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# APPRECIATION OF PRODUCTIVE PARAMETERS OF HOLSTEIN AND RED HOLSTEIN DAIRY COWS MAINTAINED ON A SEMI-INTENSIVE FARM

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## ***Abstract***

*The study aimed to assess the productive potential of Red Holstein (RH) cows maintained in semi-intensive system. The monitoring of productive parameters was performed on 52 Holstein and 43 Red Holstein cows on their first lactation. The animals were subjected to the official milk production control. The duration of the lactation was 304.5 days. The following productive results were obtained: 6673.05 kg milk on normal lactation, 4.13% fat and 3.57% protein. The somatic cells count was  $341 \times 10^3$  germs/ml. In the conditions of maintenance on a semi-intensive farm, Red Holstein cows have manifested their productive potential at 73.3%.*

***Keywords:*** Red Holstein, semi-intensive system

## ***Introduction***

The Romanian Spotted cow, of the Simmental type, with dual production, for meat and milk, is the main country's cattle breed, being mostly grown in semi-intensive farms (1). In our country, the current trend is to form, by reproductive isolation, cows with a high gene pool, with an increased milk production.

In Romania, the Red Holstein breed is grown for crossing with the Romanian Spotted, in order to increase the milk production of this local breed. The aim of the research was to monitor the productive performances of Red Holstein (RH) cows maintained in semi-intensive system.

## ***Material and methods***

The study was conducted in a dairy farm from Prahova County, on 52 Holstein (H) and 43 Red Holstein (RH) cows on their first lactation, being 2 years old and weighing  $576 \pm 9.84$  kg for Holstein and  $562.7 \pm 13.45$  kg for Red Holstein.

The animals had been included in Official Control of Performances; they were monitored in 2016-2017 during 13 inspections. The individual control of the milk production was realized by recording the production of each cow at given intervals (periodic control).

The milk production was evaluated quantitatively and qualitatively. The monitored parameters were lactation length, milk yield (appreciated on total and normal lactation) and quality of milk (percent of fat and protein, somatic cells count). The quantitative evaluation was realized using the gravimetric method and the production of milk was expressed in kilograms. Milk quality analyses were done in an authorized laboratory, using MilkoScope Expert.

The productive performance data were statistically processed using ANOVA.

### ***Results and discussions***

Data concerning the lactation length are shown in Figure no. 1.

