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The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2022; SP-11(6): 655-658

www.thepharmajournal.com
Received: 10-04-2022
Accepted: 27-05-2022

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Studies on extension and preservation of canine semen by addition of catalase at refrigeration temperature

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Abstract

The objective of this experiment was to study the effect of Catalase on the extension and preservation of canine semen. The semen parameters like per cent individual motility, per cent viability, and per cent sperm abnormalities were evaluated at refrigeration temperature (5 °C) after 0, 24, 72, and 120 hrs of collection and dilution. Six ejaculates per dog were collected by digital manipulation making a total of 36 ejaculates from six stud dogs of private owners and extended with Tris Egg Yolk Glucose Citrate (TEYGC) extender. Catalase (CAT @ 150 IU) was added to each ml of extended semen and control group (without any addition of antioxidant). The results of the present experiment reveal that the addition of catalase 150 IU improved sperm cell parameters such as per cent individual motility, per cent livability, and also decreased per cent total sperm abnormalities when compared to the control group during liquid storage of canine semen up to 120 hrs at refrigerator temperature (5 ºC). In the present study, all semen parameters significantly ($P \leq 0.05$) differ between test and control groups.

Keywords: Canine semen preservation, Catalase

1. Introduction

Artificial insemination (AI) in dogs has come a long way since the first successfully reported vaginal insemination with fresh semen performed by Fr Abbe´ Lazzaro Spallanzani in Italy, in 1780, resulting in the birth of three beagle puppies. Advances in canine physiology knowledge, as well as new advances in canine sperm technology, have made AI available and practicable worldwide. AI has been widely used in cattle for decades, but only in recent years, it has found a niche amongst dog breeders. It is a useful way of decreasing the stress on parent stock separated by large geographical distances. It is becoming popular as a management tool in canine breeding by helping in widening the gene pool of certain dog breeds where low numbers exist.

With the increase in demand for AI among dog breeders and owners, semen collection and preservation is a management tool in canine breeding, the ill effect, i.e., inbreeding within breeds can be reduced. Breeders can now select purebred dogs from all over the world to improve their kennel genetics without experiencing transport-related stress. Besides, semen from precious dogs can be saved and stored in semen banks to be used in further generations despite their death or after the peak reproductive age.

Cold storage of semen is used to reduce metabolism and to maintain sperm viability over an extended period. But the quality of semen deteriorates during this extended storage period. One of the main causes for such decline is due to the action of the reactive oxygen species (ROS) generated by the cellular components of semen, namely a superoxide anion radical (O^{2-}) and hydrogen peroxide (H_2O2).

Sperm cells are particularly sensitive to oxidative stress because of their relatively high content of polyunsaturated fatty acids in the plasma membranes that can be easily oxidized. An increase in ROS concentration in the seminal plasma or the extender during preservation is responsible for cell membrane damage and semen deterioration in different species including dogs (Michael *et al*., 2009) [22].

To prevent this oxidative stress, to increase the survival and functional integrity of gametes, the presence of antioxidants like Catalase in the extender is must.

2. Materials and Methods

A total of 6 healthy sexually matured, reproductively sound and proven male dogs aged between 2 to 4 years belonging to pet owners were chosen for the current study. The semen was collected by digital manipulation in absence of a teaser bitch, (Kutzler, 2005) [18].

A total of 6 ejaculates were collected from each male dog, with a one-week interval between collections (Johnston *et al.*, 2001) [15].

2.1 Experimental design

The collected semen was randomly divided into two groups namely laboratory prepared Tris Egg Yolk Glucose Citrate (TEYGC) extender with antioxidant (Catalase@ 150 IU/ml), Basic TEYGC Extender without the addition of antioxidants (acts as control).

The sperm rich fraction of semen was separately collected and utilized for further processing, while the pre-spermatic and prostatic fractions were discarded. Semen samples from Aliquot 1 and 2 were diluted with three volumes of laboratory prepared TRIS Egg Yolk Glucose Citrate (TEYGC) extender to achieve a final 1:4 dilution.

