Research Article

The Motility and Viability of Kintamani Dogs Semen in Different Cooled Transport Duration

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Abstract | Various efforts have been made to conserve the Kintamani dog, and one such approach is through artificial insemination (AI). To ensure optimal results in artificial insemination, understanding the factors that influence its success is crucial. This study aimed to investigate the impact of different transport times (0 hours, 1 hour, 2 hours, and 3 hours) on the motility and viability of chilled semen on Kintamani dogs. The study used a completely randomized design, with four treatments and six replications. In experiment 1, sample examined immediately after added extender, experiment 2, 3 and 4 were. The results of the study found that the average motility percentages for semen transported at 0 hours, 1 hour, 2 hours, and 3 hours were 91.00% ± 0.89, 90.83% ± 0.75, 90.17% ± 0.75, and 89.83% ± 0.75, respectively. Similarly, the average viability percentages for the same transport times were 82.00% ± 0.89, 81.66% ± 1.03, 81.66% ± 1.03, and 80.67% ± 1.03. Although there appeared to be a decline in semen quality, statistical analysis did not reveal significant differences. In conclusion, chilled semen from Kintamani Dogs transported for up to 3 hours remains suitable for artificial insemination.

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Introduction

Kintamani dogs are one of Indonesia's native dog breeds that were first recognized as provisional breeds by the Fédération Cynologique Internationale (FCI). Kintamani dogs are mountain-type dogs whose natural habitat is the Sukawana village area, District of Kintamani, Bangli Regency, Bali. The popularity of Kintamani dogs is increasing nowadays. Various efforts have been made to preserve or maintain the Kintamani dog population, both by the government and among the Kintamani dog lovers (Sulabda *et al.*, 2022).

Until now, the mating process for Kintamani dogs is carried out by bringing the male to the location of the

female to be mated, but this method is considered less effective because it can cause stress to the dog and is less practical. One of the steps taken to overcome the problems that arise in natural mating in Kintamani dogs is through the implementation of artificial insemination (Puja *et al*., 2019).

Artificial Insemination (AI) is one of the biotechnology techniques in reproduction that facilitates mating without requiring the presence of a male. In the process of artificial insemination, assisted semen transfer will be carried out into the reproductive tract of female animals (Mason., 2016; Dutta and Dutta, 2020), which allows the union of male gamete cells with female gamete cells (Jain *et al*., 2015; Patel *et al*., 2017). The application of artificial insemination is to be more promising when compared to natural mating (Sulabda and Puja, 2010).

Insemination can be performed using three types of canine semen: fresh, fresh-chilled, and frozen. Out of the three kinds of semen, chilled semen is the most widely used, especially in developed countries (Puja *et al*., 2023). The process of providing, transporting, and using chilled semen does not require special equipment or processing, making it easier to applied artificial insemination using chilled semen (Rijsselaere *et al*., 2011).

In artificial insemination, semen quality must be maintained because it is an important factor in ensuring the success of the fertilization process and the subsequent development of the fetus. The duration time as factors which significantly affected the quality of dog semen with chilled extended semen (Lojkic et al., 2022). Based on the description above, it is necessary to research the effect of duration time of transportation on motility and viability of chilled semen Kintamani Dogs. This research was carried out to find out the effect of semen delivery using a styrofoam transport box in an ice pack on the motility and viability of spermatozoa.

Materials and Methods

Animal

The animal used in this study was clinically healthy, 2-3 years old, and had no history of pathological disorder in reproduction. The dogs were taken from Sato Dog Kennel, Tegalalang, Gianyar, and Bali. Dogs are kept in a 2 x 4 m cage, fed a mixture of meat and commercial feed, and given drinking water *ad libitum*.

Research design

The design used in this study was a completely randomized design (CRD) with four treatments and six replications. The sample was assigned to the control (T0), semen examination immediately after adding diluent, while the experimental treatments T1, T2, and T3 were examineded 1,2, and 3 hours after adding diluent.

Preparation of diluent

In this study, the diluent used was egg yolk tris-based extender diluent. The compositions used are 3.025 mg TRIS, 1.7 mg citric acid, 1.25 mg fructose, 1 mg streptomycin, 1000 IU/ml penicillin, 20 mg egg yolk, and dissolved in 100 ml of distilled water. For egg yolk used is the plasma part, the plasma part is obtained by centrifuging the yolk at 900 rpm for 30 minutes (Puja *et al*., 2023).

Semen collection

The semen was collected by manual stimulation methods (Puja *et al*., 2019). Only the second fraction of the ejaculates was collected for the experiments. Semen samples were used in this experiment with a minimum of 70 motility and normal morphology of spermatozoa of ≥ 70% (Hermansson *et al*., 2021).

Motility examination

Sperm motility was evaluated according to the procedures of Johnston et al. (2001). Motility was assessed on a pre-heated stage at 37 °C. A total of 200 spermatozoa were evaluated with 100x microscope magnification. In the examination of motility that is considered is the motility of spermatozoa that move progressively and those that do not (Ajadi and Gazal, 2016).

Viability examination

The percentage of live spermatozoa was evaluated using a light microscope. Semen samples were smeared on the slide and stained with eosin negrosin citrate solution. A total of 200 spermatozoa were evaluated using a microscope with a magnification of 400x. Live spermatozoa will look clear because they do not absorb color. After all, the cell wall is still intact (Cheema *et al*., 2011). Meanwhile, dead spermatozoa will be red because they absorb eosin color.

Data analysis

The data obtained were evaluated using the analysis

of variance (ANOVA) method. If the treatment has an effect, the Least Significant Difference (LSD) test was applied to compare the differences means (Heath, 2000).