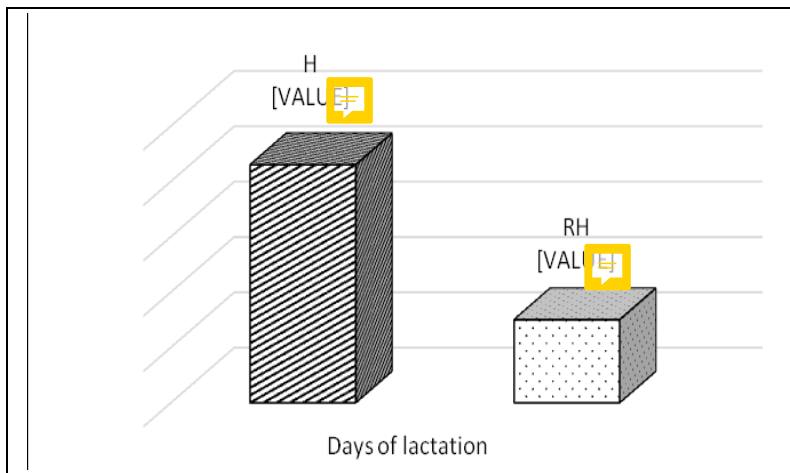


Figure no. 1. *The Lactation Length*

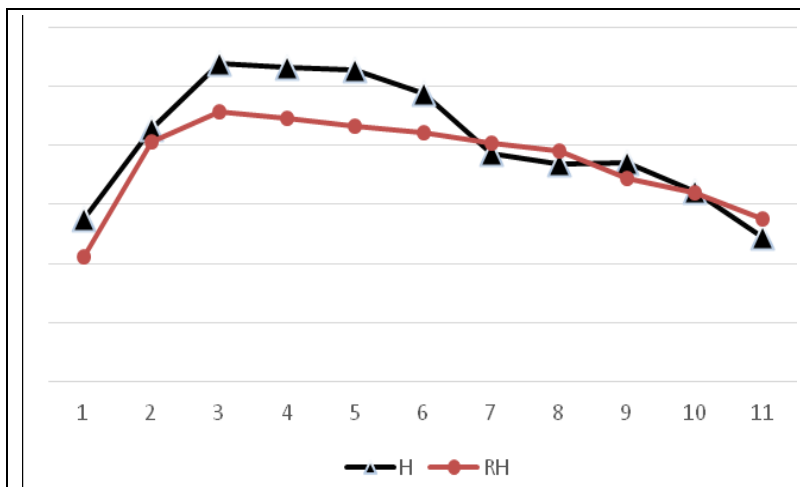


Figure no. 2. *The Lactation Curve (in Months)*

The lactation length was  $307.3 \pm 4.65$  days for Holstein cows and  $304.5 \pm 7.02$  days for Red Holstein, the differences being not significant ( $p \geq 0.05$ ). Literature showed that Lactation curve provides valuable information about the pattern of milk production during lactation. There were negative relationships between lactation length and annual production of milk and milk solids (milk fat + protein), but losses were small up to 16 months (1, 2). The present results can estimate a high productive potential in both breeds.

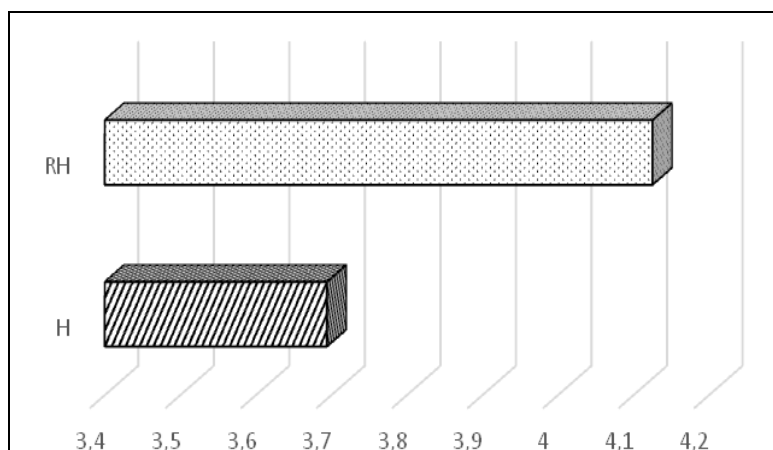
It was observed that, in both breeds, the milk peak production was achieved after 3 months of lactation, and the plateau stage is equally long (Figure no. 2).

Data concerning the quantity and quality of milk are presented in Table no. 1.

**Table no. 1. Productive parameters for milk yield**

Parameter	H	RH	Significance <sup>1</sup>
Milk yield, kg	8452.76 $\pm$ 131.53	6673.05 $\pm$ 148.26	** (p = 0.008)
Milk yield, kg/day	27.51	21.91	** (p = 0.008)
% milk fat	3.69	4.12	* (p = 0.026)
% milk protein	3.57	3.33	NS (p = 0.052)
Somatic cells count/ ml	134.5 x 10 <sup>3</sup>	127.1 x 10 <sup>3</sup>	NS (p = 0.940)

<sup>1</sup> NS  $p \geq 0.05$ ; \*  $p \leq 0.05$ ; \*\*  $p \leq 0.01$ ; \*\*\*  $p \leq 0.001$



**Figure no. 3. The Percentage of Milk Fat**

The milk production has been affected by the breed, being lower by 21.1% in Red Holstein cows. The differences were very significant ( $p \leq 0.01$ ).

The percentage of milk fat (Figure no. 3) was highest for Red Holstein cows with 11.1% ( $p \leq 0.05$ ).

In terms of the percentage of milk protein, this indicator was 3.57 for Holstein and 3.33 % for Red Holstein. The differences were not significant ( $p \geq 0.05$ ).

The somatic cells count did not show differences between breeds, being  $134.5 \times 10^3/\text{ml}$  in Holstein and  $127.1 \times 10^3/\text{ml}$  in Red Holstein ( $p \geq 0.05$ ). Due to human health and animal welfare concerns, several countries in EU set 400,000 cells/ml as the upper limit for SCC in milk. The results obtained in this study have been in line with the legislative requirements. Although some research showed differences between breeds (3, 4, 5), the present results did not differ between Holstein and Red Holstein cows.

The Red Holstein cows of this study showed their productive potential at 73.3%.

### **Conclusions**

1. There were no significant differences between Holstein and Red Holstein cows in the following indicators: lactation length, percent of milk protein and somatic cells count.

2. The milk production was significant lower at Red Holstein cows, while the percentage of milk fat was highest on this breed.

3. Red Holstein breed can be grown in semi-intensive system, but farm management needs to be improved, so that productive potential to be more than 73.3%.