Group 1: Semen sample from aliquot 1 was extended with three volumes of laboratory prepared TEYGC. Hereafter the group was referred as "Control".

Group 2: Semen sample from aliquot 3 was extended with three volumes of TEYGC and Catalase @ 150 IU/ml was added. Hereafter, the group was referred as the "Catalase" group.

2.2 Evaluation of fresh semen

The fresh semen was analyzed immediately after collection for pH, macroscopic and microscopic evaluation. The macroscopic evaluation consisted of volume, colour and consistency, while the microscopic evaluation consisted of mass motility, individual motility, sperm concentration, total sperm count, live & dead count and sperm abnormalities.

3. Results

3.1 pH

The mean pH of sperm rich fraction of canine semen was recorded as 6.341 ± 0.034 with a range from 6.0 to 6.8.

3.2 Volume

The mean volume of the sperm rich fraction of canine semen was recorded as 0.709 ± 0.021 ml with a range from 0.5 ml to 1.0 ml. There was significant difference in the mean volume of the sperm rich fraction of the semen obtained per ejaculation between the stud dogs.

3.3 Colour

The colour of the sperm rich fraction of canine semen was milky white to creamy white in the present investigation.

3.4 Consistency

The consistency of the sperm rich fraction of canine semen was thin milky.

3.5 Mass Motility

The mass motility of the sperm rich fraction of canine semen collected was observed to vary from 3.333 ± 0.421 to 4.000 ± 0.365 with an overall mean of 3.611 ± 0.160 out of 5 scale without any significant difference.

3.6 Individual Motility

The mean per cent of individual motility in the semen ranged between 71 and 86 with an overall mean of 79.805 ± 0.596. Statistical analysis revealed that there was no significant variation in individual motility.

3.7 Sperm Concentration

The sperm concentration was ranged from 264 to 441 x 10^6 per ml with an overall mean of 348.916 ± 7.025 x 10^6 per ml. Statistical analysis revealed that there was no significant ($p \leq 0.05$) variation in the sperm concentration.

3.8 Viability

The per cent live sperm in the sperm rich fraction of semen was ranged between 70 to 84. The overall mean value of live sperm per cent in sperm rich fraction of fresh semen was 79.611 ± 0.376. Statistical analysis revealed that there was significant ($p \leq 0.05$) variation in per cent live sperm among them.

3.9 Sperm Abnormalities

The overall per cent of total abnormality in the sperm rich fraction of fresh semen of stud dogs was 11.527 ± 0.256 with a range between 7 to 15 per cent. The per cent of head, mid piece and tail abnormalities recorded in the sperm rich fraction of fresh semen was 4.861 ± 0.149, 1.055 ± 0.137 and 5.611 ± 0.150, respectively, while the per cent of head, mid piece and tail abnormalities ranged between 3 to 6, 0 to 3 and 4 to 7 respectively.

Table 1: Macroscopic attributes of fresh semen collected from stud dogs

pH (Mean ± S.E.)	6.341 ± 0.034
Volume (Mean ± S.E.)	0.709 ± 0.021
Colour	Milky white to creamy white
Consistency	Thin Milky

Table 2: Microscopic attributes of fresh semen collected from stud dogs

Mass Motility	3.611 ± 0.160
Per cent Individual Motility	79.805 ± 0.596
Per cent Viability	79.611 ± 0.376
Sperm Concentration (Millions / ml)	348.916 ± 7.025
Head Abnormalities	4.861 ± 0.149
Mid Piece Abnormalities	1.055 ± 0.137
Tail Piece Abnormalities	5.611 ± 0.150
Total Sperm Abnormalities	11.527 ± 0.256

3.10 Evaluation of extended semen

The extended semen from two aliquots was assigned to two different groups (Control and Catalase groups) and stored in the refrigerator at 4 to 5 °C for 5 days. A portion of refrigerated semen was removed at 0 (after cooling to 4 to 5 °C), 24, 72 and 120 hours of storage from the refrigerator and rewarmed to 37 °C and evaluated for various microscopic parameters: Individual motility, per cent viability (or) live and dead count, per cent abnormal spermatozoa

