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Vaginal cytology and receptivity to mating behaviour of Kintamani bitch during the proestrus to estrus phase

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Abstract

The breeding and rearing of Kintamani Bali dogs have evolved from just keeping them as pets to a dog farming industry. Individual variation in female dog fertility must be detected to manage to breed successfully. Vaginal smear cytology is a low-cost and simple method of determining dog's estrus cycle stages. This study aims to determine the vaginal cytology and receptivity to mating behavior in Kintamani Bali bitch during the proestrus to the estrus phase. The investigation started on the first day of proestrus and continued until the first day of receptivity of mating with a male naturally. The results of this study showed that from the beginning of serosanguinous discharge to early estrus, characterized by a gradual change from initially parabasal and intermediate cells (small then larger) and finally to superficial cells. The onset of estrus is characterized by a predominance of superficial cells and their nucleus disappears and is accompanied by a peak of fully cornified cells. In conclusion, was observed a significant difference in cornified cell identification between the proestrus and estrus phases.

Keywords: Estrus, Kintamani Bali bitch, mating, proestrus, vaginal cytology

Introduction

Kintamani Bali dogs are currently being maintained and bred in large numbers. The Kintamani Bali Dog is a native Indonesian dog breed that originated from the village of Sukawana, Kintamani, Bangli Regency, Bali (Puja *et al.*, 2018) ^[1], and was recognized globally as an emerging breed from Indonesia by the FCI in 2019. This group of dogs is very popular among Indonesian people (Puja *et al.*, 2019) ^[2]. Kintamani dog breeding has evolved from just keeping dogs for pets to a dog farming industry. This is because the demand for dogs as pets grows year after year. Kintamani dogs are a fundamental pillar in the daily life of Balinese people. Hence the importance of dogs in Balinese, not only as companion animals but for other beneficial activities, such as religious ceremonies. The goal of every living thing is to ensure the survival of its kind. To accomplish this, they must develop reproductive functions, which consist of each living thing's ability to produce new similar individuals (Calderon *et al.*, 2020) ^[3].

The results of gynecological examinations are very important in supporting breeding management. Furthermore, a vaginal smear examination is a standard gynecological examination because it allows insight into the potential effect of estrogen and vaginal health status (Reckers *et al.*, 2022) ^[4]. Examination of vaginal cytology may provide valuable information about the stage of the ovarian cycle. Vaginal cytology examination offers a cheap, fast, and accurate clinical method to evaluate the stages of the estrus cycle in bitches. Vaginal smear cytology is a very useful examination technique in dogs due to its simplicity, commonly available equipment, and the possibility of obtaining quick results (Antonov, 2017) ^[5]. While changes in vaginal cytology reflect the role of endocrine hormones underlying the oestrus cycle, vaginal cytology is therefore almost always a better predictor of fertility than behavioral or physical signs.

Vaginal cytology is the most applied procedure to verify the status of the female reproductive system (Sharma and Sharma, 2016) ^[6]. In bitch, the examination can be performed during the proestrus, estrus, diestrus, and anestrus phases (Liu *et al.*, 2020) ^[7].

Several types of epithelial cells were discovered during the vaginal swab, including parabasal cells, intermediate cells, superficial cells, and total cornified cells (Antonov, 2017) [5]. The aim of this study was to investigate the vaginal cytology and receptivity to mating behavior in Kintamani Bali bitch during the proestrus to the estrus phase.

Materials and Methods

Animal

Three bitch of Kintamani Bali breed, aged 2-5 years were used in this study. The bitch were housed in individual cages in 2x4 m and fed balanced commercial food. All bitch was healthy. The investigation started on the first day of proestrus and continued daily until the first day of cytological estrus. Daily examination of vagina cytology until the female was receptive to mating with a male naturally. The use of these animals in this study was reviewed and approved by the Animal Ethics Committee, Faculty of Veterinary Medicine, Udayana University.

Sample collection and Staining

Vaginal cytology samples were taken using a cotton swab that had been moistened with physiological NaCl solution. The vulva hole is opened with a finger, and a cotton swab is inserted at the top and swabbed in a clockwise circle on the vaginal wall of the bitch. The cotton swab was rolled on the vaginal wall to obtain epithelial cells, then the cotton swab was rolled on a glass object. After the smear preparation, it is allowed to air dry. Then the smear was fixed with methanol-

acetic acid (3:1) for 5 minutes. The fixed smear was stained with Giemsa.

Evaluation of Vaginal Smear

The vaginal smear was examined under a light microscope for analysis during the proestrus and estrus stages of the estrous cycle. Vaginal cytology was evaluated and recorded according to cell type, with a calculated proportion of 100 cells. The cell from the vaginal is classified as parabasal, intermediate, superficial, and fully cornified cell or anuclear cell.

