Original Research

The Role of Estrogen and Progesterone Hormone on Vaginal Cytology in Bitch

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Rec. Date:	Nov 17, 2017 14:54
Accept Date:	Apr 21, 2018 08:41
DOI	10.5455/ijlr.20171117025427

Abstract

The aim of the present study was to determine the stages of the reproductive cycle of the bitch by using the exfoliative vaginal cytology technique and its relationship with hormonal level. A total of 12 bitches (n=12) were selected and vaginal cytology along with hormonal estimation was done to identify the different stages of the reproductive cycle. The vaginal samples were collected as per the cotton swab technique and stained with Leishman stain and examined microscopically for differential count of exfoliated vaginal epithelial cells. On the same day blood samples were also collected aseptically for hormonal estimation. The percentage of parabasal cell, intermediate cell and superficial cells were 7.04±0.28, 22.33±0.28, and 62.32±0.28 in proestrus; 0.56±0.03, 5.68±0.03 and 92.29±0.03in estrus and 32.56±0.28, 35.08±0.28 and 12.83±0.28 in diestrus respectively. Vaginal smears were characterized by presence of massive numbers of erythrocytes and disappearance of neutrophils in proestrus, clear background of smear and absence of neutrophils with scanty erythrocytes in estrus, infiltration of neutrophils and dirty background in diestrus. The estrogen level (29.50 ±0.28) was statistically high during proestrus and highest progesterone level (39.50 ±0.28) during diestrus. On conclusion, hormonal changes in different stages of reproductive cycle presented mainly by the effect of elevated estrogen hormone that associated with increase in superficial cells count and cornification, with reduction in neutrophil and parabasal cell in estrus and Progesterone hormone showed increased towards diestrus level with increasing intermediate cells.

Key words: Bitch, Exfoliative Vaginal Cytology, Estrogen, Progesterone, Reproductive Cycle

How to cite: Haji, M., Ahmed, F., Lalrintluanga, K., Talukdar, D., Doley, P., Behera, S., & Sarma, K. (2018). The Role of Estrogen and Progesterone Hormone on Vaginal Cytology in Bitch. International Journal of Livestock Research, 8(9), 241-247. doi: 10.5455/ijlr.20171117025427

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DOI 10.5455/ijlr.20171117025427

Introduction

The reproductive cycle of the dog is very unique from that of other domesticated animals. The different reproductive stages in bitch can be determined through behavioral, endoscopic, hormonal and cytological observation (Feldman and Nelson, 1996; Johnston *et al.,* 2001). The cyclic cellular changes of vaginal epithelium of the dog during different reproductive stages have been associated with levels of estrogen and these changes can easily be demonstrated by vaginal exfoliative cytological technique (Mshelia *et al.,* 2001). Vaginal exfoliative cytology has been described as simple and reliable tool for monitoring the stages of reproductive cycle and also helpful in the prediction of optimum mating time in the bitch (Wright and Parry, 1989). The present study was carried out to determine the stages of the reproductive cycle of the bitch by using the exfoliative vaginal cytology technique and its relationship with hormonal level.

Materials and Methods

A total of 12 clinically healthy mature bitches presented at the Veterinary Hospital, Teaching Veterinary Clinical Complex, College of Veterinary Sciences & Animal Husbandry, Central Agricultural University, Selesih, Mizoram, India were selected for this study to determine different stages of the reproductive cycle. All the 12 animals were used to study the vaginal cytology at proestrus, estrus and diestrus.

Collection and Preparation of Vaginal Smear

The vaginal smears were obtained from the respective bitches by introducing sterile cotton tipped swab into the caudal part of vagina after gently separating the lips of vulva (Fig.1).

Fig. 1 Collection of vaginal smear by cotton swab technique

Fig. 2 Preparation of vaginal smear on a clean microscope glass slide

The vaginal exfoliative cytology was obtained as per the technique demonstrated by Feldman and Nelson (1996). The smear was collected after rubbing the swab against the vaginal wall in clockwise and anti-clock wise directions once each and gently withdrawn afterwards as described by Phemister *et al.* (1973). Following collection the cells were transferred to a clean grease free glass microscopic slide by gently rolling the swab tip from one end to the other (Fig. 2). The smear was allowed to air dry and stained with a

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Leishman and Giemsa stain separately as per the protocol. After staining and drying, the slides were examined under 40X followed by oil immersion trinocular fluorescent microsope (100X). A minimum of 10 observation fields were examined as described by Bowen (2006). Vaginal cytological evaluations were performed as described by Ola *et al.* (2006).

