



COMPARATIVE STUDY ON PLASMA FIBRINOGEN AND CERULOPLASMIN CONCENTRATIONS DURING PREGNANCY AND POSTPARTUM PERIOD IN BULGARIAN NATIVE GOATS

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Summary

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The goal of this study was to determine the plasma concentrations of the acute phase proteins fibrinogen and ceruloplasmin during the pregnancy and postpartum period in goats. The experiment was carried out with six clinically healthy Bulgarian native goats, aged 4–6 years, weighing 49–56 kg, reared at the Biobase of the Faculty of Veterinary Medicine – Stara Zagora. Blood samples for assay of plasma concentrations of the studied acute-phase proteins during pregnancy were collected over 7-day intervals, from the 14th to the 147th day of pregnancy. During the postpartum period, samples were obtained at 3-day intervals between the 1st and 15th postpartum day, as well as once on the 20th and 30th day after the parturition. The results indicated that throughout the first trimester of the pregnancy, the highest average plasma fibrinogen values were established on the 14th day, while significantly lower values ($P < 0.05$) were measured on the 21st day. At this period, the values of plasma ceruloplasmin were the lowest on the 28th day and significantly higher ($P < 0.01$) at the end of the first pregnancy trimester. During the second trimester of the pregnancy there was a clear tendency towards a decrease in the levels of the examined acute phase proteins. At the last pregnancy trimester, an increase in the levels of both acute phase proteins was observed. During the postpartum period there was a significant decrease ($P < 0.05$) in the levels of ceruloplasmin and fibrinogen on the 20th day, compared to the first postpartum day. The established average values of the examined acute phase proteins could be used for comparison purposes in cases of pathological conditions during the pregnancy and post partum period of goats.

Key words: ceruloplasmin, fibrinogen, goats, postpartum period, pregnancy

INTRODUCTION

Acute phase proteins (APPs) are considered to be non-specific immune factors and the change in their concentration reflects disturbances of homeostasis in animals (Murata *et al.*, 2004). These proteins include protease inhibitors (alpha 1 anti-trypsin, alpha 1 antichymotrypsin), coagulation proteins (fibrinogen, prothrombin), complement proteins (C2, C3, C4, C5, etc.), transport proteins (haptoglobin, ceruloplasmin, hemopexin) and some other proteins like C-reactive protein, serum amyloid A, serum amyloid P, acid glycoprotein etc. (Pradeep, 2014).

The synthesis of APPs by hepatocytes is induced from a number of inflammatory mediators (cytokines): IL-1, IL-6, TNF α . These cytokines are mainly produced by macrophages in response to various external and internal stimuli (Nakagawa-Tosa *et al.*, 1995; Alsemgeest *et al.*, 1996; Yoshioka *et al.*, 2002). Extrahepatic APPs production is also possible in most mammals (Uhlar & Whitehead, 1999; Vreugdenhil *et al.*, 1999; Ceciliani *et al.*, 2012).

Inflammatory process or tissue injury are the most common causes of cytokine release, which cause APPs synthesis (Parra *et al.*, 2005). In addition to inflammatory conditions, they change their plasma levels in normal physiological conditions, such as pregnancy (Kustritz, 2005). Various authors have studied APPs levels with respect to pregnancy diagnosis in the dog (Eckersall *et al.*, 1993; Vanucchi *et al.*, 2002; Kuribayashi *et al.*, 2003; Ulutas *et al.*, 2009).

Increased plasma levels of IL-6 and APPs were observed in humans and experimental animals exposed to psychological and physical stress (Deak *et al.*, 1997; Nukina *et al.*, 2001). The physical stress can cause changes in APPs levels in cattle (Murata & Miyamoto, 1993; Al-

semgeest *et al.*, 1995). A number of studies have shown that stress may provoke an increase of APPs levels in healthy cows (Alsemgeest *et al.*, 1993; Uchida *et al.*, 1993) and mares (Yamashita *et al.*, 1991; Taira *et al.*, 1992) during the last week of the pregnancy, as well as in dogs during parturition (Vannucchi *et al.*, 2002). Normal pregnancy is associated with increased oxidative stress and changes in levels of certain steroid hormones (Wand *et al.*, 1991; Vannucchi *et al.*, 2002). It has been shown that hormones such as estrogen and progesterone instigated cytokine synthesis during pregnancy in humans (Luppi, 2003).

