The Haryana Veterinarian (March, 2024) **63(SI),** 22-25 ISSN 0033-4359 (P) **Research Article**

COMPARATIVE STUDY OF TRIS-EGG YOLK-GLUCOSE AND COCONUT WATER-BASED EXTENDERS FOR COOLED STORAGE (4º C) OF CANINE SEMEN

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ABSTRACT

Four ejaculates per dog were collected by digital manipulation to obtain 24 ejaculates. All fresh ejaculates were evaluated for macroscopic and microscopic semen evaluation tests. The semen samples were collected and extended at the ratio of 1:4 in TRIS-Egg yolk-glucose and Coconut water-based extenders by split sample technique. The results indicated that individual sperm motility percentage is better in TRIS-Egg yolk-glucose than Coconut water-based extender. Live sperm percentage between TRIS-Egg yolk-glucose dilutor and Coconut water-based extender non-significant at 0, 24 and 48 hours but significant difference at 72 hours. The abnormal percentage was significantly ($p<0.05$) lower in TRIS-Egg yolk-glucose dilutor at 24, 48 and 72 hours of preservation. In TRIS-Egg yolk-glucose, there was a non-significant difference in intact plasma membrane percentage within the group at 0, 24 hours and a significant difference at 48, 72 hours. In Coconut water-based extender group there was a significant difference in intact plasma membrane percentage at 0, 24, 48 and 72 hours.

Keywords: Canine semen, Cooled storage, Coconut water-based, Tris egg yolk glucose

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Egg yolk is the principal constituents of dilutors which protects the sperm by preventing LDL from sequestering lipid-binding proteins and improve all sperm motility in canine sperm (Iguer-Ouada, 2001). An another alternative in form of coconut water, is inexpensive and easily prepared enriched with complex organic molecules utilised for artificial insemination programs in several species (Barros and Toniolli, 2011). During cooling, temperature change induces stress on sperm membranes which causes phase changes in lipids and altered functional state of sperm membranes (Dalal *et al.*, 2018; Arjun *et al.*, 2022). The urge to replace the traditional employed egg yolk in extenders is brought on by the existence of an avian influenza outbreak (Abe *et al.*, 2008) and egg yolk impacted by microbial contamination. Therefore, the aim of the present investigation is to carry out a comparative study of tris-egg yolk-glucose and coconut water-based extenders for cooled storage (4º C) of canine semen.

MATERIALS AND METHODS

In present investigation, total 24 ejaculates sperm rich fraction @4 ejaculates/dog were collected aseptically from six privately owned healthy adultaged 01-02 years old large breed trained dogs at weekly interval by digital manipulation methods.

Experimental design: After the initial fresh macroscopic and microscopic evaluation of semen, the semen samples were extended at the ratio of 1:4 into groups TRIS-Egg yolk-glucose and Coconut water-based extenders by split sample technique (Das *et al.*, 2018). Various microscopic semen evaluation tests were carried out at 0, 24, 48 and 72 hours interval by keeping diluted sample at 4ºC refrigerator temperature.

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Statistical Analysis

Data obtained were subjected to analysis by one-way analysis of variance technique (Snedecor and Cochran, 1989) using the statistical package SPSS software 20 version.

RESULTS AND DISCUSSION

Evaluation of fresh semen

Macroscopic and microscopic characteristics of fresh semen: In this study, the overall mean±SE of reaction time and ejaculation time were 90.02±5.73 seconds and 207.30±9.74 seconds, recorded, respectively. The average volume (ml), colour, consistency and pH were 2.17±0.16, milky white, thick, and 6.35±0.03, respectively. The mass motility, individual motility, live sperm percentage, percentage of morphologically abnormal spermatozoa and total sperm concentration were 3.91±0.15, 86.45±0.97%, 87.37±0.98%, 9.08±0.25% and 353.89±11.1 million/ml, respectively for fresh semen. The variation in various findings by many authors Zorinkimi *et al.* (2017b), Shalini and Antoine (2018) may be owed to differences in body conformation, volume of second fraction and methods of semen collection.

