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Correlation between Cornification of Vaginal Epithelial Cells and Serum Progesterone Concentration during Proestrus and Estrus in She-Dogs

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ABSTRACT

The present study was conducted on fifteen she-dogs presented to the Department of Veterinary Gynaecology and Obstetrics, CSK Himachal Pradesh Krishi Vishvavidyalaya (CSKHPKV), Palampur for determination of mating time. Serum progesterone was estimated from the blood sample collected in the clot activator by cephalic venepuncture from she-dogs during the proestrus and estrus stages. The objective of the study was to establish a correlation between epithelial cells proportion (%) in vaginal epithelium with serum progesterone concentration (ng/mL) during proestrus, early estrus, and mid to late estrus in she-dogs. A significant positive correlation ($r= 0.74$; $p<0.01$) between cornified cells and progesterone concentration was recorded during mid to late estrus whereas the correlation was non-significantly negative ($p>0.05$) for non-cornified cells ($r= -0.7408$ to -0.5807). Also, the cornified cell proportion was significantly higher ($p<0.01$) during mid to late estrus as compared to proestrus and early estrus. In conclusion, the present study validated the statistical association between cornified vaginal epithelial cells and serum progesterone concentration during mid to late estrus.

Key words: Cornified cells, Linear correlation and regression, Reproduction, Serum progesterone, She-dogs.

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INTRODUCTION

Infertility in canines arises due to hormonal problems, infectious diseases and congenital or acquired defects of the genital tract. Many a time infertility is caused by inappropriate breeding management (Moxon *et al.*, 2012). Veterinarians nowadays are requested to solve problems regarding fertility in canines due to the increased demand for purebred dogs for sentimental or financial reasons (Fontbonne, 2011). The "potentially" infertile she-dog is the most common problem in canine reproduction and the majority of them are simply victims of poor management

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(Kudalkar *et al.,* 2020). Long periods of estrus behavior in she-dogs, as well as a shaky relationship between behavioral characteristics and ovulation duration, make determining the best time to breed difficult (Linde and Karlsson, 1984). The exfoliated cells reflect the hormonal, in particular the estrogenic state, of the bitch. Due to the estrogenic influence, an increase in cell layers, keratinization and exfoliation is observed in the follicular phase during proestrus, such that the 3-4 layered epithelium in anestrus becomes 20-layered during estrus. The cells change characteristically in size and nuclear morphology (Dar *et al.,* 2017). Vaginal cytology offers a rapid, inexpensive, and reliable in-clinic method to evaluate stages of the estrus cycle in the bitch (Kudalkar *et al.,* 2020). The assessment of circulating progesterone concentrations is the most used approach for detecting the luteinizing hormone (LH) surge which is found to be concomitant with the initial marked increase of the circulating progesterone (Concannon, 2011). After the LH surge, the serum progesterone (P_4) rises from around 1ng/ml, during anestrus and early proestrus to 4-5ng/ml at ovulation. In practice, a blood concentration of progesterone ≥5 ng/ml is considered indicative of ovulation (Ververidis *et al.,* 2002). The specificity progesterone assay gives a more accurate estimation of each bitch individually increasing the likelihood of successful pregnancy (Bante *et al.,* 2018). Therefore, the present study was carried out to validate the correlation analysis of vaginal epithelial cells and serum progesterone concentration during proestrus and estrus in she-dogs.

MATERIALS AND METHODS

The present study was conducted on she-dogs (N=15) presented to the Department of Veterinary Gynaecology and Obstetrics, Dr. G.C. Negi College of Veterinary and Animal Sciences, Chaudhary Sarwan Kumar Himachal Pradesh Krishi Vishvavidyalaya (CSKHPKV), Palampur (32.6°N, 76.3°E, and altitude 1290.8m). On presentation of she-dogs at the department, a complete history like age, vaccination and deworming status, any reproductive abnormalities, previous failure of conception, and last date of whelping, were recorded. The vagina was explored with a gloved finger to rule out any vaginal abnormalities. The specific reproductive examination like the onset of proestral bleeding, vulval edema, nature of vaginal discharge and colour of discharge and postural signs exhibited by she-dog like flagging of the tail in response to gentle massaging on the hind quarter was recorded.