The literature review shows that APPs are much less studied in small ruminants, than in large ruminants (Gonzalez *et al.*, 2008; Ceciliani *et al.*, 2012; Tothova *et al.*, 2014; Iliev & Georgieva, 2018; 2019). According to Tothova *et al.* (2014), despite the clinical value of their determination, the information on use in sheep and goats is scarce. APPs levels in goats have been studied in healthy animals (Heller & Johns, 2015), during the first days of the postpartum period (Janku *et al.*, 2011; Tharwat & Al-Sobayil, 2015), pregnancy toxemia (González *et al.*, 2011; Albay *et al.*, 2014), subacute rumen acidosis (Gonzalez *et al.*, 2010) experimentally induced *Staphylococcus aureus* mastitis (Fasulkov *et al.*, 2014; Sadiq *et al.*, 2019), experimental coccidiosis (Hashemnia *et al.*, 2011). There is no information for determination of the levels of fibrinogen and ceruloplasmin during pregnancy and the postpartum period in goats in Bulgaria.

The purpose of this study was to determine the plasma concentrations of the acute-phase proteins fibrinogen and ceruloplasmin during the pregnancy and postpartum period in Bulgarian native goats.

MATERIALS AND METHODS

Experimental animals

The experiment was carried out with six clinically healthy Bulgarian native goats, aged 4–6 years, weighing 49–56 kg, bred at the Biobase of the Faculty of Veterinary Medicine – Stara Zagora. The animals' feed included meadow and alfalfa hay, compound pelleted feed for small ruminants, and free access to water. The experiment was performed in compliance with the minimum requirements for protection and welfare of experimental animals according to Ordinance 20 of November 1, 2012 of the Ministry of Agriculture, Food and Forestry of the Republic of Bulgaria. In connection with these requirements, we used a minimum number of goats in the present study.

Clinical examination and ultrasound pregnancy diagnosis

A clinical examination of the goats was performed before the start of the experiment. The body temperature, pulse rate, respiratory rate, colour of visible mucous membranes and rumen movements were reported. Throughout the study, the parameters characterising the health of the animals were within the normal reference values.

Ultrasound examinations were performed using an ultrasound machine SonoScape A5 Vet (SonoScape, China) with multifrequency linear transducer (5.0–12.0 MHz) and transrectal ultrasonographic approach. All animals included in the study were diagnosed with pregnancy on day 25 after mating. The ultrasound criteria for early pregnancy diagnosis were visualisation of enlarged uterine lumen, filled with anechoic amniotic fluid, presence of hyperechoic embryo(s) with perceptible cardiac activity.

Assay of fibrinogen concentration

Plasma fibrinogen concentrations were determined by the nephelometric method of Podmore (Todorov, 1972). In a tube, 0.25 mL of plasma was mixed with 2.5 mL 10.5% Na₂SO₄. An empty control sample containing 0.25 mL plasma and 2.5 mL 0.9% saline solution was prepared. Absorbance was recorded after 3 minutes at a wavelength of 570 nm. The result was calculated by multiplying by a factor calculated from a standard curve constructed from different plasma dilutions.

Assay of ceruloplasmin concentration

Plasma ceruloplasmin concentrations were determined by the Revin method based on p-phenylenediamine oxidation (Bestujeva & Kolb, 1982). Ceruloplasmin and p-phenylenediamine formed a coloured oxidation product at pH 5.5, and the change in absorbance was determined at a wavelength of 530 nm.

Experimental design

The blood samples were obtained in sterile vacutainers from the *vena jugularis externa* of each animal, restrained by an assistant after trimming the hair and dipping the skin with 5% iodine tincture solution. Blood samples for assay of plasma concentrations of the studied APPs during pregnancy were collected at 7-day intervals, from the 14th to the 147th day of pregnancy. During the postpartum period, samples were obtained at 3-day intervals between the 1st and 15th postpartum day, as well as on the 20th and 30th day after the parturition.

Statistical analysis

The obtained results were processed by ANOVA using the Student's *t*-criterion

with statistical software StatSoft (Statistica 7, Microsoft Corp. 1984-2000 Inc.). The data were presented as mean and standard deviation. The differences in the obtained values were considered statistically significant at $P < 0.05$.

RESULTS

During the first pregnancy trimester, the highest mean values of plasma fibrinogen were found on day 14 (4.57 ± 0.8 g/L), and were significantly lower ($P < 0.05$) on the 21st day of gestation (3.51 ± 1.09 g/L). During this period, ceruloplasmin levels were the lowest on day 28 (146.1 ± 31.2 mg/L) and significantly ($P < 0.01$) higher at the end of the first pregnancy trimester (216.9 ± 53.9 mg/L) (Table 1).

During the second pregnancy trimester there was a clear tendency towards a decrease in the levels of the examined APPs. At the end of the study period (98th day of pregnancy), mean plasma fibrinogen (3.14 ± 0.8 g/L) was significantly lower ($P < 0.05$) than levels on day 56 of pregnancy. A similar pattern in the values of ceruloplasmin was found out: significantly lower ($P < 0.05$) on day 91 compared to day 56 of pregnancy (Table 2).

