

**THE USE OF PLASMA PROGESTERONE LEVELS, VAGINAL CYTOLOGY AND ELECTRICAL RESISTANCE OF VAGINAL SECRETION TO DETECT THE OESTROUS IN ANATOLIAN SHEPHERD BITCHES**

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**Anadolu Çoban Köpeklerinde östrüs tespiti için plazma progesteron seviyesi, vaginal sitoloji ve vaginal salgının elektriksel direnci ölçümünün kullanılması**

**ÖZET**

Bu çalışmada, köpeklerde plazma progesteron seviyesinin belirlenmesi, vaginal sitoloji ve vaginal salgının elektriksel direncinin ölçülerek östrüsün tespit edilmesi amaçlandı.

Materyal olarak 6 adet Anadolu Çoban Köpeği (Kangal ırkı) kullanıldı. Proöstrüs ve östrüs süresince progesteron seviyesi (ng/ml), süperfisyal hücre oranı (%), vaginal salgının elektriksel direnç değerleri (ohm) günlük olarak belirlendi. Östrüsler, vaginal sitoloji yöntemi kullanılarak belirlendi. Östrüs dönemindeki köpeklerde; plazma progesteron seviyesi (ng/ml), süperfisyal hücre oranı (%) ve vaginal salgının elektriksel direnç değerleri (ohm) sırasıyla  $2.2 \pm 0.34$  ng/ml,  $86.7 \pm 2.42$  ve  $868.0 \pm 299.0$  ohm olarak tespit edildi. Muhtemel ovulasyon günündeki süperfisyal hücre oranı (%) ve vaginal salgının elektriksel direnç değerleri (ohm) sırasıyla  $99.1 \pm 0.89$  ve  $1245.0 \pm 388.0$  ohm olarak belirlendi. Süperfisyal hücre oranı artışı ile vaginal salgının elektriksel direnç değeri artışı arasında önemli ( $p < 0.05$ ) pozitif korelasyon bulundu.

Vaginal salgının elektriksel direnç değeri değişimleri ile ovulasyon günü tespiti arasında bir ilişki gözlenmedi. Ancak, vaginal salgının elektriksel direnç değerinin ölçülerek ideal tohumlama zamanının belirlenebilmesi için günlük olarak düzenli bir şekilde direnç değerlerinin takip edilmesi ve tohumlamanın elektriksel direnç pikininin (1083-1723 ohm) düşmeye başladığı ilk günlerde yapılması önerilebilir.

**ANAHTAR KELİMELER:** Östrüs tespiti, vaginal direnç ve sitoloji, Anadolu Çoban Köpeği

**SUMMARY**

The aim of this study was to detect the estrous by measurement of plasma progesterone level, electrical resistance of the vaginal secretion along with vaginal cytology in Anatolian shepherd bitches.

A total of 6 Anatolian Shepherd bitches were used as materials. Daily blood progesterone level, superficial cell rate and vaginal resistance values of bitches were determined in pro-estrous and estrous. The estrous of bitches were determined using vaginal cytology. The mean values of plasma progesterone level, superficial cell percentage and the vaginal resistance of the bitches were  $2.2 \pm 0.34$  ng/ml,  $86.7 \pm 2.42$  % and  $868.0 \pm 299.0$  ohm in estrous, respectively. The mean superficial cell percentage and vaginal resistance values were  $99.1 \pm 0.89$  % and  $1245.0 \pm 388.0$  ohm in probable ovulation day, respectively. There was a significant correlation ( $P < 0.05$ ) between the increase of the superficial cells and the electrical resistance of the vaginal secretion.

There was no relationship between the changes of vaginal resistance and probable ovulation day. However, it was considered that measurement of the electrical resistance values daily would be of great importance in order to determine the ideal insemination time when the peak electrical resistance begins to decline to between the 1083-1723 ohms.

**KEY WORDS:** Estrous detection, vaginal resistance and cytology, Anatolian Shepherd Bitches

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## INTRODUCTION

Determination of estrous and ideal insemination time is a prerequisite to obtain optimum fertility. The fertilizing capabilities of spermatozoa are less than 24 hours in the uterus when frozen sperm is used (Concannon and Battista 1988). Therefore, precise determination of estrous and mating times is crucial when artificial insemination is performed with frozen semen.

While plasma progesterone level is 0.5-1.0 ng/ml during the pro-estrous, it increases to 15-60 ng/ml level during the diestrus phase of the estrous cycle (Feldman and Nelson 1996). Bouchard *et al.* (1991) and Wright (1991) found that ovulation occurs 48 hours after the peak level of LH in dog. Mean plasma progesterone concentration was 5.4 ng/ml at the time of ovulation reported by several researchers (Concannon *et al.* 1977, Wright 1991).

