Comparison of Vaginal Flora, Vaginal Cytology, Blood Values and Hormone Level of Cats in Different Reproductive Periods

Levent Termelioğlu[1,a], Hakan Kalender[2,b*], Serkan Erat[3,c]

[1]Sevgi Veterinary Clinic, Ankara, Turkey
[2]Kırıkkale University, Faculty of Veterinary Medicine, Department of Obstetrics and Gynecology, Kırıkkale, Türkiye
[3]Kırıkkale University, Faculty of Veterinary Medicine, Department of Animal Breeding and Husbandry, Kırıkkale, Türkiye

[a]ORCID: 0000-0002-5453-5559; [b]ORCID: 0000-0002-8145-7330; [b]ORCID: 0000-0002-9549-8694

Abstract

The aim of this study was to investigate vaginal cytology, haematological and hormonal values, the presence of bacteria in the vagina, and the relationship between these findings in different reproductive periods in cats. The study consisted of 30 healthy non-geriatric female cats that had reachedto puberty. The cats were divided into 3 equal groups (each having 10 cats) as estrus, anestrus and pregnant. The vaginal samples for microbiological and cytological examination andthe blood samples for hormonal analysis and hemogram were taken at the same time. A total of 100 vaginal epithelial cells were counted from the random areas of thevaginal cytology samples on the slide. The distributions of the percentages of the counted cells according to the groups were subtracted and compared. While there was no bacterial growth in 9 (30%) animals, bacterial growth was observed in 21 (70%) animals. There were no bacterial growth in 3 (30%), 4 (40%) and 2 (20%) animals inestrus, pregnant and anestrus groups, respectively. Estradiol (E2) level (42.64 ± 10.62 pg/ml) in estrus animals was significantly higher (P<0.001) than E2 level in pregnant and an estrus animals. The progesterone (P4) level of the pregnant group (12.22±9.35 ng/ml) was higher (P<0.001) than the P4 levels of the anestrus (0.84±0.25 ng/ml) and the estrus group (0.58±0.28 ng/ml), while the P4 levels of the estrus and the anestrus groups were similar. Significant differences were detected only in MCV, MCH and MCHC, within 19 blood parameters. MCV values were found to be lower in estrus animals (45.68±3.75femtoliter) than only in pregnant (51.21±4.99femtoliter) animals (P=0.007). The difference in MCH values between the estrus group (14.37±0.84 pg) and the pregnant group (15.62 ± 1.18 pg) (P=0.003) and the difference in MCHC values between the pregnant group (30.66±1.17 g/dl) and the anestrus group (32.42±1.04 g/dl) (P<0.001) were statistically significant. The presented results may help in the planning of future studies and the comparison of the obtained values.

Keywords: Blood value, cats, hormonal level, reproductive periods, vaginal cytology, vaginal flora.

INTRODUCTION

Social lives are changing day by day and the number of people who adopt pets increases while the relationship between people is getting weaker. Sarı (2019) reported that the rate of pet adoption has increased by 25% in the last seven years. There is no study examining this increase in the Covid 19 pandemic period in Turkey. However, veterinarians clearly observe that the number of people who have pets during the Covid 19 pandemic period has increased exponentially. Among those who want to have pets, the cat is the most preferred for many reasons such as being easier to care at home. As cats are increasingly entering our homes, their share in the pet animal market is increasing rapidly. On the other hand, it is observed that the interest shown to cats in academic research is more limited. It is thought that more studies should be done in cats in order to compare the values obtained in the studies and to make the data more reliable. In addition, the results obtained in many studies related to regional factors such as the investigation of bacteriological flora or sexual cycles due to climate have regional characteristics. For this reason, it is extremely important to obtain findings in the region (Bjurström et al., 1992).

Microorganisms are present in all natural environments in which animals live. Bacteria can also be found at different locations in bodies of animals; these commensally organisms, for instance, inhabit the gastrointestinal and urogenital tracts. In animals with signs of a reproductive disorder, the presence of a resident bacterial population in the genital tract makes the diagnosis of contributing infections difficult (Holst et al., 2003). The role of bacterial infections in many other reproductive disturbances has not been established in cats. As a consequence, owners of cats with reproductive disorders often want to have their cats treated with antimicrobials, especially when bacteria are detected in vaginal swab specimens. Characterization of the normal bacterial population of the genital tract in adult cats is therefore important (Lawler et al., 1991; Holst et al., 2003). In healthy cats, vaginal flora can be confused with many uterine or vaginal infections. *Escherichia coli (E. coli),* Coagulase negative *Staphylococcus* ssp., *Streptococcus canis (S. canis), Nonhemolytic Corynebacterium* spp. and *Haemophilus* spp. are the most frequently isolated agents in bacteriological cultivations from the vagina in cats. It can be found at a rate of 13% in anaerobic bacteria such as *Peptostreptococcus* spp. and *Bacteroides* spp. (Ekici and Canoğlu, 2013).

Holst et al. (2003) took bacteriological samples from 66 clinically healthy female cats in order to examine the presence and distribution of vaginal bacteria in 2 groups of catsas estrus and non-estrus. They found that

bacteriological positivity in animals in estrus (90%) was higher than bacteriological positivity in non-estrus (73%) animals. The most commonly identified bacteria were *E. coli, S .canis* and *Staphylococcus* spp. Five of 10 cats in estrus showed growth from cultured vaginal bacteria samples belonging to the *Pasteurellaceae* family, while 2 (4%) of non-estrus cats showed growth and the difference was significant (P<0.001). *S. canis* was detected in 3 of 10 cats in estrus and 7 of 56 cats in non-estrus, but this difference was not significant. It was emphasized that *E. coli* showed an equivalent distribution in both female groups (5/10 cats in estrus, 26/56 cats in non-estrus). *Staphylococcus* spp. grew in 11 samples (20%) from 56 non-estrus cats; however, this difference was not significant. They reported that animals in estrus and non-estrus differed from each other by the positivity of bacteriological growth and the distribution in bacterial species. It was determined that bacteriological positivity in estrus animals did not differ between mating and non-mating animals, and it was stated that high positivity was not due to mating. At the same time, it was reported that there was no difference in terms of bacteriological positivity and distribution between animals that were used progesterone (animals with high progesterone levels) and those that did not.