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# CONSIDERATIONS REGARDING THE MORPHOLOGY AND THE CYTOLOGICAL DIAGNOSTIC OF MALIGNANT LYMPHOMA IN CANIDAE

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## ***Abstract***

*The research focused on the morphology of stage IIIB multicentric lymphomas, as they are the most recurrent form of lymphoma, presenting a complex clinical tableau.*

*In order to reach a definitive diagnostic, for most cases of lymphoma in dogs we observed the presence of a predominantly homogenous population of large, immature lymphocytes (prolymphocytes and/or lymphoblasts). They can be easily identified compared to other tumour cells due to the increased nucleo/cytoplasmic ratio (N/C), and the intensely basophilic cytoplasm.*

*The lymphoma diagnosis can be reached when the cytological exam shows a homogenous cellular population of immature lymphocytes in lymph node preparations.*

***Keywords:*** malignant lymphoma, morphology, cytological diagnostics

## ***Introduction***

Lymphoma is the generic term that defines the malignant cancer that occurs through the proliferation of the cells present in the lymphatic and hematopoietic organs (lymphocytes T and B) (1).

These tumours, although quite different amongst themselves, behave aggressively and tend to spread throughout the body. This type of cancer generally appears in the lymphatic and hematopoietic organs (lymph nodes, spleen, hematogenic bone marrow), but can be found in any other type of tissue (3).

Researchers identified a connection between the development of lymphoma and herbicide usage of the phenoxyacetic acid type, and in particular of the 2,4 dichlorophenoxyacetic acid (2,4 D). Initially, the study investigated the Kansas farmers who frequently used herbicides for their crops (2).

The dogs whose owners used 2,4D to spray lawns were more predisposed to develop malignant lymphoma, the risk reportedly being doubled when the herbicides were sprayed twice or more per year. Subsequently, researchers looked at dogs that were exposed to herbicides within the last 7 days, and found that the 2,4D concentration in urine was most often equal or greater than 50µg/l. The highest concentration was recorded two days following exposure (2).

The most recurrent forms of malignant lymphoma are: multicentric, cardio-mediastinal, gastro-intestinal and coetaneous. Extranodal forms, which can appear in locations other than the lymphatic system, include ocular, nervous (CNS), bone, testicular, bladder, cardiac and nasal cavity manifestations (4).

### ***Materials and methods***

Research regarding the morphology and diagnostic in canine lymphoma took place in the clinic of the Veterinary Medicine Faculty of the *Spiru Haret* University in Bucharest, over a period of two years and the study included ten cases.

The purpose of the research was to highlight the macroscopic and microscopic morphology of stage IIIB multicentric canine lymphoma.

In order to outline the clinical signs, general examination techniques were used due to their ease to employ in medical practice: inspection, palpation, percussion, auscultation, gauging body temperature. They were preceded by the drawing up of a medical history of the patient and were followed up by paraclinical exams (haematological blood exam).

The definitive morphological diagnosis resulted from the cytological exam, using smears from fine needle aspirates from lymph nodes.

### ***Results and discussion***

The general clinical exam in stage IIIB multicentric lymphoma revealed that, with regards to the lesions involved, all ten cases presented as lymphadenopathy (generalized lymphoreticulosis). Upon palpation of the submandibular, prescapular and popliteal lymph nodes, volume enlargement and indurations were observed (Figure no. 1 and Figure no. 2).

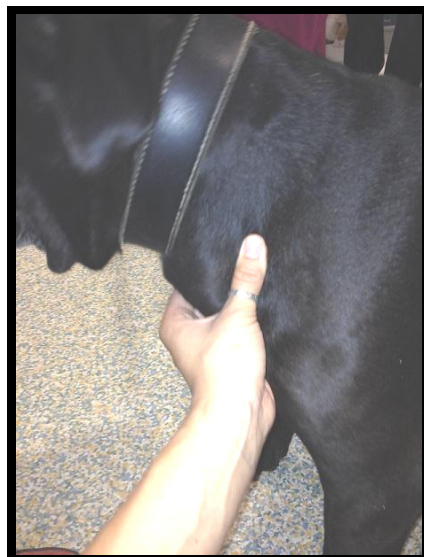
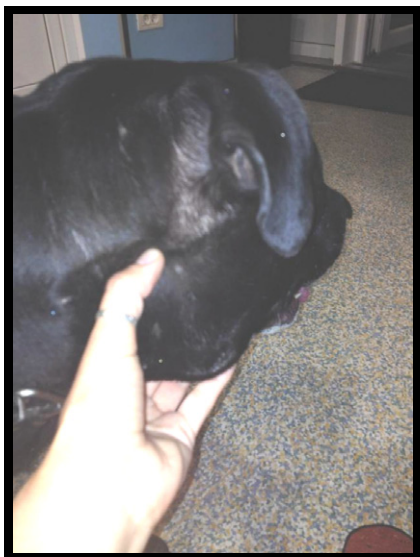


