

## OPTIMIZATION OF REPRODUCTIVE BIOTECHNOLOGIES IN BITCHES BY IMPROVING THE PROTOCOLS REGARDING ESTABLISHMENT OF THE OVULATION TIMING

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### Abstract

*Determining the optimal time of mating in bitches has become a routine in veterinary practice. Endogenous LH has an important role in ovulation induction and the beginnings of fertile period in bitches but its dosing is difficult and veterinary clinics don't apply it widely. The indirect methods of establishing fertile period are: examination of vaginal smear, determination of progesteron blood levels, vaginoscopy, or the simple examination of clinical changes of external genital segment and characteristics of vulvar discharge and receptivity. The purpose of this study is to determine the connection between estrus clinical signs and the examination methods most frequently used to assess the exact time of ovulation (vaginal smear, vaginoscopy, serum progesterone dosing).*

**Key words:** *Ovulation timing, Exfoliative citology, Vaginoscopy, P4 assay*

### INTRODUCTION

Establishing the exact ovulation moment as accurately as possible is the most important factor when an artificial insemination is made, especially when using frozen/thawed semen since sperm cells survive short periods of time in female genital tract after insemination.

Knowing exactly the physiology of sexual cycle and its variations in bitches is essential. Practically, the fertile period in bitches in which oocytes are fully mature and ready to be fertilised it lasts only 2-3 days during oestrus (Phemister, Holst &

Spano, 1973; Verstegen, Silva, & Onclin, 2001).

The variable length of proestrus and estrus in bitches can cause errors regarding ovulation onset if the ovulation moment and beginning of fertile period is based only on appearance of vulvar discharge and its modifications and on behavior changes of the bitch.

Use of vaginal cytology, hormone dosage or vaginal endoscopy are very important methods that allow us to estimate as accurately as possible the appearance moment of preovulatory LH peak and beginning of fertile period.

## MATERIALS AND METHODS

There were evaluated 46 females from different breeds presented in the Clinic of Obstetrics and Gynecology - Faculty of Veterinary Medicine from Bucharest in order to determine the optimal time of mating. All females were evaluated in order to determine the best mating time by using vaginal smear, vaginoscopy and serum progesterone assays and receptivity. Female receptivity was determined by history the owners without any further investigation.

Vaginal smears were made for all females starting the third day since vaginal discharge occurred. The frequency of making smears was once every 2-3 days during the monitoring period. Citovaginal smears were made using sterile cotton-tipped swabs to take samples on the surface of vaginal epithelium. Samples obtained were applied on a glass microscope slide and after drying they were May Grunwald Giemsa stained (MGG). Microscopic examination of dry and stained smears was made using 20X and 40X objective lens to evaluate the percentage of anucleated keratinized superficial cells.

Vaginoscopy was performed using a rigid endoscope the moment samples were taken for citovaginal smears. The endoscopic examination's purpose is to identify the changes of vaginal mucosa appearance and its folds produced under the influence of ovarian steroids during follicular phase until ovulation.

The dosage of serum progesterone concentration was made in Synevovet laboratory by chemiluminescence. Blood samples were sent and processed in the same day, the results being expressed in ng/ml. Testing of serum progesterone

concentration was performed when anucleate superficial epithelial cells appeared in vaginal smear in a rate over 70%.

## RESULTS AND DISCUSSION

By history, female receptivity was observed since the sixth day after the thrush infection until the 14th day.

In proestrus, citovaginal smear was dominated from the beginning by the presence of parabasal cells of small sizes and round shape and intermediate cells of various sizes. At the beginning of proestrus the smear shown small nucleated intermediate cells, round or oval shaped, with slightly irregular contour. In middle and late proestrus, citovaginal smear appearance tends to modify. The number of parabasal cells is significantly reduced and intermediate big cells with squamous aspect and irregular contour are present almost exclusively. Some superficial large cells with irregular contour and pyknotic nuclei appear too. During entire estrus period a large number of erythrocytes were present probably as a result of progressive estrogenic stimulation that leads to erythrodiapedesis phenomenon on endometrium and vaginal mucosa in a smaller rate ( Johnston, Root Kustritz, & Olson, 2001 ). Increased number of neutrophils is present in early and middle proestrus probably due to the presence of an increasingly number of desquamated cellular debris and bacteria. In late proestrus neutrophils disappear from citovaginal smear probably due to an intense mitotic activity of estrogens over vaginal mucosa that leads to its thickening so that neutrophils can't cross anymore the big number of cell layers, remaining

trapped at lower levels of thickened vaginal epithelium ( Concannon, 2010 ).

In estrus, for all females the general aspect of smear is dominated almost exclusively by the presence of big superficial cells with irregular contour, anucleated and keratinized. Neutrophils are absent during estrus.

In some cases erythrocytes can be seen in the citovaginal smear at the beginning of estrus, probably because of estrogen increased concentrations. But their number decreases and they even disappear until the end of estrus (Concannon, 2010).

