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Original Research Article

DYNAMICS OF SOME VAGINAL PARAMETERS IN NON-PREGNANT BITCHES AFTER MID-LUTEAL AGLEPRISTONE TREATMENT

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Summary: The aim of our study was to investigate the effects of the antiprogestin aglepristone after its application in bitches with high serum progesterone concentrations on some specific vaginal parameters. Twelve non-pregnant female dogs from different breeds and ages were included in the study. The bitches were divided into two groups. Group I (n = 6) received aglepristone (10 mg/kg, subcutaneously) injected twice, 24 h apart, on days 29 and 30 after the estimated day of ovulation, which was determined by progesterone assays. Group II (n = 6) served as a control group and received a placebo. The electrical resistance of vaginal mucus, vaginal pH and serum P_4 levels were determined on days 29, 30, 33, 36, 39, 42 and 45 after ovulation. Additionally, vaginal smears were performed to evaluate the changes in vaginal cells. Partial luteolysis was detected at day 32.5 ± 2.26 (mean ± SD) and 44 ± 1.73 in treated and control bitches, respectively ($p < 0.001$). Complete luteolysis ($P_4 < 2$ ng/ml) was observed on day 41.5 ± 2.26 in treated bitches. Beginning on the day after the first treatment, a decrease in electrical resistance of vaginal mucus was measured in the experimental group. A significant reduction ($p < 0.05$) occurred on day 41.5±2.26 compared with day 29. The pH of vaginal secretions of all bitches in Group I increased during the period starting from the day after the first treatment. A significant difference ($p < 0.05$) was found on day 32.5 ± 2.26. No changes were detected for either parameter in the control group. The cell populations in vaginal smears of all animals were similar. In conclusion, aglepristone administration to dogs during the mid-luteal stage influenced vaginal pH and the electrical resistance of vaginal mucus.

Key words: vagina; bitch; aglepristone; electrical resistance; pH

Introduction

The vagina and the vestibulum are important structures for the reproductive health of female dogs. Determination of specific vaginal parameters such as electrical resistance, pH and type of cell populations could provide some information about the local or general health of the animal.

Over the past decade, the use of antiprogestins has increased. They are competitive progesterone antagonists, which bind to specific receptors, causing their structural transformation, preventing hormonal biological effects (1).

The progesterone antagonist aglepristone is commonly used for induction of abortion or parturition in bitches, as well as for treatment of various gynaecological disorders associated with high serum progesterone levels. There are many studies on the use of aglepristone, involving induction of abortion (2), conservative treatment of pyometra (2-6), cystic endometrial hyperplasia (7), acromegaly, insulin resistance (8) and mammary GH-induced IGF-1 secretion (9). Although the

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effect of antiprogestogens on the uterus, ovarian function and mammary gland in the bitch has been investigated, little is known about their effect on external genitalia, especially on the vagina.

The aim of the present study was to investigate the effects of the antiprogestogen aglepristone on some specific vaginal parameters after its administration to bitches with high serum progesterone concentrations.

Materials and methods

Twelve non-mated bitches from different breeds (American Cocker Spaniel-2, English Cocker Spaniel-2, Samoyed-2, Golden retriever-1, German Hunting Terrier-1, mixed breeds-4) and ages (3-6 years, mean ± SD 4.08 ± 1.16) in diestrus without any reproductive problems were included in the study. All animals were clinically and gynaecologically healthy. The study has started 29 days after ovulation day, which was indicated by serum progesterone (P4) levels between 4-10 ng/ml during the last estrus.

The animals were divided into treatment (Group I) or control (Group II) groups. On day 29 and 30 after ovulation, Group I (n = 6) received 10 mg/kg body weight aglepristone (Alizine®, Virbac Laboratories, Carros, France) subcutaneously, while Group II (n = 6) received saline solution (0.3 ml/kg body weight, subcutaneously).

Blood samples were collected from each bitch on days 29, 30, 33, 36, 39, 42 and 45 after ovulation by venipuncture of the cephalic vein. Blood vials without anticoagulant were used and centrifuged (3000 X g for 15 min). The sera were stored at -20°C until assayed for serum progesterone levels. Serum progesterone levels were measured by an enzyme immunoassay (EIA) using a progesterone kit (Human, PROG ELISA, GmbH, Germany). The analytical sensitivity of the progesterone ELISA test was 0.03-0.07 ng/ml (range: 0 - 40 ng/ml) with an intra-inter assay coefficient of variation < 10%.

