ELECTRICAL RESISTANCE OF VAGINAL SECRETION IN THE BITCH

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ABSTRACT

Five multiparous and four nulliparous bitches had the electrical resistance of vaginal secretions measured by an ohmmeter. The bitches were examined daily, starting on the first day of vulval bleeding and continuing until the first day when exfoliated vaginal epithelial cells became predominantly noncornified.

In contrast to cattle, sheep, and swine, the electrical resistance of the vaginal secretions in the bitch increases during pro-oestrus and continues to be high while the bitch is showing oestrous behaviour and a high percentage of eosinophilic superficial cells in the vaginal smear. Ovulation, as determined by a rise of serum progesterone above 5 ng/ml, occurs within the period of elevated resistance. Optimal conditions for conceptions coincide with the last 3 d of elevated resistance.

Keywords: dog, oestrus, vaginal secretion, electrical resistance, cytology, conception optimum

INTRODUCTION

Researchers of the biology of reproduction in the bitch have shown that the protracted oestrous cycle is associated with an extreme variability of the different processes involved. For example, the temporal correlation of oestrous behaviour and follicular development until ovulation varies between bitches as well as from heat to heat. Diagnosis of the best time for mating or insemination on the basis of behavioural signs may therefore be inaccurate.
A more accurate method to time ovulation is by using the characteristic cornification in the vaginal epithelium, since this cornification reflects the rise in follicular oestrogen. This timing may be accomplished through systematic examination of vaginal smears. According to Linde and Karlsson (1), the first day of maximum cell cornification in the vaginal smear coincided with the time of ovulation in about 70% of the bitches. In the study by Linde and Karlsson, ovulation coincided with a serum progesterone concentration of 5.44 ng/ml. This value is similar to that reported by Concannon et al. (2).

The duration of maximal cornification—defined as 80% or more superficial cells with pyknotic nuclei or complete nuclear loss—varied from 1 to 6 d, with an average of 2.6 d.

From research done in other species, it is known that vaginal secretions also undergo characteristic changes related to the circulating hormones. During oestrus, values of the electrical resistance in the vaginal secretion of cattle, sheep, and swine are lower than at any other time in the estrous cycle (3). In cattle, the preovulatory luteinizing hormone (LH) peak is correlated with the lowest value in electrical resistance (4).

Measurement of the electrical resistance has been used routinely to determine the best breeding time in foxes (5-7). In the vixen, follicular development (pro-oestrus) is accompanied by a gradual increase of the electrical resistance within 6 d, from basic levels between 100 and 200 ohm to maximum levels between 400 and 1000 or more ohm. After this peak, which lasts 24 to 48 (at most 96) h, resistance drops to initial values within 3 to 4 d. Matings or inseminations performed during the period of declining resistance result in high conception rates.

Researchers who analyzed the hormonal status in the peripheral blood serum of foxes (8) learned that the LH surge coincides with the appearance of maximum oestradiol-17β levels and with the first acceptance of the male. The electrical resistance has its peak about 2 d later. The first significant rise of peripheral progesterone concentrations occurs simultaneously with the LH peak and then increases continuously during the period of acceptance (oestrus) as is typical for the bitch as well (2, 9).

The electrical resistance of the vaginal secretion during pro-oestrus and oestrus of the bitch has been examined by Klötzer (10). Klötzer observed conditions similar to those reported in the fox, with a continuous rise from the basic values in anoestrus (70±9 ohm) to maximum values (250 to 1216 ohm) at the last 2 d of pro-oestrus or the first day of oestrus. However, because the behaviour toward the male was taken as the orientation point, a great variability between animals was observed. Generally, the electrical resistance of the vaginal secretion declined until the end of oestrus.

This study was done to determine the relationship of changes in electrical resistance to Day 1 of diestrus as defined by Holst and
Phemister (11). The latter definition appeared to be currently the most accurately defined and consistent event in the canine reproductive cycle.