Figure no. 1. *Submandibular and Prescapular Lymphadenopathy*

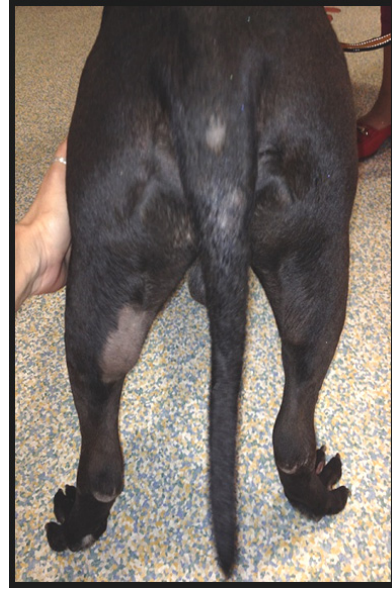


Figure no. 2. *Lymphadenopathy of Popliteal Lymph Nodes in Stage IIIB Multicentric Lymphoma*

Only one case had ocular involvement, with neoplastic uveitis (Figure no. 3). Furthermore, difficulty with deglutition and white deposits were observed on the right eye, with purulent ocular discharge.



Figure no. 3. *Neoplastic Uveitis in Stage IIIB Multicentric Lymphoma*

From a cytological perspective, monomorphous lymphoid cells with numerous malignancy criteria were observed: increased nucleocytoplasmic ratio, presence of nucleoli, intensely basophilic cytoplasm, numerous lymph node corpuscles, anisocytosis, anisokaryosis (Figures no. 4, 5, 6).

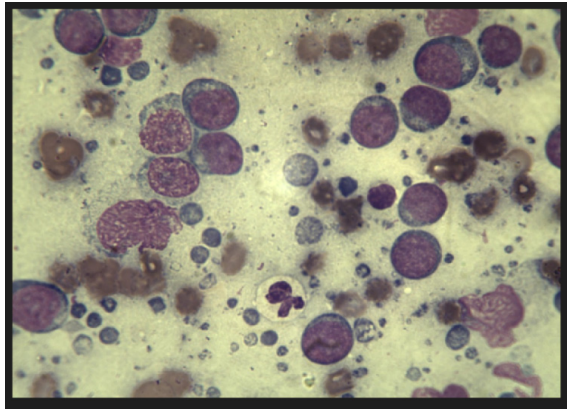


Figure no. 4. *Blood Smear in Multicentric Lymphoma*

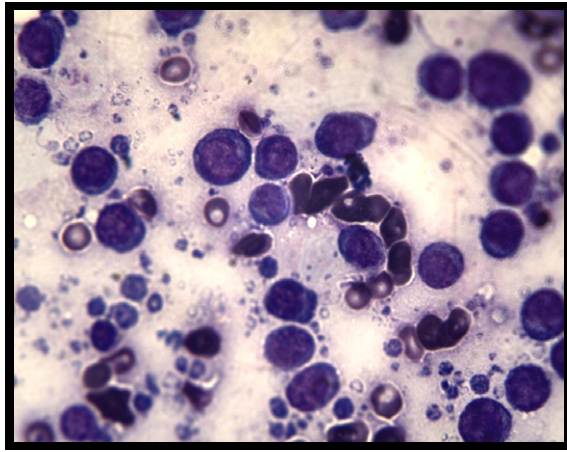


Figure no. 5. *Blood Smear in Multicentric Lymphoma*

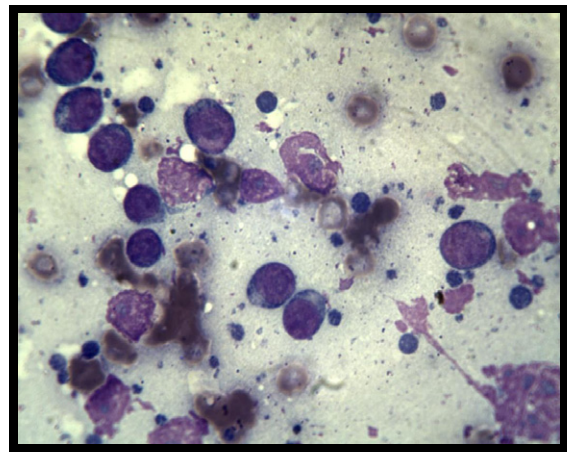


Figure no. 6. *Blood Smear in Multicentric Lymphoma*



Moreover, the cytological exam findings included the presence of numerous young lymphocytes (of the blastic type), which presented malignancy criteria (Figure no. 7).

