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# Effect of sexual rest period of ejaculation on semen quality in Kintamani Bali dog

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**Abstract**
The success of artificial insemination in dogs is influenced by many factors, including good semen preparation and handling and the number of sperm cells per dose. Good semen quality is influenced by many factors, one of which is the frequency of ejaculation. Ejaculation frequency that is too frequent will cause a decrease in libido and semen quality. This study aimed to compare semen quality parameters (motility, viability, concentration) during ejaculation rest periods of between 3 days and 5 days. A total of four Balinese Kintamani dogs were divided into 2 groups, namely Group A whose semen was taken on day 0 and between the 3rd day, and Group B whose semen was taken on day 0 and between the 5$^{th}$ day. The results showed that the average spermatozoa concentration on day 3 and day 5 was 3102 ± 1.74 and 5712 ± 1.72 million/ml, respectively. The average motility taken on day 3 and day 5 respectively was 90.13 ± 1.50 and 92.88 ± 1.62 and the average viability on day 3 and day 5 respectively was 87.75±1.61 and 93.06±1.61. The results showed that there was an increase in semen quality during the 5th day of ejaculation rest. In conclusion, the ejaculation rest period on day 5 showed better semen quality compared to the ejaculation rest period on day 3.

**Keywords:** Balinese Kintamani dog, ejaculation, motility, concentration, viability

## Introduction
The Balinese Kintamani Dog is a native Indonesian dog breed that originates from Kintamani, precisely in Sukawana Village, Bangli Regency, Bali Province. The Balinese Kintamani dog has a beautiful appearance so this dog has become a mascot for the fauna in Bangli Regency (Puja, 2007) [14]. In 2012, the Balinese Kintamani Dog was officially recognized by the Asian Kennel Union (AKU) as an Asian breed of dog and in 2019 it became the first Indonesian breed of dog to be recognized in the world by the Federation Cynologique Internationale (FCI) [3]. With this recognition, the status of Kintamani dogs has been equalized with other types of dog breeds.

The beautiful and attractive appearance of the Balinese Kintamani Dog causes increasing demand from the public to keep these dogs (Gunawan *et al*., 2012) [6]. The large demand from the public to maintain and make the Balinese Kintamani Dog a part of the dog breeding industry means that the population of the Balinese Kintamani Dog must be increased. To increase the population of the Bali Kintamani Dog, various ways can be done, one of which is the application of reproductive technology in the form of artificial insemination (Puja *et al*., 2023) [14].

Artificial insemination is a technique for assisted reproduction in animals and humans. In artificial insemination, semen is collected manually and inseminated in the female so that the fertilization process can occur without natural mating (Payan-Carreira *et al*., 2011; Mason, 2018) [11, 10]. The advantage of using artificial insemination is that it can be used especially for couples who are mostly unable to mate due to differences in body size (Kalkan and Ucer, 2022) [7], the use of genetically valuable stud dog semen throughout the world, to reduce transportation stress and inbreeding (Dutta and Dutta, 2020) [2].

The success of artificial insemination in dogs is influenced by many factors, including good semen preparation and handling and the number of sperm cells per dose (Payan-Carreira *et al*., 2011) [11], semen quality and insemination time (Kim *et al*., 2007) [9]. Good semen quality is influenced by many factors, one of which is the frequency of ejaculation.

Repeated ejaculation in a short time will produce a low semen volume (Ali Hasan *et al*., 2021) [1]. Ejaculation frequency that is too frequent will cause a decrease in libido and semen quality (Taha *et al*., 2008) [20]. Intensive semen collection appears to be less effective in maintaining good sperm quality. However, for breeding purposes or artificial insemination, an interval of 48 hours between collection sessions is recommended for semen collection in dogs (Salvado *et al*., 2024) [17].

Research has been carried out on the quality of semen at different resting times on different species, but no research has been done on dog species, especially the Balinese Kintamani Dog. For this reason, research needs to be carried out to see the effect of the sexual rest period of ejaculation for taking semen on the quality of the Bali Kintamani Dog semen.

## Materials and Methods

### Animals

In this study, the objects used were four Balinese Kintamani dogs, adult males aged 2.5 years. The Kintamani dogs used are clinically healthy and kept in 2x4m cages at the Sato Kennel, Gianyar. The samples taken were semen, using a massage method. This research has received approval from the Ethics Commission of the Faculty of Veterinary Medicine, Udayana University, Denpasar, Bali, Indonesia.

