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## The effect of teaser on the semen quality during semen collection in Kintamani Bali dogs

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### Abstract

The Kintamani Bali Dog is an emerging breed dog from Indonesia. To increase the population, artificial insemination technology needs to be used. In practice, artificial insemination requires good-quality semen. Semen can be collected with or without a teaser from a female in heat, but the presence of a female in heat is preferred. If there are no females in heat, a vaginal swab from the female in heat can be used. This study aims to determine the effect of a teaser vaginal swab of estrous bitch on the quality of semen. In this study, tests were carried out to see the motility, viability, and concentration of spermatozoa. The research design was a Crossover Study Design with two treatment groups. The data obtained were analyzed using the independent T-test. The result of the study showed that the use of a vaginal swab teaser from estrus bitch had no significant effect ( $p>0,05$ ) on the motility, viability, and spermatozoa concentration of Bali Kintamani Dogs.

**Keywords:** Kintamani Bali dogs, spermatozoa, teaser, vaginal swab

### Introduction

The Kintamani Bali dog is a purebred dog native to Indonesia, precisely originating from the Kintamani area, Sukawana Village, Bangli Regency, Bali. This mountain dog has a beautiful appearance, different from the mongrel dogs in Bali (Puja, 2007) <sup>[13]</sup>. The Kintamani Bali dog is a germplasm that has great potential for development. The Kintamani Bali dog is known for its loyal character and attractive appearance. This causes many people to be interested in getting dogs to keep them. The increasing popularity of the Kintamani Bali dog also increases its economic value and influences the existence of the Kintamani dog.

To increase the population of the Kintamani Bali dog, breeders still use natural mating between dogs. Some owners choose to mate their dogs with males from distant locations. However, transporting dogs to long distances is very stressful for dogs. To overcome this problem, it is necessary to use artificial insemination technology (Puja *et al.*, 2019) <sup>[14]</sup>. In dogs, apart from increasing the population, artificial insemination functions to improve the quality of offspring without having to mate with male and female dogs (Payan-Carreira *et al.*, 2011) <sup>[11]</sup>. Some of the advantages of artificial insemination include obtaining dogs of the desired breed, obtaining offspring from superior males who cannot mate naturally, obtaining offspring from males who are far from females, and minimizing the risk of injury during mating (Junaidi, 2006) <sup>[7]</sup>.

In recent years, the implementation of artificial insemination technology has shown rapid development and has become a current biotechnology phenomenon. Artificial insemination of Kintamani dogs has been successfully carried out (Sulabda *et al.*, 2023) <sup>[16]</sup>. In practice, artificial insemination requires good-quality semen.

Currently, semen collection can be done in three ways, namely digital manipulation, artificial vagina, and using electrical techniques for ejaculation (Baran, 2015) <sup>[1]</sup>. Semen can be collected well in the absence of a female in heat. However, the presence of a female dog in heat is preferable. In research conducted by Woszczyło *et al.* (2023) <sup>[19]</sup>, the availability of females in heat during semen collection allows for psychological sexual arousal in males. This is due to the release of semi-chemicals by the female during heat. This release is a potential source of eustress and sex pheromones, which act as natural enhancers.

The presence of a female dog in heat will quickly stimulate the male to have an erection. However, dogs in heat may not always be available. If there is no female dog in heat, frozen vaginal swabs of the female in heat can be used. This vaginal swab is used to stimulate male dogs into heat. Therefore, research regarding the effect of teasers using vaginal swabs needs to be carried out to determine its effectiveness in collecting semen from Kintamani Bali dogs.

## Materials and Methods

### Animal

The research objects used in this study were four male Kintamani dogs aged 2,5 years in clinically healthy condition. Dogs were obtained from Sato Kennel and were kept in cages measuring 2x4 m. Semen for each dog was collected using the digital massage method (Jahangirbasha *et al.*, 2018; Kumar *et al.*, 2023) [4, 8].