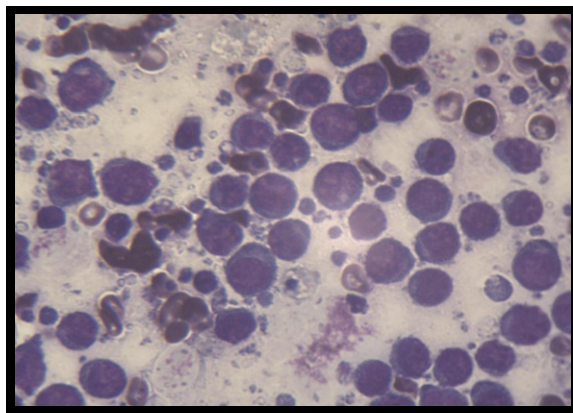


Figure no. 7. *Blood Smear in Multicentric Lymphoma*

### ***Conclusions***

1. Malignant lymphoma in dogs is characterized, from a lesion point of view, by generalized lymphadenopathy.
2. Other lesional aspects were rare and were represented by ocular lesions, manifested as neoplastic uveitis.
3. The cytological exam revealed numerous lymphoblasts that presented malignancy criteria: high nucleo/cytoplasmic ratio, the presence of nucleoli, intensely basophilic cytoplasm, and numerous cells in the mitosis stage.

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# **FINDINGS REGARDING THE EPIDEMIOLOGICAL SITUATION OF LIVESTOCK FROM A RESTRICTED AREA IN EASTERN ROMANIA**

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## ***Abstract***

*The following paper aimed to assess the epidemiological situation of livestock (bovine, caprine and ovine) from a specific veterinary sanitary circumscription area in Eastern Romania, based on the samples collected on the field, in accordance with the Strategic Programme, or based on a specific necessity, which were analyzed by the Veterinary Sanitary and Food Safety Laboratory from the respective county.*

***Keywords:*** farm animals, brucellosis, animal health

## ***Introduction***

Following the process of becoming a member of the European Union, a series of changes took place concerning disease prevention and mitigation in humans and animals respectively (2, 4).

Respecting the biosecurity principles of livestock through a series of organizational measures that aim to prevent the introduction and spread of pathogenic agents is paramount in stopping the occurrence of transmissible diseases (1, 3).

The elimination of previously mandatory preventative vaccinations and bestowing more freedom on farmers with regards to zootechnical and veterinary management led to the increase of the risk of occurrence of diseases with considerable impact on the national economy.

Vaccination also ensures the quality guarantee of the zootechnical products, the hygiene of food products of animal origin, and the protection of public health.

## ***Material and methods***

The determination process was carried out in a small area in Eastern Romania, with collected samples being analyzed within the county LSVSA. We aimed to monitor the health status of animals by detecting germs from different microbial species, from deceased animals, samples taken from production units and from households, by carrying out necropsies and detecting antibodies through laboratory tests.

Furthermore, serological exams were carried out following blood samples being collected from different animal species (ovine, caprine, bovine), in accordance with the Strategic Programme. The determination process took place throughout 2016.

### ***Results and discussions***

The following actions took place within the three locations pertaining to the respective veterinary circumscription:

- collecting blood samples for testing Brucellosis in cattle (Table no. 1);
- collecting blood samples for testing Bovine Enzootic Leukosis (LEB) (Table no. 2);
- collecting blood samples for testing Brucellosis in ovine (Table no. 3);
- collecting blood samples for testing Brucellosis in caprine (Table no. 4).

**Table no. 1. Samples Collected from Bovines for Testing Brucellosis – 2016**

Location	No. samples analyzed	Positive samples	Negative samples
A	309	0	309
B	265	0	265
C	68	0	68
Total samples	642	0	642

The testing was carried out for the *Brucella abortus* species.

The detection for *Brucella abortus* antibodies was done through the rapid sero-agglutination reaction on a slide with antigen coloured with Bengal Pink.

**Table no. 2. Samples Collected from Bovines for Testing Bovine Enzootic Leukosis – 2016**

Location	No. samples analyzed	Negative samples	Positive samples	Percentage
A	309	297	12	3,88
B	265	257	8	3,01
C	68	68	0	0
Total samples	642	622	20	2,29

Testing was carried out for Bovine Enzootic Leukosis.