Haematological tests and determination of values are extremely important for the evaluation of the physiological and health status of animals. It is almost indispensable in many situations where veterinarians need to make a diagnosis. Haematological values are generally evaluated comprehensively as an important part of the clinical examination, indicating a specific differential diagnosis or suggesting a prognosis (Abdul-Rahaman et al., 2019). In order to ensure the correct interpretation of the analysis, reference blood values should be available for each animal species (Karagül et al., 2000; Turgut, 2000). Various factors, such as age, sex, breed, pregnancy, nutrition and season, have an effect on the observed changes in blood parameters. For example, significant changes are observed in the cardiovascular, respiratory and gastrointestinal systems and blood parameters of animals during pregnancy (Şimşek et al., 2015). These physiological changes observed in pregnancy and puerperium are mainly due to hormonal changes. Many haematological changes that occur during these periods are also physiological. Knowing these value changes is extremely important in terms of haematological evaluations (Chandra et al., 2012).

During pregnancy in humans, the total blood volume increases by about 1.5 litres, mainly to meet the demands of newly formed vascular networks and vascular dilation and to compensate for blood loss at birth. A significant part of this is found in the vascular networks of the placenta and uterus. This increase in blood volume is more evident in multiple pregnancies (Chandra et al., 2012). Anaemia (low haemoglobin) is a commonly described as haematological abnormality and is also associated with adverse pregnancy outcomes (Garn et al., 1981).

Bonelli et al. (2016) found that red blood count (RBC) and haematocrit values (known also as packed cell volume or PCV) were higher in late pregnancy compared to the time of delivery and breastfeeding period. It was determined that leukocyte counts (WBC) were higher in the birth period than in the late pregnancy and lactation periods, as in mares.

Abdul-Rahaman et al. (2019) compared blood values in pregnant and non-pregnant goats. They found that PCV were lower in pregnant goats (P≤0.05). It was reported that the blood volume increased in parallel with the increase in body weight during pregnancy and the PCV decreased at the end of pregnancy in goats. It has been stated that this condition is "Pregnant Physiological Anemia". It was also determined that the RBC was significantly higher (P≤0.05) in non-pregnant goats, and mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) values were significantly higher (P≤0.05) in pregnant goats than in non-pregnant goats. WBC was found to be significantly higher (P≤0.05) in non-pregnant goats than in pregnant goats. While neutrophil and basophile counts were significantly lower (P≤0.05), lymphocyte and eosinophil counts were significantly higher (P≤0.05) in non-pregnant goats. The difference in the number of monocytes was not significant in pregnant and non-pregnant goats.

It was observed that there were not many studies on reproductive status and haematological values in cats, so a limited number of sources could be reached in studies. Şimşek et al. (2015) compared the values of blood samples taken at different periods before and during pregnancy on Angora cats. They reported that RBC, PCV and hemoglobin (Hb) levels were higher (P<0.05) before and during pregnancy (on the 15th, 30th and 45th days) compared to the 55th day of pregnancy however, MCH levels were found to be lower (P<0.01). WBC levels were lower (P<0.05) in non-pregnant cats compared to the levels on the 30th and 55th days of pregnancy. No significant differences were observed in lymphocyte (LYM) values before and during pregnancy.

Vaginal cytology is a method that can be used to determine the phases of the estrus cycle in cats as well as dogs. On the other hand, although the use of vaginal cytology is very common in dogs, it has been more limited in cats. In cats, proestrus is rarely identifiable cytologically because the first signs of estrus are behavioral rather than cytological (Kustritz, 2020). Vaginal cytology in cats reflects hormonal changes, especially during heat, and is mostly used to determine the follicular phase (Ekici and Canoğlu, 2013).

Zonturlu et al. (2005) concluded that estrus and anestrus periods in cats can be determined by looking at the cell composition by means of vaginal cytology, but it is difficult to make any clinical distinction between proestrus and metestrus periods by means of vaginal cytology in cats.

The maximum keratinization in the vaginal epithelial cells in cats is observed when the plasma estradiol concentration reaches the maximum, and this situation differs from that of dogs (Shille et al, 1979). In the vaginal smear image during estrus, cornified superficial cells without nuclei or with pycnotic nuclei are predominant (at least 60-70%). There are two main differences in vaginal cytology during estrus compared to other cyclic periods in cats. The first of these is the cleaning of the slide surface in the intercellular spaces and the second is the proportional change of the vaginal epithelial cells (Johnston et al, 2001; Zonturlu et al, 2005; Aydın and Taşal, 2013). The ratio of vaginal epithelial cells shows a dynamic change during estrus (Aydın and Taşal, 2013). The proportion of anucleated superficial cells in the female cat vaginal cytology specimen is just over 10% of all cells on the first day of the follicular stage. Between the fourth and seventh days of estrus, this rate increases to about 40%. The number of superficial cells varies between 40 and 60% of the cells present during behavioral estrus

(Shille et al., 1979; Concannon et al., 1980; Arthur et al., 1983; Christiansen, 1984; Öcal and Aydın, 1999). It is reported that in the 12-13 days of the cycle, the rate of anucleated superficial cells decreased again to around 10%. Parabasal cells are not frequently encountered during the follicular stage, on the other hand, in this stage, 40–60% of the vaginal epithelial cells are formed by nucleated superficial cells (Aydın and Taşal, 2013). The number of intermediate cells in the smear decreases from 40% to 10% during the first 4 days of the follicular phase. Erythrocytes and leukocytes are extremely rare in the vaginal smear during estrus or proestrus. Parabasal cells comprise less than 10% of all cells in all phases of the cycle (Shille et al, 1979; Feldman and Nelson, 1996).