### Research design

The research design used is Crossover Study Design, namely a research design that allows each research subject to be given all the treatments being investigated in a certain order, thus allowing within-subject analysis, not between-subject analysis (Varghese and Chen, 2023) [18]. The Crossover Study Design allows the use of fewer subjects. In administering the intervention, subjects are divided into two groups in the first period, and then an exchange of intervention is carried out in the second period. Each individual receives all interventions in the research process.

### Research procedure

Samples were obtained using the massage method. The ejaculate was examined for motility, viability, and sperm concentration. To estimate sperm motility, a small drop of samples was placed on a slide that had been warmed at 37 °C and covered with a cover slip. The number of motile spermatozoa were examined at a 100 magnification. The sperm viability was evaluated using an eosin nigrosine stain. The slides were then air-dried and examined under a light microscope at 400x magnification. A sperm cell is considered alive if its head does not absorb color and if the head is pink it means the sperm is dead. The percentage of live spermatozoa was determined by counting at least 100 spermatozoa. The number of spermatozoa is determined by a hemocytometer.

**Data analysis:** The data obtained was analyzed using the Independent T-Test with statistical software (SPSS, version 29.0). The T-test was carried out using a significance level of 0.05 ( $\alpha=5\%$ ) (Ghozali, 2012) [3].

## Results and Discussion

The effect of a teaser on the concentration of spermatozoa is presented in Table 1. The research result showed that the concentration of spermatozoa obtained with and without teaser had normal values. By using a teaser in the form of vaginal swabs dogs in heat show a slightly higher concentration than without a teaser. Based on the results of the statistical test using the T-test, it showed that there is no significant difference in the concentration of spermatozoa with teaser and without teaser ( $p>0,05$ ).

**Table 1:** Average Concentration of Spermatozoa

Treatment	Concentration of Spermatozoa
Without Teaser	1882.93 ± 317.54 <sup>a</sup>
With Teaser	2071.25 ± 751.06 <sup>a</sup>

According to Martinez (2004) [9], normal dogs may ejaculate samples that are oligozoospermic or azoospermic due to anxiety or pain. Semen collection treatment using vaginal swabs of estrous females is likely to reduce anxiety and stress in dogs. This opinion is supported by the results of research conducted by Woszczyło *et al.*, (2023) [19], which states that manual semen collection in dogs can be considered a passive procedure since the penis is handled manually and the dog is stimulated by the handler, the lack of sexual arousal from the female and the need for active sexual behavior can be considered as the reason for the lack of adequate response from the adrenal glands, as indicated by the finding of a spike in cortisol during collection semen. In addition, dog contact with a female in heat allows for a reflex reception and release of pheromones by the females that leads to psychological sexual arousal and is potentially a source of eustress and sex pheromones, the components of which occurs in such events can be considered as natural reinforces. Pheromones themselves are chemical compounds released by emitters into the environment that can modify the behavior and psychology of signal receivers. These substances are used by females in estrus to attract males and cause mating attempts (Wyatt, 2014) [20]. However, semen collection in dogs is often carried out without the presence of a female in heat as a teaser, and the effects of this procedure on the psychology and welfare of the animal are not yet fully known.

According to Traas and Kustritz (2004) [17], the presence of estrous females during semen collection can affect semen quality, especially increasing concentration. This opinion is in line with the results of research conducted, where there was a greater average concentration of spermatozoa in the same collection treatment using a vaginal swab teaser for estrous females. However, the difference in the average concentration of spermatozoa values in this study was not significant, this could be caused by several reasons, such as too short of a dog interaction with the teaser or not enough pheromones content in vaginal swabs.

The effect of a teaser on the motility of spermatozoa is presented in Table 2. The sperm motility of Kintamani dog semen without a teaser during semen collection had an average of 83,43 ± 5,69% and with a teaser 84,06 ± 7,79%. Based on the results of the statistical test using the T-test, it showed that there is no significant difference in the sperm motility of with teaser and without teaser ( $p>0,05$ ).