The vaginal cytology could be used confidently to find out estrous in the dog (Günzel and Koivisto 1984). The cell types from the vaginal cytology reflect the effect of estrogen on vaginal epithelia, and observation of the shape and amount of cells would be valuable in the diagnoses of the pro-estrous, estrous and dioestrus phases (Wright and Parry 1989).

Measurement of the electrical resistance of the vaginal secretion could be used to detect the phase of the sexual cycle in dogs (Günzel *et al.* 1986). Determination of the amount of cervico-vaginal mucus and alterations in ion concentration would be helpful in the diagnosis of estrous (Morrow 1980). Klötzer (1974) reported that there was an increase in the electrical resistance of the vaginal mucus between 495 and 1216 ohms at the last day of the pro-estrous in dogs. Leidl and Stolla (1976) reported that bitches show a different level of vaginal resistance than that of sheep, pig and cattle in the estrous phase. The electrical resistance of 70 ohm in the anoestrus increases to 226 ohm in the pro-estrous and 373 ohm in the estrous.

There are numerous studies concerning the measurement of the vaginal secretion resistance in foxes (Boue *et al.* 2000), sows (Rezac *et al.* 2003), buffaloes (Gupta and Prohit 2001) and sheep (Bartlewski 1999), but to our knowledge there are a few studies reported in bitches.

This study was performed through measurement of plasma progesterone level, electrical resistance values of the vaginal secretions along with vaginal cytology to determine the estrous in Anatolian Shepherd bitches.

## MATERIAL and METHODS

A total of 6 Anatolian Shepherd bitches, aging 3-6 years old, weighing 30-42 kg, delivered at least once were used as materials.

**Estrous detection:** The bitches were separated from the male dogs at the end of the anoestrus season according to the last delivery dates, and observed daily. Vaginal smears, measurement of the electrical resistance of vaginal secretions and blood samples were obtained from 5 bitches, starting from the observation of pro-estrous secretion (serosanguinose), edematous swelling of the perineum and vulvae until the beginning of dioestrus. Vaginal resistance, vaginal cytology and plasma samples were evaluated daily at 15 pm. The beginning of the estrous of the bitches were determined through the findings of 80 % superficial cells as reported by Feldman and Nelson (1996), and the beginning of the diestrus was determined when the superficial cell count was decreased to 20 % as reported by Holst and Phemister (1974).

**Vaginal cytology:** Vaginal smears were performed daily via cotton swab method Feldman and Nelson (1996) beginning from the first day of the pro-estrous bleeding until the beginning of the diestrus. The parabasals, intermediates, intermediate superficiales, and superficial (pycnotic nucleus and anuclear keratinized) cells were counted in the Giemsa stained smears in order to determine superficial cell percentages. Totally, 100 cells were counted and the superficial cell rate were determined each sample.

**Blood sample collection:** Blood samples were collected into anticoagulated tubes from vena sephana parva at the beginning of the pro-estrous until the beginning of the diestrus daily. Plasma samples were stored at -20 °C until assayed. Plasma progesterone levels were determined by EIA technique modified by Haliloğlu (1998).

**Vaginal secretion resistance measurement:** Electrical resistance of the vaginal secretion was measured daily with electroconductivimeter (Estron, Animaltec-Inc., USA) starting from the beginning of the pro-estrus until the beginning of the dioestrus.

**Statistics:** The correlation between the superficial cell rate and the electrical resistance change in the pro-estrous and estrous was determined. Statview Statistics program was used for correlation analysis.

## RESULTS

The values of the vaginal resistance, the superficial cell percentage and plasma progesterone levels during the pro-estrous and estrous of the 6 bitches were summarized in Table 1a (between day 1 and 10), Table 1b (between day 11 and 20) and Table 1c (average values between day 1 and 20). The correlation levels between the superficial cell rates and the vaginal resistance values during the pro-estrous and estrous were summarized in Table 2. The average values of the

