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# Dynamics of vaginal pH in the bitch during proestrus and estrus

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**Abstract:** The aim of this study was to investigate the dynamics of the pH of vaginal secretions during proestrus and estrus in bitches and its association with the optimal time for insemination and the values of steroid hormones. Sixteen bitches from different breeds and age were included in this investigation. Vaginal pH of each bitch was measured once daily, starting from the first day of vulval bleeding and continuing until the first day of cytological diestrus. In each bitch vaginal smears were taken to determine the onset of estrus and diestrus and blood samples were taken to determine serum progesterone (ng/ml) and estradiol-17 $\beta$  (pg/ml) for detection of the onset of ovulation and their influence on values and the dynamics of vaginal pH. Vaginal pH values in the bitches at the first day of proestrus were between 7.4 and 8.7. This parameter decreased with the progress of the proestrus and the entering into the estrus. Compared to the rest of the estrus, at the time of ovulation and on the next day, a consistent decrease of mean values (6.61 $\pm$ 0.15 and 6.63 $\pm$ 0.15 respectively) was observed ( $p < 0.01$ ). Individually at the time of ovulation vaginal pH ranged between 6.5 and 6.8. In the days following ovulation a gradual increase in mean values was detected. At the first day of diestrus vaginal pH values were measured significantly higher than those in estrus ( $p < 0.001$ ) and were between 7.4 and 8.3 (7.74 $\pm$ 0.26). In our study we found that the vaginal pH values in the bitch are lowest at the time of ovulation, with individual variations and exceptions.

**Keywords:** Bitch, Vagina, Ph, Progesterone, Estradiol

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## 1. Introduction

pH is defined as the negative logarithm of the activity of the hydroxonium ion [1].

Until now, there have been a limited number of studies on the pH values of vaginal secretions in the bitch. The results obtained from the different authors vary irrespective of whether the measurement is made with a pH-meter or an indicator paper.

References [2] and [3] investigated the pH of the vaginal secretions in the bitch at the different stages of the sexual cycle and found that during proestrus and estrus pH values vary from 5.5 to 6.5. According to [4], the vaginal pH in the bitch at the different stages of the sexual cycle ranges between 6.2 and 7.6, being from 6.8 to 7.6 during the proestrus and between 6.4 and 7.4 during the estrus. Performing daily measurements in the proestrus, [5] found values between 7.5 and 8.5. During estrus, the pH values were in the same range

with one exception (pH = 7.0). Based on a decrease of 0.866 $\pm$ 0.286 in 93,3 % of the animals during the fertile period after ovulation for 2.333 $\pm$ 1.534 days it was concluded that the pH measurement is appropriate for determining the optimal time for insemination in the bitch. References [2] and [3] measured vaginal pH values in diestrus from 7.0 to 8.5 and during anestrus-between 7.8 and 8.5, while the results obtained in [4] were in the range of 6.2 to 7.6 and 6.4 to 7.6 respectively.

During the different stages of the sexual cycle, the pH values of the vaginal secretions are influenced by the steroid hormone levels in the female organism [5]. Many of the remaining control mechanisms, as well as the biological role of this vaginal parameter are not yet fully understood.

The aim of this study was to investigate the dynamics of the pH of the vaginal secretions during proestrus and estrus in bitches and its correspondence with the optimal time for insemination and the values of steroid hormones.

## 2. Material and Methods

Sixteen bitches (12 multiparous and 4 nulliparous) of different breeds, aged 2-5 years, weighing between 5.2 and 27 kg were presented at the "Small Animal Clinic" of the Faculty of Veterinary Medicine, Trakia University, Stara Zagora, Bulgaria. The animals were found healthy on the basis of routine clinical examinations.

The investigation started on the first day of proestrus (appearance of serosanguineous vulvar discharge) and continued daily until the first day of cytological diestrus. All the bitches had typical clinical signs of the onset of proestrus (vaginal discharge, vulval oedema, attractiveness to male dogs), estrus ("straw colored" vaginal secretions, softening of the vulva, "flagging" of the tail) and diestrus (suspension of secretions, avoiding close contact with males). Daily measurement of the pH of vaginal fluid was made and vaginal smears were taken. Additionally, blood samples were taken to determine progesterone and estradiol-17 $\beta$  levels. During the estrus stage bitches were not mated by a male dog.

