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Original Research Article

Effect of Transport Media in Different Postmortem Storage Time on Epididymal Sperm Quality of Bali Mongrel Dogs

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Abstract: This research aimed to analyze the effect of transport media and post-mortem storage time on the quality of Bali mongrel dogs spermatozoa. Coconut water and saline solution were used as the transport medium for the epididymal samples, and sperm quality parameter analysis was carried out as well as after one hour and two hours. Testicles from 18 (2-4 years old) mongrel dogs were collected via a castration program were used in this study. From each dog, the testicles were transported in different transported media to the laboratory. One testicle was maintained in sterile saline solution, where the other testicle was in coconut water, and sperm quality parameter analysis was carried out as well as after one hour and two hours. Epididymal sperm was harvested through flushing using an egg yolk tris extender. The motility of sperm was evaluated by adding a drop of semen on a warm glass slide covered with a glass slip and viewed at a magnification of x40. Liveability and abnormality of sperm were assessed by the eosin–nigrosin stained technique. Motility and viable spermatozoa could be recovered after two hours in coconut water and saline solution, although motility decreases significantly after two-hour storage time. It is concluded that coconut water and saline solution have a protective effect on epididymal sperm. Motile and viable spermatozoa can be recovered from the epididymides after storage at transport media for two hours.

Keywords: Coconut water, epididymis, spermatozoa, motility, viability, mongrel dog.

INTRODUCTION

Through the ages, people have claimed dogs as one of their pets and the best companion animal. Between dogs and humans, there is a very deep relationship dating back thousands of years. There is the reason, dogs call them man's best friend. Recently, when their dogs die unexpectedly, the owner doesn't have much to do, the dog is buried. The genetic material potential of a dog is lost with death.

Cryopreservation of epididymal sperm is currently a topic of interest in an attempt to exploit genetically superior animals from being killed or slaughter [1, 2] and that suddenly die or undergoing orchidectomy for medical reasons [3]. Sperm can now be salvaged and cryopreserved via epididymal semen extraction [4]. The use of epididymal spermatozoa has been reported resulting from the pregnancy and live birth in several mammalian species such as in ovine [5], bison [6], Also in the dog [7]. Recovery of the epididymal spermatozoa of a dead animal is a viable option that helps to ensure that the genetic materials of a higher-priced or valuable animal are available for future use. However, sometimes, epididymal sperm collection is not easy especially in areas far from the epididymis retrieval area.

The epididymis is the male reproductive tract that plays a role in the storage of spermatozoa until the ejaculation process occurs. The epididymis is a duct-like organ that is responsible for Sperm maturation during epididymal transit [8]. It is known that live spermatozoa can be collected after animal death [9.10], and also showed fertilizability [11]. However, after an animal dies, the spermatozoa in the cauda epididymis undergo rapid degeneration. Some studies have been conducted to determine the rapid degeneration of post-mortem epididymal spermatozoa. The speed of spermatozoa degeneration is influenced by the length

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time of the spermatozoa collection after death [1] and environmental temperature [9].

Protocol to be used for recovery of viable sperm from epididymis in canine after death, it must be developed so that it can be maintained and used when needed. This research aimed to analyze the effect of transport media and post-mortem storage time on the viability of canine spermatozoa recovered post-mortem.

MATERIALS AND METHODS

Collection of testicles

Testicles from 18 (2-4 years old) Bali mongrel dogs were collected via castration program. From each dog, the testicles were removed and transported in different transported media to the laboratory. One testicle was maintained in sterile saline solution, where the other testicle was in coconut water.

Testicle preparation and sperm recovery

Semen was obtained from all testicles from the cauda epididymis thorough flushing using an egg yolk tris extender. Thereafter, a semen extender is passed through the cauda under gentle pressure and spermatozoa were collected in a Petri disk.

Quality of sperm assessment

The motility of sperm was evaluated by adding a drop of semen on a warm glass slide covered with a glass slip and viewed at a magnification of x40. Only sperm cells moving in progressive motility were including in the rating, Morphology, and viability of sperm were assessed by analyzing eosin–nigrosin stained.

Data Analysis

The data included in the model were analyzed using descriptive statistics (mean ± SD). The differences effect of two diluent transport media on quality and quantity of sperm were analyzed by the Student T-test. Statistical analysis was performed using IBM SPSS statistics 20 for Windows.

RESULT

Epididymal spermatozoa transported with different transport media show no significant difference in the percentage of motility. In contrast, sperm motility decreased significantly ($P < 0.05$) between the postmortem storage time (Table 1). Sperm motility shows no significant difference ($P>0.05$) was observed between sterile saline solution and coconut water transport media after one and two hours postmortem storage. Storage time observed has a significant effect on the motility of spermatozoa in both young coconut water and solution saline transport media ($P<0.05$). A significant decrease was observed after hours of postmortem storage in comparison to the one hour storage.