Table 1a. The electrical resistance values, progesterone levels and superficial cells between the pro-estrous and dioestrous in bitches (between day 1 and 10)

| Day intervals at the beginning of pro-oestrus and oestrus |      |   |      |      |      |      |       |       |       |      |      |
|---|------|---|------|------|------|------|-------|-------|-------|------|------|
| Bitch no  | Days | 1   | 2    | 3    | 4    | 5    | 6     | 7     | 8     | 9    | 10   |
| 1   | R    | 143   | 155  | 175  | 140  | 345  | 558   | 1034* | 1046  | 1528 | 1723 |
|   | SR   | 3   | 10.5 | 15.5 | 25.5 | 56   | 62    | 86.5* | 96    | 100  | 100  |
|   | P    | 0.09  | 0.09 | 0.11 | 0.15 | 0.18 | 0.95  | 1.11  | 4.91  | 5.71 | 6.93 |
| 2   | VR   | 156   | 187  | 260  | 430  | 615  | 940   | 1045  | 1110* | 1570 | 1410 |
|   | SR   | 4.5   | 9    | 10.5 | 22   | 46   | 53.5  | 74.5  | 88*   | 95.5 | 98   |
|   | P    | 0.05  | 0.06 | 0.09 | 0.12 | 0.14 | 0.87  | 1     | 2.08  | 3    | 5.96 |
| 3   | VR   | 130   | 123  | 145  | 218  | 437  | 578   | 654*  | 723   | 705  | 740  |
|   | SR   | 5.5   | 9.5  | 14   | 36   | 62   | 77    | 91*   | 96.5  | 98   | 98.5 |
|   | P    | 0.09  | 0.1  | 0.13 | 0.15 | 0.16 | 0.96  | 2.21  | 3.34  | 5.21 | 6.24 |
| 4   | VR   | 185   | 195  | 230  | 285  | 323  | 310   | 360*  | 400   | 640  | 715  |
|   | SR   | 4.5   | 10.5 | 24   | 29   | 52   | 75    | 85.5* | 94    | 100  | 99.5 |
|   | P    | Blood samples were not collected in bitch 4 |      |      |      |      |       |       |       |      |      |
| 5   | VR   | 160   | 163  | 280  | 490  | 650  | 1083* | 1100  | 1270  | 1025 | 790  |
|   | SR   | 4   | 10   | 16.5 | 30   | 66   | 84.5* | 97.5  | 99    | 100  | 99.5 |
|   | P    | 0.04  | 0.07 | 0.5  | 0.8  | 0.98 | 1.75  | 2.43  | 5.6   | 6.1  | 7.81 |
| 6   | VR   | 135   | 130  | 253  | 390  | 403  | 467   | 760   | 967*  | 1120 | 1245 |
|   | SR   | 5.5   | 9.5  | 12.5 | 23   | 36   | 51.5  | 66    | 85*   | 94   | 98.5 |
|   | P    | 0.03  | 0.06 | 0.1  | 0.3  | 0.4  | 0.64  | 0.78  | 2.24  | 3.56 | 4.02 |

Table 1b. The electrical resistance values, progesterone level and superficial cells between the pro-estrous and dioestrous in bitches (between day 11 and 20)

| Day intervals at the beginning of pro-oestrus and oestrus |      |   |      |       |       |      |       |       |       |       |       |
|---|------|---|------|-------|-------|------|-------|-------|-------|-------|-------|
| Bitch no  | Days | 11  | 12   | 13    | 14    | 15   | 16    | 17    | 18    | 19    | 20    |
| 1   | R    | 1534  | 1510 | 1553  | 1544  | 1180 | 1083  | 755   | 557   | 303*  |       |
|   | SR   | 98.5  | 98.5 | 100   | 100   | 99   | 99.5  | 100   | 40.5  | 21.5* |       |
|   | P    | 8.43  | 9.71 | 9.9   | 12.98 | 13.8 | 15.51 | 15.67 | 19.82 | 20.1  |       |
| 2   | VR   | 1680  | 1240 | 1430  | 840   | 810  | 435   | 210*  |       |       |       |
|   | SR   | 98.5  | 100  | 97    | 98    | 90   | 85    | 15.5* |       |       |       |
|   | P    | 5.89  | 6.78 | 9.63  | 12.4  | 14   | 16.3  | 17.35 |       |       |       |
| 3   | VR   | 1083  | 1045 | 1060  | 680   | 268  | 236   | 209*  |       |       |       |
|   | SR   | 98  | 100  | 98.5  | 97.5  | 93   | 81.5  | 24.5* |       |       |       |
|   | P    | 6.81  | 7.4  | 7.84  | 7.9   | 14.3 | 19.98 | 20.3  |       |       |       |
| 4   | VR   | 985   | 1183 | 1563  | 990   | 772  | 549   | 488   | 273*  |       |       |
|   | SR   | 99.5  | 98.5 | 99.5  | 100   | 100  | 99    | 78.5  | 23*   |       |       |
|   | P    | Blood samples were not collected in bitch 4 |      |       |       |      |       |       |       |       |       |
| 5   | VR   | 890   | 640  | 421   | 577   | 303  | 301   | 299   | 191*  |       |       |
|   | SR   | 100   | 100  | 97    | 97.5  | 100  | 79.5  | 80    | 21*   |       |       |
|   | P    | 9.3   | 10.3 | 10.35 | 14.2  | 16.4 | 19.37 | 19.45 | 24.06 |       |       |
| 6   | VR   | 1040  | 840  | 883   | 970   | 549  | 438   | 285   | 246   | 283   | 180*  |
|   | SR   | 100   | 99.5 | 100   | 99    | 100  | 100   | 96.5  | 95    | 93    | 28.5* |
|   | P    | 5.3   | 7.84 | 8.1   | 13.7  | 14.2 | 17    | 17.6  | 17.8  | 20.4  | 23.3  |