Evaluation of Vaginal Smears

The stages of the reproductive cycle of the bitches were determined by the identification of the cell types present in the vaginal smear as per the method described by Bell *et al.* (1973). Vaginal cytology was differentiated into parabasal, intermediate, superficial and squamous cells as per standard method (Maneke, 2002; Johnson, 2006; Antonov, 2016). Different vaginal epithelial cells were identified in the smear and expressed in percentage during proestrus, estrus and diestrus.

Hormone Estimation

Collection of blood (10 mL) for examination of progesterone and estrogen levels conducted during the oestrous cycle begins on day 0 (time of oestrus) and ends at the next oestrus. Serum was recovered by centrifugation (15 minutes at 2500 rpm) and stored at −20°C until being assayed for serum progesterone and estrogen levels using commercial progesterone and estrogen ELISA kit (DRG Instruments GmbH, Germany).

Results and Discussion

Different kind of cells viz. parabasal, intermediary and superficial epitheliums were observed in the vaginal cytology (Fig 3). Parabasal cells (Fig. 3B) looked like small, O-shaped oat cereal pieces; intermediate (Fig. 3A) looked like fried eggs; superficial ("cornified") cells (Fig. 3C, D & E) seen like corn flakes and neutrophils were segmented (Fig.3F). Vaginal smears were characterized by presence of massive erythrocytes in proestrus, clear background of smear in estrus and dirty background in diestrus. This observation was similar to the reports of Najamudin *et al.* (2010). The proportion of each epithelial cell at different stages of the reproductive cycle is shown in Table 1 and Fig. 4. Based on the standard and characteristic of exfoliated vaginal epithelial cells in proestrus, estrus and diestrus phase, the percentages of parabasal, intermediate and superficial cell were presented in the Table 1. The observed proportion was similar with Widiyono *et al.* (2011) and Gupta *et al.* (2012) in which the largest epithelial cell proportion in the estrus phase was the superficial cells. The results showed that the proportion of the superficial cells was higher during proestrus (62.67%) and increased during estrus (93.50%). The parabasal and intermediate cells count were very low in the vaginal smears of the estrus bitches.

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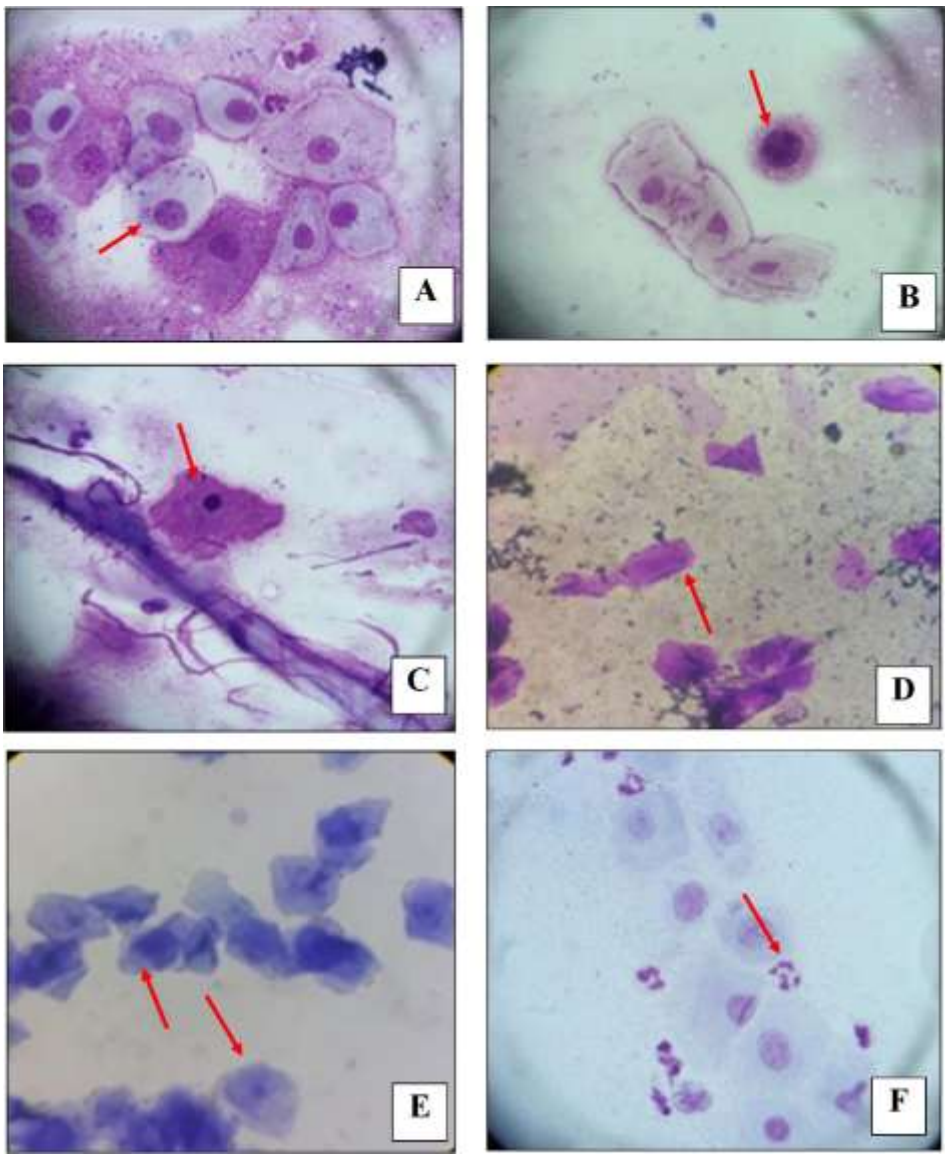

