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Relationship of progranulin response with some physio-biochemical parameters in Akkaraman sheep

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Progranulin (PGRN) is a multifunctional protein that is critical in the retention of embryos in the endometrium of the uterus and the protection of cervical tissue during fetal growth. In case of PGRN release and dysfunction, cervical tissue may remodel (regeneration) and lead to the risk of abortion. Serum PGRN level in sheep and its variation according to different physiological periods and its relationship with some biochemical parameters are unknown. In this study, the relationship between early lactation, early and late pregnancy serum PGRN levels and some biochemical parameters in Akkaraman sheep was investigated for the first time. In the literature search, no similar studies were found on the relationship between early lactation, early and late pregnancy serum PGRN levels and some biochemical parameters in Akkaraman sheep. The research was carried out on 90 Akkaraman sheep reared in the same herd under the same care and feeding conditions. In the study, Akkaraman ewe were divided into 3 groups as early lactation, early pregnancy and late pregnancy. 1st group is early lactation period ewe (first 60 days of lactation), (n = 30), 2nd group is in the first period of pregnancy (on the 0–90th day of the first pregnancy (n = 30)), 3rd group is in the late pregnancy of pregnancy (100–150 days of pregnancy (n = 30)) was created. Blood samples from *vena jugularis* were taken before morning feeding from the animals that formed the study groups. PGRN levels in serum were determined by ELISA method. In addition, glucose, calcium (Ca), magnesium (Mg), phosphorus (P), total cholesterol (TC), triglyceride (TG), low density lipoprotein (LDL), high density lipoprotein (HDL), very low density lipoprotein (VLDL) levels were analyzed in biochemistry autoanalyzer. As a result of the study, lactation and early pregnancy and late pregnancy mean serum PGRN levels were determined as 1.93, 1.78, 1.33 ng mL^{-1}, respectively. The serum PGRN, glucose, Ca, Mg, P, TC, TG, LDL, HDL and VLDL levels of sheep with late pregnancy, early lactation and early pregnancy periods, which constitute the study groups, were found to be statistically significant ($P<0.05$). Our results have shown that due to the physiological role of PRGN in keeping the embryo in the endometrium of the uterine and in preserving the cervical tissue during fetal growth, there may be a useful parameter in the pursuit of the pregnancy process.
Keywords: Progranulin, lactation, pregnancy, biochemical parameters, ewe

INTRODUCTION

Progranulin (PGRN) is a pluripotent growth factor in glycoprotein structure consisting of 593 amino acids encoded by the Granulin gene located on chromosome 17q21.32 and having a molecular weight of 68–88 kDa (Zhou *et al*., 1993; He and Baterman, 2003; Sun *et al*., 2022; Ventura *et al*., 2023). It has got a variety of names such as PGRN, GP88, granulin epithelium precursor, proepithelin, acrogranin, and PC cell growth factor (He and Baterman,2003; Bateman and Bennett, 2009). Various cell types express PGRN, including skeletal muscle, adipose tissue, chondrocytes, hematopoietic

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cells, epithelial and endothelial cells, neurons and macrophages (Korolczuk, 2017).

PGRN is a key actor and regulator of immune response and inflammation, as well as tissue regeneration (Wu and Siegel, 2011; Jian *et al.,* 2013; Korolczuk, 2017). PGRN, acts as a growth factor, anti-inflammatory agent or adipokine, depending on the target tissue (Liu and Bosch, 2012; Korolczuk, 2017; Bateman *et al*., 2018; Pogonowska *et al*., 2019). The role of PGRN in inflammation is associated with the tumor necrosis factor receptor (Matsubara *et al.*, 2012) and in adipose tissue, it is reported that it is mediated by stimulation of IL–6 expression (Tang *et al.,* 2011). Angiogenesis is a physiological process critical to mammary gland development during lactation and pregnancy (Andres and Djonov, 2010). PGRN, which has a role as a factor in angiogenesis, is reported to stimulate expression by endothelial cells (Xu *et al.,* 2020). PGRN is critical for the retention of the embryo, which develops due to its anti–inflammatory effect, to the endometrium of the uterus and to the protection of cervical tissue during fetal growth. It is also reported that high levels of PGRN are present in the placenta during angiogenesis (He and Baterman, 2003; Desmarais *et al.,* 2008). In case of PGRN release and dysfunction, cervical tissue may remodel and lead to the risk of abortion (Samejima *et al.,* 2021). However, no studies have been found on how serum levels change in maintaining pregnancy in terms of elucidating PGRN physiology during pregnancy and during early lactation. Therefore, our current research has the distinction of being the first in the literature.