Vaginal electrical resistance and pH levels were determined and vaginal smears were performed on the same days, immediately following the blood collection.

Vaginal electrical resistance was measured using the "Draminski Dog Ovulation Detector" (Draminski®, Poland). The vulva was cleaned with a dry paper towel and the probe was inserted into the vagina. The button was then pressed three times with full rotation (360˚), so that the electrodes came into full contact with the vaginal mucus. The result was recorded on a checklist. Vaginal pH levels were determined using two types of strips (4.0-7.0 and 6.5-10.0) (Merck KGaA). A speculum was inserted and indicator strips were placed on the vaginal wall for at least 3 seconds. The changes in the colour of the indicator were compared with the reference table for both strips.

Vaginal smears were collected with sterile cotton swabs (size Ø2.5x170) to evaluate the changes in vaginal cells, and stained with Haemacolor® (Merck KGaA). A minimum of 10 fields of view were observed with a light microscope at magnifications of 160 to 400 X. Cells from the vaginal wall were differentiated as basal, parabasal, intermediate, superficial or keratinized (10).

Partial luteolysis was defined on the basis of a 50% decrease in serum P_4 concentrations from the mean pre-treatment value, while complete luteolysis was defined at P_4 concentrations below 2 ng/ml (11).

The results were expressed as mean ± SD and analysed using ANOVA for repeated measures. $P \leq 0.05$ was considered significant.

Results

The results for aglepristone treatment effects on P_4 levels, vaginal electrical resistance and vaginal pH are presented on Figures 1, 2 and 3, respectively.

Before treatment, the mean serum progesterone concentrations in treated (Group I, n = 6) and control (Group II, n = 6) bitches were 60.33±27.39 and 57.29±24.21 ng/ml, respectively. Partial luteolysis was observed at day 32.5±2.26 and 44±1.73 in the treated and control groups, respectively ($p<0.001$). Complete luteolysis (P4<2 ng/ml) was observed at day 41.5±2.26 only in Group I.

The electrical resistance of vaginal mucus of all animals in Group I decreased, starting on the day after the first treatment and continued to decrease after the second administration; a statistically significant difference ($p<0.05$) was observed in all Group I bitches on day 42 after ovulation, i. e. after the complete luteolysis (41.5±2.26). In Group II bitches, vaginal electrical resistance was not reduced.

Vaginal pH values increased in all Group I bitches, beginning on the day after the first and

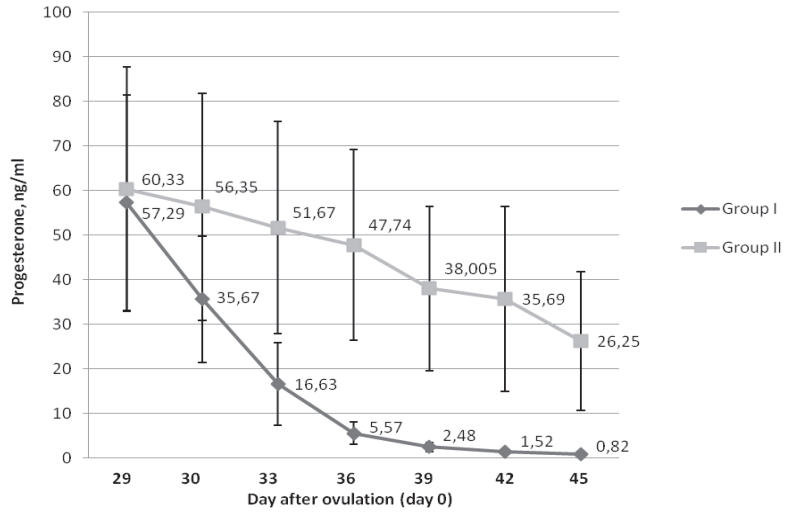

Figure 1: Serum P_4 levels in treated (Group I, n = 6) and control (Group II, n = 6) bitches