### Research design

The research design used was a Completely Randomized Design (CRD), with 2 treatments at different intervals for the ejaculation rest of Bali Kintamani Dog semen, namely taking the 3rd day and the 5th day interval. A total of 4 dogs were divided into 2 groups, namely Group A whose semen was taken on day 0 and between the 3rd day, and Group B whose semen was taken on day 0 and between the 5th day. Semen collection was carried out at the same time in succession until 32 semen samples were collected

### Semen collection

Semen is taken using a massage method from the preputium to the bulbous glands. When massaging, the male will respond by lifting one of his legs. Next, massage is carried out on the bulbous glands so the male will make rhythmic movements as if inserting his genitals into the female's reproductive tract. After that, hold the bulbus glandis tightly and the penis will begin to erect accompanied by ejaculation. The semen that manages to come out is collected in a glass funnel (Puja *et al*., 2023) [14].

### Segment evaluation

Examination of spermatozoa motility is carried out by dripping about one drop of Kintamani dog semen on a glass object. Then the semen that has been dripped on the object glass is covered using a cover glass. The object glass and cover glass used must be warmed to a temperature of 37 ºC. After that, the semen was observed under a microscope with 100x magnification. The total number of spermatozoa motility is based on seeing the movement of spermatozoa.

Examination of spermatozoa viability can be carried out using the eosin nigrosin citrate staining technique by dripping one drop of diluted semen on a glass object and then making a smear by pressing and pushing using another glass object. The semen is then dried, once dry, add carnoys as a fixative then let it sit for 5 minutes. Next, rinse with water and dry. Then add eosin, let it sit for 15 seconds, and continue by adding nigrosin then let it sit for 15 minutes. After that, rinse with water and dry. When it is dry, observe under a microscope with 400x magnification to see the percentage of live and dead spermatozoa.

The concentration check is carried out by sucking the semen with an erythrocyte pipette to a scale of 0.5 and then sucking in a 3% NaCl solution to a scale of 101. Then, close the pipette using the thumb and middle finger then homogenize the semen together with the 3% NaCl solution by swinging it to form a number. 8. After that, drop semen into the hemocytometer counting chamber which has been covered using a cover glass and then let it flow until the counting area is filled. Then, observe the counting chamber under a microscope with 400x magnification (Puja *et al*., 2023) [14].

### Data analysis

The data obtained was analyzed using the Independent Sample T-Test in the SPSS application. The t-test was carried out by looking at the significance level of 0.05 (Ghozali, 2012) [5].

## Results and Discussion

The effect of ejaculation rest on semen collection on the spermatozoa concentration of Kintamani Bali dogs is presented in Table 1. The mean concentration of spermatozoa collected at 3-day intervals was 3102 ± 1.74 million/ml and 5712 ± 1.72 million/ml for 5-day intervals. The results of research related to concentration show that the collection with a rest period of 3 shows a decrease in concentration, while the collection with a rest period of 5 days shows an increase in concentration compared to 3 days (Table 1). The results of statistical analysis showed that there was a significant difference between semen collection with a rest period of 3 days and 5 days ($p<0.05$).

**Table 1:** Mean spermatozoa concentration of Kintamani dogs given ejaculation rest for 3 days and 5 days.

| Length of Rest Time | Concentration ($x10^6$) (mean±SD) |
|---|---|
| Hari ke 3 | 3102±1,74 |
| Hari ke 5 | 5712±1,72 |

Research on ejaculation rest periods of 3 days and 5 days provides results stating that a 5-day rest period has better results compared to a 3-day rest period. However, from the results obtained whether the ejaculation rest period was 3 days or 5 days, both were still said to have a normal semen concentration range and were suitable for use for artificial insemination.

The average percentage of motility during ejaculation rest for 3 days was 90.13% and for 5 days was 92.88% (Table 2). The results of statistical analysis showed that there was a significant influence of ejaculation rest time on spermatozoa motility in Kintamani Bali dogs ($p<0.05$). The motility of spermatozoa from Kintamani dogs with an ejaculation rest period of 5 days was better than with an ejaculation rest period of 3 days. The results show that there is an increase in spermatozoa motility if the ejaculation rest period is carried out for 5 days (Table 2).