\*: The days found as to determine the beginning of oestrous and dioestrous by vaginal cytology. VR (ohm): Vaginal resistance, SR (%): Superficial cell rate, P (ng/ml): progesterone, MVR (ohm): Mean vaginal resistance MSR (%): Mean superficial cell rate, MP (ng/ml): Mean progesterone

Table 3. The average values of the electrical resistances, progesterone level and superficial cell rates on the estrous, possible ovulation and diestrous day

|                        | MVR        | MSR       | MP        |
|------------------------|------------|-----------|-----------|
| Estrous                | 868±299    | 86.7±2.42 | 1.88±0.47 |
| Possible Ovulation Day | 988±605    | 99.3±0.88 | 5.5±0.28  |
| Diestrous              | 227.7±49.0 | 22.3±4.30 | 21.0±2.71 |

MVR (ohm): Mean vaginal resistance MSR (%): Mean superficial cell rate, MP (ng/ml): Mean progesterone

Table 1c. The average values of the electrical resistances, progesterone level and superficial cell rates between the pro-estrous and dioestrous in bitches (days 1-20)

| Days       | 1                     | 2                     | 3                     | 4                     | 5                      | 6                     | 7                      | 8                      | 9                     | 10                   |
|------------|-----------------------|-----------------------|-----------------------|-----------------------|------------------------|-----------------------|------------------------|------------------------|-----------------------|----------------------|
| MVR (ohm)  | n:6<br>151.5<br>±20.1 | n:6<br>158.8<br>±29.2 | n:6<br>223.8<br>±52.8 | n:6<br>325.5<br>±134  | n:6<br>462.2<br>±138.5 | n:6<br>656<br>±295    | n:6<br>826<br>±289     | n:6<br>919<br>±312     | n:6<br>1098<br>±394   | n:6<br>1104<br>±419  |
| MSR (%)    | n:6<br>4.5<br>±0.9    | n:6<br>9.8<br>±0.6    | n:6<br>15.5<br>±4.7   | n:6<br>27.6<br>±5.2   | n:6<br>53.0<br>±10.9   | n:6<br>67.2<br>±13.5  | n:6<br>83.3<br>±11.7   | n:6<br>93.1<br>±5.4    | n:6<br>97.9<br>±2.6   | n:6<br>99.0<br>±0.8  |
| MP (ng/ml) | n:5<br>0.06<br>±0.03  | n:5<br>0.08<br>±0.02  | n:5<br>0.19<br>±0.18  | n:5<br>0.30<br>±0.29  | n:5<br>0.37<br>±0.36   | n:5<br>1.03<br>±0.42  | n:5<br>1.51<br>±0.76   | n:5<br>3.63<br>±1.58   | n:5<br>4.72<br>±1.36  | n:5<br>6.19<br>±1.41 |
| Days       | 11                    | 12                    | 13                    | 14                    | 15                     | 16                    | 17                     | 18                     | 19                    | 20                   |
| MVR (ohm)  | n:6<br>1202<br>±324   | n:6<br>1026<br>±300   | n:6<br>1152<br>±542   | n:6<br>933<br>±340    | n:6<br>647<br>±346     | n:6<br>507<br>±303    | n:6<br>374.3<br>±212.5 | n:4<br>316.7<br>±163.8 | n:2<br>293<br>±14.1   | n:1<br>180<br>±0.0   |
| MSR (%)    | n:6<br>99.1<br>±0.9   | n:6<br>99.4<br>±0.9   | n:6<br>98.7<br>±1.4   | n:6<br>98.6<br>±1.2   | n:6<br>97.0<br>±4.4    | n:6<br>90.7<br>±9.7   | n:6<br>66.0<br>±36.4   | n:4<br>44.9<br>±34.5   | n:2<br>57.3<br>±50.6  | n:1<br>28.5<br>±0.0  |
| MP (ng/ml) | n:5<br>7.15<br>±1.69  | n:5<br>8.41<br>±1.52  | n:5<br>9.16<br>±1.12  | n:5<br>12.24<br>±2.52 | n:5<br>14.54<br>±1.06  | n:5<br>17.63<br>±1.95 | n:5<br>18.07<br>±1.83  | n:3<br>20.56<br>±3.19  | n:2<br>20.25<br>±0.21 | n:1<br>23.30<br>±0.0 |