Akkaraman ewe is a breed resistant to diseases that can live in poor care and feeding conditions and variable climatic conditions. It has the ability to walk long distances and evaluate poor pastures. In this study, the effects of serum PGRN and some biochemical parameters (Glucose, Ca, Mg, TC, TG, LDL, HDL, VLDL) levels in Akkaraman sheep in early lactation, early pregnancy and late pregnancy were investigated.

MATERIALS AND METHODS

Before starting the current study, ethics committee approval was obtained from the Bayburt University Local Ethics Committee (Decision date and number: 02.07.2018/2018/9). The research was carried out in line with ethical principles and rules, protecting animal welfare and rights. The study was carried out in a ewe farm in Demirözü, Bayburt, which was raised under intensive conditions (40° 9' 45.1332" N; 39° 53' 34.2564 "E) (Fig.1).

The animals used in the research were raised in the herd under the same care and feeding conditions, and each group was created using a randomized trial order within itself, and 90 ewes materials were used. In the study, Akkaraman breed ewe were divided into 3 groups: lactation, early pregnancy, and late pregnancy . Ewe in group 1 in early lactation (n=30); Group 2, ewe in the first period of pregnancy (0–90 days of gestation) (n = 30); The third group consisted of ewe in the late pregnancy of pregnancy (100–150th day of pregnancy) (n=30), (Fig. 2).

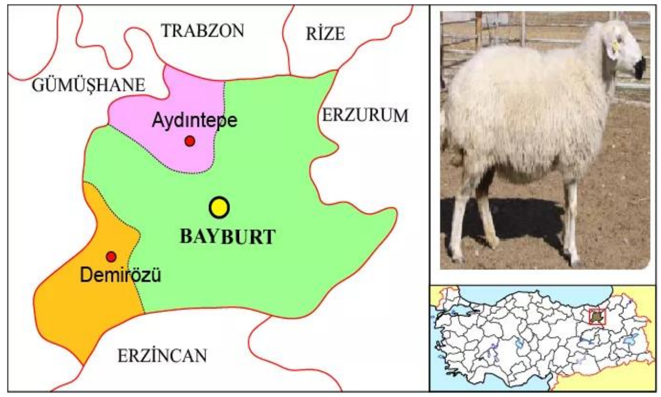

Figure 1. Investigation of the relationship between serum PGRN and some biochemical parameters in Akkaraman sheep bred in different physiological periods in Bayburt, Türkiye.

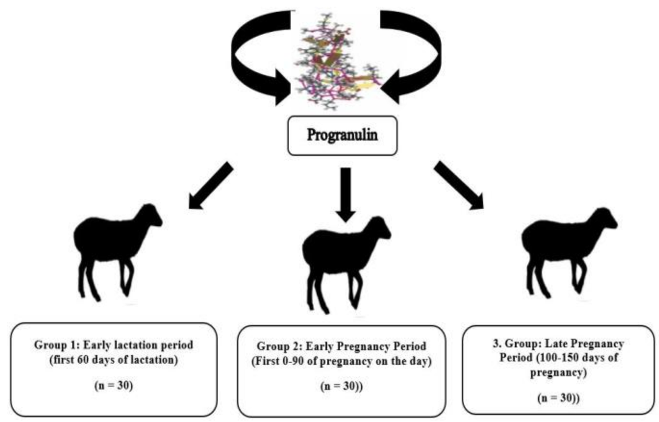

Figure 2. Diagram showing the trial protocol and study groups of the research

Blood samples were taken from the *vena jugularis* before morning feeding from the animals in the study groups. Serum samples obtained from blood samples were stored at –80 °C. PGRN levels in serum were determined by ELISA method. In addition, Glucose, Ca, P, Mg, TC, TG, LDL, HDL, VLDL levels were analyzed in biochemistry autoanalyzer. During the research, care was taken to ensure that the environment and feed factor were the same.