### 2.1. Determination of vaginal pH

The vaginal pH levels were determined using two kinds of strips (4,0-7,0 and 6,5-10,0) (Merck KGaA). Speculum was inserted and the indicator strips were touched to the vaginal wall for at least 3 sec. Determination was done after comparing the changes in the colour of the indicator and the appropriate table for both strips.

### 2.2. Vaginal cytology

Vaginal smears were taken with sterile cotton swabs (size  $\varnothing 2,5 \times 170$ ) to evaluate the changes in vaginal cells, which were stained with Haemacolor<sup>®</sup> (Merck KGaA). A minimum of 10 fields of view were observed with a light microscope and the magnification of 160 to 400 times. Cells from the vaginal wall were differentiated like basal, parabasal, intermediate, superficial and keratinized [6].

Estrus was defined when keratinized epithelial cells in the vaginal smears were more than 80% of the total [7]. Occurrence of cytological diestrus was registered when the percentage of keratinized epithelial cells abruptly decreased and parabasal and intermediate cells appeared which sometimes exceeded 50% of the total amount in the vaginal smear [7].

### 2.3. Hormone Analysis

To determine the levels of progesterone (P4) and estradiol-17 $\beta$  (E2), blood samples (~2 ml) were collected from each bitch by venipuncture of the cephalic vein. Upon collection, the blood samples drawn into tubes without anti-coagulant, were centrifuged (3000 x g for 10 min) and the sera stored at -20°C until assayed for progesterone and estradiol-17 $\beta$ .

Serum progesterone levels were measured by an enzyme immunoassay (EIA) using progesterone kit (Human, PROG

ELISA, GmBh, Germany). The analytic sensitivity of the progesterone ELISA test was 0.03-0.07 ng/ml (range of 0 - 40 ng/ml). Serum estradiol-17 $\beta$  levels were measured by an enzyme immunoassay (EIA) using estradiol kit (Human, Estradiol ELISA, GmBh, Germany). The analytic sensitivity of the estradiol ELISA test was 3-6 pg/ml (range of 0 - 2000 pg/ml).

Ovulation was determined at progesterone levels between 4 and 10 ng/ml [8].

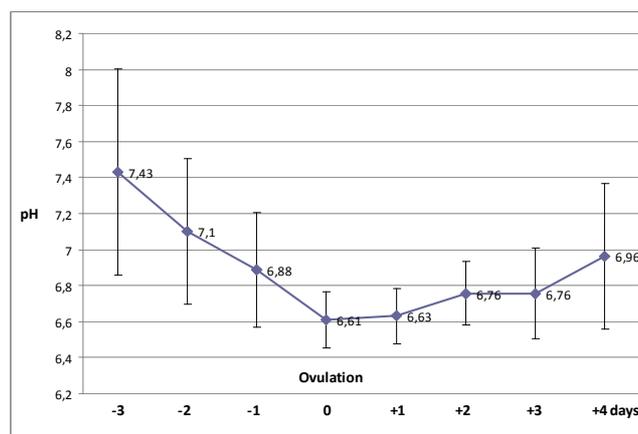
### 2.4. Statistics

The results of our trial were analyzed by one-way ANOVA (StatSoft 1984-2000 Inc. Copyright © 1990-1995 Microsoft Corp.) and are expressed as means $\pm$ SD.  $P \leq 0,05$  was considered significant.

## 3. Results

The duration of the proestrus ranged from 7 to 10 days ( $8.62 \pm 0.96$  days), the estrus - from 6 to 10 days ( $8.12 \pm 1.02$  days) and the onset of the cytological diestrus started between 17 and 20 days ( $17.87 \pm 1.02$  days) after the onset of the proestrus.

