

ETOLOGICAL AND ENDOCRINE COORDINATES IN CORRELATION WITH ESTRAL CYCLE PHASES IN BITCHES

COORDONATE ETOLOGICE ȘI ENDOCRINE ÎN CORELAȚIE CU FAZELE CICLULUI ESTRAL LA CĂȚELE

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ABSTRACT | REZUMAT

The physiology of reproduction in bitches is more distinctive than in other domestic species because the length of the heat cycle is considerably longer than in other species. Female dogs belonging to several breeds and aged between 2 and 6 years were examined daily to detect the appearance of vulva edema and blood tinged discharge, indicating the beginning of the proestrus. Also, the interpretation of the vaginal smear consisted of classifying the cells, assessing the appearance and quantity of mucus, and establishing the cyto-vaginal indices.

A special emphasis has also been put on the study of sexual behavior with the passage from one phase of the estral cycle to another, following the intensity of vaginal discharge, the interest in the male and the reflex of tail withdrawal, which was observed in 80% of these, while at 20%, this behavior specific for the estrus period, did not occur.

Estrogens, progesterone and the pituitary gonadotropic hormones (FSH și LH), were dosed with high precision by the immunochemoluminescence assay. In 30% of the bitches, FSH pre-ovulatory wave preceded LH with about 12 hours, while in 70% of cases, the two preovulatory waves occurred concurrently. The plasma level of estrogens records high mean values at the beginning of the proestrus, rising sharply one day before the LH preovulatory wave. With regard to plasma progesterone dosing before the LH preovulation wave, were recorded values below 1 ng/ml.

Keywords: estral cycle, vaginal smear, hormones

Fiziologia reproducerii la cățele este mai aparte, deoarece lungimea ciclului de călduri este considerabil mai lungă decât la celelalte specii domestice. Cățelele care aparțineau mai multor rase și aveau vârste diferite (între 2-6 ani) au fost examinate zilnic pentru depistarea apariției edemului vulvei și a scurgerilor sero-sanguinolente, semne care indică începutul proestrului. De asemenea, interpretarea frotiului vaginal a constat în clasificarea celulelor întâlnite, evaluarea aspectului și cantității de mucus, și stabilirea indicilor citovaginali.

Un accent deosebit s-a pus și pe studiul comportamentului sexual, odată cu trecerea dintr-o fază în alta a ciclului estral, urmărindu-se intensitatea scurgerilor vaginale, interesul față de mascul dar și reflexul de retragere a cozii, care a fost observat la 80% dintre cățelele luate în studiu, în timp ce la 20% dintre acestea, comportamentul specific perioadei de estru, nu se manifesta.

Estrogenii, progesteronul și hormonii gonadotropi hipofizari (FSH și LH), s-au dozat cu mare precizie prin testul de imunochemoluminescență. La 30% dintre cățele, valul preovulator de FSH l-a precedat pe cel de LH, cu aproximativ 12 ore, în timp ce la 70% din cazuri, cele două valori preovulatorii au apărut concomitent. Nivelul plasmatic al estrogenilor, înregistrează valori medii ridicate la începutul proestrului, crescând brusc, cu o zi înaintea valului preovulator de LH. În ceea ce privește dozarea progesteronului plasmatic înaintea valului preovulator de LH, s-au înregistrat valori medii sub 1 ng/ml.

Cuvinte cheie: ciclu estral, examen citovaginal, hormoni

The bitch is an animal, which heats occur diestric but generally appear in spring and autumn.

Many breeds emerged lately and quite often adapt to other environmental conditions than specific ones, leading in some cases to reduce or even abolish certain instincts, including the sexual one. Due to the large va-

riations in different breeds of dogs of origin, maintenance and different ages, we considered it appropriate to establish average values of the reference parameters for spontaneous estral cycle at different stages of the estral cycle, and subsequently to evaluate and statistical interpretation of the data obtained.