The detection for LEB antibodies was done through an indirect ELISA (immunoenzymatic) assay.



**Table no. 3. Samples Collected from Ovine for Testing Brucellosis – 2016**

Location	No. samples analyzed	Positive samples	Negative samples
A	377	0	377
B	353	0	353
C	356	0	356
Total samples	1086	0	1086

Testing was carried out for the *Brucella melitensis* species.

The detection for *Brucella melitensis* antibodies was done through the rapid sero-agglutination reaction on a slide with antigen coloured with Bengal Pink.

**Table no. 4. Samples Collected from Caprine for Testing Brucellosis – 2016**

Location	No. samples analyzed	Positive samples	Negative samples
A	247	0	247
B	192	0	192
C	81	0	81
Total samples	520	0	520

Testing was carried out for the *Brucella melitensis* species.

The detection for *Brucella melitensis* antibodies was done through the rapid sero-agglutination reaction on a slide with antigen coloured with Bengal Pink.

**Table no. 5. Preventative on-Demand Tests Carried Out on Bovines (Youth, 12-18 Months), Location A – 2016**

Test	No. samples analyzed	Positive samples	Negative samples
Bluetongue	1	0	1
LEB	1	0	1
Leptospirosis	1	0	1
Brucellosis	1	0	1

Detecting the Bluetongue virus was done through ELISA immunoenzymatic assays, competitive method (Tables no. 1, 5).

Detecting the LEB virus antibodies was done through ELISA immunoenzymatic assays, indirect method.

Detecting the *Brucella abortus* antibodies was done through the rapid sero-agglutination reaction on a slide with antigen coloured with Bengal Pink.

Detecting the *Leptospira spp.* was carried out through the lysis micro-agglutination test.

### ***Conclusions***

1. Tests carried out in accordance with the Strategic Programme confirmed the status of exempted area with regards to Brucellosis, both in large and small ruminants, throughout the monitored period.

2. With regards to Bovine Enzootic Leukosis, testing revealed the presence of the virus in certain cattle stocks, which led to the removal of the positive animals through slaughter.

3. Following on-demand testing of reproduction bulls, Paratuberculosis cases were identified and the positive animals were removed from reproduction.

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# WHEN DO WE NEED TO DOSE THE PROGESTERONE FOR A FEMALE DOG?

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## **Abstract**

*We often have situations where the financial resources of the owners are limited and we, as doctors, must diagnose as accurately as possible and as quickly as possible.*

*Behavioural or sex-related disorders in female dogs are very commonly caused by variations in the progesterone and its dosing should be the first intention in many cases and could exclude costly laboratory examinations or unnecessary surgical procedures.*

*The study was conducted on a number of 10 females aged 2 to 13, between January 2017 and February 2018 in the USH clinic and two private clinics in Bucharest.*

**Keywords:** progesterone, dog, female, dosage, gestation, sterilized

## **Introduction**

The most frequent disorders in the reproduction area for dogs are determined by the values of the progesterone that do not correspond with the normal numbers. These are quantifying in:

- ❖ postoestrus bleeding (with the alteration of the scale of oestrogen/progesterone);

- ❖ anovulatory oestrus or subtle etc.;

- ❖ extension of the oestrus period;

- ❖ unsuccessful breeding.

Sometimes, rare at dogs, increased values of the progesterone may be caused by persistent luteal corpses, the existence of endometrial infections that can manifest with vaginal leaks and changes of the general condition.

In the case of sterilized females with clinical signs of heats, loss of appetite, oedema of the vulva, accepting the males etc., lastly, the level of progesterone is needed when the gestation is unable to be maintained in different stages.

Alongside the hypothyroidism, one of the main causes of miscarriages is the low level of progesterone and the correct diagnostic may help for future gestations.

## **Material and methods**

For the dosage of progesterone, approximately 2 millilitres of peripheral venous blood are harvested in a vacutainer without additive. After this, we leave it

30 minutes at room's temperature for the curd to form, then it's centrifuged and the separation of the serum is made in a tube without additive. It can then be preserved at the refrigerator for maximum 24 hours until the laboratory will take it.

Do not refrigerate whole blood until the separation with the serum has been done, because it was found that the level of progesterone is decreased (especially when the refrigeration happens in the first 2-3 hours after harvesting).

Also, a prolonged contact between the serum and the erythrocytes should be avoided or the use of a gel tube.

The animals who participated at this study were female dogs between the age of 2 and 13 and the study was carried out between January 2017 and February 2018 in the clinic of the Faculty of Veterinary Medicine, *Spiru Haret* University and in two private clinics in Bucharest.