MATERIALS AND METHODS

Nine 4- to 6-year-old Beagle bitches from different departments of the School of Veterinary Medicine in Hannover were used as experimental animals. Five of them (Bitches 1, 2, 4, 5, 7) had welped previously; the remaining four were nulliparous. The bitches were kept separated from male dogs either in groups (Bitches 1-6, 8) or in individual cages (Bitches 7, 9) and fed a well-balanced commercial food.

The investigations were performed from October 1982 to February 1984. Each bitch was placed on the study on the first day of vulval bleeding (defined as the first day of pro-oestrus) and was observed daily until early dioestrus. One bitch (Bitch 3) was started in late pro-oestrus because onset of pro-oestrus was missed. Daily measurements of electrical resistance of vaginal secretions were done prior to other manipulations to avoid bias.

The instrument used for the measurements was the prototype of equipment developed by Metzger et al. (12) and modified for similar use in the fox (5, 7). This equipment consisted of an unbreakable probe (length 15.0 cm, diameter 0.8 cm) with two gilded brass electrodes at the apical segment (interelectrode distance 0.5 cm) and an ohmmeter with a measuring scale from 0 to 1000 ohm (Figure 1). The instrument was contained in a metal box (15 x 10 x 8 cm).

Figure 1. Equipment for measuring electrical resistance of vaginal secretion in the bitch.

Electrical resistance was measured with alternating current to prevent possible polarisation of the electrodes. There was direct
reading on a linear scale (error < 3%), and readings were independent of
the variations in temperature and the supply of voltage. The instrument
was powered by battery, operating with control of the battery voltage.

Before each measurement, the probe was cleaned with a mild dis-
infec tant (Aciderm plus, Th. Goldschmidt AG Chemische Fabriken, 4300
Essen, West Germany).

After complete drying, the probe was inserted into the vagina until
electrodes were at the cranial third of the vagina and brought into
good contact with the mucosa.

To evaluate and interpret changes in the electrical resistance
during the reproductive period, the first day of dioestrus, as defined
by Holst and Phemister (11), was referred to as orientation point and
marked as Day 0. This day was identified by the abrupt decrease in the
proportion of superficial cells (by at least 20%) and a concomitant
increase of intermediate (noncornified) cells to above 10% and some-
times as high as 50%.

To determine the onset of dioestrus, each measurement of the
electrical resistance was followed by taking a swab from the cranial
vagina using a speculum. Smear preparation was performed with pre-
stained slides (Testsimplets*, Boehringer Mannheim GmbH, 6800 Mannheim,
West Germany) according to the method described by Günzel and Koivisto
(13). In three bitches (Bitches 1, 2, 4), an additional smear was pre-
pared from each swab on a clean slide and stained according to the
method of Papanicolaou (14). This method involves using a polychromatic
stain that differentiates cornified (eosinophilic) and noncornified
(cyanophilic) cells and is therefore suitable to show the increasing
cornification of the vaginal cells during pro-oestrus and oestrus. This
method was used to verify the relationship between the cell cornifica-
tion rate and the electrical resistance of the vaginal secretion during
heat.

In another four bitches (Bitches 5, 6, 7, 8), the macroscopic consis-
tency of the vaginal secretion was examined and noted daily to compare
further the parameter to the electrical resistance. The classification
from Lindsay' (15) was used:
1 - slightly moist (thin layer of transparent mucus)
2 - moderate glistening moist (in pro-oestrus, red secretion)
3 - slightly moist to sticky
4 - sticky

In two bitches (Bitches 3, 9), blood samples were taken daily from
the vena cephalica to analyse the progesterone concentration in serum.
The serum samples were stored at -20°C until quantification of
progesterone by radioimmunoassay.

