P-ISSN: 2304-3075; E-ISSN: 2305-4360
International Journal of Veterinary Science
www.ijvets.com; editor@ijvets.com
Research Article https://doi.org/10.47278/journal.ijvs/2022.171

Dynamic of Vaginal pH and Ovary Ultrasound Imaging of Kintamani Bali bitch during Proestrus to Estrus Phase

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Article History: 22-617 Received: 17-May-22 Revised: 03-Jun-22 Accepted: 10-Jun-22

ABSTRACT

Dogs are one of the pets that have many useful benefits for humans. According to this, many people consider getting a dog, especially the Kintamani Bali dog, so it is important to know how the reproductive physiology of the dog works. This study aims to find a new simple method for determining the right time for mating seen from the vaginal pH and the correlation with ovary ultrasound in the Kintamani Bali bitches. This research is an observational study to determine vaginal pH levels and the connection with ovary ultrasound images in Kintamani Bali bitches during the proestrus phase to the estrus phase. Vaginal pH levels were checked every day from the first day of proestrus, when there was a blood discharge until the female ovulates. Ultrasound examinations were taken four times, when the first bleeding (proestrus phase), seven days later (end of proestrus) when the female accepted the male (estrus), and three days later (ovulation). The result showed that vaginal pH decreased from proestrus to estrus. At the beginning of the estrus phase, the average pH level was 7.1, while the highest was 7.8. Ovulation occurred on the 10th to 12th day from the first blood discharge, and the pH level was between 6 to 6.5 with an average of 6.2. after ovulation, the average pH level increased to 6.83. There is a relationship between the dynamics of vaginal pH and the ultrasound image of the ovaries in the Kintamani Bali bitches during the proestrus phase until estrus.

Key words: Kintamani Bali Dog, Vaginal pH, Ultrasound imaging of ovaries, Proestrus, Estrus.

INTRODUCTION

Dogs are carnivorous mammals and have been domesticated from the wolves around 15.000 years ago. High intelligence, loyal character, and various types, shapes, and sizes make dogs very popular pets. Aside from being a pet, dogs are also frequently used to help humans, such as guide dogs for blind people, search and rescue dogs, police dogs, and others. According to Headey et al. (2008), dog owners generally have better sleeping conditions than people who do not have dogs. Dogs are often called man's best friends and help humans a lot. The Kintamani Bali dog was recognized as a new breed of dog by the Federation Cynoloqique Internationale (FCI). This dog originating from the Kintamani mountains, is a native Indonesian dog breed that is categorized as an ancient dog (Puja et al. 2018). Kintamani Bali dog is categorized as a companion dog (companion dog) and Kintamani Bali dog was considered a descendent from Balinese feral dog with a loss of genetic diversity (Sawitri et al. 2021). This excellent dog group is very popular among the people in Indonesia (Puja et al. 2019). This reason makes the demand for dog breeding increase significantly, especially the Kintamani Bali dogs. So, it is very important to know the physiological features of the dog's reproduction (Saleh and Shamkhi 2018).

The bitches have several phases in the repeating cycle in their reproductive life. This cycle is known as the estrus cycle. It is divided into several phases, proestrus (the initial period), estrus (the period of sexual acceptance), diestrus/metestrus (the period when sexual activities are slowing down) and anestrus (the rest period). In bitch, there are many changes in every phase, such as in the ovary (Junaidi 2021). The changes in ovarian structure can be observed during the estrus phase. Observations can be done using diagnostic imaging such as ultrasonography. Besides the ovary, other changes can be observed directly, for example, the changes in the external genital system, such as the vulva and vagina.

Cite This Article as: Utomo BGR, Puja IK and Gunawan IWNF, 2023. Dynamic of vaginal pH and ovary ultrasound imaging of Kintamani Bali bitch during proestrus to estrus phase. International Journal of Veterinary Science 12(2): 242-247. https://doi.org/10.47278/journal.ijvs/2022.171

Besides the physical changes, chemical changes can also occur at ovulation, one of which is a change in the vaginal pH level. pH (Power of hydrogen) is the degree of acidity and is usually used to determine how acidic or basic a solution is. In this case, it is the secretion produced by the cervix and excreted through the vagina. The secretion produced by the cervix has a rheological feature that can show the quality values and it can be used to measure the successful mating process (Makmun et al. 2017).

There has been no research on the dynamics of vaginal pH levels in Kintamani Bali Dogs during the proestrus to estrus phase and associated with the ultrasound imaging of the ovaries. If the dynamics of vaginal pH can be observed during the estrus phase and it has associated with the changes in ovaries. It is possible to use it as a new, cheap, and simple method for determining the estrus phase in dogs.