Shille et al. (1979) examined the relationship between estrus behaviors, vaginal smear and estradiol 17-β level in their study. Theydetermined that the day when the plasma estradiol 17-β level rises above 20pg/ml is the beginning of the follicular phase and the day when it falls below 20 pg/ml is the end of the follicular phase. They determined the estrus behavior and vaginal smear findings for 5 days before the follicular phase, during the follicular phase (7 days) and for five days after the end of the follicular phase. They reported that cytologically and clinically, proestrus begins with the clear appearance of the smear base area on vaginal cytology and ends when the female cat accepts to mate with the male cat.

According to Feldman and Nelson (1996), the anestrus period is like a long interestrus period. During this period, plasma estrogen and progesterone concentrations remain at basal levels, and pituitary hormone concentrations fluctuate slightly. It is in harmony with the interestrus period in vaginal cytology. Cell distributions in vaginal smear in cats in anestrus period consist of less than 10% parabasal cells, 40–70% intermediate cells, and 30-40% nucleated superficial cells. The smear is covered with mucus and crumbs at its base and does not show a clean appearance. Johnson et al. (2001) reported the cell distribution in vaginal smears from cats in anestrus as 9.7% parabasal cells, 87.4% intermediate cells, 2.7% nucleated superficial cells, 0.2% anucleated superficial cells and 3% neutrophils.

Zonturlu et al. (2005) found the rate of all superficial cells to be 92.57% in the estrus period, 51.90% in the proestrus period, 53.22% in the metestrus period and 8.28% in the anestrus period. Basal and parabasal cell ratios were reported as 76.81% during anestrus, 30.68% during metestrus, 30.23% during proestrus, and 1.63% during estrus. In addition, erythrocyte was not found in any of the smears examined during proestrus and it was stated that only 4 of 29 samples had neutrophil leukocytes in the estrus period.

Especially in recent years, it is observed that the number of cats among domestic animals has increased tremendously. Cats have an important place in huge commercial pet sector. However, it has been observed that cats, which stand out in many aspects, remain at a limited level in academic studies. This is because either no studies have been made and some values have not been reached, or there have been few studies on certain subjects.

The aim of this study was to investigate vaginal cytology, haematological and hormonal values, the presence of bacteria in the vagina, and the relationship between these findings in different reproductive periods in cats.

MATERIALS AND METHODS
Animals
The study consisted of 30 healthy non-geriatric female cats that had reached to puberty. The cats were divided into 3 equal groups as estrus (n=10), anestrus (n=10) and pregnant (n=10). The oldest cats were 6 years old and the youngest was 10 months old.

Collection and Evaluation of Samples for Vaginal Flora
No anesthetic or sedative medication was used during the collection of vaginal samples from the cats. First, microbiological samples were taken in order to prevent contamination. Swabs with gel was used to take samples (FıratmedStuart® Transport Media, Ankara, Turkey). The sterilized otoscope cannula was placed in the vagina, and the samples were taken by rubbing against the vaginal walls in the dorsal direction from the base of the vagina without contacting the vulva skin with the swab stick, which is prevented from contacting the environment through the cannula, and placed in the gel tube. It was kept in a refrigerator at +4ºC until it was sent to the laboratory without waiting at room temperature. The preserved microbiological samples were sent to a private laboratory while maintaining the cold chain, and the isolation was performed. Laboratory samples were evaluated for *Actinomyces* spp., *Arcanobacterium* spp., *Bacillus* spp., *E. coli, Enterobacter* spp., *Klebsiella* spp, *Staphylococcus* spp., *Streptecoccus* spp., *Staphylococcus aureus (S. aureus) Staphylococcus epidermidis (S. epidermidis), Corynebacterium* spp. and *Pseudomonas* spp.

Collection and evaluation of vaginal cytology samples
A port cotton slightly moistened with 0.9% isotonic NaCl solution was used for sampling. The port cotton was entered the vulva and advanced in the dorsal direction and rubbed into the vaginal walls and the cells were taken on cotton. Then, by rolling the port cotton in circular motion on the slide, the cell samples were transferred to the slide. After the procedure, the slides were dried and stained. Diff Quick dye set (Diff Quick Stain Set®, İstanbul, Turkey) was used for staining vaginal cytology specimens. Later, cover slips were pasted with Entellan (EntellanMerck®, Kenilworth, USA) and vaginal cytology samples were prepared. The microscope (Olympus®, CHK2-F-GS, Tokyo, Japan) was used for the examination of vaginal cytology samples (Figure 2.3.). The objective and ocular of the microscope used were adjusted to 10X magnification, and the cells were counted with a 100Xmagnification. The percentages of cell distributions in the samples were determined.

Collection, Processing and Evaluation of Blood Samples
Two separate blood samples were taken from the cats. The first sample was taken into a 2ml tube with K3 EDTA (Ayset Tube Edta® 3K, Adana, Turkey) and used to determine the hemogram (whole blood count) values. The second sample was taken into a sterile red capped blood collection tube (Hema&Tube Plain®, Ankara, Turkey) and serum samples were obtained. The animals were kept in the proper position, the hairs on the medial saphenous vein were shaved and the skin was disinfected with alcohol before entering the vein with a thin cannula during blood samples collection. Approximately 0.5-1 ml and 4-5 ml of blood samples were collected for hemogram and hormone analysis, respectively. The blood sample staken into a

sterile blood collection tube with a red cap were centrifuged at 3500 rpm for 5 minutes (Centrifuge® Model 800D, Zhejiang, China), and serum samples were obtained. Hemogram samples were evaluated with an automatic blood count device (Mindray®, BC-2800 VET, Shenzhen, China). The serum samples were taken into Eppendorf tubes at a minimum level of 1 ml, and stored in a refrigerator at +4ºC until they were sent to the laboratory.

Statistical Analysis
The preliminary analysis was first performed to see if the data met the parametric test assumptions (Shapiro Wilk test for normality test and Levene test for homogeneity of variance). In addition, the histogram, box-whisker plot and QQ plot were visually examined. The skewness and kurtosis of the data were also checked. As a result, it was seen that the data did not meet the parametric test assumptions. The Kruskal Wallis testwas performed to see if there was a difference between the groups in terms of the measured parameters. Pairwise comparisons in the groups were made with the Mann-Whithney U test. A new P values of <0.017 was accepted as statistical significance level after Bonferroni correction. All analyzes were performed using IBM SPSS v25 (IBM Corp®, Armonk, USA).