Fig. 3: Vaginal exfoliative cytology of the bitches showing different epithelial cells (arrow mark**)**

A. Intermediate cells
B. Parabasal cells
C. Superficial cells (nucleated)
D. Anuclear superficial cells
E. Anuclear and Nuclear superficial cells
F. Neutrophils

This finding was in agreement with the finding of Mshelia *et al.* (2001) who also reported appearance of the parabasal cells and intermediate cells were as low as 7% in estrus bitches. In estrus phase, estrogen hormone increased activeness of uterine wall and caused hypersecretion of epithelial cells of the uterus and vagina resulting predominant of the superficial cells in this phase (Siregar *et al.*, 2016).

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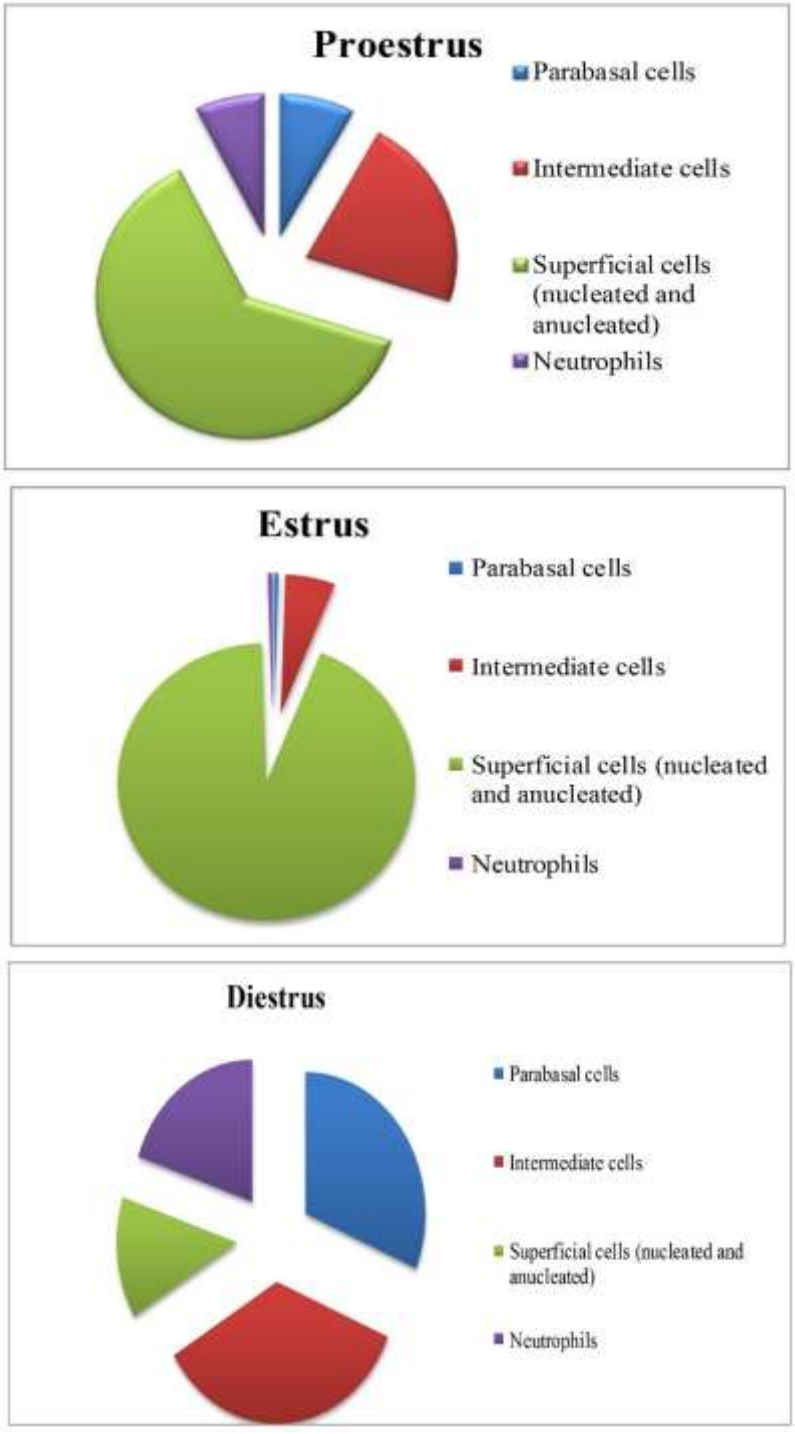

Fig. 4: Characteristic and proportion of exfoliated vaginal epithelial cells of the bitches at different stages of the reproductive cycle

During estrus, there was presence of scanty erythrocytes and absence of neutrophils which was also supported by Mshelia *et al.* (2001). In the present study, the vaginal smears of the pregnant bitches revealed

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that the superficial cells declined to 13% which was similar with the finding of Mshelia *et al.* (2001) who observed as 15% in pregnant bitches. This might be due to increase in cells of the deeper layer and a rise in serum progesterone levels above 5ng/ml as reported by Mshelia *et al.* (2001). During pregnancy, reappearance of the neutrophils was also observed and found to be 19.83 % in the present study. Neutrophils are often abundant in smears taken during pregnancy and are not uncommon at other stages though rare during estrus. Moderate numbers of neutrophils are a common though not consistent feature of normal canine vaginal smears and not by themselves indicative of vaginitis (Bowen, 2006). The level estrogen (pg/ml) and progesterone (ng/ml) during proestrus, oestrus and diestrus were presented in the Table 1.