MATERIALS AND METHODS

Ethical Approval

This study was conducted following the principal and guidelines of the animal ethics committee Faculty of Veterinary Medicine Udayana University, Denpasar, Bali, Indonesia.

Object of Research

This study used three Kintamani Bali bitches that had given birth and a normal estrus cycle as a sample. The bitches are maintained by Surya Kennel in Bangli District, and ages range from 2 to 5 years. The condition looks healthy, and all bitches have been regularly vaccinated. Examination of the vaginal pH level and ultrasound images of ovarian development during proestrus to estrus phases were performed. Samples were taken every day from the first day of a blood discharge (proestrus phase) until the ovulation occurred.

Vaginal pH Measurement

The vaginal pH test is carried out using litmus paper that has fourteen color indications. At the first bloody discharge, the measurement is tested every day until the bitch ovulates, which would be confirmed through an ultrasound image of the ovaries. Litmus paper is placed vertically on the surface of the vagina that has mucous for about three seconds. After a few seconds, the litmus paper color will be changed and immediately compared with the color standardization and the vaginal pH level is determined. Some dogs do not secrete mucous during a certain phase in the estrus cycle, so a cotton bud is used. A cotton bud is used to push the paper slightly into the vulva so that the mucous can stick to the litmus paper (Antonov et al. 2014).

Ultrasound Examination

Ultrasound examination was started from the first day of bleeding (the beginning of the proestrus phase). During proestrus, follicles may be detected from day 2-7 and in some cases, they are found to be more than 1cm in diameter as ovulation approaches (O'brien and Barr, 2009). The bitch was positioned on her back (dorsal recumbency) and handled to control the dog's movement. The fur around the abdomen was shaved using a pet clipper to get a maximal quality of ultrasound images. After the area was clean, the ovaries were examined.

From the anatomy, ovaries are located caudal to the kidney, so the technique for examining the ovaries is similar to the kidneys (Noviana et al. 2018). This study used two different ultrasound machines, a PT50C Vet "Touch Color Doppler Ultrasound Scanner System" with a micro convex- array probe. Another machine is Ultrasound Portable Mindray 2D Black and White DP-10 Vet with a macro probe. This ovarian examination used both the sagittal and transversal plane and transabdominal window, and the frequency is about 7 to 8.5 MHz. to get the best result (Kinns and Nathan, 2017). The examination was carried out four times, on the first day of bleeding (proestrus), the following week (end of proestrus), when the female is willing to accept the male (estrus), and two to three days after estrus or at the time of ovulation.

Data Analyses

Ultrasound image of the ovaries during the proestrus to estrus phase was collected, including their shape, echogenicity, and size were noted. The vaginal pH level is presented graphically. The result was then analyzed descriptively so that a conclusion was obtained on the vaginal pH level when the ovaries have ovulated. It was used as a parameter to determine the right mating time.

RESULTS

In this study, the vaginal pH level of all samples was found normal and is neither too acidic nor too alkaline. This result indicated that the dogs were healthy and had no abnormalities. The duration of the proestrus phase is from 6 to 8 days, and ovulation occurs from days 10^{th} to 12^{th} from the beginning of the proestrus phase. The difference in ovulation time is because the individual's response to the estrogen hormone may differ.

The pH level of vaginal discharge during the proestrus phase ranged from 6.2 to 8.5, with a mean of 7.3 (Fig. 1). This pH level is relatively decreased when it enters the estrus phase and continues until ovulation. On the first day of the proestrus phase, the highest pH level was 8.5 and the lowest was 7.5, with an average of 8.17. At the end of the proestrus phase, the average pH level decreased to 6.83 until the beginning of the estrus phase or when the female accepted the male, the pH levels of two of the three samples used (66.7%) increased. At the beginning of the estrus phase, the average pH level was 7.1, while the highest was 7.8. Ovulation occurred between days 10-12 since the first discharge. The pH level of vaginal discharge ranged from 6-6.5, with an average of 6.2. After ovulation, the average pH level increased which was 6.83.

An ultrasound examination of the ovaries is performed to confirm that the bitch has ovulated. Ultrasonographic images obtained from all samples were normal and no abnormalities were found in size, shape, or echogenicity. At the beginning of proestrus, the ovary looked oval and tended to be anechoic, but there was also a slight hyperechoic appearance. The ultrasound image of the ovary in the proestrus phase was the smallest in size of the other phases (estrus and ovulation) (Table 1). During proestrus, the average size of the right ovary was 0.8 x 1.37 cm, with the smallest size being 0.73 x 1.27 cm. While the left ovary is 0.83 x 1.15 cm, with the smallest size 0.66 x 0.9 cm. The size of the ovaries on each bitch did not differ far enough.