The maximal concentrations of estradiol-17 $\beta$  in individual animals varied between 50.13 and 123.47 pg/ml and were detected between 5 and 9 days ( $7.5 \pm 1.15$  days) after the onset of the proestrus or 2-5 days ( $3.56 \pm 0.81$  days) before the ovulation, which occurred between 9 and 13 days from the first day of the proestrus ( $11.06 \pm 1.24$  days) and from 0 to 2 days ( $1.25 \pm 0.77$  days) after the onset of the estrus. At the day of ovulation (Day 0), the estradiol-17 $\beta$  levels were significantly lower ( $p < 0.001$ ) compared to their peak and varied between 22.58 and 29.53 pg/ml. On the first day of the estrus the progesterone values ranged from 2.64 to 4.37 ng/ml and on the first day of the diestrus - between 31.62 and 33.90 ng/ml.



**Figure 1.** Dynamics of mean values of vaginal pH in bitches in days around ovulation.

During proestrus, the pH of vaginal secretions ranged between 6.5 and 8.7 ( $7.59 \pm 0.45$ ). At the first day of this stage, the pH values varied from 7.4 to 8.7 ( $7.96 \pm 0.31$ ).

After that a significant decrease was detected ( $p < 0.001$ ), reaching their lowest levels at the last day of the proestrus at values between 6.5 and 7.7 ( $7.09 \pm 0.33$ ).

The trend of a reduction of the vaginal pH continued during estrus. The values at this stage ranged from 6.1 to 7.7 ( $6.81 \pm 0.26$ ). The average pH value ( $6.81 \pm 0.26$ ) was significantly lower ( $p < 0.001$ ) compared to that during the proestrus. At the first day of the estrus, the vaginal pH in all of the bitches was between 6.5 and 7.1 ( $6.82 \pm 0.2$ ). At the time of the ovulation, the pH was significantly lower ( $p < 0.01$ ) than the average values in estrus and it reached levels between 6.5 and 6.8 ( $6.61 \pm 0.15$ ). Fig. 1 graphically presents the dynamics of the mean values of the pH of vaginal secretions of animals in days around the ovulation.

At the first day after the ovulation, the mean pH values were not statistically different from the ones of the time of ovulation and ranged between 6.5 and 6.8 ( $6.63 \pm 0.15$ ). For the following days, the pH values increased significantly and ranged from 6.5 to 7.1 ( $6.76 \pm 0.17$ ) ( $p < 0.01$ ) on the second day, from 6.1 to 7.1 ( $6.76 \pm 0.25$ ) ( $p < 0.05$ ) on the third day and from 6.5 to 7.9 ( $6.96 \pm 0.4$ ) ( $p < 0.01$ ) on the fourth day.

The differences between the mean values were statistically significant between the first and the second ( $p < 0.05$ ), as well as between the third and fourth day ( $p = 0.05$ ) after the ovulation.

These trends, however, do not reflect on some differences among the bitches. The lowest pH values were measured in 11 of the 16 examined animals (68.75%) during ovulation. In comparison to the first day of estrus the medium decrease in pH value was  $0.21 \pm 0.17$  ( $p < 0.01$ ). The other five (31.25%) bitches showed major individual variations. In one of the animals, the vaginal pH at the time of ovulation coincided with the value of the day before (6.5), while in another – with the one of the day after the ovulation (6.8). In two bitches (13.5%), the lowest values (6.5 in both animals) were observed respectively on the first and the second day after the ovulation (which occurred at pH=6.8). The lowest measured pH value of vaginal secretions in this study was 6.1. It was detected in one animal (6.25%) on the 3rd day after the ovulation, which occurred at pH=6.5.

During the first day of the cytological diestrus, all of the examined animals (100%) displayed a significant increase ( $p < 0.001$ ) in pH of the vaginal fluid and the values varied between 7.4 and 8.3 ( $7.74 \pm 0.26$ ).

## 4. Discussion

This study is an important contribution specifying the dynamics of pH of the vaginal secretions in the bitch during proestrus and oestrus.

The importance of determining this parameter has different aspects. Firstly, it is to clarify certain biological patterns. The pH values show some specific characteristics among species. Until now there are several studies in various animal species. In cows vaginal pH during proestrus has been found to be 7.4, during estrus values decrease to 6.9 and in metestrus and diestrus they increase to 7.2 and 7.4

respectively [9]. In dairy cows [10] and [11] measured the lowest pH values on the first day of the estrus, followed by an increase. Reference [12] found in sows that the rapid decrease in the vaginal pH values from 7.2 to 6.3 during estrus lasted for 3 days, which coincided closely with the sexual receptivity to a boar and they considered it appropriate for determining the heat in this species. Also in sheep, the pH of vaginal and cervical secretions decreases during estrus [13, 14]. The lowest pH values during the sexual cycle in mares were detected at the time of ovulation [15]. In all these species the lowest pH values of vaginal secretions coincide with the stage of the sexual cycle when estrogen levels are highest.