Table 3: Effect of Duration of Preservation and Extender on various canine spermatozoa parameters at Refrigeration Temperature (5 °C)

Parameter	At 0 Hr of Incubation		At 24 Hr of Incubation		At 72 Hr of Incubation		At 120 Hr of Incubation	
	Catalase	Control	Catalase	Control	Catalase	Control	Catalase	Control
Individual Motility	77.694 ± 0.563	76.277 ± 0.572	70.833 ± 0.348	61.388 ± 1.319	53.027 ± 1.241	46.055 ± 0.982	34.888 ± 0.910	29.861 ± 0.835
Per cent Viability	77.611 ±	77.833 ±	68.388 ±	65.138 ±	55.972 ±	40.138 ±	34.611 ±	29.527 ±

	0.361	0.343	0.442	0.388	0.629	0.555	0.913	0.686
Per cent Head Abnormalities	5.167 ± 0.129	5.500 ± 0.152	5.750 ± 0.156	6.000 ± 0.12	6.223 ± 0.106	7.723 ± 0.181	7.667 ± 0.203	8.667 ± 0.203
Per cent Mid Piece Abnormalities	1.334 ± 0.09	1.473 ± 0.123	1.473 ± 0.102	1.695 ± 0.088	2.223 ± 0.127	2.362 ± 0.139	3.973 ± 0.13	4.195 ± 0.154
Per cent Tail Piece Abnormalities	5.778 ± 0.16	6.362 ± 0.134	6.945 ± 0.149	7.139 ± 0.145	10.223 ± 0.302	11.223 ± 0.302	11.667 ± 0.301	13.389 ± 0.348
Per cent Total Sperm Abnormalities	12.278 ± 0.225	13.334 ± 0.192	14.167 ± 0.221	14.834 ± 0.217	18.667 ± 0.322	21.306 ± 0.401	23.306 ± 0.363	26.25 ± 0.411

Semen parameters significantly ($P \leq 0.05$) differ between catalase and control groups

The mean individual motility of the canine extended semen in the catalase group was 77.694 ± 0.563, 70.833 ± 0.348, 53.027 ± 1.241 and 34.888 ± 0.910 per cent at 0, 24, 72 and 120hrs of preservation at refrigerated temperature, respectively which is in agreement with the studies of Luvoni *et al*, (2000) [19] using 200 IU of catalase. However, lower values were reported by Michael *et al*, (2007) [21] using 300 IU of catalase, Beccaglia *et al*, (2009) [5] using 150 IU and 450 IU of catalase extended with Tris Egg Yolk Fructose Citrate with 0.04 per cent soya bean lecithin, while higher values were reported by Michael *et al*, (2009) [22] using 100 IU of catalase added to Tris Egg Yolk Glucose Citrate extender, Kmenta *et al*, (2011) [17] using 150 IU of catalase to Tris Egg Yolk Fructose Citrate Lecithin extender, Thiangtum *et al*, (2012) [27] using 100, 400 and 1600 IU of catalase added to Egg yolk Tris-fructose citrate extender, Del Prete *et al*, (2018) [8] using 15 IU of catalase supplemented to EYT-G extender and Cheema and Kaur (2021) [7] using 200 IU of catalase supplemented to Tris Egg Yolk Citric acid Fructose extender.

The mean viability of the canine extended semen in the catalase group was 77.611 ± 0.361, 68.388 ± 0.442, 55.972 ± 0.629 and 34.611 ± 0.913 per cent at 0, 24, 72 and 120hrs of preservation at refrigerated temperature, respectively. But, lower values were reported by Michael *et al*, (2007) [21] using 300 IU of catalase, while higher values were reported by Michael *et al*, (2009) [22], Kmenta *et al*, (2011) [17], Thiangtum *et al*, (2012) [27] and Cheema and Kaur (2021) [7].