Table 1: The ovarian size mean and ultrasound imaging

Examination Time	Ovarium Size (cm)				Description
	Right Ovary		Left Ovary		
Proestrus	0.8	1.37	0.83	1.15	Hypoechoic, Oval Shape
Estrus	1.01	1.73	1.13	1.85	Anechoic, Slightly Round shape
Ovulation	0.87	1.22	1.04	1.55	Anechoic

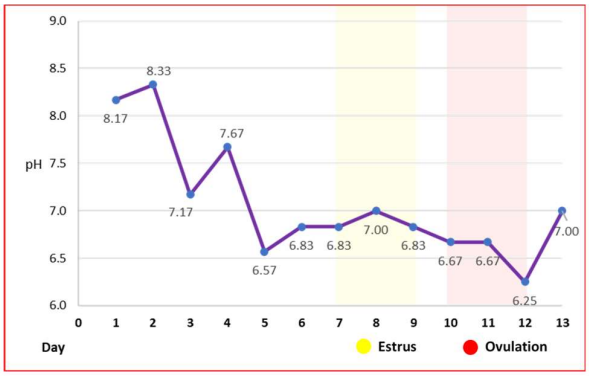

Fig. 1: Graphic of the vaginal pH average from day 1 to 13.

The estrus phase was signed by accepting the female when mounted by the male and occurred between the seventh and ninth day. During estrus, there was an increase in the size of the ovaries in all samples. The ultrasound image of the ovary looked more anechoic during the estrus phase than the proestrus phase. Moreover, in one bitch, there was a twofold increase in the size of the left ovary. In this phase, the average ovarian size was 1.01 x 1.73 cm on the right ovary and 1.12 x 1.85 cm on the left. During ovulation, a decrease in ovarian size was observed, but in one bitch, there was a slight increase in the size of the left ovary. This decrease was caused because the ovaries have released eggs into the fallopian tubes. The average right ovary is 0.87 x 1.22 cm, while the left ovary is 1.04 x 1.55 cm.

DISCUSSION

This research on the dynamics of vaginal pH levels associated with ultrasound images of the ovaries during the proestrus to estrus phase is important to provide a new simple method for determining mating time. Antonov et al. (2014) stated that the vaginal pH value varies between species and shows some special characteristics in these species. Many studies have been conducted on vaginal pH in several animal species.

At the beginning of the proestrus phase, vaginal pH levels ranged from 7.5 to 8.5. This result is similar to the previous study (Antonov et al. 2014), where the vaginal pH in bitches at the beginning of proestrus ranged from 7.4 to 8.7 with an average of 7.96. The pH level decreased from the end of the proestrus phase to the estrus phase. In rodents (rats), the pH level in the proestrus phase is 5.57 and decreases in the estrus phase to 4.53 (Ganesan and Kadalmani 2016). According to (Fesseha and Degu 2020), vaginal pH levels in cows decreased during estrus and tended to be constant or stable during the diestrus phase. The pH level decreased from 7.0 to 6.72 the day before entering the estrus phase. At the beginning of the estrus phase, the pH level reached 6.54. Research to Layek et al. (2011), in cows, the lowest pH level occurred before ovulation, which was 6.45.

This phenomenon of decreasing pH levels also occurs in horses, Polak and Kammlade Jr (1981), in their research, stated that vaginal pH levels for three days before ovulation were 7.40, 7.38 and 7.36, respectively. During ovulation, the pH level of the vagina is the lowest compared to the other phases, namely 6.81; after ovulation, the pH level has increased, which is the same as the data from this study. This study found that the lowest pH results occurred when the dog was in the ovulation phase, which is similar to the results of studies conducted by Schulz (2002) and Ross (2005).

Changes in pH levels at each phase in the estrus cycle are influenced by levels of estrogen, which decrease significantly before ovulation, and the hormone progesterone, which increases. Brabin et al. (2005) stated that fluctuations in steroid hormones indirectly affect vaginal pH in mammalian species. Normally, when the behavior of the animal (sheep) has shown symptoms of estrus, there will be an increase in body temperature and vaginal pH levels as well (Rasad and Setiawan 2017). The vaginal discharge becomes clearer (colorless) when entering the estrus phase than during the proestrus phase. According to Widayati et al. (2018) in their research on Etawah Goats, this could be an indication of changes in the ionic composition of the vagina. The decrease in vaginal pH is influenced by the accumulation of ions such as hydrogen, sodium, and chloride present in the vagina (Gaafar et al. 2005).