Feed: Feed: The basal diet used in the study consisted of 18% crushed barley, 18% bran, 27% vetch straw and 37% oat or alfalfa grass.

Collection of Serum Samples: Blood was taken from the neck vein (*vena jugularis*) of all ewe included in the study. Blood samples were taken into 10 mL tubes (VACUETTE® TUBE

9 mL Z Serum Clot Activator) without anticoagulant. The blood taken was centrifuged at 3000 rpm for 10 minutes in a refrigerated centrifuge (NÜVE NF 1200, Türkiye) in the laboratory. The obtained sera were transferred to sterile eppendorf tubes. It was stored in deep freezers set at – 80 °C until laboratory analysis was performed.

Measurement of serum PGRN level: The Ewe Progranulin (PGRN) species–specific ELISA Kit Product code: SG–70706, CHINA) kit was used to measure the PGRN hormone level in the serum of ewe. The process was carried out in accordance with the procedure specified in the manufacturer's catalogue.

Statistical analysis: The data obtained as a result of the research was statistically analyzed in IBM SPSS v25 statistical program with General Linear Model Univariate. The variables examined and their interactions with the statistical model are given below:

$Y_{ijkl} = \mu+\alpha_i +\beta_j +\gamma_k + (\alpha\beta)_{ij} + (\alpha\gamma)_{ik} + (\beta\gamma)_{jk} + (\alpha\beta\gamma)_{ijk} + \varepsilon_{ijkl}$.

where, Y is μ: mean, α is early lactation, β is early pregnancy and γ is the late pregnancy effect. The difference between the group means was determined by the Duncan Multiple Comparison test (Steel and Torrie, 1980).

RESULTS

The data on serum PGRN, Glucose, Ca, P, Mg, TC, TG, LDL, HDL, VLDL levels of Akkaraman ewe in different periods of early lactation, early pregnancy and late pregnancy, which make up the study group, are given in Table 1. Mean serum PGRN hormone levels of the groups in early lactation, early pregnancy and late pregnancy s were determined as 1.93, 1.78, 1.33 ng mL^{-1}, respectively. Likewise, mean serum glucose levels of early lactation, early pregnancy and late pregnancy were 64.30, 61.39, 56.64 mg/dl (Table 1). In addition, the serum Ca, P and Mg levels in the early lactation, early pregnancy and late pregnancy are 103.4, 53.31, 23.1 mg/dl, respectively, while the lowest levels are respectively in the early lactation period in ewe with the highest levels in the late pregnancy period. It was determined as 74.6, 52.79, 17.1 mg/dl, respectively, in ewe in the period (Table 1).

Serum TC, TG and VLDL levels were found to be 75.36, 39.28 and 7.85 mg/dl, respectively, in ewe with the highest levels in late pregnancy. Likewise, the lowest levels were determined as 55.67, 25.7, and 5.14 mg/dl in ewe in early lactation period, respectively. On the other hand, the mean serum HDL level was highest in the early lactation period (37.52 mg/dl) and the lowest in the early pregnancy period (4.88 mg/dl) in ewe. Likewise, the highest mean serum LDL levels were determined in the late pregnancy period (32.4 mg/dl) and the lowest level in the early gestational period (28.28 mg/dl) ewe (Table 1).