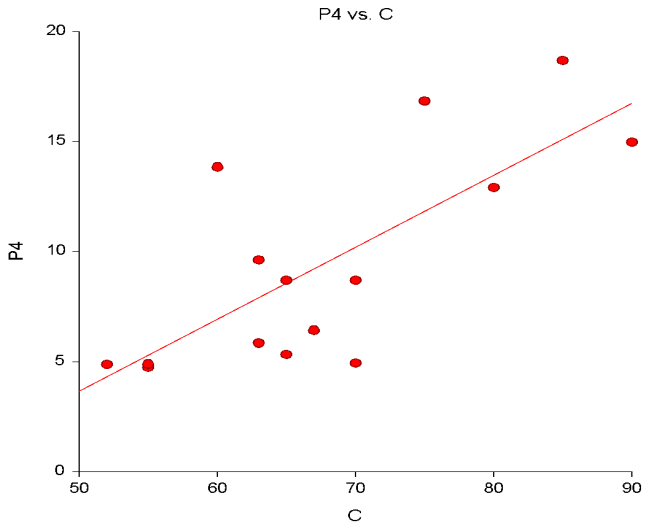
For exfoliative vaginal cytology, the samples (n=33) were collected using a cotton swab technique in which a sterile cotton-tipped swab was used after moistening with 2-3 drops of sterile normal saline. The samples were collected from the anterior vagina. The smear was prepared immediately after the withdrawal of the swab by rolling (not sliding or rubbing)the cotton tip along the length of a glass microscope slide. Two parallel tracks were rolled on a single slide just to duplicate the smear followed by fixation with 70% alcohol and fixed smears were subjected to Giemsa, Leishman's and Modified Shorr staining methods. Stained smears were examined microscopically and interpreted. Based on shape, size and morphology, the cells were categorized into parabasal, small intermediate, large intermediate, superficial or cornified, together with information on the presence of red blood cells, neutrophils and other types of cells. A total of 200 exfoliative vaginal epithelial cells were counted and the percentage of each type of cell was calculated.

The blood sample for estimation of serum progesterone concentration was collected in a clot activator by cephalic venepuncture from she-dogs. Samples were collected on the day of the presentation of she-dogs for exfoliative vaginal cytology. The collected samples were allowed to clot and then centrifuged at 2500 rpm for 5 minutes. Using a micropipette, serum samples were collected and transferred to Eppendorf tubes and stored at -20°C until analyzed.

The obtained data was statistically analyzed using one-way ANOVA (analysis of variance) and linear correlation, and regression analysis with NCSS 2021, USA (Version 21.0.1).

RESULTS AND DISCUSSION

The present research took note of analyzing the correlation between the cornification of vaginal epithelial cells and serum progesterone concentration during proestrus and estrus in she-dogs. A perusal of Table 1 indicated a significant positive correlation ($p=0.0014$) between the percentage of cornified cells and progesterone concentration (ng/mL) during mid to late estrus (Figure 1) whereas a negative correlation ($p=0.0016$) was recorded between percent non-cornified cells and progesterone concentration (ng/mL) at the same stage (Figure 2). The correlation between the percentage of cornified cells and progesterone concentration (ng/mL) was found to be positive but non-significant ($p<0.01$) during proestrus (Figures3 and 4) and early estrus (Figure 5) whereas the correlation between the percentage of non-cornified cells and progesterone concentration was non-significantly negative ($p>0.05$) at the early estrus (Figure 6). Also, the cornified cell proportion was significantly higher ($p<0.01$) during mid to late estrus as compared to proestrus and early estrus (Table 1).



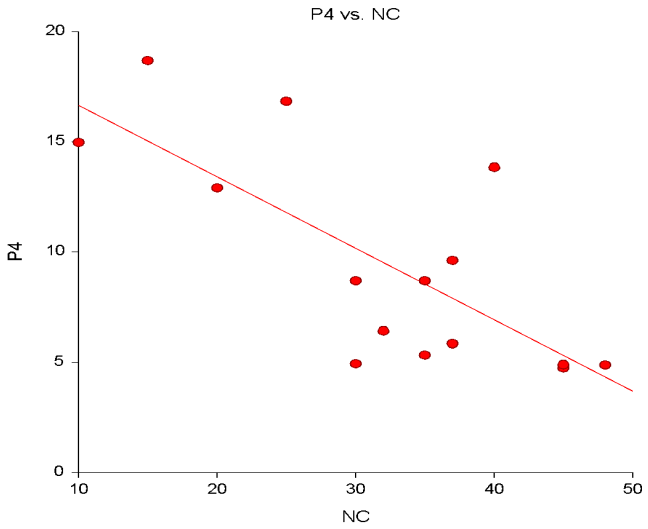
P_4- Serum progesterone concentration (ng/ml);
NC- Percentage of cornified cells

r=0.7447; p=0.0014;

P_4 = (-12.6521) + (0.3262) C

(n=15)

Fig.1: Linear correlation and regression analysis of serum progesterone concentration (ng/mL) cornified epithelial cells during mid to late estrus stage of the estrous cycle in she-dogs