\*: The days found as to determine the beginning of oestrous and dioestrous by vaginal cytology. VR (ohm): Vaginal resistance, SR (%): Superficial cell rate, P (ng/ml): progesterone, MVR (ohm): Mean vaginal resistance MSR (%): Mean superficial cell rate, MP (ng/ml): Mean progesterone

Table 2. The correlation levels between the superficial cells and the vaginal resistances during the pro-oestrus and estrous

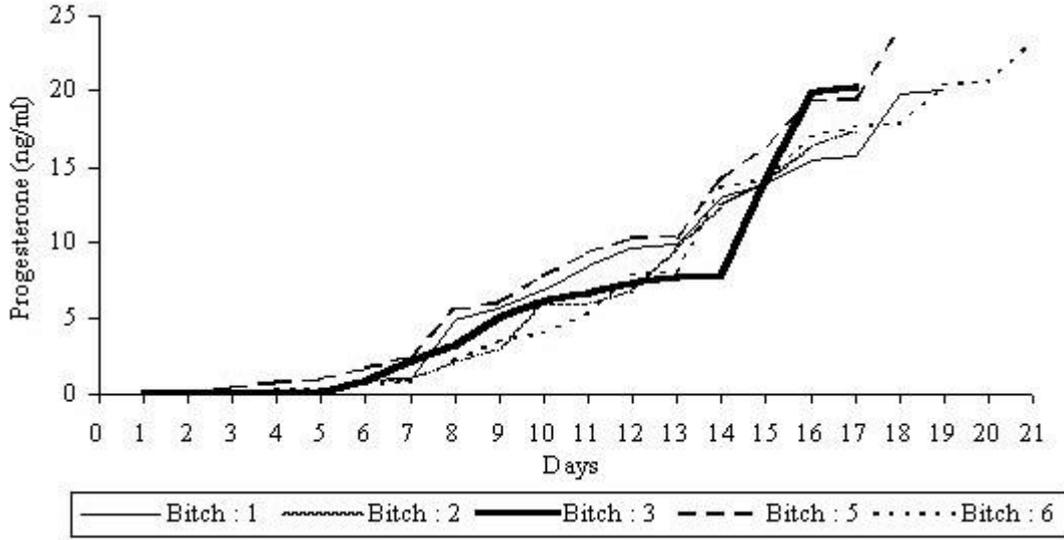
| Bitches | Pro-oestrus ( SC-VR) | Estrous ( SC-VR)  |
|---------|----------------------|-------------------|
| 1       | r = 0.91 (P<0.01)    | r = 0.79 (P<0.01) |
| 2       | r = 0.98 (P<0.01)    | r = 0.68 (P<0.05) |
| 3       | r = 0.98 (P<0.01)    | r = 0.63 (P<0.05) |
| 4       | r = 0.89 (P<0.05)    | r = 0.52 (P>0.05) |
| 5       | r = 0.95 (P<0.01)    | r = 0.50 (P>0.05) |
| 6       | r = 0.95 (P<0.01)    | r = 0.37 (P>0.05) |

Table 4. Correlation analysis among the average values of the electrical resistances, progesterone level and superficial cell rates on the estrous, possible ovulation and dioestrous day.

|       | MOSR              | MOVR          | MOP           | MOVSR         | MOVVR          | MOVP           | MDSR          | MDVR          |
|-------|-------------------|---------------|---------------|---------------|----------------|----------------|---------------|---------------|
| MOVR  | r -0.18<br>p 0.72 |               |               |               |                |                |               |               |
| MOP   | r 0.31<br>p 0.62  | -0.41<br>0.49 |               |               |                |                |               |               |
| MOVSR | r -0.76<br>p 0.08 | -0.11<br>0.83 | -0.49<br>0.39 |               |                |                |               |               |
| MOVVR | r 0.12<br>p 0.82  | 0.52<br>0.29  | -0.59<br>0.29 | -0.28<br>0.59 |                |                |               |               |
| MOVP  | r -0.24<br>p 0.69 | 0.82<br>0.09  | -0.49<br>0.39 | 0.08<br>0.89  | 0.89<br>0.04*  |                |               |               |
| MDSR  | r -0.15<br>p 0.78 | -0.35<br>0.49 | 0.26<br>0.67  | 0.38<br>0.45  | -0.91<br>0.01* | -0.88<br>0.04* |               |               |
| MDVR  | r -0.02<br>p 0.97 | -0.32<br>0.54 | -0.87<br>0.06 | 0.47<br>0.35  | 0.34<br>0.52   | 0.39<br>0.51   | -0.19<br>0.72 |               |
| MDP   | r -0.64<br>p 0.24 | -0.01<br>0.99 | 0.03<br>0.96  | 0.40<br>0.50  | -0.57<br>0.32  | -0.51<br>0.38  | 0.66<br>0.22  | -0.40<br>0.51 |

