SHORT COMMUNICATION

Effects of Coconut Water-Based Extender on Motility, Viability, Morphology and Membrane Integrity of Refrigerated (4°C) Canine Spermatozoa

Abhay Kumar Meena*, Chandra Shekher Saraswat, Sumit Prakash Yadav

Abstract

The objective of this study was to determine the effect of a coconut water-based extender on the quality parameters of dog semen (n=24 ejaculates) during refrigeration (4°C) preservation up to 72 h. Four ejaculates per dog were collected from 6 dogs by digital manipulation. All fresh ejaculates were evaluated for macroscopic and microscopic semen evaluation tests. The semen samples were then extended in a coconut water-based extender using a split sample technique. The diluted semen was kept in a refrigerator for preservation at 4°C and evaluated at 0, 24, 48, and 72 h for sperm quality parameters. The sperm motility significantly reduced over a period of time from 0 to 72 h. The live sperm percentage did not differ significantly at 0, 24 and 48 h of preservation. However, there was a significant drop in live sperm percentage at 72 h. The percent sperm abnormalities were significantly increased from 24, 48 to 72 h of preservation. In coconut water-based extender there was a significant difference in intact plasma membrane percentage at different time slots (0, 24, 48 and 72 h). It was concluded that coconut water-based extender can preserve canine sperm for up to 48 h while maintaining sperm motility and viability above 50%. Sperm abnormalities remained within 20% and plasma membrane integrity above 60% both were maintained up to 72 h of semen preservation.

Key words: Canine semen, Coconut water-based semen extender, Cooled storage

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Introduction

The popularity for proven breeds with known pedigrees, such as Labradors, German Shepherds, Beagles and Cocker Spaniels, has been demanding. Natural or planned mating can sometimes fail due to a number of factors (low libido to senility, musculoskeletal issues). Semen storage and artificial insemination for a brief period of time are required in such circumstances. Coconut water (*Cocos nucifera*) has antioxidant qualities (Mantena *et al.,* 2003) that are present in addition to essential elements like sugar, vitamins, minerals, potassium, magnesium, fibre and proteins (Silva and Bamunuarachchi, 2009).Coconut water seems to be suitable as a semen extender in canines due to its isotonic properties, being cheap, effective, and easy to use (Cardoso *et al.,* 2003). Tender coconut water was also reported to be a highly successful sperm extender for dogs (Cardoso *et al.,* 2003). Coconut water extender is adequate for preservation of canine semen at 37°C for 180 min (Uchoa *et al.,* 2002). Coconut water contains carbohydrates, amino acids, minerals, and vitamins, as well as cryoprotectants and antioxidants (Yong *et al.,* 2009). Coconut water is low in phospholipids and high in complex organic compounds such as proline, glycine, glutamic acid, and indole-acetic acid (IAA), which prolongs and protects the life of spermatozoa. Its molecular structure is reinforced by cell membrane protection (Nunes and Combarnous, 1995). This study was aimed to evaluate the effects of coconut water-based extender on motility, viability, morphology, and membrane integrity of canine spermatozoa at 0, 24, 48, and 72 h of preservation under refrigeration temperature (4°C).

Department of Veterinary Gynaecology and Obstetrics, Post-Graduate Institute of Veterinary Education and Research, Jaipur, Rajasthan University of Veterinary and Animal Sciences, Bikaner-334001 (Rajasthan), India

Corresponding Author: Abhay Kumar Meena, Department of Veterinary Gynaecology and Obstetrics, Post-Graduate Institute of Veterinary Education and Research, Jaipur, RAJUVAS, Bikaner-334001 India. e-mail: abhaykumarmeena007@gmail.com

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Materials and Methods

Semen was collected from 6 dogs (4 German shepherd, 1 Labrador and 1 Siberian husky) at weekly interval for a total of 24 ejaculates by digital manipulation. The fresh sperm-rich fraction was examined for macroscopic examination included volume, colour, consistency and pH, while the

microscopic examination included mass motility, individual sperm motility, sperm concentration, sperm abnormalities, live count and hypo-osmotic swelling test (HOST). Following the preliminary evaluation, the sperm-rich fraction of the sperm sample was diluted 1:4 in Coconut water-based extender at room temperature. The composition of extender contained Tris hydroxymethyl aminomethane 3.025 g, Citric acid 1.7 g, D-Glucose 0.18 g, Sodium Penicillin 0.65 mg/mL and Streptomycin 1 mg/mL in 100 mL DW, to which 20 mL Coconut water was added. The samples of extended semen were placed in a beaker filled with water at 37 °C, and then cooled to 4 °C in a refrigerator. At 0, 24, 48, and 72 h, diluted samples were tested for individual sperm motility, viability, abnormalities (Payan-Carreira *et al*., 2011), and the spermatozoa (%) with intact plasma membrane were determined using the HOST (Jayendran *et al.,* 1984).

Data were subjected to analysis by completely randomized design (CRD) by one way analysis of variance technique (Snedecor and Cochran, 1994) using the statistical package SPSS software 20 version. The means of different experimental groups were tested for statistical significance by Duncan's multiple range test.