Ethics Committee Approval
The study was conducted with the approval of Kırıkkale University Animal Experiments Local Ethics Committee (Date 06.11.2019 / Issue 2019/12).

RESULTS
While there was no bacterial growth in 9 (30%) animals, bacterial growth was observed in 21 (70%) animals. When evaluated according to the groups, there were no bacterial growth in 3 (30%), 4 (40%) and 2 (20%) animals in estrus, anestrus and pregnant groups, respectively. The distribution of bacteria grown by groups is given in Table 1.

Table 1. The distribution of bacteria growth by groups.

AnimalOrder No	Estrus	Pregnant	Anestrus
1	*Escherichia coli*	*Staphylococcus aureus, Peptostreptococcu* spp.	No bacterial growth
2	*Escherichia coli Peptostreptococcu* spp. *Staphylococcus aureus Streptococcus* spp.	No bacterial growth	*Escherichia coli*
3	No bacterial growth	No bacterial growth	*Escherichia coli*
4	*Staphylococcus aureus*	*Staphylococcus aureus*	*Escherichia coli Staphylococcus aureus*
5	*Escherichia coli Staphylococcus aureus*	*Escherichia coli Staphylococcus aureus*	No bacterial growth
6	No bacterial growth	*Escherichia coli*	*Escherichia coli Staphylococcus aureus*
7	*Enterococcus* spp.	*Escherichia coli*	*Staphylococcus epidermidis*
8	No bacterial growth	*Streptococcus spp.*	*Staphylococcus aureus*
9	*Escherichia coli*	No bacterial growth	*Enterobacter* spp.
10	*Escherichia coli*	No bacterial growth	*Staphylococcus aureus*
Total	70% bacterial growth	60% bacterial growth	80% bacterial growth

When the bacterial distribution was evaluated in the samples with bacterial growth, *E. coli* was the most isolated bacteria with a value of 57.1% followed by *S. aureus* (47.6%), *Peptostreptococcus spp.* (9.5%), *Streptococcus spp.* (9.5%), *S. epidermidis* (4.7%), *Enterococcus spp.* (4.7%) and *Enterobacter spp.* (4.7%).

When all animals included in the study, *E. coli* was again the most isolated bacteria with a value of 40% followed by *S. aureus* (33.3%), *Peptostreptococcus* spp. (6.6%), *Streptococcus* spp. (6.6%), *S. Epidermidis* (3.3%), *Enterococcus* spp. (3.3%) and *Enterobacter* spp. (3.3%) (Table 2).

Table 2. Distribution of bacterial growths.

Bacterial growths	Estrus	Pregnant	Anestrus	Rate of growthpositive (%)	Rate in allanimals (%)
Escherichia coli	5	3	4	12/21 (57.1%)	12/30 (%40)
Staphylococcus aureus	3	3	4	10/21 (47.6%)	10/30 (%33.3)
Staphylococcus epidermidis	0	0	1	1/21 (4.7%)	1/30 (%3.3)
Streptococcus spp.	1	1	0	2/21 (9.5%)	2/30 (%6.6)
Enterococcus spp.	1	0	0	1/21 (4.7%)	1/30 (%3.3)
Peptostreptococcus spp.	1	1	0	2/21 (9.5%)	2/30 (%6.6)
Enterobacter spp.	0	0	1	1/21 (4.7%)	1/30 (%3.3)

E2 level in estrus animals (42.64±10.62 pg/ml) was significantly higher (P<0.001) than in pregnant animals (5.48 ± 4.88 pg/ml). Similarly, the E2 level of animals in estrus was significantly higher (P<0.001) than the E2 level of animals in anestrus (3.20 ± 1.24 pg/ml). Although the mean E2 level in the pregnant group was higher than the anestrus group, this difference was not statistically significant. The P4 level of the pregnant group (12.22 ± 9.35 ng/ml) was higher (P<0.001) than the P4levels of the anestrus (0.84 ± 0.25 ng/ml) and the estrus group (0.58 ± 0.28 ng/ml), while the P4 levels of the estrus and the anestrus groups were similar (Table 3).

Table 3. Comparison of the groups in terms of estradiol (E2) and progesterone (P4).

	Estrus Mean±SE (MeanRank) [Median]	Pregnant Mean±SE (MeanRank) [Median]	Anestrus Mean±SE (MeanRank) [Median]	P
E2 (pg/ml)	42.64±10.62[a] (15.50) [42.71]	5.48 ±4.88[b] (11.80) [3.91]	3.20±1.24[bc] (9.20) [2.95]	P<0.001
P4 (ng/ml)	0.58±0.28[b] (7.60) [0.61]	12.22±9.35[a] (25.50) [9.60]	0.84±0.25[b] (13.40) [0.91]	P<0.001

[a,b,c] Values in same row with different superscripts differ significantly at P<0.001

Hemogram was performed to reveal the differences between the groups. The significant differences were detected only in 3 parameters, MCV, MCH and MCHC, among the 19 parameters compared. MCV values were found to be lower in estrus animals (45.68 ± 3.75 femtoliter) than only in pregnant (51.21 ± 4.99 femtoliter) animals (P=0.007). The difference in MCH values between the estrus group (14.37 ± 0.84pg) and the pregnant group (15.62 ± 1.18pg) (P=0.003) and the difference in MCHC values between the pregnant group (30.66 ± 1.17 g/dl) and the anestrus group (32.42 ± 1.04 g/dl) (P<0.001) were statistically significant (Table 4).

Table 4. Comparison of blood values according to groups.