Results and Discussion

In this study, the difference in storage times of the cooled transport box, the result showed that the handling time after 1, 2, and 3 hours did not affect the motility and viability of Kinamani dog semen. Mean Sperm motility after treatment is shown in Table 1. The results of the chilled semen motility examination after 0 hours, 1 hour, 2 hours, and 3 hours in a cooled transport box were 91.00±0.89; 90.83±0.75; 90.17±0.75; 89.83±0.75%, respectively.

Table 1: *Average motility of kintamani dog chilled semen at different times of storage in a cooled transport box.*

Transportation time	Motility (%) (mean±SD)
0 hour	91,00±0.89[a]
1 hour	90.83±0.75[a]
2 hours	90.17±0.75[a]
3 hours	89.83±0.75[a]

Notes: The same notation indicates no significant difference (P>0.05).

The dead sperm did not substantially change their values during the periode of stored in cooled transport box (Table 2). The mean viability after 0 hours, 1 hour, 2 hours, and 3 hours in the cooled transport box were 82.00±0.89; 81.66±1.03; 81.66±1.03; 80.67±1.03% respectively. Both examination results showed a slight decrease, but statistically not significantly different (P≥0.05).

Table 2: *Average Viability of Balinese Kintamani Dog Cold Semen at different times of storage in a cooled transport box.*

Transportation time	Viability (%) (mean±SD)
0 hour	82,00±0.89[a]
1 hour	81.66±1.03[a]
2 hours	80.83±0.75[a]
3 hours	80.67±1.03[a]

Notes: The same notation indicates no significant difference (P>0.05).

This study is the first study conducted with a single focus on observing the semen quality of Kintamani dog breeds with the hope of contributing to a better understanding of the reproductive capacity of Kintamani dog breeds. Research on the effect of the length of transportation time on the motility and viability of chilled semen of Kintamani Dogs was conducted to know the effect of direct semen delivery using styrofoam transport boxes cooled with icepack. During the transportation period, there can be a decrease in the quality of spermatozoa (Puja *et al.*, 2023). This can also be seen in the results of this study, where the average results of sperm motility after 0 hour transportation time is 91%, 1 hour transportation time is 90.83%, 2 hours transportation time is 90.17%, and 3 hours transportation time is 89.83% were obtained. Although it has decreased when compared to the standard motility requirements for artificial insemination where the total motility of spermatozoa that are said to be normal is ≥ 75% (Pipan *et al.*, 2020) or must be more than 70% (Dalmazzo *et al.*, 2017), the semen from this study is still suitable for use in artificial insemination.

Similar to the results of motility examination, the examination of viability (Figure 1) or the number of live and dead spermatozoa also obtained results that decreased with increasing length of transportation time. The results obtained are the average viability with the treatment of 0 hours transportation time is 82%, 1 hour transportation time is 81.66%, 2 hours transportation time is 80.83%, and 3 hours transportation time is 80.67%. With the smallest average viability is 80.67%.

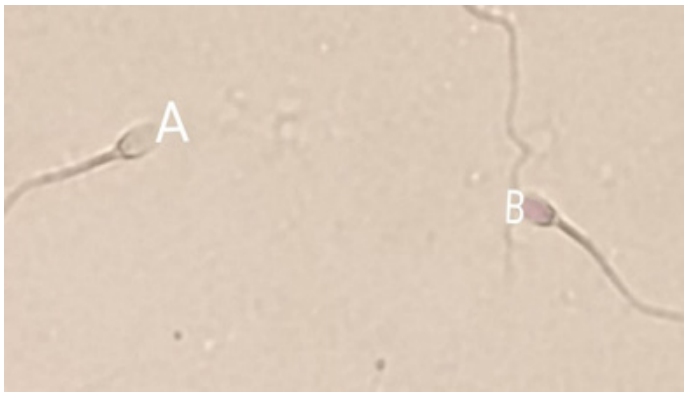

Figure 1: *Viability examination (live spermatozoa, colorless (A), dead spermatozoa, colored (B) (400x).*

Another important factor that influenced the data obtained in this study was the form of semen storage and the application of diluent. To maintain the quality of spermatozoa for longer time, semen cooling was carried out. The choice of chilled semen is also due to the consideration of the treatment of the longest transportation time is quite short and there are no special techniques or tools needed in semen cooling

technology so it is easy to apply (Rijsselaere *et al*., 2011). The application of diluent can be a solution to extending the shelf life of chilled semen. The major advantages of using chilled semen are that processing and shipping are easy and cheap as no special equipment is required. Semen is placed into a diluent before being stored at low temperatures, then the life of spermatozoa can be maintained longer (Puja *et al*., 2023).

Conclusions and Revommendations

Based on the results of the research it can be concluded that the length time of transportation from 0 hours to 3 hours does not have a significantly different effect on the motility and viability of Kintamani Dog semen. By obtaining an average percentage of motility above 89% and an average percentage of viability above 80%, the chilled semen of Kintamani Dogs after 3 hours of storage in a cooled transport box is still suitable for artificial insemination

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Novelty Statement

This is first report on effect of transport duration on the motility and viability of Kintamani dog spermatozoa

Author's Contribution

Mariana Isti Dwiningsih: Developed the research idea, conducted an analysis of data, and drafted the manuscript.
I Ketut Suatha and I Ketut Puja: Responsible for manuscript preparation, preparing, and critical checking of this manuscript.
All authors contributed equally in writing the final manuscript.

Conflict of interest
The authors have declared no conflict of interest.

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