

## Original Article

Veterinary  
MedicineDetection of Standing Heat In Bitches:  
Application of Vaginal CytologyOlufisayo LEIGH<sup>1</sup>, Lukman RAJI<sup>2\*</sup>, Ejiroghene DIAKODUE<sup>1</sup>

## ABSTRACT [ENGLISH/ANGLAIS]

Vaginal cytology studies were carried out to detect standing heats in twelve adult bitches. The predominating superficial cells observed were Large Intermediate Epithelial Cells (LIEC) and Giant Anucleated Cells (GAC) at  $38.0 \pm 21.6$  hours before and at standing heats. Student t-test analysis revealed significance differences between LIEC before ( $21.58 \pm 14.41$ ) and at standing heat ( $38.08 \pm 12.08$ ) ( $p = 0.00$ ); LIEC before and GAC before ( $37.00 \pm 18.40$ ) standing heats respectively ( $p = 0.03$ ); LIEC before and GAC at standing heat ( $44.58 \pm 24.18$ ) ( $p = 0.02$ ); and the mean percentages of GAC before (45.30%) and GAC at standing heat (54.68%). However, there were no significance differences between the mean percentages of LIEC before (49.17%) and at standing heat (50.81%); GAC and LIEC before standing heat; GAC and LIEC at standing heats. Also, the differences between GAC before and at standing heat ( $p = 0.23$ ); GAC and LIEC at standing heats ( $p = 0.49$ ); and LIEC at standing heat and GAC before standing heat ( $p = 0.89$ ) were not significant. These findings indicate that vaginal cytology is a useful tool in detecting standing heat in bitches.

**Keywords:** Standing heat, bitches, cytology, superficial cells

## RÉSUMÉ [FRANÇAIS/FRENCH]

C Des Études de la cytologie vaginale ont été réalisées pour détecter des chaleurs chez douze chiennes. Les cellules superficielles prédominantes observées étaient de grandes cellules épithéliales intermédiaire (LIEC) et des cellules géantes anucléées (GAC) à  $38,0 \pm 21,6$  heures avant et pendant la durée des chaleurs. L'analyse du test-t a révélé des différences significatives entre LIEC avant ( $21,58 \pm 14,41$ ) et pendant les chaleurs ( $38,08 \pm 12,08$ ) ( $p = 0,00$ ) ; LIEC avant et GAC avant ( $37,00 \pm 18,40$ ) chaleurs respectivement ( $p = 0,03$ ) ; LIEC avant et GAC pendant les chaleurs ( $44,58 \pm 24,18$ ) ( $p = 0,02$ ) , et les pourcentages moyens de GAC avant ( $45,30\%$ ) et GAC pendant les chaleurs ( $54,68\%$ ) . Cependant, il n'existe aucune différence de degré d'importance entre les pourcentages moyens de LIEC avant ( $49,17\%$ ) et pendant les chaleurs ( $50,81\%$ ) de ; GAC et LIEC avant chaleurs ; GAC et LIEC à chaleur fixe. En outre, les différences entre GAC avant et pendant les chaleurs ( $p = 0,23$ ) , GAC et LIEC pendant chaleurs ( $p = 0,49$ ) , et LIEC pendant les chaleurs et GAC avant les chaleurs ( $p = 0,89$ ) n'étaient pas significatives. Ces résultats indiquent que la cytologie vaginale est un outil utile dans la détection des chaleurs chez les chiennes.

**Mots-clés:** La chaleur fixe, femelles, v cytologie, les cellules superficielles

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## INTRODUCTION

The canine industry in Nigeria is witnessing a surge in the number of pet owners and breeders. This has been attributed to three major reasons which includes, pleasure, protection/security and profit making [1, 2]. Hence, fertility in the bitch is considered to be of great socio-economic importance. However, it has been observed that majority of the bitches presented for fertility investigations are fertile [3]. The apparent infertility is often related to lack of understanding of the oestrous cycle and accurate monitoring of the optimum mating time (standing heat) in these bitches [4, 5]. Vaginal Cytology is a simple technique that has been used to

characterize the different stages of the oestrous cycle in the bitch [6, 7]. But its use to determine standing heat has been controversial [8]. This study was therefore undertaken to investigate the usefulness of Vaginal Cytology to determine standing heat or optimum mating time in bitches.