Blood Parameters	Estrus	Pregnant	Anestrus	P
WBC	11.96±3.38	16.18±7.05	12.64±7.25	
Lenf	4.15±2.21	4.77±5.06	3.12±2.33	
Mon	0.53±0.17	0.93±0.94	0.52±0.28	
Gran	7.28±2.21	10.48±3.70	9.00±5.70	
Lenf %	33.99±14.10	26.21±14.75	27.24±11.69	
Mon%	4.72±1.36	5.35±2.36	4.86±1.60	
Gran %	61.26±14.24	68.44±16.99	67.90 ±12.28	
RBC	9.58±1.35	8.42±1.34	8.50±1.10	
HGB	13.78±1.85	13.15±1.99	13.07±1.70	
HTC	43.50±5.22	42.78±5.86	40.51±5.10	
MCV	45.68±3.75[b]	51.21±4.99[a]	47.78±1.79[ab]	P =0.007
MCH	14.37±0.84[b]	15.62±1.18[a]	15.44±0.69[a]	P =0.003
MCHC	31.60±1.14[ab]	30.66±1.17[b]	32.42±1.04[a]	P <0.001
RDW	14.38±0.62	14.13±0.76	13.89±0.95	
PLT	203.80±106.42	196.80±169.64	339.70±235.63	
MPV	10.36±0.88	10.77±1.31	11.05±1.07	
PDW	15.42±0.61	16.40±1.25	15.60±0.79	
PCT	0.21±0.12	0.22±0.19	0.29±0.17	
Eos%	6.29±4.20	4.87±3.99	3.77±2.65	

[a,b] Values in same row with different superscripts differ significantly at P<0.01.

A total of 100 vaginal epithelial cells were counted from random areas on the slide. The slide surface was very clean in the vaginal smear samples obtained from the estrus group (the inability to determine the mucus layer that takes dye in the intercellular spaces), and the clear observation of the cells enabled easy differentiation of animals in estrus. However, such a clean slide surface was not observed in the smears obtained from the pregnant group and animals in anestrus (Figure1). The distributions of the percentages of the counted cells according to the groups were subtracted and compared. Parabasal cells were not detected in any of the animals in the estrus group. The percentage of parabasal cells was significantly higher in pregnant (71.60 ± 20.25%) and anestrus (81.20 ± 10.08%) groups than in estrus group (0%) (P<0.001). However, the percentage of parabasal cells in the pregnant group was lower than the animals in the anestrus group, the difference between the two groups was not statistically significant. The percentage of intermediate cells was significantly different (P=0.004) between the estrus group (5.20 ± 3.50%) and the pregnant group (16.70 ± 11.33%). Although the percentage of intermediate cells in the pregnant groups was higher than the anestrusgroup, the difference was not significant. The percentage of superficial cells in the estrus group (58.40 ± 5.34%) was significantly higher than the pregnant (9.30 ± 9.95%) and anestrus group (P<0.001). The percentage of keratinized superficial cells (35.80 ± 9.90%) in the estrus group was significantly (P<0.001) higher than the pregnant (9.30 ± 9.95%) and the anestrus group (1.40 ± 0.97%) (Table5).
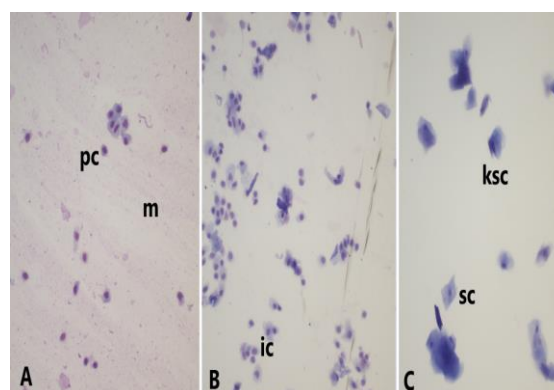
Figure1. Vaginal epithelial cells. **A:** anestrus, **B:** pregnant, **C:** estrus, **m:** mucus layer, **pc:** parabasal cells, **im:** intermediate cells, **sc:** superficialcells, **ksc:** keratinized superficial cells

Table 5. Percentage distribution of cell types by groups.

Distribution of cell types(%)	Estrus	Pregnant	Anestrus	P
Parabasal cell	0.00[b]	71.60±20.25[a]	81.20±10.08[a]	P<0.001
Intermediate cell	5.20±3.50[b]	16.70±11.33[a]	11.60±7.47[ab]	P=0.004
Superficial cell	58.40±5.34[a]	9.30±9.95[b]	5.20±3.74[b]	P<0.001
Keratinized superficial cells	35.80±9.90[a]	2.30±2.21[b]	1.40±0.97[b]	P<0.001

[a,b] Values in same row with different superscripts differ significantly at P <0.001.

DISCUSSION AND CONCLUSION

In the present study, *E. coli, S. aureus, S. epidermidis, Streptococcus* spp., *Peptostreptococcus* spp., *Enterococcus* spp. and *Enterobacter* spp. were detected by bacteriological analyses of vaginal swab specimens, which were collected from healthy cats. This result was compatible with the results presented by previous researchers (Holst et al., 2003; Ekici and Canoğlu, 2013). However, the bacteria, such as *E. coli*, *Streptococcus* spp. and *Staphylococcus* spp., were also commonly isolated from the cats with pyometra (Kenney et al. 1979; Lawler et al. 1991). These factors are in full agreement with the present study. Bjürström and Linde-Forsberg (1989) determined that *E. coli, β haemolytic Streptococcus* and *Pasteurella multocida* were the most isolated in 900 samples obtained from dogs, while the same bacteria were also found in animals with reproductive problems. Therefore, they concluded that vaginal bacterial examination is of low diagnostic value for dogs with normal reproductive function. In another study conducted in dogs, some of the factors that make up the flora are present in the flora in every period of the sexual cycle, and some of them are not in certain periods. It has been reported that bacteria can grow in the vagina in dogs in the estrus stage and this does not affect the pregnancy outcome (Fındık et al. 2003). In the present study, it was determined that the bacterial results obtained from the vaginas of healthy female cats in different reproductive periods were exactly the same as the results obtained in studies conducted in female cats with genital tract infection. Therefore, it has been concluded that bacteriological examinations of the vagina have an insignificant diagnostic value in examinations to detect genital tract infections in cats that appear healthy and do not have reproductive problems. As a result of this study, it was determined that bacterial growth in pregnant cats did not prevent them from having a healthy pregnancy process and giving birth in a normal way. In this regard, this situation should be taken into consideration in bacteriological examinations performed for the diagnosis of infectious infertility in clinically healthy cats that do not become pregnant. In the present study, bacteriological growth was not observed in 9 (30%) of 30 cats, while bacteriological growth was observed in 21 (70%) cats. Holst et al. (2003) reported that negative bacterial culture results in 15 of 66 cats (23%), while 51 cat (77%) had positive result. The bacterial flora of the vagina, which has a sensitive balance, is very sensitive to the endogenous and exogenous changes. Although hormonal status and sexual period are the most important factors on changes in the bacterial flora of the vagina in diseases, drug therapy such as antibiotics, immunological status and microbial interactions, microbial interactions can be determinative on the balance. In addition, genetic differences between breeds, oxygen, pressure, pH, humidity and amount of epithelial debris can take place in other factors affecting the flora balance (Bjurström et al., 1992). A compatible result was presented by Holst et al. (2003), who found 77% culture positive result in cats. Similarly, 70% positive result were also obtained in the presented study.