Sperm function test of fresh semen: In present findings, fresh semen samples have the sperm with intact plasma membrane percentage assessed by HOST was 89±0.94% with a range of 80-95%. A lower and higher percentage of

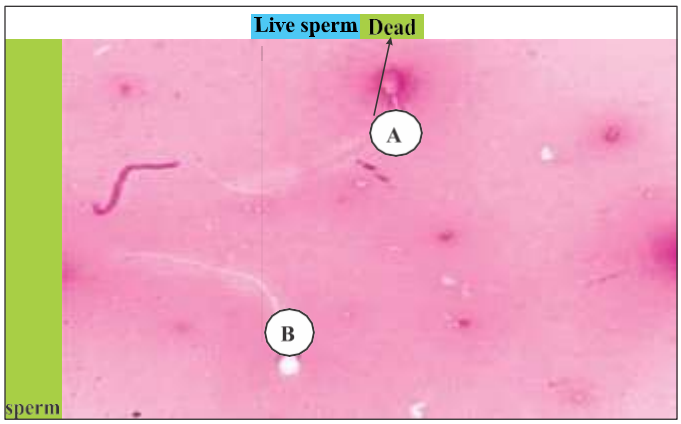

Fig. 1. A-Dead sperm (Pink coloured), B-Live sperm (White coloured)

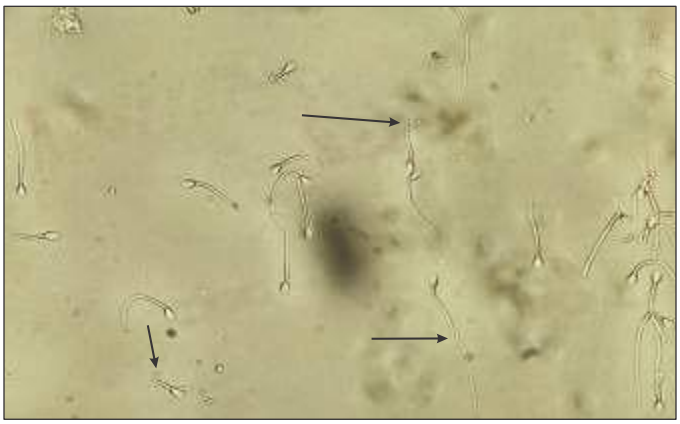

Fig. 2. Sperm with curling tail as sperm having intact plasma membrane (40X)

Fig. 3. Double Heads

Fig. 4. Bend Tail

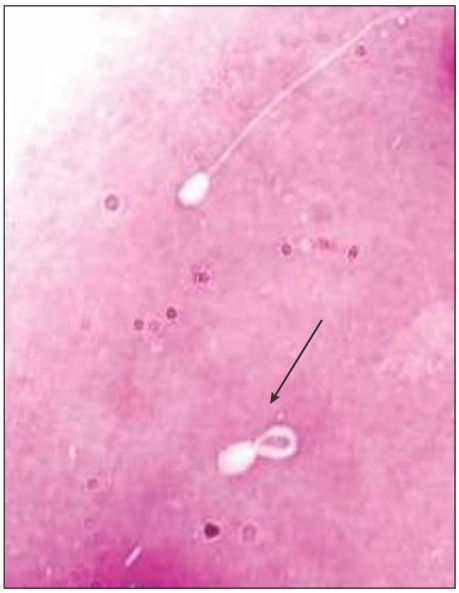

Fig. 5. Dag defect

Fig. 6. Proximal Protoplasmic Droplets

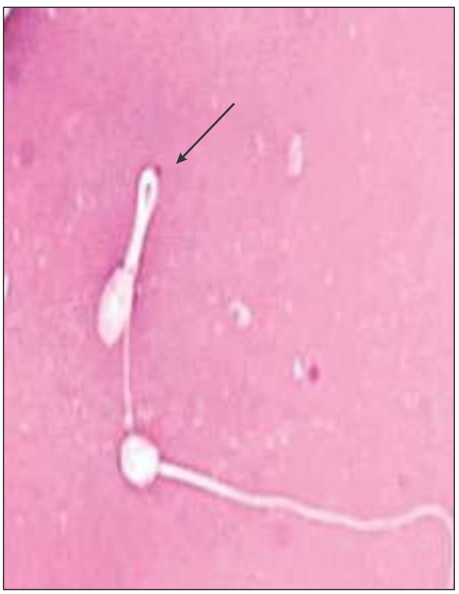

Fig. 7. Bent tail

Fig. 8. Coiled tail

intact plasma membrane was reported by Ray *et al.* (2019) and Arunmozhi *et al.* (2021), respectively.