The mean per cent of total sperm abnormalities of the canine extended semen in the Catalase group was 12.278 ± 0.225, 14.167 ± 0.221, 18.667 ± 0.322 and 23.306 ± 0.363 at 0, 24, 72 and 120 hrs of preservation at refrigerated temperature, respectively. But higher values were reported by Michael *et al*, (2009) [22] and Kmenta *et al*, (2011) [17].

4. Discussion

The variation in the individual motility, viability and sperm abnormalities could be attributed to the type of extender, storage of catalase, Soyabean Lecithin combination. Individual variation, breed variation, age of the stud dog, environmental factors mainly temperature, presence of exogenous and endogenous antioxidants. Catalase deficiency can be noticed in stud dogs suffering from protein deficiency and with chronic fluoride exposure (Gibb *et al.*, 2021) [11].

Contrary, Hatamoto *et al*, (2006) [13] did not find catalase activity in any of the canine seminal plasma samples examined, whereas, catalase activity has been determined in the seminal plasma of normozoospermic dogs (Kawakami *et al.*, 2007) [16]. Detection and quantification of catalase is difficult, as it is mainly intracellular (Miesel *et al.*, 1997) [23] and the presence of exogenous catalase in the extracellular compartment (diluent/extender) might not be sufficient for improving semen quality during chilling.

In this study, the addition of catalase an intracellular enzyme (Miesel *et al.*, 1997) [23] might have protected the spermatozoa against reactive oxygen species (ROS) damage (Fernandez *et al.*, 2007) [10], which inhibit enzymatic activity and cellular function due to its toxic effect (Aitken *et al.*, 1989) [1]. Besides, protecting the plasmalemma of the spermatozoa, it might have maintained the integrity of normal acrosome (Maxwell and Stojanov, 1996) [20], mitochondrial membrane integrity by rearranging lipids and proteins (Holt, 2005 and Nizanski *et al.*, 2012) [14] and cytoskeletal structure of flagella in this study.

Apart from this, catalase is also believed to protect SOD, GSH and TAC. Efflux of cholesterol from sperm membrane etc. might have prevented premature capacitation and acrosome reaction (Witte and SchäferSomi, 2007) [28] in this study. Besides, this catalase might have reduced the deleterious effect of cooling on motility (Camara *et al.*, 2011). The addition of catalase also is beneficial to spermatozoa within the female reproductive tract (Del Prete *et al.*, 2018) [8]. The better results with catalase might be due to the above facts in the present investigation.

Likewise, the addition of catalase to the semen extender has a positive effect on chilled semen of ram (Camara *et al.*, 2011), Dog (Kmenta *et al.*, 2011) [17], Mithun (Peruma *et al.*, 2013) [25], Cock (Partyka *et al.*, 2015) [24], Buffalo bull (Bansal *et al.*, 2016) [4], Stallion (Delprete *et al.*, 2019) [9], Holstein bull (Hakoueu *et al.*, 2019) [12] and Buck (Ranjan *et al.*, 2021) [26]. But few authors stated that the catalase did not have any effect as mentioned above (Aurich *et al.*, 1997; Ball *et al.*, 2001; Hatamoto *et al.*, 2006; Beccaglia *et al.*, 2009 and Thiangtum *et al.*, 2012) [2, 3, 13, 5, 27] but also had toxic effect @ 200 IU/ ml (Maxwell and Stojanov, 1996) [20] or by increasing production of superoxide anion (Michael *et al.*, 2007) [21].

5. Conclusion

The present study concluded that the canine semen could be preserved well in laboratory prepared extenders up to 72 hrs without compromising the sperm motility and viability. Further, it is also concluded that the addition of Catalase @ 150 IU/ml to Tris Egg Yolk Glucose Citrate extender might be useful for keeping quality of canine semen up to 72 hours.

6. Acknowledgements

The authors thank P. V. Narsimha Rao Telangana Veterinary University, Hyderabad for providing the necessary financial support and infrastructure to carry out the study.

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