MATERIALS AND METHODS

For the relevance of the study and interpretation correlated with statistical results, we considered sam-

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pling from 10 bitches clinically healthy, belonging to several breeds (Boxer, Beagle, German Shepherd, Rottweiler, Dalmatian, Husky and Mix breed), with different ages (between 2-6 years), under the proestrus phase of the estral cycle. Bitches studied were examined daily to detect vulva edema and serosanguineous discharge, signs of actually beginning proestrus. On the first day of the onset proestrus at all bitches, vaginoscopic examination was performed once a day, to observe occurrence of vaginal folds.

Was put on trial with special emphasis the sexual behavior of each part, when passing from one phase to another of estral cycle. Thus, the intensity of vaginal discharge, vulva edema, interest in male and reflex of tail withdrawal were followed.

To estimate the ovulation moment, the LH, FSH and progesterone were dosed to all studied bitches. Blood sampling was performed every 8-10 hours during the early follicular phase (from the first day of proestrus by observing vaginal folds, after vaginoscopic examination) and every 4-6 hours in late proestrus and beginning of the follicular phase (from the observation of the vaginal mucosa folds, following the vaginoscopic examination, up to 5 days after the day when ovulation is estimated). To estimate the moment of ovulation, plasma progesterone was dosed 3 times per week from the beginning of oestrus. It is worth noting

that the highest plasma concentration of LH detected in the preovulatory period was the "O" moment (T=0).

RESULTS AND DISCUSSIONS

In all the studied bitches, proestrus phase was characterized by the presence of vaginal bloody secretions, onset and development of vulvar edema.

As for the vaginal discharges, they have a high intensity (+++) at 30% (n=3) of all bitches selected for this group (n=10) and medium (++) at 70% (n=7) of them.

Regarding the intensity of the evolution of vulvar edema, scored on a scale of 0-3 (0 in bitches in anestrus and 3 in bitches with a maximum expression of vulvar edema), it was large / maximum (+++) at half of the cases (50%) and medium(++) to 30% (n = 3) of them. In contrast, in 2 bitches in this batch (20%), the intensity of the development of vulvar edema was low (Table 1). Thus, in this phase of the estral cycle, the sexual behavior of the bitch is represented by interest for males, but without the acceptance of the match. This may often represent the moment of sudden change from the behavior of the proestrus to the behavior of heat. In this context, at the beginning of the oestrus, 80% of the females studied were interested in males but they didn't accept the match. (Table 1 and Chart 1).

Table 1

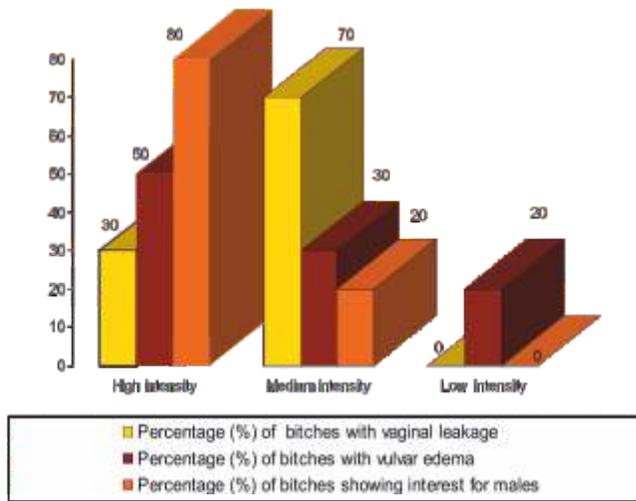
Intensity of proestrus and oestrus in bitches from control group