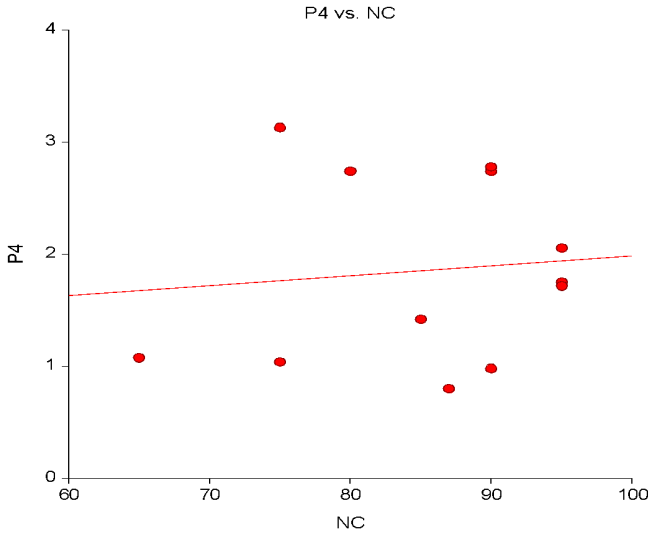
P_4- Serum progesterone concentration (ng/ml);
NC- Percentage of non-cornified cells

r= -0.7408; p=0.0016;

P_4 = (19.8908) + (-0.3245) NC

(n=15)

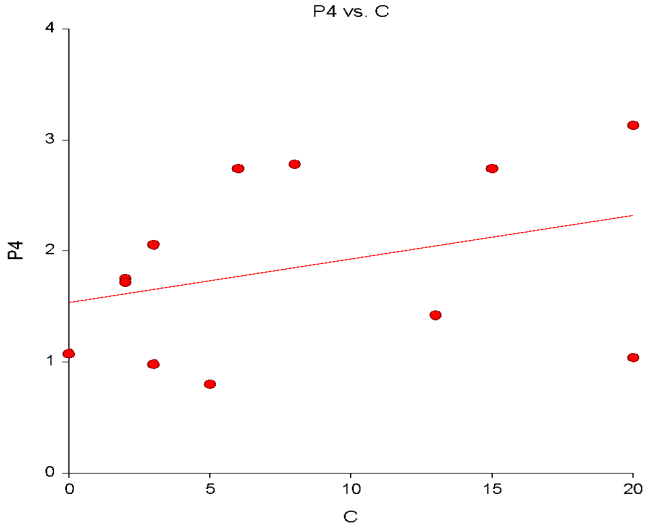
Fig. 2: Linear correlation and regression analysis of serum progesterone concentration (ng/mL) with non-cornified epithelial cells during mid to late estrus stage of the estrous cycle in she-dogs



P_4- Progesterone concentration (ng/ml);
NC- Percentage of non-cornified cells

r=0.1009; p=0.7551; P_4 = (1.1119) + (0.0087) NC(n=12)

Fig. 3: Linear correlation and regression analysis of serum progesterone (P_4) concentration (ng/mL) with non-cornified epithelial cells during proestrus stage of the estrous cycle in she-dogs

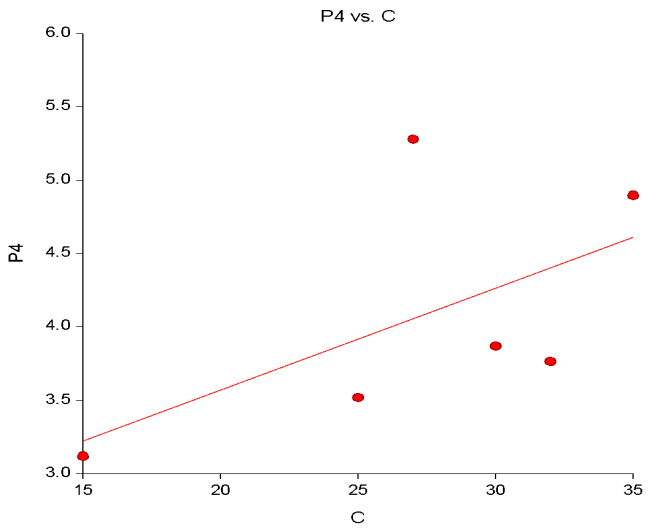


P_4- Progesterone concentration (ng/ml);
C- Percentage of cornified cells

r=0.3402; p=0.2792;

P_4 = (1.5352) + (0.0392) C(n=12)

Fig. 4: Linear correlation and regression analysis of serum progesterone concentration (ng/mL) with cornified epithelial cells during proestrus stage of the estrous cycle in she-dogs