Evaluation of Preserved Semen (4°C):

Microscopic Evaluation:

Individual sperm motility: There was a significant ($p<0.05$) and non-significant difference observed at different time intervals of preservation within both groups and between the groups, respectively. At 0 hours, In TRIS egg yolk, similar observations were made by Sanchez *et al.* (2006) and Michael *et al.* (2009) where as higher observations were reported by Iguer-ouada and Verstegen (2001). In Coconut water-based extender, similar observations were made by Gunawan *et al.* (2016) whereas higher observations were reported by Puja *et al.* (2018) which could be due to variations in individual, age, breed and environment. At 24 hours, In TRIS-Egg yolk glucose, almost similar observations were made by Ponglowhapan *et al.* (2004) and in Coconut water-based extender similar observations were made by Gunawan *et al.* (2016). At 48 hours, initial motility drops in TRIS-Egg yolk glucose dilutor which was agreement with Bouchard *et al.* (1990). However, higher observations for Tris egg yolk were reported by Sanchez *et al.* (2006) and in Coconut water-based extender by Puja *et al.* (2018). At 72 hours, the motility further dropped in TRIS-Egg yolk glucose dilutor which was in agreement with Bouchard *et al.* (1990) and Srinivas *et al.* (2022) and higher observations were reported by Sanchez *et al.* (2006) in TRIS-Egg yolk glucose dilutor. In Coconut water-based extender, higher observations were reported by Puja *et al.* (2018).

Percentage live spermatozoa: There was a significant ($p<0.05$) and non-significant difference observed at different time intervals of preservation within both groups and between the groups respectively at different time slots of preservation. However, there was a significant difference in live sperm percentage between groups at 72 hours. At 0 hour, In TRIS-Egg yolk glucose, almost similar observations were made by Sanchez *et al.* (2006). However, higher observations were reported by Michael *et al.* (2009), Das *et al.* (2018) and lower observations were reported by Srinivas *et al.* (2022). In Coconut water-based extender, higher observations were reported by Gunawan *et al.* (2016) and Puja *et al.* (2018). At 24 hours and 48 hours, In TRIS Egg yolk-glucose dilutor and Coconut water-based extender, higher observations were reported by Das *et al.* (2018) and Puja *et al.* (2018), respectively. However, lower observations at 24 hours were reported by Srinivas *et al.* (2022) in Tris egg yolk dilutor. There was a significant difference in live sperm percentage between both groups at 72 hours. In TRIS Egg yolk-glucose dilutor, however, higher observations were reported by Michael *et al.* (2009) and lower observations were reported by Srinivas *et al.* (2022). Higher observations were reported by Puja *et al.* (2018) in coconut water based extender.

Table 1. Composition of extenders

	Ingredient	Tris egg yolk Glucose extender (TEYC)	Coconut Water Based extender (CW)
Part A	Tris Hydroxy methyl amino methane	2.44 g	3.025 g
	Citric acid	1.36 g	1.7 g
	D-Glucose	0.82 g	0.18 g
	Sodium Penicillin	100.00 IU	0.65 mg/ml
	Streptomycin	100 mg	1 mg/ml
Part B		Egg yolk-20 ml	Coconut water-20 ml