No.	Breed	PROESTRUS		OESTRUS	
		Intensity of vaginal leakage	Intensity of vulva edema	Interest for males	Reflex of tail withdrawal
1	Boxer	++	++	+++	present
2	Beagle	++	++	+++	present
3	Rottweiler	+++	+++	+++	present
4	German Shepherd	+++	+++	+++	present
5	Metis	++	+	++	absent
6	Beagle	+++	+++	+++	present
7	Mix breed	++	++	+++	present
8	Husky	++	+++	+++	present
9	Dalmatian	++	+	++	absent
10	Rottweiler	++	+++	+++	present
TOTAL		+++ - n=3 ++ - n=7	+++ - n=5 ++ - n=3 + - n=2	+++ - n=8 ++ - n=2	present - n=8 absent - n=2

Legend: +++ - high intensity; ++ - medium intensity; + - low intensity

Chart 1

The dynamic of intensity of proestrus and oestrus in bitches from control group



It is worth noting that the values obtained in this study are close to those obtained by other authors (1). Another behavioural manifestation, specific to the estrogen phase and considered in this study, was the tail deflection reflex (sign of female in heat acceptance of the male), observed in 80% of these, while at 20%, this estrous-specific behavior doesn't occur (Chart 1).

For the surprise and confirmation of estral cycle phases, serial vaginal smears, along with vaginoscopy (additional imaging of the vagina), were performed during the sex cycle, allowing the visualization of the mucosa using an endoscope (15).

Interpretation of vaginal smear in bitches in this group consisted of classifying the cells, assessing the appearance and quantity of mucus, establishing cytovaginal indices, and correlating all of these data to establish the sex cycle phase (Table 2).

In all the studied bitches, the vaginal cytology at the beginning and middle of the proestrus is characterized by the presence of erythrocytes and a mixture of epithelial cell types (parabasal, intermediate and superficial), bacteria and leukocytes, in particular neutrophils (Fig. 1. a, b). The percentage of small intermediate and parabasal cells decreases towards the end of the proestrus, increasing the percentage of superficial, keratinized ones.

With regard to the estrus phase in studied bitches, vaginal cytology (Fig. 2.a) revealed a percentage of over 85% superficial, keratinized, epithelial vaginal cells (with sharp margins, angular, small nucleus, pyknotic or most often without nuclei, having the appearance of fish scales or corn flakes), values closed to those mentioned by the literature (2,13).

In oestrus, following vaginoscopy in all the bitches in this group, there is a reduction in edema and the appearance of pale, primal folds.

In females under study, after examination of vaginal smears in metoestrus, it can be observed that they contained a very large amount of mucus and had an intense basophilic coloration predominant of nucleated cells and polymorphonuclear leucocytes as a result of the reduction in the number of layers of the vaginal mucosa (11).

After performing vaginoscopy, it was observed in all bitches in metoestrus, the disappearance of the mucosal folds, this getting a white-pink striped color (rosette appearance). In the anestrus, which is a period of complete sexual silence without specific clinical signs, vaginal secretions were absent in all the animals under study, and during vaginoscopy the vaginal mucosa was without folds in all animals in this batch.

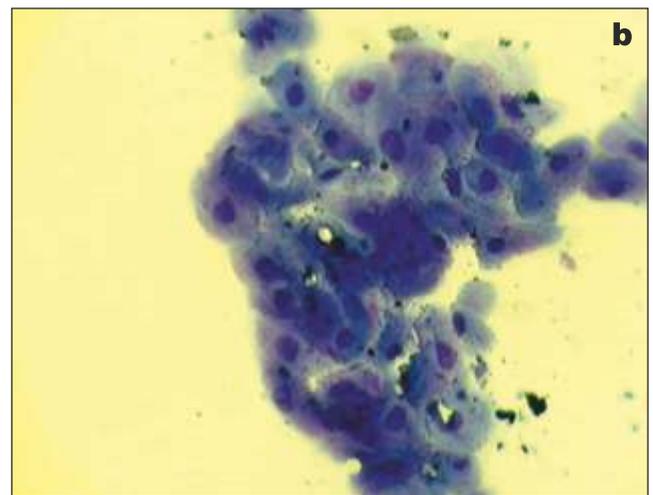
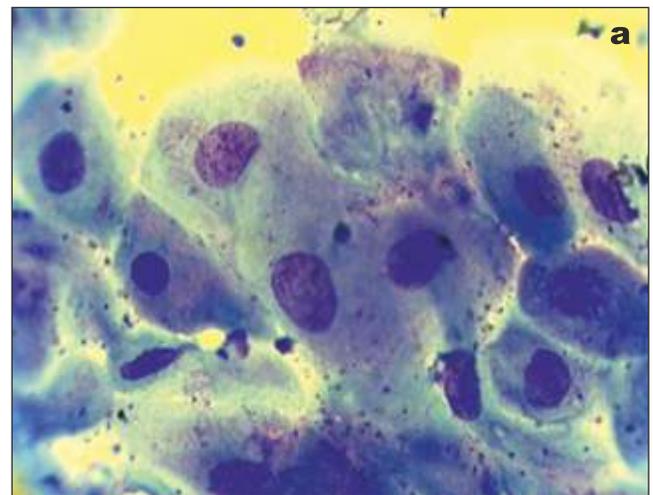