Data Analyses

The appearance of vaginal epithelial cells was determined by examining the morphology of epithelial cells during the proestrus to estrus phase. Data from the examination of the number of epithelial cells were grouped every day from the proestrus to estrus phase and tabulated in percentage (0-100%). The results of the study were then presented descriptively.

Results

The vaginal smear from the Kintamani bitch revealed that all types of cells were found during the proestrus to estrus phase (Fig 1). The vaginal smears collected from the proestrus bitches revealed that the nucleated epithelial cells appeared at a higher percent during proestrus, and estrus cornified squamous epithelial cells (Table 1).

Table 1: The Number of Vaginal Smears Collected from Kintamani Bitches that were categorized by type of cell

	Vaginal Mucosal Epithelial Cells				
	Parabasal Cell (%)	Small Intermediate Cell (%)	Large Intermediate Cell (%)	Superficial Cells (Nucleated) (%)	Fully Cornified Cell (Anucleated) (%)
Day 1	23,67	33,67	30	8	4,67
Day 2	15,33	28	37	12	7,67
Day 3	8,67	24	46,33	14	7
Day 4	4,67	14,33	50,67	19	11,33
Day 5	0	3,33	44	27,33	25,33
Day 6	0	0,67	30	36,33	33
Day 7	0	0	4,33	36,33	59,33
Day 8	0	0	2	44,33	53,67
Day 9	0	0	0,67	34,67	64,67
Day 10	0	0	1,67	39	59,33
Day 11	0	0	0	38,5	61,5
Day 12	0	0	0	55,5	44,5

The parabasal cells decreased in numbers during proestrus and disappear on day 5, and the fully cornified cell (Anucleated) increased until day 9. The vaginal smear on day 1, revealed parabasal cells, large intermediate cells, superficial cells, fully cornified cells, and small intermediate cells in greater numbers than other cells. The bitch exhibited

receptivity to mating on day 9. On day 9, there was a peak and dominance of fully cornified cells (64.67%), with no large intermediate cells identified. On this day, was revealed the dominance of fully cornified cells was observed (61.5%) and the percentage of superficial cells was 38.5%.

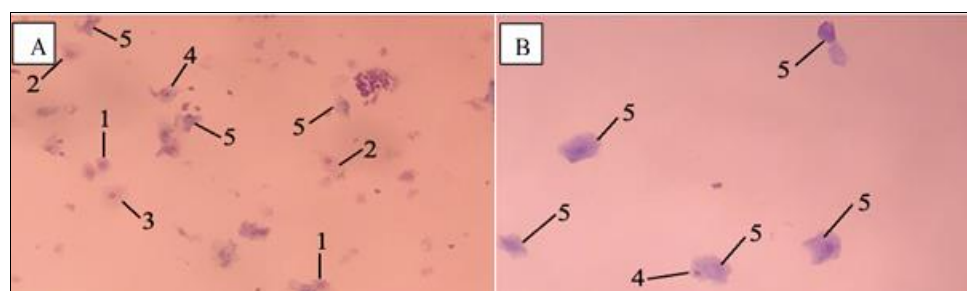


Fig 1: Vaginal Smear of Kintamani Bali Bitch (A= Proestrus, B=Estrus). The classification of vaginal smear examination. (1) Parabasal cell, (2) Small Intermediate cell, (3) Large Intermediate cell, (4) Superficial cell, (5) Anuclear cell

From day 1 to day 6 of after appearance vaginal discharge, the Kintamani Bali bitch refused males. The tail was observed covering the vulva, avoiding and attacking the males. On day 9 of after appearance vaginal discharge, the Kintamani bitch was allowed a male for mating.

Along with the history appears to be serosanguinous for about 9 days, indicating that the bitch is in the late proestrus to early estrus stages of the cycle.

Discussion

The Kintamani Bali bitch, which was used in this study, was found to have a normal vaginal cytology and exhibit behavior response to the male dog during the proestrus to estrus phase. In this study, the onset of the proestrus phase in Kintamani Bali bitch was marked by the appearance of serosanguinous secretions on the vulva for a duration of 8 days. While the estrus phase begins on day 9 after beginning vaginal discharge. The beginning of estrus a normal bitch is days after the first appearance of vaginal discharge (Todorov *et al.*, 2020) [8]. From the first appearance of serosanguineous discharge on the vulva, indicating the start of the proestrus phase. Estrous is the phase when the ovulation occurs and the bitch is eager to accept the male dog and mate with him (Orlandi *et al.*, 2020) [9]. The phases of proestrus and estrus can be determined by sexual behavior, physical signs (vulvar swelling, vaginal bleeding), or by vaginal cytology (Dar *et al.*, 2017) [10]. During estrus, bitches showed a higher percentage of superficial cells (Orlandi *et al.*, 2020) [9]. Estrous cycle generally refers to the phase characterized by clinical manifestation, which includes vulvar edema, and sanguineous vaginal discharge, male attractiveness, receptivity to mating, and a cornified vaginal smear.