Table 1: Effect of transport media and postmortem storage on epididymal sperm motility

Transport Media	Postmortem Storage Time	
	one hour	two hours
Saline solution	71.25 ±5.85	63.00 ±1.82
Coconut Water	71.70 ±5.73	65.50 ±1.29

No significant decrease in liveability was observed. The mean sperm liveability between saline solution and coconut water media transport in one hour and two hours storage time was presented in Table 2. The sperm liveability between one hour and two hours storage time was not significantly different ($P>0.05$).

Table 2: Effect of transport media and postmortem storage time on epididymal sperm Liveability

Transport Media	Postmortem Storage Time	
	one hour	two hours
Saline solution	77.0± 1.82	76.25 ± 1.50
Coconut Water	78.25±2.87	75.75± 1.50

Epididymal spermatozoa transported with different transport media show no significant difference ($P>0.05$) in the percentage of abnormality (Table 3). After two hours of postmortem storage time, the percentage of total abnormalities was no significant difference with one hour postmortem storage ($P>0.05$). The percentage of total abnormalities increase in two hours postmortem storage at both transported in saline solution and coconut water media transport.

Table 3: Effect of transport media and postmortem storage time on percentage of sperm abnormalities

Transport Media	Postmortem Storage Time	
	one hour	two hours
Saline solution	7.75± 0.95	8.25 ± 1.50
Coconut Water	7.00± 0.81	8.25 ± 1.89

DISCUSSION

The implementation of artificial insemination in the domestic animal industry, semen from animals routinely collected from ejaculation. However, many studies show, epididymis has been used as a source of semen. Semen collected from epididymis is used as an alternative tool particularly on abnormal animals and also dead animals. Spermatozoa collected from the epididymis of dead animals are still motile and fertile for several days [5], and suitable for use for cryopreservation and artificial insemination [6]. Recent studies demonstrated that viable spermatozoa were determined after the dead animal, however, this varies according to condition and temperature [12], to time of epididymal spermatozoa storage [13].

In this study, the sperm quality was affected by time after orchiectomy. After two hours postmortem storage in transport media show decrease motility, liveability, and increase abnormality. Spermatozoa collected from the epididymis showed variations in

individuals. After one hour of postmortem storage, the sperm motility was 71.25 % in saline solution and 71.70% in coconut water. Regarding the motility of spermatozoa collected from the epididymis at one hour of storage in two transport media, which can be considered excellent. Semen that was classified as having poor motility (<30% progressive), as good (30-65% progressive), and excellent (>65% progressive) [14]. The value of sperm motility in this study was compared, demonstrating lower sperm motility for the flushing and mincing methods [15].

After two hours storage, the value of sperm motility slightly decreases when compared to one hour storage. The results of this study are lower when compared to the results of ejaculation sperm in dog [16]. However, the value of this study considered acceptable percentage for live spermatozoa for artificial insemination in dogs. The minimum sperm motility that can be used for artificial insemination in dogs was 60% [17]. The data of this study indicate that the epididymal spermatozoa already have motility capabilities that are equivalent to the motility of ejaculatory spermatozoa so that they can be used for artificial insemination purposes [18]. Recent studies have demonstrated that epididymal spermatozoa have been viable for a determined time after the death of the animal, however, this varies according to the time of epididymal spermatozoa storage.

The current study shows no significant differences in sperm liveability in the different storage times and transport media. After two hours of storage, mean epididymal sperm liveability reach 75.75 %. It is conceivable that even after two hours of storage of canine epididymal spermatozoa at different transport media, the spermatozoa could still be viable enough to be used in assisted reproductive technologies. The data obtained in this research is similar to those obtained in Nigerian Local dog [10]. It was found that mean epididymal sperm motility between the 0th, 12th,24th, and 48th hour in Nigerian Local dog, obtained mean of 86.4 ± 1.5, 78.0 ± 3.1, and 75.0 ± 1.8 respectively.

The sperm abnormalities, do not show significant differences during storage time on the transport medium. After two hours of storage show, the percentage of abnormalities slightly increase than in one hour of storage time. This increase shows no significant difference. The data obtained in this research lower to those obtained in Nigerian dog [10]. The percentage of sperm morphological abnormality increased with storage time. This data obtained similar when compared to those obtained in bovine [1].

The results of this study demonstrate that the transport medium has a protective effect on epididymal sperm. The transport medium used able us to obtain good quality of sperm. The beneficial effect of this medium may be explained by the potency of coconut water and saline solution as a buffering agent. Coconut water is a refreshing drink with electrolytes (ionic mineral) similar to human plasma [19], and water resembles the intracellular fluid [20]. Coconut water has properties and is characterized by has the highest antioxidant activity [21], as expressed by the phenolic compounds [22]. Coconut water has sufficient nutrients, vitamins, minerals, amino acids, and phytohormones [23]. This study shows that coconut water has properties that can maintain a stable pH. The coconut water and saline solution as a transport medium for the epididymal provide good protection against the condition of local dog spermatozoa.

CONCLUSION

The current study has shown that coconut water and saline solution have a protective effect on epididymal sperm. Motile and viable spermatozoa can be recovered from the epididymides after storage at transport media for two hours. The beneficial effect of this medium may be explained by the potency of coconut water and saline solution as a buffering agent.

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