Table 1. Percentage of exfoliated vaginal epithelial cells and hormonal level of the bitches at different stages of the reproductive cycle

Stages	Proestrus (n=12)	Estrus (n=12)	Diestrus (n=12)
Parabasal cells (%)	7.04±0.28	0.56±0.03	32.56±0.28
Intermediate cells (%)	22.33±0.28	5.68±0.03	35.08±0.28
Superficial cells (%)	62.32±0.28	92.29±0.03	12.83±0.28
Neutrophils (%)	6.90±0.28	0.51±0.03	16.65±0.28
Estrogen (pg/ml)	29.50^{a} ±0.28	12.03^{b}±0.03	9.25^{c}±0.28
Progesterone (ng/ml)	1.04^{a}±0.28	5.25^{b} ± 0.03	39.5^{c} ± 0.28

Means bearing different superscript in a row differed significantly (P<0.01)

In proestrus and estrus phase, estrogen hormone will increase activeness in uterus wall; it causes hypersecretion in epithelial cells of the uterus and vagina, so that the superficial cells were increased. In this research, the estrogen concentrations in proestrus, estrus, and diestrus phase were 29.50 ±0.28, 12.03±0.03 and 9.25±0.28 pg mL^{-1}, respectively. Increasing concentrations of estrogen in proestrus and estrus phase may be related to the high proportion of superficial cells. The average concentration of progesterone was different on the stage of cycle. At the time of proestrus, progesterone level was 1.04±0.28 ng mL^{-1}. The concentration reached its highest level during diestrus phase length with average concentration of progesterone being 39.5 ± 0.28 ng mL^{-1}. When progesterone becomes dominant diestrus phase, the number of larger cells was sharply reduced (Zohara *et al.*, 2014) so that the epithelial cells were dominated by parabasal cells. In this study, the proportion of parabasal cells in diestrus phase higher in comparison with proestrus and oestrus phase.