In the present study, progesterone and estrogen hormone levels of female cats in different reproductive periods were measured and the differences between groups were evaluated. According to many studies, (Feldman and Nelson, 1996; Hillier, 2001; Johnston et al., 2001) during the follicular phase in adult female cats, the plasma estrogen concentration spikes abruptly and remains high for 3-4 days and then slowly begins to decline. One day before the onset of the follicular phase, the plasma estrogen level is 12-15pg/ml. It increases to approximately 25 pg/ml on the first day of the follicular phase and 45 pg/ml on the third day. The plasma estrogen level, which rises slightly above 50 pg/ml on the fifth day, decreases to 20-25 pg/ml on the 7th day and 10 pg/ml on the 8th day. Assuming that ovulation no longer occurs on the 8th day, it can be considered as the first day of the interestrus period (Feldman and Nelson, 1996; Hillier, 2001). In the present study, the mean E2 level in estrus cats was determined as 42.64 ± 10.62 pg/ml. The obtained values were found to be compatible with previous studies. As expected, E2 level in cats in estrus was significantly higher than the E2 level in pregnant cats (P<0.001). The E2 values determined in these two groups are in agreement with the report of Johnston et al. (2001) in which the value peaks during estrus and estradiol decreases to basal level after the 5th day after mating (8-12 pg/ml) and remains low until 58-62 days of pregnancy. Although not significant, the mean E2 level (5.48 ± 4.88 pg/ml) in the pregnant group was nearly twice as high as in the anestrus group (3.20 ± 1.24 pg/ml) and this can be explained by the fact that there is a follicular development, albeit limited, in the ovaries of pregnant animals.

In cats, 1-2 days after ovulation or 2-3 days after mating, the plasma progesterone level rises above the basal (<1.0 ng/ml) value and exceeds 2 ng/ml. In the following 10-12 days, it rises to the level of 15ng/ml. The rise continues until 25-30 days of pregnancy and reaches an average of 15-30 ng/ml. Significant individual differences are observed between peak serum progesterone levels in pregnant cats (Jewgenow, 2012). Progesterone levels decrease in the later stages of pregnancy and decrease to 4-5 ng/ml in the last days (Schmidt et al. 1983). In the present study, the mean serum progesterone value was 12.22 ± 9.35 ng/ml in samples taken from animals after the second half of pregnancy. According to individual serum progesterone analyses, the highest and lowest values were 30.73 ng/ml and 3.37 ng/ml, respectively. The lowest progesterone level in a cat was associated with to be at the end of pregnancy period. Although, all of the pregnant cats were in the second half of pregnancy period, they were probably in the different periods of pregnancy. This might be a reason for their variable progesterone level intervals. This finding was compatible with the previous findings reported by Jewgenow (2012) and Schmidt et al. (1983). The progesterone level of the pregnant group (12.22 ± 9.35ng/ml) was higher than the progesterone level of the

anestrus group (0.84 ± 0.25ng/ml) (P<0.001) and the progesterone level of the estrus group (0.58 ± 0.28ng/ml) (P<0.001) in the present study. This is a natural consequence of the presence of an active corpus luteum in the pregnant group, and the progesterone level is below the basal level in the anestrus and estrus groups. The fact that the mean progesterone levels obtained in both the estrusand the anestrus group were below the basal level was found to be compatible with previous studies.

Shille et al. (1979) determined estrus behavior and vaginal smear findings during 5 days before the follicular phase, at the follicular phase (for 7 days) and five days after the end of the follicular phase. They noted that external symptoms of estrus were observed by 70-90% after the third day of the follicular phase. On the same days, parabasal cells were not observed, while intermediate cells were less than 10%, superficial cells 60% and keratinized superficial cells were observed at a rate of 40%. It has been reported that clinical symptoms of estrus were observed at 80-90% after 4 days of the follicular phase and 90-100% after 5 days (Shille et al., 1979). In the presented study, the rate of observing external signs of estrus was 100%, since both the mean estradiol level of the cats in the estrus stage was 42.64 pg/ml and the cat owners' animals came to the clinic showed estrus. These findings suggest that cats in estrus are in the period after 3 days of the follicular phase. In the present study, while parabasal cells were not detected in the vaginal cytology samples of the cats in the estrus group, the rate of intermediary cells was 5.20 ± 3.50%, the rate of superficial cells was 58.40 ± 5.34%, and the rate of keratinized superficial cells was 35.80 ± 9.90%. These values were similar with the ones obtained in this study by Shille et al. (1979). It has been revealed in many studies that the rate of anucleated superficial cells in the female cat vaginal cytology sample was slightly above 10% on the first day of the follicular stage and increased to about 40% on the 4th-7th days of estrus (Shille et al, 1979; Concannon et al, 1980; Arthur et al, 1983; Christiansen, 1984; Öcal and Aydın, 1999). These findings were very similar to the results obtained in the presented study. In the present study, the slide surface was very clean in the vaginal smear samples obtained from the estrus group (the inability to determine the mucus layer that takes dye in the intercellular spaces), and the clear observation of the cells enabled easy differentiation of animals in estrus. However, such a clean slide surface was not observed in the smears obtained from the pregnant group and animals in anestrus. This situation was similar to previous studies. Two main differences were observed in vaginal cytology in cats during estrus compared to other cyclic periods; the first of these was the cleaning of the slide surface in the intercellular spaces and the second was the proportional change of the vaginal epithelial cells (Johnston et al, 2001; Zonturlu et al, 2005; Aydın and Taşal, 2013).