Fig. 1. Proestrus (a, b). Are present parabasal, intermediate and superficial cells, erythrocytes and neutrophils (original)

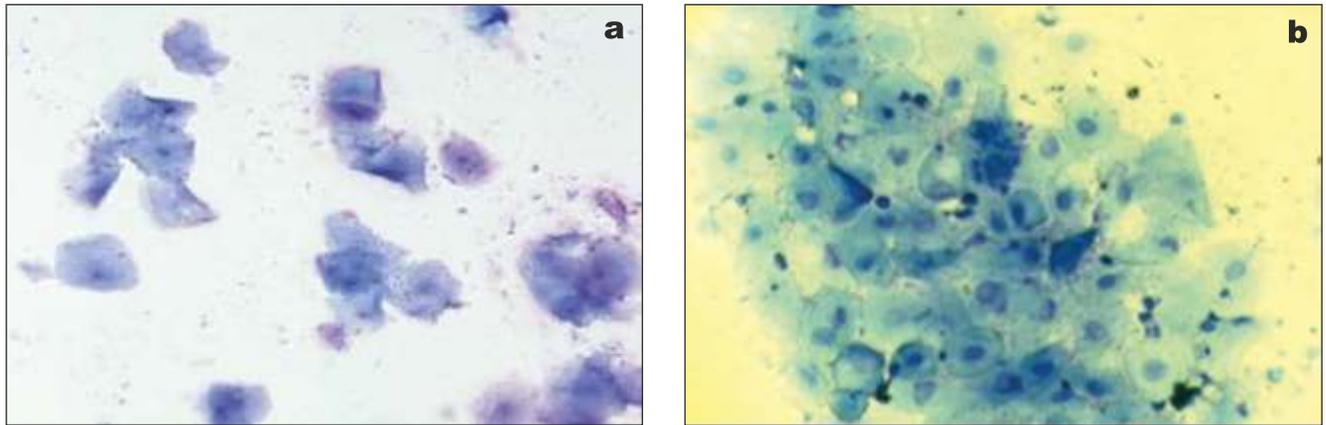


Fig. 2. a. Estrus. Numerous superficial vaginal epithelial cells, keratinized (original); b. Diestrus. Predominant parabasal and intermediate cells with basophilic coloration (original)

In the bitches from this group, in anestrus, in the vaginal smear (Fig. 2.b) predominated the parabasal and intermediate cells with basophilic staining and the vaginal mucosa was thin, smooth, red. Similar histological, anatomical and ethological correlations have been made by many researchers (6,10).

It is worth noting that the dosage of serum levels of some hormones (FSH, LH, progesterone and estrogens) comes to complement the physiology of reproduction in the bitch. Pituitary gonadotrophins play an

essential role in the induction of follicular phase and ovulation in bitches. Specialty literature mentions that ovulation is preceded always by a LH preovulatory wave (4, 7, 8).

