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Plasmatic Lipid Profile in Dog During Pregnancy and Lactation

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***Abstract*—Examination of plasma lipid concentration in dogs has a clinical interest since lipid profile is a very important tool for the research and diagnosis of some pathologies associated with abnormal lipid metabolism. In this study, we evaluated the effect of pregnancy and lactation on the lipid profile in dogs of different breeds. Blood was collected from the cephalic veins of 12 female dogs in two different periods: during the last pregnancy period and after the second week of lactation. 12 clinically healthy female dogs with similar body weight and age to the first group were used as a control group.**
Blood samples were collected with EDTA and centrifuged for 10 minutes at 3000 rpm. The plasma was separated and used for examination. The examined parameters were: triglycerides, total cholesterol, HDL, LDL and VLDL. To measure these parameters, the EMP-168 biochemical analyzer was used by applying the kit instructions accordingly. Statistical analysis of the results obtained from laboratory examinations showed that pregnancy and lactation could affect the values of some lipid blood parameters.
In pregnant dogs, the concentration of cholesterol and triglycerides was higher (P <0.05) due to increased concentration of very low density lipoproteins (VLDL). We found that lactation was characterized by higher concentrations of triglycerides and VLDL compared to non-pregnant.
During the last stage of pregnancy and the lactation period, dogs exhibit fluctuations in the lipid profile, so these physiological conditions should be taken into consideration when interpreting the results of laboratory examinations.

Keywords— lipid profile, pregnancy, lactation, dog

I. INTRODUCTION

Lipid profile is a useful tool for the research and diagnosis of some pathologies associated with an abnormal lipid metabolism [1]. On the other hand, lipemic serum can positively or negatively interfere with the quantitative analysis of other serum analytics, determined by colorimetric methods. Hyperlipidemia can lead to hemolytic in vitro resulting from changes in the erythrocyte membrane fragility, giving rise to false results of different tests.

The reference values for serum lipids vary greatly depending on various factors as well as biological and environmental specific laboratory methodology. So often recommended to use laboratory-specific reference values for the species where possible.

Cholesterol, as a component of the plasma membrane, the precursor of all steroid hormones, bile acids, and vitamin D in the body is used to detect health problems that may be encountered during pregnancy and to assess the nutritional status of animals [2]. Blood parameters, including lipidogram, vary in a pathological condition, as well as in different physiological stages. Pregnancy and lactation are physiological conditions that lead to obvious changes in hematologic and biochemical parameters in all species.

Pregnancy and lactation are recognized as the most critical periods in animal feeding. The energy requirements of pregnant animals increase during the last pregnancy period. Fetus growth and development depend on breast feeding with balanced and calcium-rich portions. The activity of maternal metabolites is altered during pregnancy and lactation, and a variety of substances and metabolites pass from mother to fetus or go to milk production. All of these changes are expected to be reflected in the values of the maternal blood parameters.

In order to prevent metabolic disorders or eventual subclinical diseases during these periods, it is necessary to determine the physiological variability of blood parameters in healthy bitches and systematically monitor these parameters [3].

II. MATERIAL AND METHODS

In order to study the influence of pregnancy and lactation on the lipidogram in dogs, 24 healthy female dogs of different breeds were selected and divided into two groups. The first group (the control group) was represented by 12 non-pregnant female and the second group consisted of 12 female animals at the last stage of the pregnancy, with a more or less similar body weight and age to the group of non-pregnant females. Blood from the second group was collected during the last pregnancy period and after the second week of lactation.

The "healthy" status was clinically based on the following criteria: no apparent signs of disease were found during the examination, normal temperature, normal pulse and respiratory rate. To determine the condition/state of pregnancy in the pregnant women's group, we were based on the date of matting and the manual methods for its control.

Blood was collected from the cephalic vein of animals kept for at least 8 hours in starvation. The samples were collected with EDTA and centrifuged for 10 min at 3000 rpm. The plasma was separated and used for examination. According to [4], EDTA is recommended as an anticoagulant for performing the lipidogram, because it stops the lipoprotein-lipase activity and maintains the correct concentration of lipoproteins.

Methods for lipidogram determining: The separated plasma from all samples was observed with a naked eye. Samples that resulted to be turbid underwent the chylomicrons test, allowing the plasma sample to remain in the refrigerator for 12 to 14 hours. Through this test, samples with elevated levels of chylomicrons, very low density lipoprotein (VLDL), or both, were separated.

The concentrations of triglycerides (TG), total cholesterol (TC) and high density lipoprotein (HDL) were measured with the EMP biochemical analyzer, using the methods defined in the manufacturer's instructions reagents. For other cholesterol fractions, the Friedewald method [17] was used. For the calculation of VLDL, the triglyceride values were divided by 5. As for the calculation of LDL (low density lipoprotein), the formula stated below was used:

LDL cholesterol = Total cholesterol - HDL cholesterol - VLDL cholesterol.

The results obtained from the laboratory tests of the blood samples of groups of animals were submitted to statistical processing. For different parameters measured it was determined the average ± sd, minimum and maximum values of fluctuation, the significance difference between two populations (tD). Results were evaluated statistically using the Student test, t-test and $P < 0.05$ were considered significant.

III. Results and Discussions

From the results obtained (Table 1 and 2), fluctuations of blood values in female animals were observed during the second month of pregnancy.

The level of triglycerides, total cholesterol and VLDL was higher ($P < 0.05$) in pregnant animals, compared with the values obtained in those not pregnant (Fig. 1 and 2). Concentration of HDL resulted higher in the second group, but these differences were not statistically verified when compared to the first group. There were no apparent fluctuations in LDL values. Similar findings have been reported by [5], [6], [7].