Serum progesterone (ng/ml blood serum) was determined in the
hormonanalytic laboratory of the Clinic of Obstetrics and Gynecology in
the School of Veterinary Medicine in Hannover, according to the method
described by Hoffmann et al. (16) and slightly modified by Linneweber
(17).
For the use with dog serum, an anti-progesterone serum was produced by immunization of rabbits with 11-hydroxyprogesterone-hemisuccinate:BSA. Its crossreactivity was 100% with progesterone (further: less than 0.01% with dehydroepiandrosterone; 0.19% with androstendione; 1.26% with 17-hydroxyprogesterone; less than 0.01% with hydrocortisone, 17β-oestradiol, oestrone, and oestriol; 1.48% with 11-deoxycorticosterone; 0.07% with testosterone; 0.03% with pregnenolone; 0.79% with corticosterone; less than 0.01% with cortisone; 1.2% with 4-pregnen-20α-ol-3-one; 2.8% with 5α-pregnan-3.20-dion; 5β-pregnan-3.20-dion).

The antiserum dilution was 1:20,000, and the tracer was 1,2,6,7-(n)3H-progesterone. Incubation was performed with 10,000 cpm per tube. The sensitivity of the standard curve was 10 pg. The intraassay and interassay coefficients of variance for the dog serum progesterone determination were 3.3% (n=7) and 4.4% (n=7).

The samples were examined repeatedly if the difference between duplicate samples exceeded 12%.

RESULTS

Because the duration of the heats differed individually (dioestrus Day 0 was used as orientation point; Table 1) and because the values of Bitch 3 were not complete, there were some inconsistencies in the number of values (n) during the initial phase of heat.

A gradual increase from 120 to 220 ohm at the beginning of pro-oestrus to peak values of 700 to 980 ohm was observed. In Bitches 1, 2, 3, 4, 6, 7, and 8, maximum electrical resistance of 900 ohm or more lasted 4 to 11 d, building a more or less stable plateau. In Bitch 5, only one value of 960 ohm was found, while the measurements 4 d before or after this peak were between 600 and 650 ohm. The electrical resistance of the vaginal secretion of Bitch 9 never reached more than 700 ohm and varied from 100 to 200 ohm during the period of maximum values.

Within the phase of high electrical resistance, the variability decreased gradually and was least from Days -8 to -4. The mean values and low standard deviations on Days -1 and 0 make clear that in these final two days, a rapid decline of the electrical resistance of the vaginal secretion might be a typical and reproducible symptom for the end of oestrus.

The drop to 500 ohm or less occurred in four of the nine bitches (Bitches 3, 4, 7, 8) immediately from Days -2 to -1, in three (Bitches 3, 5, 6) from Days -3 to -2, and in two (Bitches 1 and 9) from Days -4 to -3. The fall from the high plateau to the low values of Day 0 (100 to 200 ohm) occurred within 2 to 4 d.

Holst and Phemister (11) found the period from Days -3 to -10 to be the phase of highest conception rates after a single mating during oestrus. In the present investigations, the period from Days -3 to -10 corresponded with the phase of high average resistance values (690 to 756.7 ohm).
Table 1. Electrical resistance of vaginal secretion during heats of nine bitches

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Rising electrical resistance values (Figure 2) were accompanied by an increasing visible viscosity of the vaginal mucus. During the period of highest resistance, the secretion appeared either slightly moist to sticky or sticky. Toward Day 0, the declining resistance corresponded with an increasing moistness in the vaginal cavity.

Figure 2. Mean profile and standard deviations (†) of the electrical resistance (ohm/-) and consistency (1-4/---) of the vaginal secretion during heat of Bitches 5, 6, 7, and 8.
The percentage of cornified cells (Figure 3), indicating the rising oestrogen production of the ripening follicles, increased gradually from Day -14 and reached more than 80% on Day -7. Maximum cornification (average 83.3 to 88.3%) was found over 4 d. Thereafter (from Days -4 to 0), the cornified cells disappeared within 4 d. In comparison, the rise of the electrical resistance occurred 2 to 3 d earlier, running nearly parallel with the percentage of cornified cells.

