal integrity, DNA integrity and mitochondrial membrane potential at 24 hours post thaw and presented in Table.

Progressive motility

The mean percentage of progressively motile sperms presented in this study were 44.81 ± 0.84 and 54.44 ± 1.16 for semen added

with EY and LDL extender, respectively, at 24 h post thaw, and the differences was statistically significant (P<0.01). Belala *et al*. (2016) compared between egg yolk plasma (EYP) and LDL extenders and reported non-significant difference in post thaw motility between two extenders in canine semen. The highly significant difference (P<0.01) obtained in the present study might be due to high concentration of LDL used and use of EY (including cryoprotective antagonists) instead of EYP. LDL attenuates the toxicity of glycerol during cryopreservation. The significant difference between EY and LDL might also be due to the endotoxins produced by egg yolk damaging the spermatozoa structure and function (Akhter *et al*., 2011)

Viability

The mean percentage of viable spermatozoa evaluated in the present study were 47.19 ± 1.06 and 55.25 ± 0.81 per cent for semen extended with EY and LDL, respectively, at 24 h post thaw and the differences were statistically significant (P<0.01). The significant difference recorded in the present study might be due to the fact that LDL adheres to cell membrane during freezing and thawing process and preserves the membrane integrity (Bergeron *et al*., 2004).

Table. Morphological and functional characteristics of spermatozoa with EY and LDL extender at 24 h post-thaw (Mean ± SE)

Variables	Tris-Glucose bound extender containing 20 % egg yolk (EY) (n=16)	Tris-Glucose bound extender containing 8 % low-density lipoprotein (LDL) (n=16)	t-test	P- Value	Signifi-cance
Progressive motility	44.81 ± 0.84	54.44 ± 1.16	6.67	0.0000	**
Live percentage of spermatozoa	47.19 ± 1.06	55.25 ± 0.81	6.01	0.0000	**
Plasma membrane integrity	54.88 ± 1.39	60.94 ± 0.96	3.57	0.0012	**
Acrosomal integrity	49.81 ± 1.27	58.31 ± 1.02	5.18	0.0000	**
DNA Integrity	89.56 ± 0.63	91.38 ± 0.63	2.03	0.0515	NS
Mitochondrial membrane potential (MMP)	44.69 ± 0.68	52.63 ± 0.46	9.63	0.0000	**

** Significant (P<0.01); NS- Not significant (P>0.05)

Plasma Membrane Integrity by Hypo-Osmotic Swelling Test (HOST)

The mean percentage of spermatozoa showing plasma membrane integrity in the present study were 54.88 ± 1.39 and 60.94 ± 0.96 per cent for semen extended with EY and LDL, respectively, at 24 h post thaw and the difference was statistically significant (P<0.01). The significant results can be explained by the fact that LDL replaces the phospholipids lost or damaged by thermal shock and also reduces the transition temperature phase of sperm membrane (Bencharif and Dordas-Perpinya, 2020)

Acrosomal Integrity

The mean percentage of acrosomal integrity of spermatozoa recorded in the present study were 49.81 ± 1.27 and 58.31 ± 1.02 per cent for semen added with EY and LDL, respectively and the difference was statistically significant (P<0.01) which were in accordance with Prapaiwan *et al*., (2016), who has also reported significant difference between the two extenders.

DNA Integrity

The mean percentage of DNA integrity of spermatozoa in the present study were 89.56 ± 0.63 and 91.38 ± 0.63 per cent for semen added with EY and LDL, respectively at 24 h post thaw but the difference was statistically non-significant. No statistical difference was observed in the present study between EY and LDL extenders because the sperm nucleus has more stable packing and highly condensed DNA organization (Yanagimachi, 1994).

Mitochondrial membrane potential (MMP)

The mean percentage of mitochondrial membrane potential of spermatozoa in the present study were 44.69 ± 0.68 and 52.63 ± 0.46 per cent for semen extended with EY and LDL, respectively, at 24 h post thaw and the difference was statistically significant (p<0.01). As LDL has potential advantages like protecting spermatic cells by their incorporation within plasma membrane and replacing loss or damaged phospholipids due to thermal shock and also reduces transition temperature phase of plasma membrane. In addition, LDL also forms a protective film on the surface of plasma membrane thus providing stability during cryopreservation (Bencharif and Dordas-Perpinya, 2020).

To conclude, addition of LDL at 8 per cent concentration in Tris-glucose bound extender increases motility and survivability of frozen-thawed canine spermatozoa by forming a protective film on the surface of plasma membrane thus providing stability during cryopreservation.

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