MOVR : Mean vaginal resistance in the estrous phase, MOP : Mean progesterone in the estrous phase, MOSR : Mean superficial cell rate in the estrous phase, MOVVR : Mean vaginal resistance in the possible ovulation day, MOVP : Mean progesterone in the possible ovulation day, MOVSR : Mean superficial cell rate in the possible ovulation day, MDVR : Mean vaginal resistance in the dioestrous phase, MDSR : Mean superficial cell rate in the dioestrous phase, MDP : Mean progesterone in the dioestrous phase

Figure 1. Plasma progesterone levels between the beginning of the pro-oestrous and dioestrous in five bitches



Figures 2, 3, 4, 5, 6 and 7. The changes of the electrical resistances and superficial cells between the pro-oestrous and dioestrous in bitches (\*:  $p < 0.05$ ).

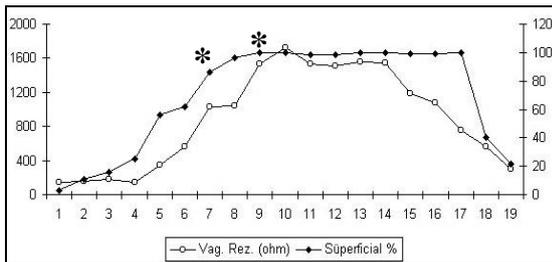


Figure 2, Bitch 1

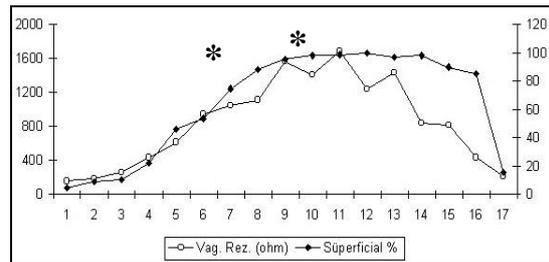


Figure 3, Bitch 2

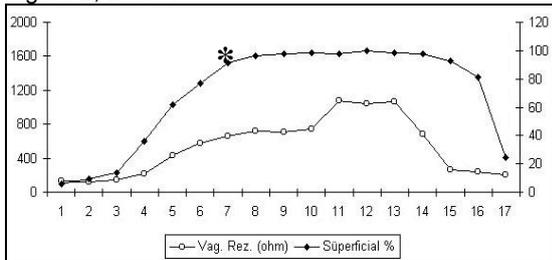


Figure 4, Bitch 3

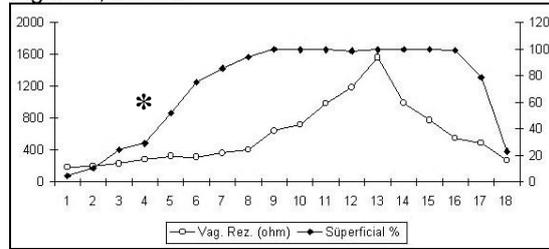


Figure 5, Bitch 4

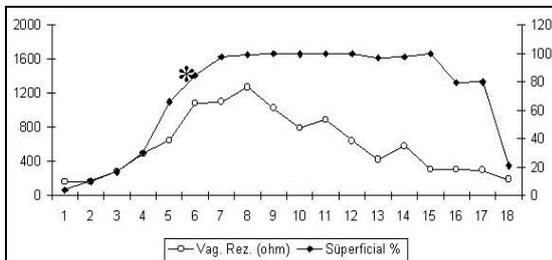


Figure 6, Bitch 5

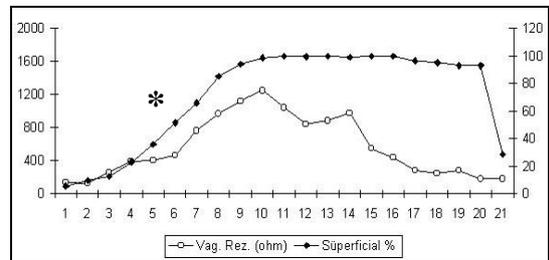


Figure 7, Bitch 6

electrical resistances, progesterone level and superficial cell rates on the estrous, possible ovulation and diestrous day were cited in Table 3.