Table 1. Comparative serum PGRN, Glucose, Ca, P, Mg, TC, TG, LDL, HDL, VLDL mean values (ng mL^{-1}) of the Akkaraman sheep in Türkiye

Parameters	Groups	Value	P
PGRN (ng mL^{-1})	Early Lactation	1.93 ± 0.02^a	0.01
	Early Pregnancy	1.78 ± 0.02^b	
	Late Pregnancy	1.33 ± 0.03^c	
Glucose (mg/dl)	Early Lactation	64.30 ± 0.24^a	0.00
	Early Pregnancy	56.64 ± 0.38^c	
	Late Pregnancy	61.39 ± 0.65^b	
Ca (mg/dl)	Early Lactation	74.60 ± 0.34^c	0.00
	Early Pregnancy	83.90 ± 0.38^b	
	Late Pregnancy	103.4 ± 0.21^a	
P (mg/dl)	Early Lactation	52.79 ± 0.20^c	0.04
	Early Pregnancy	53.76 ± 0.22^a	
	Late Pregnancy	53.31 ± 0.17^{ab}	
Mg (mg/dl)	Early Lactation	17.1 ± 0.12^c	0.00
	Early Pregnancy	17.96 ± 0.15^b	
	Late Pregnancy	23.1 ± 0.16^a	
TC (mg/dl)	Early Lactation	55.67 ± 0.18^c	0.00
	Early Pregnancy	67.21 ± 0.13^b	
	Late Pregnancy	75.36 ± 0.22^a	
TG (mg/dl)	Early Lactation	25.7 ± 0.14^b	0.00
	Early Pregnancy	24.42 ± 0.11^c	
	Late Pregnancy	39.28 ± 0.14^a	
LDL (mg/dl)	Early Lactation	31.42 ± 0.16^b	0.00
	Early Pregnancy	28.28 ± 0.12^c	
	Late Pregnancy	32.40 ± 0.16^a	
HDL (mg/dl)	Early Lactation	37.52 ± 0.24^a	0.00
	Early Pregnancy	35.28 ± 0.15^b	
	Late Pregnancy	34.02 ± 0.23^c	
VLDL (mg/dl)	Early Lactation	5.14 ± 0.03^b	0.00
	Early Pregnancy	4.88 ± 0.02^c	
	Late Pregnancy	7.85 ± 0.03^a	

*PGRN (Progranulin), Ca (Calcium), Mg (Magnesium), TC (Total cholesterol), TG (Triglyceride), LDL (low Density Lipoprotein), HDL (High Density Lipoprotein), VLDL (Very Low Density Lipoprotein). Means within a column showing different superscripts are significantly different (p<0.05): SEM = standard error of the mean.

DISCUSSION

PGRN is an adipokine that plays a key role in many physiological and pathological processes, such as autocrine growth factor, inflammation, glucose and lipid metabolism, as well as its regulating effect on insulin resistance (Liu, 2011; Matsubara *et al.,* 2012; Sun *et al*., 2022; Ventura *et al*., 2023). PGRN, a growth factor, has a role in mammary gland development in lactation, embryogenesis during pregnancy, preservation of cervical tissue, and fetal growth (He and Baterman,2003; Desmarais *et al*., 2008; Andres and Djonov, 2010; Stubert *et al*., 2012). The mean serum PRGN level of our study is consistent with the results of the study reporting that it is high in early pregnancy and low in late pregnancy (He and Baterman, 2003; Andres and Djonov, 2010; Desmarais *et al*., 2008; Todoric *et al*. 2012; Samejima *et al*., 2021). Although our study is the first to evaluate the changes in serum PGRN levels during pregnancy and lactation, it has limitations. We think that it is formed due to the high serum PGRN level in the early pregnancy period, and its physiological role in keeping the embryo in the endometrium of the uterus and protecting the cervical tissue during fetal growth.

Examining the biochemical parameters of different physiological periods such as lactation and pregnancy in sheep is very important for evaluating the metabolic profile of sheep, providing correct nutrition management and preventing or following diseases related to reproductive problems. Glucose is a very important molecule for placental, fetal oxidative metabolism and tissue development in sheep. In addition, 50-70% of maternal glucose use is used by the uteroplansenta in the late pregnancy of pregnancy in sheep (Schlumbohm and Harmeyer, 2008). Low glucose levels should be monitored at the end of pregnancy, as a decrease in serum glucose levels in sheep leads to pregnancy toxicity and death in hypoglycemic sheep. It is thought that the low serum blood glucose level occurring in the last period of pregnancy may be due to the increase in glucose use by the uterus and placenta due to the growth of the fetus. When the serum glucose levels of the sheep constituting the study groups are examined, it is compatible with the results of the research (Hu *et al*., 1990; Antunović *et al*., 2002; Schlumbohm and Harmeyer 2008; Soliman, 2014), but it differs with some research results (Khatun *et al*., 2011; Wang *et al*., 2021; Wang *et al*., 2023).We think that the reason for this is due to fetal growth rate and energy consumption during pregnancy and metabolic changes during the lactation period.