In the present study, when the distribution in vaginal epithelial cells was compared according to the reproductive status, it was revealed that there were significant differences between the groups. The percentage differences between the pregnant group and estrus group were significant in all cell types (P<0.001). In the estrus and the anestrus group, the percentage difference between all the remaining cells, except for the intermediary cells, was also significant (P<0.001). Although the average of the percentage of intermediate cells in the anestrus group was more than twice that of the estrus group, the difference was notsignificant. However, when the anestrus and pregnant groups were evaluated, no difference was found in terms of percentage distribution in any cell group. Although it is very easy to distinguish between pregnant and anestrus cats with vaginal smear findings in cats, this is not the case for pregnant and anestrus cats. The distribution of all types of vaginal epithelial cells in the estrus group, where the estrogen level is significantly higher, is significantly different from the other groups in which the estrogen level is at basal levels. On the other hand, no difference was observed between the pregnant group with a peak progesterone level and the anestrus group with a basal progesterone level. This situation was evaluated as progesterone level did not have any effect on vaginal cell distribution in cats.

In the present study, it was investigated whether there were differences between the blood valuesof the cats in different reproductive periods. Significant differences were found between the groups only in 3 parameters, MCV, MCH and MCHC, among the 19 parameters compared. MCV values were found to be higher in pregnant cats (51.21 ± 4.99femtoliter) but the difference was significant only between pregnantanimals and those in estrus (P<0.01). The MCH value was the lowest in the estrus group (14.37 ± 0.84 pg). The mean valueof the pregnant group (15.62 ± 1.18 pg) was not statistically different from the anestrus group (15.44 ± 1.18pg). On the other hand, the difference between the estrus group and the anestrus group was significant (P<0.01). The MCHC value was the lowest in pregnant group (30.66 ± 1.17 g/dl). While the difference between the pregnant group and the estrus group (31.60 ± 1.14 g/dl) was not significant, the difference between the pregnant groupand the anestrus group (32.42 ± 1.04 g/dl) was significant (P<0.001) MCHC shows the average amount of hemoglobin in picograms per 100 ml of red blood cell. Therefore, MCHC is affected by both the amount of hemoglobin and the size of red blood cells.

Şimşek et al. (2015) compared the values obtained from blood samples taken from cats before conception and at different gestational periods in pregnant Angora cats. They found that the MCV value in Angora cats during the gestational period (52.59 ± 4.60 femtoliter) was higher (41.68 ± 0.60 femtoliter) than the values before conception (P<0.05), and the MCHC valueswere found to be higher throughout the pregnancy except the values after the 55th day of pregnancy. Şimşek et al. (2015) reported that MCH values were not statistically different from the values before conception during the whole pregnancy, except for the values after the 55th day of pregnancy. In the presented study, parallel results were found with the findings of Şimşek et al. (2015) with higher MCV values in the pregnant group.MCH values were not different from other groups, and MCHC values were low.

A study by Abdul-Rahaman et al. (2019) compared blood values in pregnant and non-pregnant goats and determined that MCV, MCH and MCHC values were significantly higher (P≤0.05) in pregnant goats than in non-pregnant goats. They noted that the observed increase in MCV, MCH and MCHC in pregnant goats resulted in an increase in the total oxygen carrying capacity of the circulating blood.

It is known that various factors such as age, sex, animal breed, pregnancy, nutrition and season have an effect on the changes observed in blood parameters. It was observed that there were not many studies on reproductive status and haematological values in cats. However, significant changes are observed in the cardiovascular, respiratory and

gastrointestinal systems and blood parameters of animals during pregnancy (Şimşek et al. 2015). It is thought that these physiological changes observed in pregnancy and puerperium are mainly due to hormonal changes. Many haematological changes that occur during these periods are also considered physiological. Knowing these value changes is extremely important in terms of haematological evaluations. An individual's haematological indices during pregnancy are largely indicative of her general health status (Chandra et al., 2012).

In the present study, progesterone and estrogen hormone levels, vaginal cytology, whole blood values and bacteriological cultivation results of the vagina were compared in adult female cats in different reproductive periods such as pregnant, anestrus and estrus. It was observed that the studies on these subjects in cats were very limited before the study was conducted and at the writing stage. In this respect, it is thought that the presented study, albeit limited, may help in the planning of future studies and the comparison of the obtained values. In addition, it is thought that some findings such as the distribution of bacteria in the vaginal flora may vary regionally, and it is thought that this studyrevealed a very limited distribution of bacteria. On the other hand, although it is known that the number of animals included in the study is limited to 30 due to the difficulties of obtaining more animals. It is, therefore, thought that it would be more beneficial to carry out future studies using a much higher number of cats and that the bacteriological diversity could be revealed more clearly. Similarly, in studies to be conducted with a large number of cats, blood values that can be affected by many factors can be more clearly revealed in terms of their levels and changes in different reproductive periods for cats.

Acknowledgements

Conflict of Interest
The authors declare that they have no competing interests.

Authorship contributions
Concept: L.T., H.K., S.E., Design: L.T., H.K., S.E., Data Collection or Processing: L.T., H.K., S.E., Analysis or Interpretation: L.T., H.K., S.E., Literature Search: L.T., H.K., S.E., Writing: L.T., H.K., S.E.

Financial Support

REFERENCES
Abdul-RahamanYT, Humid AO, Hamad Al-Dulaimi HS. 2019. Effect pregnant and non-pregnant on haematological and biochemical parameters of qatari goats in Iraq. Indian Journal of Public Health Research & Development, 10(10): 2743-2748.

Abdel Daim MM, Shaheen HM, Abushouk AI, Toraih EA, Fawzy MS, Alansari WS, Aleya L, Bungau S. 2018. Thymoquinone and diallyl sulfide protect against fipronil - induced oxidative injury in rats. Environmental Science and Pollution Research, 25(24): 23909-23916.