We dare say that there are certain specifics in the bitch. As mentioned in the introduction, the results of the different investigations are very heterogeneous. During proestrus we observed that vaginal pH values ranged from 6.5 to 8.7 with a tendency to a gradual decrease. The lowest pH values in our investigation were detected at the stage of estrus which coincides with the results of [4] and [5]. It was accompanied by a progressive decrease in estrogen and gradual increase of progesterone levels. This peculiarity could be regarded as species specific for the bitch. It is possible to look for the correlation between pH and other parameters, for example – the electrical resistance of the vaginal mucosa. In our previous study [16] we found that the highest values of electrical resistance coincide with the time of ovulation when the vaginal pH is in its lowest ranges.

For animal species with vaginal deposition of semen it is important for the vaginal pH to correspond with the pH of the semen. Seminal plasma has a necessary buffering capacity, which allows neutralizing small differences as a compensatory mechanism [17]. In common domestic species the pH of seminal plasma ranges from 6.7 to 7.4. In the dog the values are between 6.3 and 6.7 [18]. The vaginal pH in the bitch during ovulation and in the days afterwards when is the optimal time for insemination almost coincide with those of the sperm. We believe that this fact provides favorable conditions for the survival of spermatozoa in the female reproductive system.

Vaginal pH depends on the levels of reproductive hormones in the body and therefore they vary in different stages of the sexual cycle [5]. The cell populations in vaginal mucosa during the sexual cycle change depending on the steroid levels in the female organism [7]. Undoubtedly endocrine function directly or indirectly affects the characteristics of the sexual organs, especially during proestrus and estrus. It has been established that the injection of estrogens in animals after ovariectomy resulted in a decrease in the vaginal pH [19].

Another aspect of the importance of the vaginal pH is the determination of the optimal time for insemination in the bitch. The results of our investigation showed a significant decrease in vaginal pH values at the time of ovulation. This fact could easily find a practical application. In the bitch, primary oocytes are ovulated and fertilization cannot occur until 48 to 72 hours after ovulation [17, 20]. Therefore, the

most appropriate time for mating or artificial insemination is 2-3 days after ovulation. In our study, we found that when ovulation occurred, pH of vaginal secretion of bitches was between 6.5 and 6.8. Reference [5] also registered ovulation in the same ranges of vaginal pH.

We also found that there is a gradual increase in vaginal pH values on the second day after ovulation. In our opinion, the most important indication that ovulation is already completed is the increase in vaginal pH after its maximal decrease, making it the appropriate time for the insemination of the bitch. But we should not ignore that there are exceptions. For instance, in one of the animals the lowest pH was measured on the third day after ovulation.

Health aspect also determines the importance of vaginal pH. There is a necessity of additional investigations in this area in the future, because many questions remain unanswered. Although, according to [4] there are no hints of correlation between specific diseases and the values of vaginal pH in the bitch, we believe that it is possible certain pathological processes in the genital organs to be accompanied by changes in pH of the vaginal secretions. The reason for this could be found in the influence of the vital activity of microorganisms, inflammatory secretes and abnormal hormonal profile in the body. For example, vaginal pH of women is 5 or lower due to the vital activity of anaerobic lactobacilli that produce lactic acid, which is microbicidal for many sexually transmitted disease pathogens [17]. It is unclear what would be the importance of changing pH of the vagina as a successful therapeutic approach for the treatment of genital diseases in the bitch.

## 5. Conclusion

We could conclude that determination of vaginal pH in the bitch is an important parameter, which has to find a greater use as a part of a routine gynecological examination. For a more precise interpretation of the results it is necessary to perform a series of tests during proestrus and estrus. The lowest values of vaginal pH are associated with the occurrence of ovulation. The presence of exceptions requires comparing the results of pH values with other methods of examination.

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