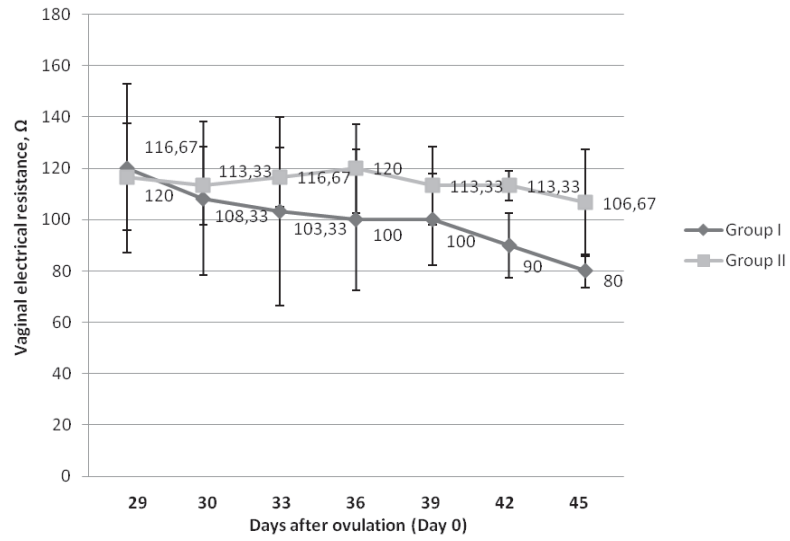

Figure 2: Vaginal electrical resistance in treated (Group I, n = 6) and control (Group II, n = 6) bitches

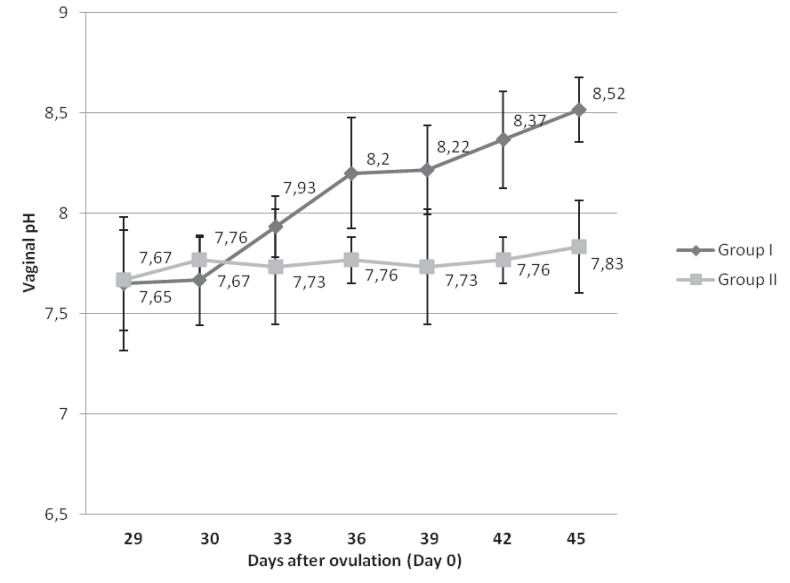

Figure 3: Vaginal pH in treated (Group I, n = 6) and control (Group II, n = 6) bitches

kept on increasing after the second treatment. A statistically significant difference ($p<0.05$) was observed in all bitches on day 33 post ovulation, i.e. after partial luteolysis (32.5±2.26) compared with day 29. This decrease persisted in the subsequent days of measurement. In Group II, vaginal pH also increased, but the difference was not significant until day 45.

The cell populations in vaginal smears of all animals in Groups I and II were similar and consisted of parabasal cells, small intermediate cells and neutrophil leukocytes.

Discussion

According to previous studies (12, 13), antiprogestogens do not have direct or indirect luteolytic effects in female dogs (in contrast to humans). Aglepristone administered to bitches during the early luteal stage decreased the interestrous interval without effect on the duration of the luteal stage (14). Injected in the mid-luteal stage, the same antiprogestogen induced early luteal regression and a decrease in serum progesterone levels in treated bitches (11). In our study, we also treated the animals during the mid-luteal stage (days 29 and 30 after ovulation) and found similar results about the luteal function.

Our study is the first investigation of the dynamics of specific vaginal changes in bitches after the termination of the biological activity of progesterone. The results confirm that these vaginal parameters depend on progesterone concentration, especially the changes in vaginal electrical resistance. Until now, most studies have only demonstrated the influence of progesterone levels and dynamic changes of this parameter depending on the LH surge and estrogen levels in females (15).

There are two key aspects in the interpretation of the results. The first is related to the time of statistically significant changes in the values of the parameters after aglepristone administration.