Vaginoscopy was performed for all females the moment samples for citovaginal smear were taken allowing an accurate assessment of changes in vaginal epithelium induced by estrogen/progesterone ratio. In early proestrus the increased level of plasma estrogen cause vaginal mucosa edema that first leads to primary vaginal folds formation that are parallel arranged to vaginal lumen. In middle and late proestrus, the increasingly higher level of plasmatic estrogen cause the accentuation of vaginal mucosal edema

leading to appearance of secondary vaginal folds that are arranged in transverse manner over the primary vaginal folds (Rehm, Stanislaus, & Williams, 2007; Jeffcoate & Lindsay, 1989). In estrus, due to early luteinizing of ovarian follicles, vaginal edema starts to reduce also leading to reduction of primary and secondary vaginal folds edema, offering vaginal mucosa a wrinkled appearance (Lindsay & Concannon, 1986). The change is progressive starting from the end of late proestrus and beginning of estrus. Maximum intensity of these specific changes can be observed between 3 and 4 days after ovulation.

During late proestrus due to preovulatory luteinizing, serum progesterone concentration started increasing progressively maintaining itself to a level between 1-3 ng/ml for a period that varies between 3 up to 14 days.

In the moment of ovulation, medium serum progesterone level was 4.61 ng/ml.

**Table 1. Results obtained after applying monitoring methods of sexual cycle in the bitch**

Case no.	Ovulation day after:			
	Citovaginal smear	Vaginoscopy	P4 dosage(ng/ml)	Receptivity
1	9	11	3,9	6
2	7	10	3,6	12
3	11	12	4,2	9
4	10	13	3,8	7
5	8	11	4,1	11
6	10	12	4,4	12
7	11	12	3,7	8
8	7	10	4,8	7
Case no.	Ovulation day after:			
	Citovaginal smear	Vaginoscopy	P4 dosage(ng/ml)	Receptivity
9	14	18	5,9	8
10	12	13	5,1	9
11	9	10	4,7	13
12	7	9	3,9	7
13	9	12	4,9	9

14	11	13	5,8	12
15	16	17	6,1	14
16	10	11	4,1	7
17	7	8	3,6	11
18	8	11	4,2	6
19	6	9	3,9	6
20	8	9	4,1	10
21	11	14	5,8	9
22	9	11	5,3	9
23	7	10	4,7	8
24	10	11	4,4	13
25	8	9	3,7	6
26	7	8	4,6	9
27	9	11	5,5	12
28	10	12	4,9	8
29	13	15	6,4	10
30	8	11	4,9	7
31	10	12	5,1	8
32	8	10	4,5	10
33	11	13	5,7	8
34	6	8	4,0	6
35	10	13	4,8	11
36	11	12	5,1	14
37	8	10	4,4	6
38	9	11	4,2	11
39	8	10	3,8	10
40	10	14	5,6	8
41	8	10	4,6	6
42	9	10	3,9	7
43	11	13	4,1	12
44	10	12	5,5	14
45	9	10	3,7	9
46	8	10	4,1	12

Based on sexual receptivity, ovulation was estimated at  $9,28 \pm 2,43$  days after onset of vaginal discharge.

Examining the citovaginal smear, ovulation and onset of fertile period in bitches was estimated between  $9,3 \pm 2,02$  days.

According to vaginoscopy, ovulation time was estimated on an average of  $11,33 \pm 2,11$  days. Progesterone dosage revealed a medium level of  $4,61 \pm 0,75$  ng/ml in the moment of ovulation.

Statistical analysis was made using the IBM SPSS – ver. 19 for Windows (IBM,

New York, USA - paired student T test and one way ANOVA. In some cases, in order to establish the degree of correlation between data groups, Pearson coefficient (R) was calculated.

Results are presented as mean values  $\pm$  standard deviation. The statistical significance level was  $P < 0,05$ .

**Table 2. Value of the Pearson correlation coefficient between the 3 evaluation methods for the ovulation time in the bitch**

	Receptivity-Vaginoscopy	Receptivity - P4 dosage	Receptivity-Vaginal cytology
Value of Pearson coefficient	0,24	0,27	0,35

-1 negative correlation; 0 uncorrelated; 1 positive correlation

Obtained results indicate a poor degree of correlation between the 3 methods of investigation and monitoring of sexual cycle in the bitch and clinical receptivity. Current implementation of these 3 methods allows a better assessment of the beginning of fertile period.

## CONCLUSION

46 bitches were subjected to monitoring their sexual cycle by making citovaginal smear, vaginoscopy and serum progesterone dosage.

Based on the examination of citovaginal smear in all 46 bitches, ovulation took place on the average of  $9,3 \pm 2,02$  days. Vaginoscopy evaluation established that ovulation takes place on an average of  $11,33 \pm 2,11$  days. Progesterone dosing revealed a medium value of  $4,61 \pm 0,75$  ng/ml in the moment of ovulation, with limits between 3,6-6,4 ng/ml. Based on sexual receptivity, ovulation was estimated at  $9,28 \pm 2,43$  days.

The obtained results indicate that there is no examination method that taken separately can accurately establish the ovulation timing. It is indicated to obtain an overview from the concomitant use and correlation of the results of several examination methods in order to determine

the time of ovulation, providing more accurate results.

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