Results and Discussion

Semen Quality of Dogs

The average ejaculate volume, colour, consistency and pH were 2.17±0.16 mL, milky white, thick, and 6.35±0.03, respectively. The mean values of mass motility, individual motility, live sperm, abnormal sperm, total sperm concentration and HOS reactive sperm were 3.91±0.15, 86.45±0.97%, 87.37±0.98%, 9.08±0.25%, 353.89±11.1 million/mL, and 89.0±0.94%, respectively, for fresh semen. The variations in the volume of the semen were caused by differences in the size of the dog, the animal's age, body weight and breed, the size of the prostate gland, the frequency of semen collection and the volume of the second fraction collection. However, Zorinkimi *et al.* (2017a) reported higher, while Filho *et al*. (2011) reported lower volume of sperm rich fraction than the present finding. Observations of colour of different semen samples were in accordance with the Barve (2014) and Srinivas Rao *et al*. (2022). The consistency of sperm rich fraction and pH with sperm concentration values in the present study were similar to the reports of Barve (2014) and Shalini and Antoine (2018). Moreover, Khye *et al.* (2021) reported lower semen pH, while Srinivas Rao *et al*. (2022) observed higher pH than our observed value. Average mass motility as observed in the present study was in accordance with the findings of Raut (2009). However, Shalini and Antoine (2018) observed lower value. The initial motility agreed fairly well with the observations made by Dobranić *et al.* (2005). However, higher sperm motility was reported by Kawakami *et al.* (2005) and Silva *et al.* (2009) and slightly lower one by Shalini and Antoine (2018) and Ray *et al*. (2019), which could be attributed to individual variation, breed and environmental conditions.

The live spermatozoa count in present study was in agreement with Zorinkimi *et al.* (2017a), whereas slightly higher values were recorded by Alamo *et al.* (2005), Kawakami *et al.* (2005) and Khye *et al.* (2021), and slightly lower one by Shalini and Antoine (2018) and Ray *et al.* (2019). Slightly lower abnormal sperm percentage than the present observation was reported by Kawakami *et al*. (2005), while higher abnormal sperm percentage was reported by Gradil *et al.* (2006). The present findings on sperm concentration are in agreement with Belala *et al.* (2016). However, Shalini and Antoine (2018) reported higher sperm concentration. These differences in sperm abnormalities and sperm concentration can be attributed to variation in individual, age, breed and environment. The present observations on the hypo-osmotic swelling test (HOST) were in accordance with those of Barve (2014). A lower percentage of sperm having intact plasma membrane was reported by Violeta and Pana (2007) and Ray *et al.,* (2019) and higher one by Michael *et al.* (2009), Zorinkimi *et al.* (2017a) and Arunmozhi *et al.* (2021).

Effect of Refrigeration Preservation

The present study aimed to compare the effects of coconut water-based extender on sperm motility, live sperm count, sperm abnormalities and membrane integrity in canine semen preservation (Table 1). Sperm motility percentages at 0, 24, 48 and 72 h of refrigeration of canine semen in coconut water-based extender were 84.09±1.22, 69.46±2.31, 58.34± 2.43 and 44.17±3.49 %, respectively. Similar observations were also made by Gunawan *et al.* (2016). However, higher values were reported by Puja *et al*. (2018) and Cheema *et al*. (2021) in Tris egg yolk extender supplemented with coconut water. The differences in the sperm motility between the published literature and the present findings could be due to the

Table 1: Comparison of microscopic evaluated parameters of canine semen in coconut water based extender at different time intervals

Time of storage	Coconut water-based extender			
	Individual sperm motility (%)	Live sperm (%)	Abnormal sperm (%)	Intact plasma membrane (%)
0 h	84.09±1.22^{d}	86.75±0.91^{d}	11.00±0.27^{a}	84.80±0.89^{d}
24 h	69.46±2.31^{c}	71.38±1.10^{c}	13.25±0.26^{c}	76.55±1.42^{c}
48 h	58.34±2.43^{b}	57.80±0.93^{b}	14.96±0.26^{d}	71.80±1.29^{b}
72 h	44.17±3.49^{a}	43.50±1.11^{a}	16.88±0.27^{e}	64.46±1.64^{a}

Values bearing uncommon superscripts (a,b,c) differ significantly between storage periods at $p<0.05$.

difference in the initial motility in neat semen before dilution. The live sperm counts at 0, 24, 48 and 72 h of preservation in coconut water based extender were 86.75±0.91, 71.38±1.1, 57.8± 0.93 and 43.5±1.11 %, respectively. However, higher sperm viability was reported by Gunawan *et al.* (2016) and Puja *et al*. (2018) in coconut water-based extender. Total morphological abnormalities of sperm at 0, 24, 48 and 72 h in current coconut water-based were 11±0.27, 13.25±0.26, 14.96±0.26, 16.88±0.27 %, respectively, which was similar to Vicente *et al*. (2018) in Bali bulls. Furthermore, intact plasma membrane percentages at 0, 24, 48 and 72 h were 84.8±0.89, 76.55±1.42, 71.8±1.29, 64.46±1.64 %, respectively. Using coconut water-based extender nearly similar observations were made by Cheema *et al.* (2021) and Zorinkimi *et al.* (2017b).

The study concludes that coconut water seems to be suitable as a semen extender in canines due to its isotonic properties, being cheap, effective, and easy to use. Coconut water-based extender can store canine sperm for up to 48 h while maintaining initial motility and live percentages above 50%. Sperm abnormalities remain below 20% and plasma membrane integrity above 60% till 72 h of semen preservation at 4°C.

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