### ***Results and discussions***

The dosage of progesterone is a simple, cheap and relevant method. Over the course of the sexual cycle and gestation, the progesterone has values between 0.9 and 80 ng/ml.

The physiological values of progesterone are (Figure no. 1):

- anoestrous: 0.9-1 ng/ml;
- the final of the anoestrous: 1.2 ng/ml;
- proestrous: 3-4 ng/ml;

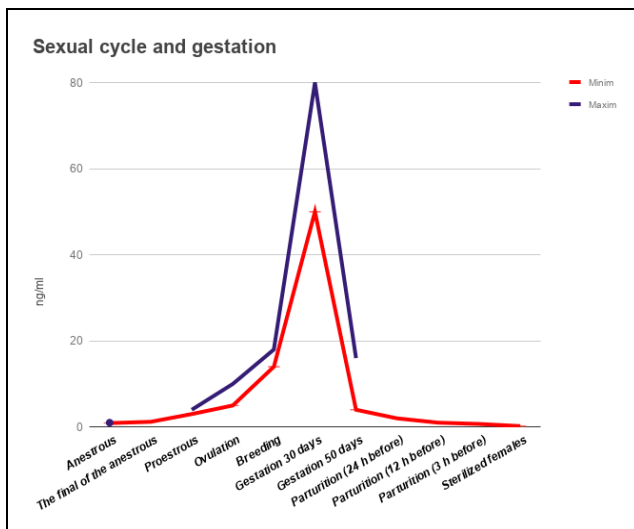


Figure no. 1. *Physiological values of progesterone*

- ovulation: 5-10 ng/ml (after the top of the preovulation of LH for 48 hours the breeding can be made);
- breeding (the best moment for it): 14-18 ng/ml (the moment can be delayed until the value of the progesterone is maximum 20 ng/ml);

- gestation at 30 days: 50-80 ng/ml;
- gestation at 50 days: 4-16 ng/ml;
- parturition (24 hours before it): approximate 2 ng/ml;
- parturition (12 h before it): 1 ng/ml;
- parturition (3 hours before it): 0.7 ng/ml;
- sterilized females: maximum 0.2 ng/ml.

The owners of the animals that participated at this study wanted to dose the progesterone for the following reasons:

- spontaneous abortion in the 41<sup>st</sup> day of gestation: 1 case (a 2 years old female);
- spontaneous abortion in the 32<sup>nd</sup> day of gestation: 1 case (a 3 years old female);
- the failure of the mount between day 9 and the day 13 at of the oestrous: 3 cases (females with the age between 2.3 and 3.5);
- being in heat after the ovariectomy: 4 cases (females with the age between 5 and 13);
- the prolongation of the anoestrous (delayed heats): 1 case (a 2.8 years old female).

After harvesting the samples and sending them to the laboratory following the conditions described above, the result were.

### **Case I**

*Sheppard dog, female:* Her first abortion took place in the 41<sup>st</sup> day of gestation, and the dosage of the progesterone was made the following day after abortion. The result was 8.5 ng/ml, representing approximately 25% from the biological value necessary in the last third of the gestation period. This value was insufficient for maintaining the gestation.

### **Case II**

*Pekinese, female, 3 years old:* Similar with the first one, the patient had her abortion in the 32<sup>nd</sup> day of gestation and the value of progesterone was 35 ng/ml, that representing approximately half of the necessary needed for the first half of the gestation, meaning 50-80 ng/ml.

### **Case III, IV, V**

*Half breed, females, 2, 3 and 3 years old:* These 3 females, despite repeated mounts, did not remain pregnant. The first and the second one were mated in the 10<sup>th</sup> and the 12<sup>th</sup> day from the first signs of the oestrous and the third one in the 9<sup>th</sup>, 11<sup>th</sup> and 13<sup>th</sup> day from the start of the heat period. At the next cycle of heat the dosage of progesterone was made. In the 9<sup>th</sup> day from the start of the oestrous it was found that the values of 3.9 ng/ml, 4.6 ng/ml and 4.1 ng/ml were corresponding with a prolonged proestrus, the perfect moment for the mount requiring values of progesterone 3 times larger. The re-dosing of the progesterone in the all three cases was repeated at 24 hours and 72 hours and the values were

12.5 ng/ml, 13.9 ng/ml and 14.6 ng/ml at the third dosage. The first mount was made at 24 hours after the third dosage, repeating itself at 24 hours and 48 hours. In total, there were 3 mounts, every one of them after the 13<sup>th</sup> day from the moment the heat started and 2 of the 3 females got pregnant (Figure no. 2).