This timing discrepancy occurs because the individual response to the hormone estrogen differs. Changes in cytology during the dog's estrus cycle correspond to changes in estrogen concentration in the blood. When the concentration of estrogen rises, the vaginal mucosa thickens and the cell layer proliferates. As the mucosa thickens, the surface cells change in size, shape, and characteristic staining and become larger, irregularly shaped, and eventually form a nucleus. Due to the obvious thickening of the vaginal mucosa during proestrus, neutrophils cannot enter the vaginal lumen, whereas abundant red blood cells can enter the lumen via diapedesis due to the estrogenic action. Estrogen levels rise before and during proestrus, then fall as preovulatory luteinizing hormone levels rise. Rising estrogen levels cause the "cornification" seen in the reviews studied during estrus. Ovulation takes place two days after the LH surge. During proestrus, serum estrogen concentrations rise, inducing capillary breakdown and red blood cell leakage through the uterine epithelium, as well as vaginal epithelial proliferation (Dar *et al.*, 2017) [10].

The vaginal smear examination showed a gradual transition from intermediate and parabasal cells to superficial cells from the beginning to the end of proestrus (Dar *et al.* 2017) [10]. This is by the results of this study. On day 1 after the appearance of vaginal discharge (proestrus phase), the highest percentage of epithelial cells was small intermediate cells, large intermediate cells, parabasal cells, and superficial cells. Then on the 5th day, no parabasal cells were seen in the vaginal examination, and superficial cells began to dominate. The results of this examination differ from the study conducted on clinically healthy adult female dogs by Haji *et al.* (2018) [11] and Gupta *et al.* (2012) [12], which showed that

the proportion of superficial cells was higher during proestrus. Differences in the percentage of cells in the proestrus phase also occurred with research on Labrador dogs and German Shepherds conducted by Rao *et al.* (2011) [13]. Vaginal examinations from female Labrador and German shepherd dogs revealed that superficial cells occurred in a larger percentage during proestrus, the percentage of intermediate cells was observed in almost the same number during proestrus and parabasal cells appeared occasionally during the proestrus phase. Differences in vaginal smears in female dogs during proestrus occur due to the thickening of the vaginal mucosa and proliferation of different cell layers between individuals. As the mucosa thickens, the surface cells change in size, shape, and characteristic staining and become larger, irregularly shaped, and eventually anucleated. Large numbers of red blood cells can enter the lumen through diapedesis (Silva *et al.*, 2020) [14].

The predominance of superficial cells is the defining feature of the cytologic estrus phase (Dar *et al.*, 2017) [10]. The Kintamani Bali bitch allowed males for copulation on the 9th days. It is characterized by the peak of fully cornified cells on vaginal smear examination. On the 9th day of vaginal discharge, fully cornified (anucleated) cells dominated as much as 64.67%, and the percentage of superficial cells (nucleated) was 34.67%. According to Kustritz (2012) [15], not all female dogs are entirely cornified, and vaginal smear examination consists of nucleated superficial cells, which is consistent with the results of vaginal smear examination of the Kintamani Bali bitch. Thus, on two to three alternate days the bitch should to go for breeding.

The status of vaginal epithelial cells is closely related to the body's response to estrogen levels. Variances in estrogen concentration in dogs are caused by differences in the hormonal content of female dogs' blood, differences in the breeds studied, the number of eggs ovulated in each individual, and the age of the animals. The variation in peak estrogen levels is also caused by environmental conditions, including the presence of male animals during the initial time of observation. This boosts the activation of the hormone estrogen formation. There is no external effect once the female animal is separated from the male, therefore the increase in the hormone is only due to the physiology of the animal itself (Nalley *et al.*, 2011) [16]. Cytological content can also be influenced by various factors such as management, feeding, weather, and environmental temperature.

Conclusion

Vaginal cytology is a good aid in the diagnosis of the stage of the estrus cycle in Kintamani bitch. The procedure can reliably be used for diagnosing the stage of oestrus and the optimum time for mating in Kintamani bitch. Vaginal cytologic examination allows determining the type and changes of superficial vaginal epithelial cells. In the estrus phase appearance of keratinized and the rest are large intermediate cells where the majority of the cells. The onset of estrus is characterized by receptivity to mating. The proestrus is characterized by the appearance Proestrus is characterized by the appearance of small parabasal and intermediate cells and these cells tend to decrease.

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