**Table 2:** Average Motility of Spermatozoa of Kintamani Dogs with Ejaculation Rest Periods of 3 Days and 5 Days.

| Length of Rest Time | Motility (%) (mean±SD) |
|---|---|
| Hari ke 3 | 90,13±1,50 |
| Hari ke 5 | 92,88±1,62 |

The viability of spermatozoa of Kintamani Bali dogs is influenced by ejaculation rest time. The average percentage of viability during ejaculation rest for 3 days was 87.75% and for 5 days was 93.06%. The results of statistical analysis showed that there was a significant influence of ejaculation rest time on the viability of spermatozoa in Kintamani Bali dogs ($p<0.05$). Viability decreases with increasing frequency of ejaculation in Kintamani dogs (Table 3).

**Table 3:** Mean Viability of Spermatozoa of Kintamani Dogs with Ejaculation Rest Periods of 3 Days and 5 Days.

| Length of Rest Time | Viability (%) (mean±SD) |
|---|---|
| Hari ke 3 | 87,75±1,61 |
| Hari ke 5 | 93,06±1,61 |

The findings from this research are important in the reproductive management of Balinese Kintamani dogs. This is the first study to focus on observing the semen quality of Kintamani dogs with different ejaculation rest times. Research results have shown that a 5-day ejaculation rest period shows better semen quality compared to a 3-day ejaculation rest period.

The average total number of spermatozoa in this study was still within the range according to Root Kustritz (2007) [16] who reported that the total number of spermatozoa was on average between 300 million and 2 billion/ejaculate. The exact range of total number of spermatozoa in dog ejaculate is not specifically known. This amount depends on the size of the testicles and the frequency of semen collection (Raza and Andrabi, 2022) [15].

This study showed that the spermatozoa motility of Kintamani dogs was significantly higher in semen collection with a 5-day ejaculation break compared to a 3-day break. Spermatozoa motility in this study was above the normal range. This statement can be supported by the results of research conducted by scientists, where motility is normal and good if the spermatozoa cell movement is ≥75% moving progressively (Pipan *et al*., 2020) [12]. The research results for Kintamani dogs were higher than for German Shepherd dogs, namely 83.3 ± 0.79% (Shalini and Antoine, 2018) [19]. However, it is very close to the results reported by Sulabda *et al*., (2022) [19] on Kintamani Dogs which stated that the motility of fresh Kintamani Dog spermatozoa was in the range of 89 -94%

Viability in this study was determined by calculating the percentage of live dead spermatozoa using the eosin-nigrosin citrate staining method (Pipan *et al*., 2020) [12]. In this study, the percentage of viable and dead spermatozoa was above the normal range value. According to Puja *et al*. (2023) [14], the percentage of dead spermatozoa in each ejaculate is between 15%-20%, while for a good semen concentration if the spermatozoa cells number 200-600 x 106 spermatozoa/ml. The results of this study are slightly smaller than the results reported by Sulabda *et al*., (2022) [19], who reported that the percentage range for survival and death of Kintamani dog spermatozoa was 90-95%.

The parameters used to assess semen quality during ejaculation rest periods of 3 days and 5 days showed that there was a significant difference ($p<0.05$), indicating that the ejaculation rest period greatly influenced the semen quality of Kintamani dogs. According to Vágenknechtová *et al*., 2011 [21], the quality of dog semen is influenced by internal factors, namely the sequence of semen collection and the age of the dog. Apart from that, factors that can influence semen quality are the size of the testicles, age, and sexual activity, where if ejaculation of semen is too frequent it can cause the quality of semen to decrease because the semen reserves in the epididymis have been depleted.

Ejaculation frequency that is too frequent without being given a limit or rest time for a male will cause a decrease in the quantity and quality of the semen produced. Reducing the gap between ejaculations can improve semen quality and the minimum sexual rest time required for a dog to maintain good semen quality is 3 days (Raza and Andrabi, 2022) [15]. According to Kaya *et al*., 2002[8] stated that a high frequency of ejaculation will have an impact on specific changes in spermatozoa so that it will affect the fertility of spermatozoa when they are deposited in the female reproductive tract by artificial insemination (AI) or natural mating. Research from Foote, 1964 [4] stated that in general, giving ejaculation rest for 5 days is mandatory before collecting and evaluating semen. The quality of semen decreases during the ejaculation rest period of 3 days. No research has been found that specifically explains the causal factors. However, it is possible that a time interval that is too short or an ejaculation frequency that is too frequent can cause a decrease in semen quality.

## Conclusion

Based on the results of the research conducted, it can be concluded that ejaculation rest periods of 3 and 5 days affect the quality of the semen of Kintamani dogs. The percentage of motility, viability and concentration of the semen of Kintamani dogs given ejaculation rest for 3 days and 5 days is still above the normal value for dog quality. A rest period of 5 days shows better quality than a rest period of 3 days.

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