The correlation levels among the average values of the electrical resistances, progesterone level and superficial cell rates on the estrous, possible ovulation and diestrous day were summarized in Table 4. Plasma progesterone values of the 5 bitches were summarized in Figure 1. The correlation between the superficial cell rates and the vaginal resistance values during the pro-estrous and estrous for each bitch was illustrated in Figures 2, 3, 4, 5, 6 and 7. The parameter of each bitch is as follows:

**Bitch 1:** Plasma progesterone level, superficial cell percentage and vaginal resistance values were 1.11 ng/ml, 86.5%, 1034 ohm in the estrous day (7<sup>th</sup> day) and 5.71 ng/ml, 100%, 1528 ohms in the possible ovulation day (9<sup>th</sup> day) (Table 1a). There was a significant correlation between the superficial cell percentage and the vaginal resistance values during the pro-estrous ( $P<0.01$ , 7<sup>th</sup> day) and estrous ( $P<0.01$ , 9<sup>th</sup> day) (Table 2 and Figure 2).

**Bitch 2:** Plasma progesterone level, superficial cell percentage and vaginal resistance values were 2.08 ng/ml, 88 %, 1110 ohm in the estrous day (8<sup>th</sup> day) and 5.89 ng/ml, 98.5 %, 1680 ohm in the possible ovulation day (11<sup>th</sup> day) (Table 1b). There was a significant correlation between the superficial cell percentage and the vaginal resistance during the pro-estrous ( $P<0.01$ , 8<sup>th</sup> day) and estrous ( $P<0.05$ , 11<sup>th</sup> day) (Table 2 and Figure 3).

**Bitch 3:** Plasma progesterone level, superficial cell percentage and vaginal resistance values were found as 2.21 ng/ml, 91 % and 654 ohm on the 7<sup>th</sup> day and 5.21 ng/ml, 98 % and 705 ohm on the 9<sup>th</sup> day (Table 1a).

The correlation between the superficial cell percentage and the vaginal resistance values were found to be significant ( $P<0.01$ , 7<sup>th</sup> day) during the pro-estrous but it was insignificant ( $P>0.05$ , 9<sup>th</sup> day) during the estrous (Table 2 and Figure 4).

**Bitch 4:** This bitch had a low mean resistance value of 360 ohm ranging from 1563 to 273 ohms between the middle and the end of the oestrus, respectively, which was different from those of other bitches (Table 1a, Table 1b, Figure 5). Superficial cell percentage and vaginal resistance values were found as 85.5 % and 360 ohms, respectively on the 7<sup>th</sup> day. There was a significant positive correlation ( $P<0.05$ ) between the superficial cell percentage and the vaginal resistance values in the pro-estrous. It was insignificant ( $P>0.05$ ) during the estrous (Table 2 and Figure 5).

**Bitch 5:** Vaginal cytologic method revealed that plasma progesterone level was 1.75 ng/ml on the 6<sup>th</sup> day of the estrous. Vaginal resistance and superficial cell rates were 1083 ohms and 84.5 %, respectively

on the sixth day. Progesterone level was 5.60 ng/ml on the 9<sup>th</sup> day of the possible ovulation day. Electrical resistance value was 1270 ohm and superficial cell percentage was 99 % on the 9<sup>th</sup> day of the possible ovulation day (Table 1a).

There was a positive correlation ( $P<0.01$ ) between the vaginal cytology and vaginal resistance during the pro-estrous, but no significant correlation ( $P>0.05$ ) was found in the estrous (Table 2 and Figure 6).

**Bitch 6:** Plasma progesterone level, superficial cell rate and vaginal resistance value were found as 2.24 ng/ml, 85 % and 967 ohms, respectively at the beginning of the estrous. Plasma progesterone level, electrical resistance and superficial cell percentages were 5.30 ng/ml, 1040 ohm and 100 %, respectively on the possible ovulation (11<sup>th</sup> day) (Table 1b).

Statistical correlation between the vaginal cytology and the vaginal resistance was significant ( $P<0.01$ ) in the pro-estrous, and it was not significant ( $P>0.05$ ) in the estrous (Table 2 and Figure 7).

## DISCUSSION

Measurement of the plasma progesterone level is one of the best method for predicting the estrous in bitches. (Günzel and Koivisto 1984, Morton and Bruce 1989). In our study, all the bitches had 1.75-2.70 ng/ml of progesterone levels at the beginning of the estrous which was similar to those reported by others (Guerin *et al.* 1997, Jeffcoate 1997).