The changes in vaginal impedance after aglepristone treatment occurred only after a statistically significant decrease in serum progesterone levels. Substantial changes in vaginal fluid pH were found even earlier, on the 4th day after the first aglepristone administration, when serum progesterone levels were still relatively high. It is well known that the environmental pH influences the development of microorganisms and the effect of some therapeutic agents. The increase in pH resulting from the aglepristone treatment should be taken into consideration in decision-making for optimal therapy for diseases of the genital organs.

According to other reports (15-20) as well as previous works of our group (21, 22), vaginal electrical resistance and pH during proestrus and estrus are extremely dynamic parameters that vary over a very short interval of time: within 1-2 days. It is believed that this is a response to constantly changing levels of progesterone and estrogen and to the LH peak (15). Measurement of vaginal resistance and pH during estrus helps determining the optimal time for insemination. Changes in these parameters following aglepristone administration during the diestrus depend on the period of time after progesterone receptors are blocked, which is ultimately followed by a drop in progesterone levels. Similarly, the occurrence of abortion is 6-7 days after antiprogestin administration (23). It is well known that progesterone is required to maintain pregnancy.

The second aspect involves the direction of the changes in studied parameters following the administration of aglepristone. It was detected that the vaginal resistance decreases and in the meantime the pH increases. Similar changes occur during estrus, after ovulation, when the levels of progesterone are constantly increasing (16, 18, 21, 22). Future studies should address the exact mechanisms of these changes in vaginal resistance and pH.

The absence of changes in the vaginal cell populations after the use of antiprogestins during diestrus confirm the dominant role of estrogens in the changes of vaginal wall's cell populations.

In conclusion, the present study demonstrates that the treatment of bitches with the antiprogestogen aglepristone during diestrus results in statistically significant decrease in vaginal electrical resistance on the 14th day after the 1st injection, when the serum progesterone levels were lower than 2 ng/ml, as well as a significant increase in the pH of vaginal fluids on the 4th day after the 1st injection, when partial luteolysis was detected. A change in the vaginal cell populations was not detected.

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DINAMIKA NEKATERIH PARAMETROV NOŽNICE PRI NEBREJIH PSICAH PO TRETIRANJU Z AGLEPRISTONOM PO SREDNJI LUTEALNI FAZI

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Povzetek: Namen naše raziskave je bil proučiti učinke aplikacije antiprogestina aglepristona psicam z visoko koncentracijo progesterona v serumu, na nekatere specifične nožnične parametre. V raziskavo je bilo vključenih dvanajst nebrejih psic različnih pasem in starosti. Psice so bile razdeljene v dve skupini. Skupina I (n = 6) je prejela aglepristone (10 mg/kg subkutano), injicirano dvakrat v 24 urah, 29. in 30. dan po predvideni ovulaciji, ki je bila določena z merjenjem progesterona. Skupina II (n = 6) je služila kot kontrolna skupina in je prejemala placebo. Električna upornost nožnične sluzi, nožnični pH in serumski nivo P4 so bili izmerjeni 29, 30, 33, 36, 39, 42 in 45 dni po ovulaciji. Poleg tega so bili odvzeti nožnični brisi za vrednotenje sprememb v celicah nožnice. Delna luteoliza je bila odkrita 32,5. ± 2,26 dan (povprečje ± SD) pri skupini zdravljenih psic in 44. ± 1,73 dan pri skupini kontrolnih psic ($p < 0,001$). Popolna luteoliza ($P_4 < 2$ ng/ml) je bila opažena 41,5. ± 2,26 dan pri zdravljenih psicah. Na dan po prvem tretiranju je bilo izmerjeno zmanjšanje električnega upora vaginalne sluzi, izmerjene v eksperimentalni skupini psic. Značilno zmanjšanje ($p < 0,05$) je bilo zaznano 41.5. ± 2,26 dan v primerjavi z 29. dnem. pH nožničnih izločkov vseh psic v skupini I se je povečal v obdobju od dneva po prvem tretiranju. Značilne razlike ($p < 0,05$) so bile vidne 32,5. ± 2,26 dan. V kontrolni skupini niso zaznali sprememb pri vseh parametrih. Populacije celic v vaginalnih brisih vseh živali so bile podobne. Iz opisane raziskave lahko zaključimo, da dodajanje aglepristona psicam v srednji lutealni fazi vpliva na nožnični pH in na električno upornost nožnične sluzi.

Ključne besede: vagina; psica; aglepristone; električna upornost; pH