![Figure 3. Mean profile and standard deviations (⁻) of the electrical resistance (ohm/-) of the vaginal secretion and mean percentage of cornified cells (%/-) during heat of Bitches 1, 2, and 4.](image)

In these three bitches (1, 2, and 4), high resistance values were reached on Day -10 and ran nearly constant until Day -4. The last 4 d of this plateau (Days -7 to -4) coincided with the period of maximum cornification. The final drop of resistance toward Day 0 was closely related to the disappearance of cornified cells from the vaginal smear. The progesterone concentrations (Figure 4), determined in the peripheral blood serum of Bitches 3 and 9, exceeded 1.0 ng/ml on Days -9 (Bitch 3) and -10 (Bitch 9).
Figure 4. Electrical resistance patterns (ohm/—) of vaginal secretion and progesterone profiles (ng/ml/—) during heat of Bitches 3 and 9.

A concentration of 5.44 ng progesterone/ml serum, indicating the time of ovulation according to Concannon et al. (2), was found on Days -7 to -6 (Bitch 3) and on Days -7 to -8 (Bitch 9). In both animals, the time of ovulation lay within the phase of maximum electrical resistance.

DISCUSSION

The results obtained in our investigations are similar to observations made by Klötzer (10), who found that the increase of the electrical resistance of the vaginal secretion of the bitch during pro-oestrus was opposite to the conditions in other species (3). Nevertheless, Klötzer could not demonstrate a plateau of maximum resistance that lasted several days as shown here. Since Klötzer (10) used the first day of acceptance as the orientation point for evaluating his data, he could not show any reproducible results and in his opinion the measurements of the electrical resistance of the vaginal secretion did not have any diagnostic value.
Choosing the first day of dioestrus as the reference day (Day 0) made it possible to give some reproducible information about the changes in the electrical resistance of the vaginal secretions during pro-oestrus and oestrus. Since in the international literature, the definition for the stages of the oestrus cycle of the bitch are varied, the first day of dioestrus should be explained for better understanding. Holst and Phemister (11) found this day to be the most constant date in the otherwise variable oestrus cycle of the bitch. This day was more closely correlated than any other time with the conception rate, litter size, and length of gestation. Dioestrus was classified as the period during which the corpora lutea are fully functional. Its beginning was recognized by a typical shift in the vaginal cytology.

Comparison of the electrical resistance of the vaginal secretion and the conception rate after a single mating during oestrus (11) leads us to suggest that the breeding period is principally indicated by maximum resistance. For practical use, if the bitch accepts the male, there may be a good chance for conception when the vaginal mucus has a high electrical resistance.

The percentage of cornified cells was parallel to the electrical resistance, increasing with a delay of 2 to 3 d. Cell cornification is provoked by the rising amounts of oestrogens produced by the maturing follicles during pro-oestrus. Oestrogens may also cause changes in the electrical resistance of vaginal secretion. As Linde and Karlsson (1) showed, the oestradiol peak preceded the day of maximum cornification by at least 3 to 6 d. Thus the electrical resistance reacts to oestrogen earlier than does the vaginal epithelium. Maximum cell cornification served as an indicator for the breeding time, which coincided with the last 4 d of maximum resistance values.

The comparison between the clinical appearance and the electrical resistance of the vaginal mucus points to a direct dependence of the latter upon viscosity. High resistance values were found in the case of either a moist to sticky or a sticky secretion.

The bloodserum progesterone concentrations provided an indication of the ovulation time, which in the two bitches examined took place on Day -7 to -6 and -8 to -7. These results are similar to the findings of Holst and Phemister (18), who timed the ovulation on Day -6. According to their research work as well as that by Tsutsui and Shimizu (19), the period offering the best conditions for conception lasts from Days -4.5 to -1.5. This phase coincides with the last 3 d of high electrical resistance (Table 1).

Although the previous data are based on a small number of animals, we conclude that measuring the electrical resistance of vaginal secretion can give additional valuable information about the present stage of heat. If examinations are done regularly at 2-d intervals, it may be possible to narrow the best time for conception and thus create good conditions for successful breeding or artificial insemination.
REFERENCES