## MATERIALS AND METHODS

Twelve sexually matured, healthy German shepherd bitches weighing between 28.5 and 35.0kg were selected for the study. They were all located within Ibadan city raised under the best possible conditions in terms of feeding, housing and health care. The procedure for

sample collection was such that the bitches were muzzled with mouth guards while a sterile swab was introduced at the dorsal most point of the vulva cleft angled upward at 45 degrees. It was rolled between the fingers to advance it beyond the urethral papilla, against the vaginal surface. Then pulled out, rolled firmly from one end of a clean glass microscope slide to the other [9, 10]. These samples were collected consecutively from the first day of bloody vaginal discharge (proestrus) up to the last day of acceptance of mating (oestrus). The vaginal smears were air dried and fixed in methanol for 5minutes, stained with Giemsa and then left for 45minutes. Thereafter, gently rinsed with distilled water and air dried again [11, 12]. Using a compound microscope, the glass slides were examined for epithelial cells and polymorphonuclear cells, first at low magnification ( $\times 10$ ) and later at higher magnification ( $\times 40$ ). With the higher magnification, polymorphonuclear cells, parabasal cells, small intermediate cells, large intermediate cells, and giant anuclear cells were identified and quantified in percentages in relation to one another using this formula:

$$\% \text{ of } x \text{ cells (on a slide)} = \frac{\text{Number of (x cells) on a slide} \times 100}{\text{Total number of cells on the slide}}$$

The stages of the sexual cycle of the bitches were determined according to the cell types and their proportion on the vaginal smear [13, 14]. Data of superficial cells obtained from the vaginal smears before and at standing heat were analyzed using the student-t-statistics.

## RESULTS

The predominating superficial cells at  $38 \pm 26$  hours before and at standing heat were large intermediate epithelial cells (LIEC) and giant anucleated cells (GAC). At standing heats, these cells were clumped together with presence of sperm cells when viewed under the microscope. Some bitches accepted mating at (standing heat) relatively few hours earlier than others. There were significance differences between LIEC before ( $21.58 \pm 14.41$ ) and at standing heat ( $38.08 \pm 12.08$ ) ( $p = 0.00$ ); LIEC before and GAC before ( $37.00 \pm 18.40$ ) standing heats respectively ( $p = 0.03$ ); LIEC before and GAC at standing heat ( $44.58 \pm 24.18$ ) ( $p = 0.02$ ); and the mean percentages of GAC before (45.30%) and GAC at standing heat (54.68%). However, there were no significance differences between the mean percentages of LIEC before (49.17%) and at standing heat (50.81%); GAC and LIEC before standing heat; GAC and

LIEC at standing heats. Also, the differences between GAC before and at standing heat ( $p = 0.23$ ); GAC and LIEC at standing heats ( $p = 0.49$ ); and LIEC at standing heat and GAC before standing heat ( $p = 0.89$ ), were not significant (Table 1 and 2). Figure 1 shows cell types before standing heat with LIEC and GAC, evidence of clumping but no sperm cells while Figures 2 and 3 show cell types at standing heat, with more of GAC than LIEC with evidence of clumping and presence of sperm cells.

## DISCUSSION

The study revealed that vaginal cytology is a useful tool in determining optimum mating time (standing heat) in bitches. The predominating superficial cells at  $38 \pm 26$  hours before (proestrus) and at standing heat (oestrus) were large intermediate epithelial cells (LIEC) and giant anucleated cells (GAC). This is similar to earlier reports that the proestrus and oestrus phases of the oestrous cycle in the bitch is characterized majorly by LEIC and GAC [6, 7]. There were more LEIC at standing heats than LEIC before standing heats and this difference was significant ( $p = 0.00$ ). Also, the mean percentage values of GAC at standing heats were significantly higher than before standing heats. These findings are similar to earlier reports that the percentage of these cells progressively increases from 0% to 100% during proestrus and remains at 100% during oestrus or standing heat [8]. The clumping of these cells was more pronounced (with presence of sperm cells) at standing heats than before standing heat.

**Table 1:** Table 1 shows results of student t-test analysis of the superficial cells data obtained in this study.

	Superficial cells	Mean $\pm$ SD	Sig (2-tailed)
Pair 1	LIEC BSH	21.58 $\pm$ 14.41	0.00
	LIEC AST	38.08 $\pm$ 12.08	
Pair 2	LIEC BSH	21.58 $\pm$ 14.41	0.03
	GAC BSH	37.00 $\pm$ 18.40	
Pair 3	LIEC BSH	21.58 $\pm$ 14.41	0.02
	GAC AST	44.58 $\pm$ 24.18	
Pair 4	LIEC AST	38.08 $\pm$ 12.08	0.89
	GAC BSH	37.00 $\pm$ 18.40	
Pair 5	LIEC AST	38.08 $\pm$ 12.08	0.49
	GAC AST	44.58 $\pm$ 24.18	
Pair 6	GAC BSH	37.00 $\pm$ 18.40	0.23
	GAC AST	44.58 $\pm$ 24.18	

LIEC BSH= Large Intermediate Epithelial Cell before Standing Heat; LIEC AST= Large Intermediate Epithelial Cells at Standing Heat; GAC BSH= Giant Anucleated Cell before Standing Heat; GAC AST= Giant Anucleated Cell at Standing Heat.