Until now, there are still no other aspects that significantly affect changes in vaginal pH. Several studies have been done previously are the relationship between microorganisms and vaginal pH levels. Groppetti et al. (2012) stated that the reduction in bacteria number with the progress of the follicular phase had been proven in their research. This result may depend on changes in the Ph vaginal environment, which have been shown to decrease from proestrus to estrus. The vaginal bacterial flora is suspected to be influenced by pH, oxidation-reduction potential, constituent cells, hormones, presence of mucus, presence and concentration of antimicrobial agents, and biological conditions (Noguchi et al. 2003). Based on these reasons, there may be several pathological conditions that can affect changes in vaginal pH. However, according to Ross (2005), there is no relationship between certain diseases and changes in vaginal pH in dogs.

The lowest pH level from the results of this study was recorded when the bitch ovulated. Research conducted by Khalifa et al. (2010), in goats, a sudden drop in vaginal pH after the estrus phase is an indication and character of ovulation. In this study, the most important indication that ovulation has taken place is increased vaginal pH after a maximal decrease. This increase is a sign that it is the right time to inseminate the female dog (Antonov et al. 2014). This increase also happened in this study, were on day 13, the pH level rose significantly, indicating that ovulation had occurred.

Ultrasonography is used to detect the time of estrus and ovulation in dogs. Ultrasonography appears to be a useful tool that allows the evaluation of changes in follicle size and number and the number of corpus luteum during various stages in the estrus cycle (England et al. 2009). In this study, ultrasound examination of the ovaries of the Kintamani Bali bitch was not easy; this could be due to the dog's difficult condition to handle , the dog's condition,