TABLE I. BLOOD CONCENTRATION OF CHOLESTEROL AND TRIGLYCERIDES IN THE ANIMALS STUDIED

Parameters (mg/dl)	Triglycerides	Total cholesterol
Group I Non-pregnant	99.8 ±23.4	138.7±31.6*
Group II Pregnant	153.3 ±34.5*	165.5±28.6*
Group III Lactation	166.1 ±41.3*	177.2±36.7

**Statistical difference between the average values of groups (P<0.05)*

TABLE II. BLOOD CONCENTRATION OF LIPOPROTEINS IN THE ANIMALS STUDIED

Parameters (mg/dl)	HDL	LDL	VLDL
Group I Non-pregnant	74.0±18.2	42.1±13.4	21.6±11.1
Group II Pregnant	82.3±21.9	54.3±16.9	31.2±13.4*
Group III Lactation	85.1±25.7	58.1±18.2	34.3±10.9*

**Statistical difference between the average values of groups (P<0.05)*

According to certain authors [8], [9], [10], total cholesterol, LDL, HDL and triglycerides (TG) increase in response to stimulation of estrogen and insulin resistance. While nutritional requirements of the fetus increase significantly in the last pregnancy period, maternal fat deposits are reduced as free fatty acids and glycerin are used by the liver to synthesize energy-rich fetal compounds. Passing to a catabolic state favours the use of maternal fatty tissue as energy sources, thus saving glucose and amino acids for the fetus. In addition, maternal lipids, particularly cholesterol, are available for use by the fetus in building cell membranes and as a precursor to bile acids and steroid hormones.

Fast central nervous system (CNS) maturation occurs during the last week of pregnancy [11]. Because the CNS contains quite high levels of lipid compared to other organs, it is expected that the lipid level will be increased to support the proper development of neurological structures [12], [13].

As it is reflected in Tables 1 and 2, the average total cholesterol concentration is increased from 138.7 mg/dl to 177.2 mg/dl in dogs at the lactation period. The triglycerides' concentration and the VLDL levels during the lactation period increase from 99.8 mg/dl and 21.6 mg/dl to 166.1 mg/dl and 34.3 mg/dl respectively. On the contrary, HDL and LDL levels do not show significant fluctuations between the two groups ($P > 0.05$). Statistical processing showed significant changes ($P < 0.05$) in TG and VLDL levels

among non-pregnant and lactation animals. No significant differences were found when the results of lipidogram of animals at the lactation period were compared with those of pregnant animals.

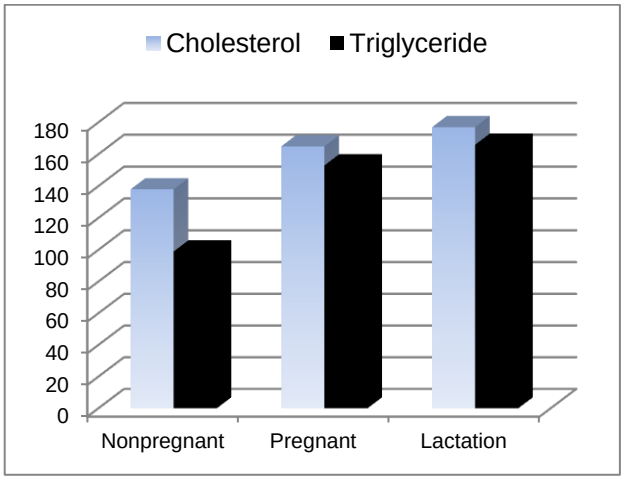

Fig. 1. *Fluctuation of TC and TG in different physiological states*

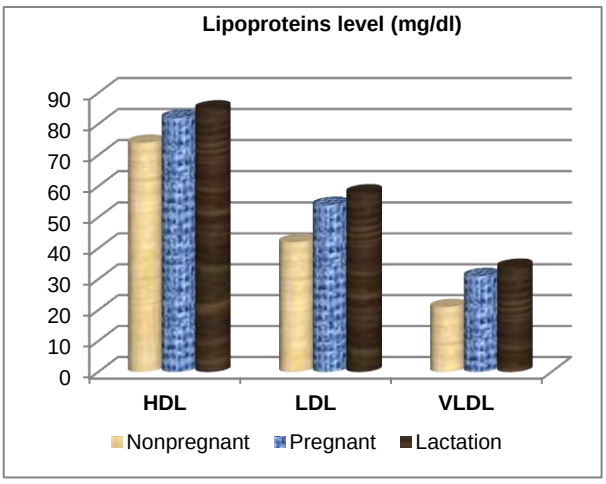

Fig. 2. *Fluctuation of lipoproteins in different physiological states*

The lipidogram changes in third group animals are similar to reports by certain researchers concluding that lactation is the period with the greatest energy demand [14],[15], [16].

In situations with a negative energy balance, such as late pregnancy and early lactation, adipocyte lipolysis in the fat deposits will increase the VLDL concentration on plasma. Although VLDL corpuscles contain more triglycerides than cholesterol, both of these substances tend to grow.

On the other hand, other authors [5] found that lactation in dogs has lower quantities of lipids and lipoproteins compared to the non-pregnant state.

Conclusions: During pregnancy and lactation, some of the blood components may undergo significant changes, preventing the correct interpretation of blood parameters in diseases or disorders that may occur during this period. The study on the physiological lipid metabolism of the dog is useful not only to better understand the metabolic mechanisms, but also for a more accurate interpretation of the lipid parameters in clinics.

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