Morphological abnormalities of spermatozoa: There was a significant difference ($p<0.05$) in abnormal sperm percentage at different time slots of preservation within the groups. The sperm abnormalities percentages were significantly higher in group II at 24, 48 and 72 hours of preservation but a non-significant difference was observed at 0 hour. At 0 hours, In TRIS-Egg yolk glucose, almost similar observations were made by Michael *et al.* (2009), higher observations were reported by Srinivas *et al.* (2022). Lower observations were reported by Das *et al.* (2018). At 0 hours, in Coconut water-based extender nearly similar observations were made by Vicente *et al.* (2018) in bulls. At 24 hours, in TRIS-Egg yolk-glucose higher observations were reported by Srinivas *et al.* (2022) but Das *et al.* (2018) reported lower observation and almost similar observation for 48 hours. In Coconut water based extender for 24 and 48 hours, nearly similar observations were made by Vicente *et al.* (2018) in Bali bull. At 72 hours, in TRIS-Egg yolk glucose, higher observations were reported by Srinivas *et al.* (2022) and nearly similar observations were made by Vicente *et al.* (2018) in Bali bull for Coconut water based extender.

Sperm Function Test: In between the groups, there was a significant difference ($p<0.05$) in intact plasma membrane percentage at 0, 24 and 72 hours of preservation but non-significant difference was noticed at 48 hours. Within the group, TRIS-Egg yolk-glucose there was a non-significant difference in intact plasma membrane percentage at 0, 24 hours and a significant difference in intact plasma membrane percentage at 48, 72 hours were observed. On the other hand, within Coconut water-based extender group there was a significant difference in intact plasma membrane percentage at different time slots (0, 24, 48 and 72 hours). At 0 hours, our findings for Tris egg yolk extender were in agreement with Zorinkimi *et al.* (2017a). However, higher and lower observations were reported by Michael *et al.* (2009) and by Sanchez *et al.* (2006), respectively. Nearly similar observations were made by El Nattat *et al.* (2017) in Coconut water-based extender. At 24 hours, almost similar observations were made by Zorinkimi *et al.* (2017a) for Tris egg yolk extender. For coconut water-based extender, similar observations were made by Cheema *et al.* (2021). At 48 hours and 72 hours,

Table 2. Comparisons of Microscopic Evaluated Parameters in two extenders Tris-egg yolk glucose extender (T) and Coconut water based extender (C) at different time intervals

Parameter (%)	Extender	0 hour	24 hours	48 hours	72 hours
Individual sperm motility	T	85.21 ± 1.145^{dA}	72.71 ± 2.128^{cA}	59.17 ± 3.48^{bA}	47.92 ± 3.958^{aA}
	C	84.09 ± 1.229^{dA}	69.46 ± 2.316^{cA}	58.34 ± 2.439^{bA}	44.17 ± 3.493^{aA}
Live spermatozoa	T	84.17 ± 1.111^{dA}	73.63 ± 0.978^{cA}	59.17 ± 1.113^{bA}	47.3 ± 1.212^{aA}
	C	86.75 ± 0.919^{dA}	71.38 ± 1.1^{cA}	57.8 ± 0.936^{bA}	43.5 ± 1.11^{aB}
Abnormal sperm	T	10.59 ± 0.282^{aA}	12.25 ± 0.271^{bA}	13.63 ± 0.268^{cA}	14.96 ± 0.298^{dA}
	C	11 ± 0.276^{aA}	13.25 ± 0.264^{cB}	14.96 ± 0.266^{dB}	16.88 ± 0.278^{eB}
Intact plasma membrane	T	88.8 ± 0.955^{cA}	85.21 ± 1.09^{cA}	79.84 ± 1.501^{bA}	73.75 ± 1.836^{aA}
	C	84.8 ± 0.899^{dB}	76.55 ± 1.427^{cB}	71.8 ± 1.294^{bA}	64.46 ± 1.643^{aB}

Mean values having different superscripts in row (A, B) and column (a, b, c, d, e) in differ significantly ($P \leq 0.05$)

intact plasma membrane percentage in TRIS-Egg yolk-glucose and Coconut water-based extender, almost nearly similar observations were made by Cheema *et al.* (2021), respectively. It is concluded that TRIS-Egg yolk-glucose is the better dilutor for canine semen to maintain sperm plasma membrane integrity.

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