In this context, the pituitary gonadotropic hormones were initially dosed from bitches under study, 72 hours before the peak of LH ($T = 0$) and then for a period of 72 hours after this peak.

Thus, the highest value of LH plasma concentration was $T=0$ or "day 0".

Table 2

The average of the LH, FSH, estrogen and progesterone plasma concentration in bitches from the control group, during the peri-ovulatory period

PERIOD		PLASMATIC HORMONES			
		LH (ng/ml)	FSH (U/l)	Estrogens (pg/ml)	Progesteron (ng/ml)
Before the LH preovulatory wave	Day -3	1,0±0,1*	1,6±0,1*	29±4*	0,6±0.1*
	Day -2	1,6±0,1*	1,8±0,1*	44±4**	0,8±0.1*
	Day -1	1,4±0,1*	1,9±0,1*	61±5***	0,7±0.1*
Day 0 (T=0)		12,8±2,2***	13,6±2,4***	22±4**	1,7±0.2**
After the preovulatory LH wave	Day 1	3,4±1,6*	8,8±1,8**	19±4*	2,9±0.2**
	Day 2	3,2±1,3*	5,6±1,6**	15±3*	8,1±1**
	Day 3	2,4±1,1*	4,6±1,4**	13±3*	-
	Day 10	-	-	9±2**	19,6±4***
	Day 25	-	-	11±3*	21,8±4***
	Day 30	-	-	-	18,9±4***
	Days 51-82	-	-	-	>1

Day 0 ($T = 0$) – the day when the plasma concentration of LH reaches a preovulatory peak.

* $p > 0,05$ – insignificant differences

** $p < 0,05$ – significant differences

*** $p < 0,01$ – distinctly significant differences

Table 3

Synthesis of the events within an oestrus cycle in bitches from the control group

SEXUAL CYCLE PHASE	Duration	EVENTS	VAGINAL SMEAR	VAGINOSCOPY	PROGESTERON (ng/ml)	Clinical signs / Behavioral manifestations
PROESTRUS	3 days-3 weeks. (average - 9 days)	Estrogen peak 61±5 pg/ml	large, nucleated, white blood cells, white blood cells and numerous red blood cells	the vaginal mucosa is thickened, edematous (white/gray) with bloody secretions	>1	vulvar edema, haemorrhagic vaginal discharge, the female does not accept the male
		LH peak 12,8±2,2 ng/ml FSH peak 13,6±2,4 U/l	numerous keratinized cells, few red blood cells		1,7±0.2	
ESTRUS	3 days-3 weeks. (average - 9 days)	OVULATION	over 85% of keratinized cells, large mucus	reduction of edema, appearance of primary folds, pale appearance (white-pink color)	8,1±1	clear, transparent vaginal discharge, the female accepts the male
		Fertile period			19,6±4	
METESTRUS		The end of the fertile period	reappearance of parabasal cells, large influx of polymorphonuclear leukocytes	disappearance of mucous folds, striped white-pink color (characteristic aspect of rosette)	21,8±4	-
		Closure of the cervix			18,9±4	
DIESTRUS	2-3 months in the absence of gestation	Estrogens - basal secretion (2-10 pg/ml)	parabasal cells, small intermediates, isolated nuclei, nonclaronuclear background	mucous thin, smooth, red	>1	period of sexual silence
ANESTRUS	1-6 months	Estrogens - basal secretion (2-10 pg/ml)				

The beginning of the preovulatory wave of LH and FSH respectively (the peak of the two hormones), was considered to be the first statistically significant difference ($p < 0.01$), the mean value of the hormone on 72-28 hours before $T = 0$.

Similarly, the end of LH and FSH preovulatory waves was determined to be the last value that exceeded the mean value of the respective plasma hormone (plus standard deviation) over the period 28-72 hours from $T = 0$. Differences between the basal plasma concentrations of LH and FSH, respectively, before and after preovulatory waves were statistically analyzed using the Student t test.