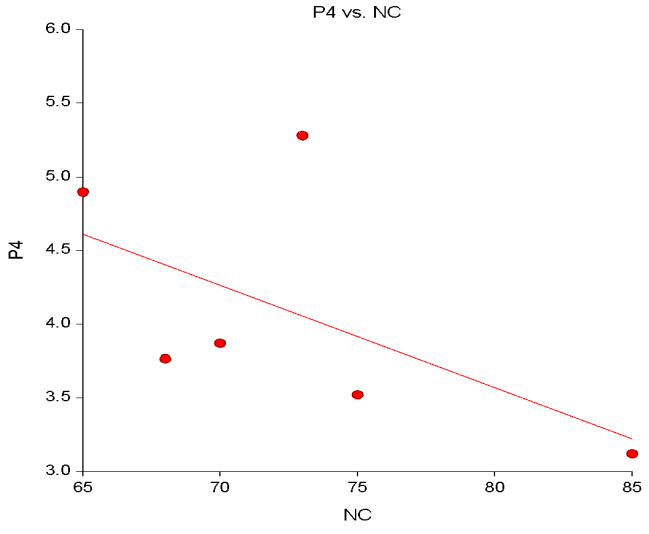
P_4- Serum progesterone concentration (ng/ml);
C- Percentage of cornified cells

r=0.58; p=0.2269;

P_4 = (2.18) + (0.07) C

(n=6)

Fig. 5: Linear correlation and regression analysis of serum progesterone concentration (ng/mL) with cornified epithelial cells during early estrus stage of the estrous cycle in she-dogs



P_4- Serum progesterone concentration (ng/ml);
NC- Percentage of non-cornified cells

r= -0.58; p=0.2269;

P_4 = (9.10) + (-0.07) NC

(n=6)

Fig. 6: Linear correlation and regression analysis of serum progesterone concentration (ng/mL) with non-cornified epithelial cells during early estrus stage of the estrous cycle in she-dogs

The estrous cycle in she-dogs can be monitored using changes in the percentage of epithelial cells in vaginal smears (Siregar *et al.,* 2011). Estrogen hormone increased the activeness of the uterine wall and induced hypersecretion of uterine and vaginal epithelial cells, resulting in the predominance of superficial cells (70-80%) during the estrus phase (Siregar *et al.,* 2011). Increased estrogen also causes a rise in vaginal epithelial cell turnover, which leads to the progressive cornification seen in vaginal cytology (Concannon, 2011). Ovarian hormones have many targets, including the vaginal epithelium. Serum estrogen concentration peaks 1 to 2 days before estrus and exfoliative vaginal cytology appear to be the reflection of serum estrogen concentration, which continues to be greater than basal concentrations, even though they are declining towards basal levels. Serum progesterone (P_4) concentrations have been reported to increase gradually from basal values with the advancement of proestrus and exhibit a distinct, rapid, and detectable increase around the time of LH surge (Concannon, 2011).

Hormonally, estrus is a period of progressively decreasing estrogen concentrations, progressively increasing progesterone concentrations and a brief burst of LH release (Feldman and Nelson, 2004).Identification of rapid distinct risein progesterone concentrations has been proposed as an indirect method of predicting the LH surge (Feldman and Nelson 2004,). Elevation of serum P_4 concentration before ovulation is reported to be peculiar to she-dogs and the source of this elevation is reported to be the preovulatory luteinized follicles (Goodman, 2001). In the present study, the continuous increase in progesterone concentration was recorded during proestrus and estrus as it begins to rise during the last 2 to 48 hours of proestrus above the critical 0.5 ng/mL plateau (Bouchard *et al.,* 1991). Serial blood samples performed every 2-3 days may be used to identify the rise in progesterone which indicates that the LH surge has occurred; routine breeding may be timed by this parameter (Feldman and Nelson, 2004). A serum progesterone concentration greater than 2ng/ml is typically accepted as an indicator of the LH surge (Bouchard *et al.,* 1991), yet a wide range of 3 to 10 ng/ml of progesterone concentrations has been reported as indicative of ovulation (Wright and Parry, 1989). These concentrations can be variable among she-dogs, laboratories, type of assay used, and methods of specimen handling, and may depend on whether progesterone is measured in plasma or serum (Volkmann, 2006). However, the correlation between the percentage of vaginal epithelial cells and progesterone concentrations (ng/mL) has not been discussed yet in the literature and the present study confirmed the statistical association between these parameters.

CONCLUSIONS

The present study recorded a significant positive correlation between cornified cells and progesterone concentration during mid to late estrus whereas the correlation was non-significantly negative for non-cornified cells during early estrus. However, the correlation was non-significant during proestrus and early estrus in she-dogs. Thus, current study findings can be used for future reference in understanding canine reproduction.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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