**Table 2:** Average Sperm Motility

Treatment	Sperm Motility
Without Teaser	83,43 ± 5,69 <sup>a</sup>
With Teaser	84,06 ± 7,79 <sup>a</sup>

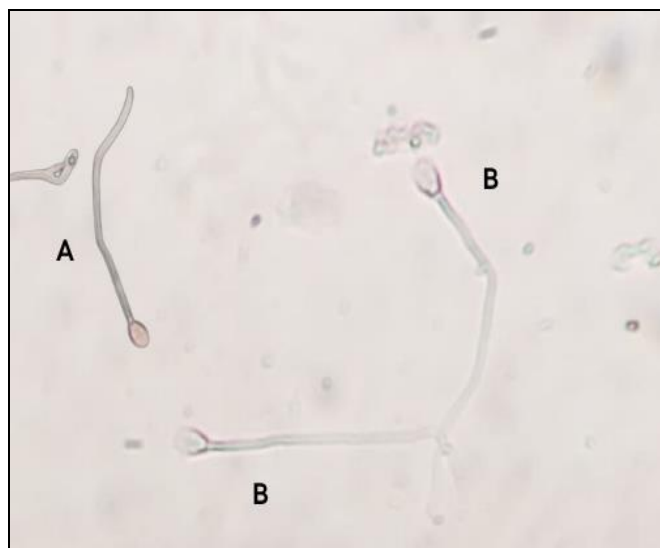
In examining semen quality, assessing sperm motility is an important aspect of determining semen quality (Sergeant *et al.*, 2019) [15]. When examining spermatozoa motility, the indicator that needs to be understood and paid attention to is the forward movement of the spermatozoa. This movement is a forward movement in a straight line and can move from one point to another. This forward movement provides an overview of qualities related to fertility. Normal progressive sperm motility is  $\geq 75\%$  (Pipan *et al.*, 2020) [12], or normal sperm motility values must be more than 70% (Dalmazzo *et al.*, 2018) [2]. Spermatozoa with motility below 60% is considered an abnormal condition (Johnston, 1991) [5].

The results of this research regarding the sperm viability in Kintamani dogs with and without teaser during semen collection showed the same results. The average sperm

viability without a teaser was  $84.62 \pm 7.14\%$ , while with a teaser it was  $85.18 \pm 7.46\%$ . The results of the Independent T-Test showed that there was no significant difference in sperm viability between with teaser and without teaser ( $p > 0.05$ ). Sperm viability is defined as the percentage of live sperm found in a semen sample. Spermatozoa with a color head are live spermatozoa, while dead spermatozoa show a red color on their heads (Figure 1). The average sperm viability of Kintamani Dog with teaser and without teaser during semen collection is presented in Table 3.

**Table 3:** The Average of Sperm Viability.

Treatment	Sperm Viability
Without Teaser	$84,62 \pm 7,14$
With Teaser	$85,18 \pm 7,46$



**Fig 1:** Sperm Viability of Kintamani Dog (400x magnification). A: dead spermatozoa, B: live spermatozoa

In this study, sperm viability testing was carried out using an eosin nigrosine stain, this is based on Pipan *et al.*, (2020) [12], which stated that eosin nigrosine is one of the best methods for examining sperm viability. Examination with eosin nigrosine is considered fast, and simple and does not require cell manipulation to determine sperm viability. By counting the number of spermatozoa that absorb the dye, can estimate the ratio of the number of live and dead spermatozoa. A good semen sample must have at least 80% normal and viable spermatozoa (Johnston *et al.*, 2001) [6], if the proportion of normal spermatozoa is below 60%, it can impact fertility (Oettle, 1993) [10].

### Conclusions

Based on the results of the research that has been carried out, it can be concluded that the use of teasers in the form of vaginal swabs from dogs in heat does not affect the percentage of motility, viability, and concentration of spermatozoa. Further research needs to be carried out to determine the impact of other types of teasers such as dogs in heat on the quality of spermatozoa in semen collections such as using synthetic teasers.

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