Comparing the levels of plasma hormones (LH, FSH, estrogens and progesterone) in the bitches from the control group ($n = 10$) during the periovulatory period, there are significant differences, associations and effects that are mentioned in Synthetic Table 3.

Thus, day 0 ($T = 0$) is considered the day when the LH plasma concentration reaches a peak (a maximum) preovulatory, mean value (12.8 ± 2.2 ng/ml) which is significantly higher ($p < 0.01$) than the plasma concentration of this hormone before, and after the LH preovulatory wave. In comparative studies, in bitches from this group, we found that the pre-ovulatory wave of LH was accompanied by a preovulatory FSH wave, which reached an average value of 13.6 ± 2.4 U/l, distinctly higher ($p < 0.01$), compared to the mean values of the pre-LH wave (14).

Also, in most of the bitches, the preovulatory

growth of luteinizing hormone (LH) occurs concurrently with preovulatory growth of follicle stimulating hormone (FSH), as mentioned by other researchers in their studies (9, 12).

In contrast, the duration of the pre-ovulatory wave of LH and FSH was different. Thus, the mean duration of the FSH wave (90 ± 6 hours) was significantly higher ($p < 0.05$), approximately three times higher than the mean wavelength of LH (32 ± 4 hours).

In all of the control group bitches, the estrogen plasma level records high mean values at the onset of the proestrus, increasing sharply one day before the LH preovulatory wave, when the concentration of these hormones reaches a peak (61 ± 5 pg/ml).

Due to the increased estrogen concentration in the proestrus, during vaginal endoscopy, mucosal edema is observed, the presence of pink and smooth folds. Because of this, the vaginal lumen was narrowed in these bitches, and in most of the patients studied (80%), the blood leak was visible after this examination.

After that, as shown in Table 2, plasma levels of estrogen decrease abruptly during the oestrus, reaching from the 4th day of the cycle ($9-15$ pg/ml), the hormonal levels during the early proestrus (5,13).

In the LH preovulatory day (Table 3), plasma progesterone dosing showed a statistically significant increase ($p < 0.05$), reaching an average of 1.7 ± 0.2 ng/ml, which increased ($p < 0.01$), reaching a peak on day 25 (21.8 ± 4 ng/ml), after which it was maintained at a high level until day 30 (18.9 ± 4 ng/ml).

CONCLUSIONS

1. In bitches from the studied group, the LH pre-ovulatory peak was recorded 2-4 days before the first signs of sexual activity were observed.

2. Following the dosing of the two pituitary gonadotropic hormones (LH and FSH), it was found that in 30% of the bitches, the FSH preovulatory wave preceded the LH by about 12 hours, while at 70% of the cases, the two preovulatory waves occurred concurrently, suggesting that in the bitch there are differences in regulating the secretion of FSH and LH, knowing that the principal regulator of pituitary gonadotrophin secretion is GnRH.

3. The relationship between estrogen, LH and progesterone levels, suggests that while the initial increase in pre-ovulatory LH stimulates steroidogenesis and progesterone secretion, inhibition of estrogen secretion occurs, resulting in decreased plasma levels of estrogen of these hormones.

4. In most control group bitches, a peak of estrogen concentration is observed 48-24 hours before the LH preovulatory peak, followed by a decrease in plasma levels, together with the occurrence of the LH peak.

5. The mean duration of the pre-ovulatory FSH wave (90 ± 6 hours) was significantly higher ($p < 0.05$), compared to the mean LH wavelength (32 ± 4 hours).

6. Following the dosing of plasma progesterone in bitches from the study group, a low mean level of this hormone can be observed before the preovulatory wave (below 1 ng/ml, with a range of $0.6-0.8 \pm 0.1$ ng/ml).

7. Each stage of the estral cycle showed particularities both in the sexual behavior of the respective females and in the intensity of the behavioral manifestations, which were within the species - specific limits.

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