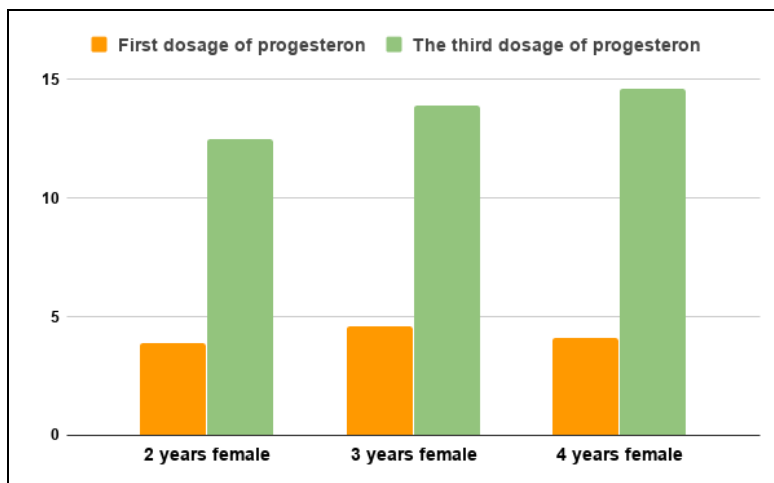


Figure no. 2. *The dosage of progesterone in the failed mounts*

#### Case VI, VII, VIII, IX

*Half breed Bichon (2 cases), Poodle, Pekinese, females, 5, 6, 11 and 13 years old:* The clinical signs manifested were the signs of heat after ovariectomy. There were found swelling of the vulva and that they were accepting the males, the females being sterilized for a minimum of 2 years in all the 3 cases. The dosage of progesterone was realized in all the cases and the following values were found: 3.9 ng/ml, 2.9 ng/ml, 3.6 ng/ml and 3.2 ng/ml, much higher values than the normal values of progesterone at sterilized females 0.2 ng/ml.

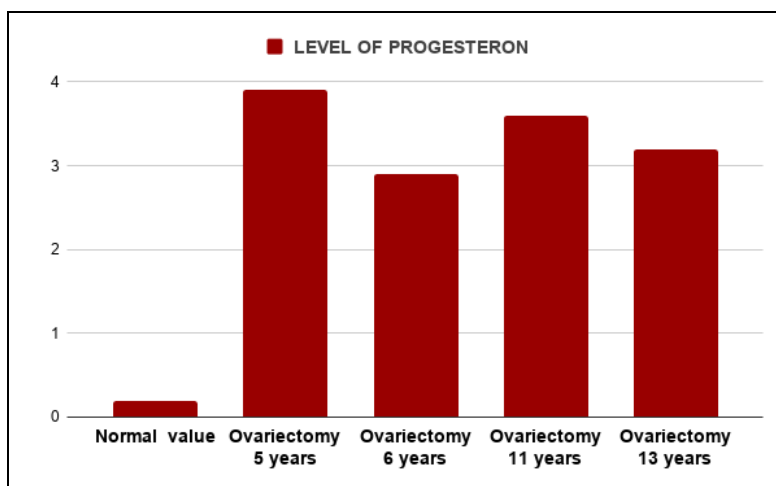


Figure no. 3. *The level of progesterone in ovariectomy cases*

Diagnostic laparotomies were executed in all 4 cases and ovarian tissue debris and numerous adhesions at the level of the castration stump have been identified. They were rigorously excised and none of the patients had any clinical signs of oestrus until the end of the study (Figure no. 3).

### **Case X**

*Bichon, female, 2.8 years old:* The clinical signs were the lack of heat, 10 months after the start of the previous oestrus. After an abdominal ultrasound, the presence of a cystic in the right ovary area of approximately 1.5 cm diameter was found. Dosage of progesterone was performed 10 months after the previous oestrous and a value of 0.9 ng/ml was observed, the value of the anoestrous period. The suspicion was that an anecdotal form with a thickened and irregular wall could only be a luteal cyst derived from the phenomenon of follicular dehiscence and at which level there was discontinuous and prolonged luteinisation of the granule.

### **Conclusions**

The dosage of the progesterone is fast, cheap, usable and it can be put in discussion in all cases with similar clinical signs as the cases presented previously.

For the females with great genetic values, this diagnosis certainly can help them initiate and maintain gestation.

In the cases of sterilized females, the dosage of progesterone can exclude different diseases that can affect the genital and the urinary area with the following symptoms: oedematous vulva and general changes, so avoiding prolonged treatments, antibiotherapie and other laboratory exams that can be costly and unjustified.

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