Electrolytes are minerals that break down into ions, which are small electrically charged particles when dissolved in water. They play an important role in many physiological processes such as fluid-electrolyte balance, osmotic pressure, cardiovascular system, nervous system and muscle functions in the body.Ca; is an electrolyte associated with many activities such as blood clotting, muscle contraction, the transmission of nerve impulses, cardiac regulation, secretion of hormones, enzyme activation and stabilization. P; plays an important role in energy transfer, DNA and RNA synthesis, and bone development. In sheep, an imbalance of the Ca and P ratio or absorption disorders also cause rickets. Mg; is vital in many metabolic processes such as energy production, regulation of blood pressure, nerve signal transmission, and muscle contraction (McClure, 2003; Kaneko *et al*., 2008; Varanis *et al*., 2021). In the study, the similarity with the studies that reported that the average serum Ca, P and Mg values of sheep during late pregnancy increased according to their values during early lactation and early pregnancy (Abdelrahman et al., 2002; Moghaddam et al., 2008; Brozostowski et al., 2015); that it has not changed (Alonso et al., 1997;Özpınar, 1997) differ with the reported study results. For this reason, we think that fetal growth during pregnancy is caused by nutrient requirements depending on milk production during lactation and due to differences in Ca, P, Mg metabolism and physiological (such as race, age, live weight, body condition score).

The storage of energy in the organism, which includes molecules such as lipids, fatty acids and their derivatives (including tri-, di-, monoglycerides and phospholipids), as well as other sterol-containing metabolites such as cholesterol, is one of the basic compounds that constitute the structural function of the cell membrane. Plasma lipids are composed of cholesterol, cholesterol esters, triglycerides and phospholipids. Lipids and lipoprotein (LDL, VLDL and HDL) profiles are used in the diagnosis of animal nutrition, health status and metabolic disorders (Nazifi *et al*., 2002). Cholesterol has the property of being a substrate for steroids (progesterone production) during pregnancy in sheep. With the decrease of insulin sensitivity, which affects adipose tissue metabolism, during late pregnancy, cholesterol and lipoprotein levels increase (Schlumbohm and Harmeyer 1997). While a significant increase was determined in serum VLDL, LDL, TG, and TC levels in the late pregnancy period of our study compared to the early lactation and early pregnancy periods ($P<0.05$), a decrease was found in the mean serum HDL level ($P<0.05$). While the results of our study are consistent with the research results reported in the literature (Schlumbohm and Harmeyer 1997; Nazifi *et al*., 2002; Zywicki *et al*., 2018), they differ from the results of some studies (Santos et al., 2011; Kandiel *et al*., 2016). As a justification for this, we foresee that it is shaped as a result of lipid mobilization in order to provide the necessary energy depending on the gestational period.

Conclusion: As a result, this study found that PGRN, Glucose, Ca, Mg, P, TC, TG, LDL, HDL, and VLDL in serum in Akkaraman ewe affected early lactation, early pregnancy, and late pregnancy at a statistically significant level ($p<0.05$). We believe that PGRN will become a new useful parameter in the evaluation and monitoring of the pregnancy process. However, more numerous and comprehensive studies are needed in order to form a general opinion.

Conflict of Interest**:** The Authors declare that there is no conflict of interest

Authors' Contribution Statements**:** Design and idea stage of the article BB, FGB; PRGN analysis part BB, GCK, BBL, BT; CT in statistical analysis; VA; In the fieldwork, ET, MG, FGB, HK, HAU, BB, SA, ZP, UÜ has taken part. All authors read and approved the final manuscript.

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