Arthur HG, Noakes DE, Pearson H. 1983. Veterinary Reproduction and Obstetrics 6th Ed, Balliére Tindall, London, pp. 689-892.

Aydın M, Taşal İ. 2013. Kedilerde Üreme Fizyolojisi. In: Kaymaz M, Fındık M, Rişvanlı A, Köker A (Eds), Köpek ve Kedilerde Doğum ve Jinekoloji. Medipres Matbaacılık, Malatya Turkey, pp. 283-294.

Bjurström L, Linde-Forsberg C. 1992. Long-term study of aerobic bacteria of the genital tract in breeding bitches. American Journal of Veterinary Research, 53(5): 665-669.

Bonelli F, Rota A, Corazza M, Serio D, Sgorbini M. 2016. Hematological and biochemical findings in pregnant, postfoaling, and lactating jennies. Theriogenology, 85(7): 1233-1238.

Chandra S, Tripathi AK, Mishra S, Amzarul M, Vaish AK. 2012. Physiological changes in hematological parameters during pregnancy. Indian Journal of Hematology and Blood Transfusion, 28(3): 144–146.

Christiansen IBJ. 1984. Reproduction in the Dog and Cat 1st Edition. Bailiere Tindall, London.

Concannon PW, Hodgson B, Lein D. 1980. Reflex LH, release in estrous cats following single and multiple copulations. Biology of Reproduction, 23(1): 111-117.

Ekici H, Canoğlu E. 2013. Genital organların muayenesi. in: Kaymaz M, Fındık M, Rişvanlı A, Köker A (eds), Köpek ve Kedilerde Doğum ve Jinekoloji, Medipres Matbaacılık, Malatya, Turkey, pp. 297-315.

Feldman EC, Nelson RW. 1996. Canine and Feline Endocrinology and Reproduction (2nd ed), WB Saunders Company, Philadelphia, pp. 741-768.

Fındık M, Maral N, Keskin O, Kalender H, Aslan S. 2003. The relationship between the stages of the sexual cycle, the pregnancy and postpartum periods and vaginal flora in Kangal breed bitches. Turkish Journal of Veterinary and Animal Sciences, 27(3): 761-765.

Garn S M, Keating M T, Falkner F. 1981. Hematological status and pregnancy outcomes. The American Journal of Clinical Nutrition, 34(1): 115-117.

Hillier SG. 2001. Gonadotropic control of ovarian follicular growth and development. Molecular and Cellular Endocrinology, 179(1-2): 39-46.

Holst BS, Bergström A, Lagerstedt AS, Karlstam E, Englund L, Baverud V. 2003. Characterization of the bacterial population of the genital tract of adult cats. American Journal of Veterinary Research, 64(8): 963-968.

Jewgenow K, Amelkina O, Painer J, Göritz F, Dehnhard M. 2012. Lifecycle of feline corpora lutea: Histological and intraluteal hormone analysis. Reproduction in Domestic Animals, 47(6): 25-29.

Johnston SD, Root-Kustritz MV, Olson PNS. 2001. The feline estrous cycle, In: Johnston SD (Eds), Canine and Feline X Theriogenology 1st Ed, W.B. Saunders Company, Philadelphia, pp. 396-402.

Karagul H, Altıntaş A, Fidancı UR, Sel T. 2001.Klinik Biyokimya, 1. Baskı. Medisan Yayınevi, Ankara, Turkey, pp. 55-167.

Kenney KJ, Matthiesen DT, Brown NO. 1987. Pyometra in cats: 183 cases (1979–1984). Journal of the American Veterinary Medical Association, 191(9): 1130-1132.

Kustritz MVR. 2020. Vaginal Cytology in the Bitchand Queen. In: Sharkey LC, Radin MJ, Seelig D. (Eds) Veterinary Cytology. John Wiley&Sons, Inc., Hoboken USA, pp. 552-559.

Lawler DF, Evans RH, Reimers TJ. 1991. Histopathologic features, environmental factors, and serum estrogen, progesterone and prolactin values associated with ovarian phase and inflammatory uterine

disease in cats. American Journal of Veterinary Research, 52(10): 1747-1753.

Öcal H, Aydın M. 1999. Anöstrus dönemindeki kedilere uygulanan GnRH'nın ovaryum aktivitesi üzerine etkisi. Fırat University Veterinary Journal of Health Sciences, 13(3): 391-398.

Sarı E (2019) Kapitalizmin Yeni Durağı Evcil Hayvanlar. Ticari Hayat 23 Kasım 2019. Erişim: [http://www.ticarihayat.com.tr/yazar/KAPITALIZMIN-YENI-DURAGI-EVCIL-HAYVANLAR/2618/] Erişim tarihi: 01.11.2020.

Schmidt PM, Chakraborty PK, Wildt DE. 1983. Ovarian activity, circulating hormones and sexual behavior in the cat. Biology of Reproduction, 28(3): 657-671.

Shille VM, Lundstrom KE, Stabenfeldt GM. 1979. Follicular function in the domestic cat as determined by estradiol-17 beta concentrations in plasma: relationt oestrous behavior and cornification of exfoliated vaginal epithelium. Biology of Reproduction, 21(4): 953-963.

Şimşek Ö, Arıkan Ş, Çınar M. 2015. Reference values for selected hematological and biochemical blood parameters from prepregnancy to advanced gestation in Angora cats. Turkish Journal of Veterinary and Animal Sciences, 39(1): 29-33.

Turgut K. 2000. Veteriner Klinik Laboratuvar Teshis. 2. Baskı. Bacıvanlar Basım Sanayi aş., Konya Turkey.

Zonturlu AK, Kaçar C, Maral N, Aslan S. 2005. Kedilerde vaginalsmear yöntemi ile siklus dönemlerinin saptanması ve ovaryumlar üzerindeki yapıların arasındaki ilişkinin araştırılması. Ankara Üniversitesi Veteriner Fakültesi Dergisi, 52: 17-21.