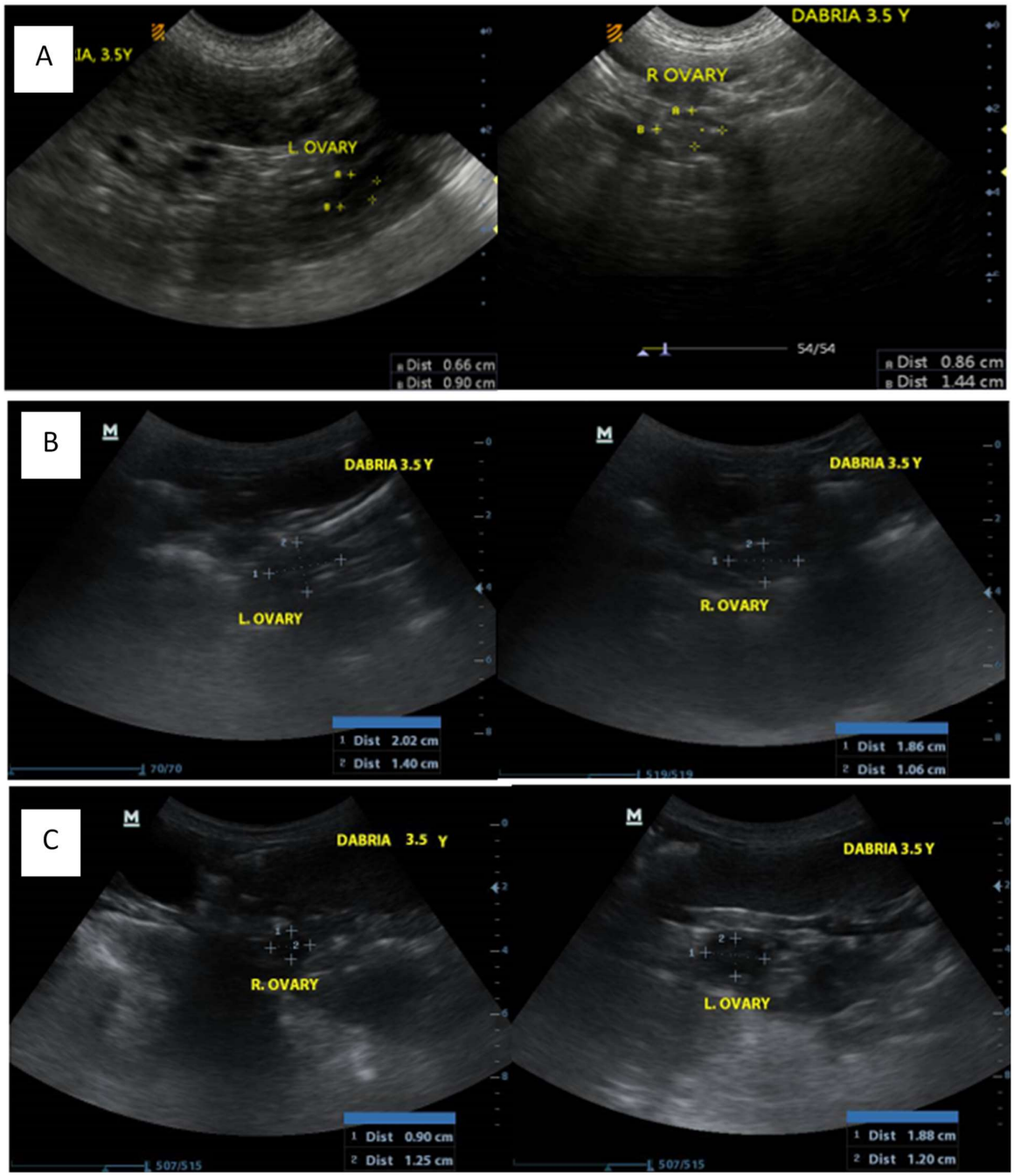

Fig. 2: Ovarian ultrasound imaging in one of the dog samples. A: The beginning of proestrus; B: Estrus phase (preovulatory) and C: After ovulation.

which was too alert when meeting new people, and the difficulty level in the ovarian examination technique itself. According to Barbosa et al. (2013), ultrasound images of the ovaries around ovulation are more challenging to analyze in bitches than in other species.

Dickie (2006) states that ultrasound images of the ovaries are generally round, filled with fluid, and look anechoic, while the corpus luteum has a homogeneous structure that may have a hypoechoic or anechoic part in the middle. The presence of active structures such as follicles and corpus luteum increases the size of the ovaries, which is why identification is easier during the estrus and diestrus phases.

On the examination that has been carried out, the ultrasound image is oval and hypoechoic on the first day of the proestrus phase (Fig. 2A). This result is by what was conveyed by Dennis (2010), where during anestrus and early proestrus, the image of the ovary is round with soft/regular margins and is hypoechoic. On the eighth day, when the end of proestrus entered the beginning of the estrus phase, the ovary's ultrasound results increased in size and the echogenicity structure became darker or anechoic (Fig. 2B). A change in echogenicity accompanies this increase in ovarian size to anechoic. These results are similar to the results of research conducted by Lévy and Fontbonne (2007), where during the pre-ovulatory or late

proestrus phase, the size of the ovary increases, with the appearance of follicles with an anechoic structure and filled with fluid, the ovaries in this phase are very easy to identify. The increase size of the ovaries is due to the increase size and number of follicles. The variation of follicles number in each ovary is 3–4 (Bergeron et al. 2013). Furthermore, Eker and Salmanoğlu (2006) reported the various of ovary size is ranged from 1.08-2.50cm in follicular phase.

Ultrasound examination was carried out again two days after obtaining an image of the ovaries in the pre-ovulatory phase. The ultrasound image showed an anechoic appearance, still oval but slightly reduced size (Fig. 2C). This decrease in the size of the ovaries, we can believe, is a sign that ovulation has occurred. It is similar to study that conducted by Eker and Salmanoğlu (2006) who reported the decrease size of the ovaries after ovulation in bitch. According to Lévy and Fontbonne (2007), ultrasound images of the ovaries at pre-ovulatory and post-ovulatory times sometimes look the same. Important checks are carried out every day during the preovulatory phase to detect ovulation with certainty.

Conclusion

From the results of the research that has been carried out, it may be concluded that there is a relationship between the dynamics of the vaginal pH of the Kintamani Bali bitch during the proestrus to estrus phase and the ultrasound image of the ovaries. The pH level continued to experience a significant decrease from the proestrus to estrus phase. The lowest pH level occurred when the dog ovulated between 6-6.5 with an average of 6.2. Ovulation ends with a marked increase in pH after a decrease and an ultrasound appearance with an anechoic structure, round shape, and a decrease in ovarian size.

Acknowledgment

The authors thank Eka Andrijan Novianto, DVM who help to collect the ultrasound image and the Surya Kennel owners, Bangli. Who contributed to this study.

Author contribution

I Ketut Puja designed the research, Bravanasta Glory Rahmadyasti Utomo collected the data. I Wayan Nico Fajar Gunawan contributed to collection and interpretation of the data. All authors wrote and reviewed the manuscript.

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