**Table 2:** Table 2 shows the mean percentages of superficial cells before and at standing heats.

Superficial cells	Mean % before standing heat	Mean % at standing heat
GAC	45.30	54.68
LIEC	49.17	50.81

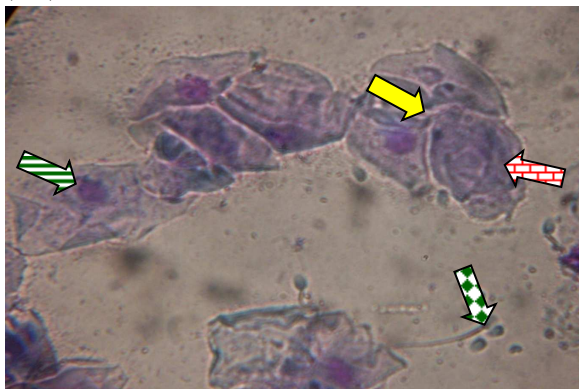
LIEC= Large Intermediate Epithelial Cell; GAC= Giant Anucleated Cell.





**Figure 1:** Before standing heat, there were LIEC and GAC and clumping of cells (× 40).



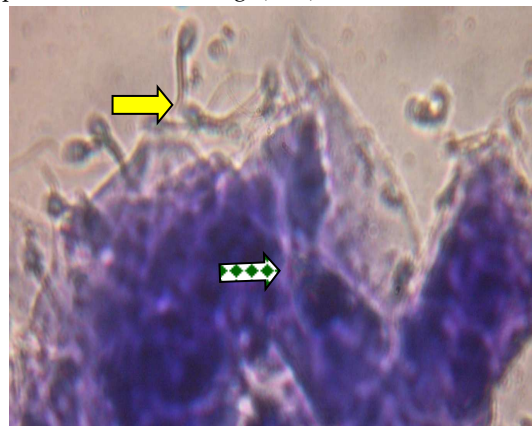
-  Giant anucleated cells
-  Large intermediate epithelial cell
-  Clumped superficial cells



**Figure 2:** At standing heat, more of GAC and LIEC and clumping of cells with evidence presence of sperm cells (×40).



-  Giant anucleated cell
-  Large intermediate epithelial cell
-  Sperm cell
-  Clumped superficial cells

**Figure 3:** At standing heat, abundance of Giant Anucleated Cells and few Large Intermediate Epithelial Cells with clumping of cells and presence of numerous sperm cells after mating. (× 40).



-  Numerous sperm cells
-  Clumped superficial cells

We also observed that the duration of proestrus and oestrus varied slightly among the bitches. Some bitches accepted mating relatively earlier than others. This is similar to earlier reports that some bitches are early ovulators while others are late ovulators [15, 16]. Dog breeders are therefore advised to be careful of using the thumb rule that the duration of proestrus and oestrus is nine days each [17, 18], so most mating are (erroneously) commenced on day ten of vaginal discharge from the vulva. This is probably one of the major reasons for reproductive failures in bitches due to miss-timed mating [19, 20]. This study findings have shown that beyond its use to characterize the various phases of oestrous cycle, vaginal cytology can be used successively to determine standing heats in bitches. We suggest further studies even in other breeds of dogs to establish these findings. With this achieved, vaginal cytology could be used alone or (as suggested by previous reports) in combination with proper clinical examination, vaginoscopy, progesterone and probably luteinizing hormone testing, to determine optimal breeding time in bitches [7, 20].

**CONCLUSION**

It is concluded that vaginal cytology is a useful tool in determining standing heats in bitches. This is a step further than its normal use for characterisation of various phases of oestrous cycle. In this study, we were able to distinguish and determine accurately standing heats using the significant differences between LIEC and GAC values

during proestrus and oestrus phases in the bitches. Adoption and use of these findings would increase pregnancy rates and minimize or prevent reproductive wastages that are associated with miss-timed mating in bitches.

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**CONFLICT OF INTEREST**

No conflict of interests was declared by authors.

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