Conclusion

The vaginal cytology is an easy and reliable diagnostic tool for staging the reproductive cycle of the bitches especially for the determination of the oestrus stage which may be helpful in the prediction of optimum mating time in the bitch.

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DOI 10.5455/ijlr.20171117025427

References

1. Antonov AL. 2016. Application of exfoliative vaginal cytology in clinical canine reproduction – A review. Bulg. J. Vet. Med. 2: 82-101.
2. Bell E, Bailey J and Christie D. 1973. Studies on vaginal cytology during the canine oestrus cycle. Research in Veterinary Science.14: 173-179.
3. Bowen R. 2006. In: Cytologic Changes through the Canine Estrous Cycle. Colorado State University, Fort Collins, U.S.
4. Feldman EC and Nelson RW. 1996. Ovarian cycle and vaginal cytology. In: Canine and Feline Endocrinology and Reproduction. 2nd ed. Philadelphia, W.B. Saunders., USA, pp. 526-46.
5. Gupta AK, Dhami AJ and Ghodasra DJ. 2012. Exfoliative vaginal cytology: An effective tool to know the reproductive status of the bitch. Indian Journal of Field Veterinarians, 8(1):61-64.
6. Johnson C. 2006. Reproductive disorders. In: Willard, M. and Tvedten, H. (eds) Loboratory diagnosis in small animal practice, Urban and Fischer ltd., Germany, pp. 377-383.
7. Johnston SD, Root KMVR, Olson PNS. 2001. Canine and Feline Theriogenology, W.B. Saunders Company, Philadelphia, USA, ISBN-10: 0-7216-5607-2, pp: 16-40.
8. Maneke N. 2002. Study to describe of vaginal cells in bitch than a contribution for objective of cycle diagnosis. Veterinary Medicine Dissertation, Berlin.
9. Mshelia GD, Amin JD and Chaudhari SUR. 2001. Oestrus detection in the Nigerian Mongrel bitch: Application of vaginal cytology. Pak. Vet. J., 21(1): 45-50.
10. Najamudin R, Sriyanto A, Agungpriyono S, and Yusuf TL. 2010. Determining the estrus cycle in java mouse-deer by changes of vaginal cells. Veterinary Journal 11(2): 81-86.
11. Ola SI, Sanni WA and Egbunike G. 2006. Exfoliative vaginal cytology during the oestrous cycle of West African dwarf goats. Reprod. Nutr. Dev., 46: 87-95.
12. Phemister RD, Holst PA, Spano IS and Hopwood ML. 1973.Time of ovulation in the beagle bitch. Biol. Reprod., 8: 74-82.
13. Siregar TN, Melia J, Rohaya TCN, Masyitha D, Wahyuni S, Rosa J, Nurhafni PB and Herrialfian. 2016. Determining Proportion of Exfoliative Vaginal Cell during Various Stages of Estrus Cycle Using Vaginal Cytology Techniques in Aceh Cattle. Veterinary Medicine International, 1:1-5.
14. Widiyono PP, Srmin P, Astuti P and Airin CM. 2011. Levels of serum estradiol and progesterone views vulva and vagina smear cytology bligon goat during estrus cycle. Journal Veterinary, Indonesia, 2(4): 263-268.
15. Wright P and Parry B. 1989.Cytology of the canine reproductive system. Veterinary Clinics of North America. Small Animal Practice.19: 851-874.
16. Zohara B. F., Azizunnesa A., Islam M. F., Alam M. G., Bari F. Y. 2014. Exfoliative vaginal cytology and serum progesterone during the estrous cycle of indigenous ewes in Bangladesh. Journal of Embryo Transfer, 29(2):183–188.

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DOI 10.5455/ijlr.20171117025427