While the mean plasma progesterone level was determined as 5.4 ng/ml at the time of ovulation by Concannon *et al.* (1977) and Wright (1991), it was reported by Bouchard *et al.* (1991), to be 4.9 ng/ml. Superficial cell rate was found to be  $99.1\pm 0.89$  (98-100 %) when the progesterone level was between 5.21-5.89 ng/ml in our study (Table 3) which would be considered as the possible ovulation day. This is in agreement with the findings of over % 90 increase of superficial or cornified cells at the ovulation stage during the early estrous (Bouchard *et al.* 1991, Wright 1991). Additionally, Negative significant correlation ( $p<0.05$ ) were determined between the mean progesterone level and the mean vaginal resistance on the possible ovulation day (Table 4).

The bitches tested by a male dog, had 84.5 – 88.0% of superficial cells at the beginning of the estrous. Superficial cells decreased to 16.5 - 28.5 % in the same bitches at the last day of the estrous. Holst and Phemister (1974) found that superficial cells decreased to 20.0 % at the first day of dioestrus. On the other hand, Bouchard *et al.* (1991) found that the superficial cells were more or less than 20%. In our study, superficial cells were 16.5% at the last day of the estrous in the second bitch, and that decreased to below 20.0 % at the beginning of the diestrous (Table 3).

In the presented study, as seen in figures 2, 3, 4, 5, 6 and 7, while all the bitches had a regular increase for the electrical resistance level during the

pro-estrous, there was a decrease on the electrical resistance in bitches 5 and 6, and there was an irregular and continued increases in bitches 1, 2, 3 and 4 in the estrous. The superficial cells between 97.5 and 100 % had a plateau pattern during the estrous in all bitches. With regard to the electrical resistance, this type of plateau was observed only in the bitches 1 and 3 during the estrous (Fig 2 and 4).

There were a significant differences on resistance values ranging from 705.0 to 1680.0 ohms ( $1245.0 \pm 388.0$  ohms) among the bitches when possible ovulation day was predicted according to the progesterone level of 5.4 ng/ml reported by Wright (1991). The bitches 1, 2, 5 and 6 had estrous when the resistance values reached to 1000 ohms, compared to the bitches 3 and 4, which had estrous when the resistance values were between 360 and 654 ohms. An irregular decrease was observed on the resistance values after 48-72 hours on the possible ovulation day (Figures 2, 3, 6 and 7), but the bitches 3 and 4 had gone to estrous with the low resistance values between 360.0 and 654.0 ohms. Electrical resistance increased at the end of the possible ovulation day and estrous (Table 1a, Table 1b, Figures 4 and 5). The decreased level of the electrical resistance was close to those of the pro-estrous at the end of the estrous.

The values of the electrical resistance of the bitches 1, 2, 5 and 6 found in our study in the pro-estrous and estrous were similar to the findings of Günzel *et al.* (1986). On the other hand, irregular changes of the electrical resistance of the bitches 3 and 4 were similar to Klötzer's (1974) findings during the estrous. Günzel *et al.* (1986) stated that ideal insemination time was the last 3 day of the highest level of the electrical resistance between 700 and 980 ohms. This was not suitable for ideal insemination of some bitches in the second half of the estrous, because possible survival duration of mature oocytes is 2-3 days in the uterus when the insemination was performed 6 days later after LH peak (Concannon *et al.* 1989).

Altered vaginal resistance values were reported in the pro-estrous and estrous by Klötzer (1974), Leidl and Stolla (1976), which is similar to our results. The alterations in the electrical resistance result from the differences of the vaginal secretion flow (Günzel *et al.* 1986), presence of blood in the vagina (Leidl and Stolla 1976), differences of estradiol 17- $\beta$  hormone level (Aboul *et al.* 1983), and both the effect of hormone concentration and chemical compound of the mucus (Aboul *et al.* 1983). However, presence of erythrocytes was reported in vaginal cytology in the early estrous (Günzel *et al.* 1986, Feldman and Nelson 1996). In this study, the amount of blood cells of mucus, the differences of level estrogen and progesterone hormones and the differences of electrolyte compound of mucus may affect the electrical resistance of vaginal secretion. In addition to these, alterations in the cell types of the vaginal wall with the resultant effect of ion and fluid composition in the cytoplasm of the superficial cells

affect the electrical resistance during the pro-estrous and estrous.

In conclusion, plasma progesterone concentration or superficial cell rate could be used safely in the estrous detection. Although no relationship was found between the changes of electrical vaginal resistance and probable ovulation day, the measurements of electrical resistance daily would be beneficial to determine the ideal insemination time when the peak electrical resistance begins to decline to between 1083-1723 ohms.

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