During the last pregnancy trimester, an increase was observed in the levels of both APPs (Table 3). Immediately before parturition (147 day of pregnancy), mean values of ceruloplasmin (231.5 ± 22.2 mg/L) were significantly higher ($P < 0.001$) than those on day 105. Plasma fibrinogen concentrations were also statistically significantly different ($P < 0.01$) before parturition compared to the beginning of the third pregnancy trimester.

Table 1. Plasma fibrinogen and ceruloplasmin concentrations (mean \pm SD; n=6) during the first pregnancy trimester in goats

Day of pregnancy	Acute phase protein	
	Fibrinogen, g/L	Ceruloplasmin, mg/L
14	4.57 ± 0.80	175.7 ± 27.1
21	$3.51 \pm 1.09^*$	184.2 ± 47.4
28	4.47 ± 1.07	146.1 ± 31.2
35	$3.60 \pm 0.90^*$	166.7 ± 40.2
42	4.47 ± 1.10	177.8 ± 44.4
49	4.32 ± 0.60	$216.9 \pm 53.9^{**}$

* $P < 0.05$ vs day 14; $P < 0.01$ vs day 28.

Table 2. Plasma fibrinogen and ceruloplasmin concentrations (mean \pm SD; n=6) during the second pregnancy trimester in goats

Day of pregnancy	Acute phase protein	
	Fibrinogen, g/L	Ceruloplasmin, mg/L
56	4.08 ± 0.7	201.7 ± 33.8
63	3.82 ± 0.5	202.8 ± 39.3
70	3.48 ± 0.6	195.6 ± 47.6
77	3.30 ± 0.8	193.4 ± 44.05
84	3.58 ± 1.0	167.8 ± 28.05
91	$3.16 \pm 0.7^*$	$156.9 \pm 30.8^*$
98	$3.14 \pm 0.8^*$	174.4 ± 40.5

* $P < 0.05$ vs day 56.

During the postpartum period there was a significant decrease ($P < 0.05$) in the levels of ceruloplasmin and fibrinogen on the 20th day, compared to the first postpartum day (Table 4).

Comparing averaged values during the different stages of pregnancy and the postpartum period, a significant decrease in fibrinogen levels was found out in the second ($P < 0.05$) and third ($P < 0.001$) pregnancy trimester compared to the first pregnancy trimester, as well as an increase ($P < 0.05$) during the postpartum period compared to the third pregnancy trimester

Table 3. Plasma fibrinogen and ceruloplasmin concentrations (mean±SD; n=6) during the third pregnancy trimester in goats

Day of pregnancy	Acute phase protein	
	Fibrinogen, g/L	Ceruloplasmin, mg/L
105	2.62±0.4	166.5±13.2
112	2.72±0.6	169.02±12.2
119	2.67±0.6	155.02±19.9
126	3.11±0.6	183.02±9.6
133	2.76±0.5	204.5±26.6
140	3.22±0.7	218.7±41.4
147	3.36±0.4**	231.5±22.2***

** P<0.01 vs day 105; *** P<0.001 vs day 105.

Table 4. Plasma fibrinogen and ceruloplasmin concentrations (mean±SD; n=6) during the postpartum period in goats

Postpartum day	Acute phase protein	
	Fibrinogen, g/L	Ceruloplasmin, mg/L
1	4.44±1.1	220.8±63.0
3	4.21±0.9	184.9±71.2
6	4.54±1.1	144.8±59.1
9	3.66±1.5	153.2±61.2
12	3.14±1.5	162.7±48.9
15	3.33±0.8	135.8±35.5
20	3.20±0.7*	112.5±31.3**
30	3.22±1.3	130.1±72.9*

* P<0.05 vs day 1; P<0.01 vs day 1.

(Fig. 1). Averaged ceruloplasmin levels were significantly (P<0.05) higher in the second and third pregnancy trimester vs the postpartum period (Fig. 2).

DISCUSSION

Physiologically, fibrinogen is a heterogeneous protein synthesised in the liver and found in the circulating blood during the acute phase of inflammation (Adler *et al.*, 2000). The analysis of the obtained results

showed that the highest values of fibrinogen in goats are observed during the first trimester of pregnancy and the beginning of the postpartum period.

According to Vanucchi *et al.* (2002) significant increases in plasma fibrinogen in pregnant bitches were observed 28 days after the LH peak, i.e. in the second half of pregnancy, with the highest values (319±133 mg/dL) being detected during the 5th week of gestation. For this reason, the authors believe that during this period the plasma concentration of fibrinogen may be used as a method for pregnancy diagnosis in dogs. In contrast, Ulutas *et al.* (2009) reported the highest fibrinogen levels during the first half of pregnancy (773±68 mg/dL) in dogs. We also believe that the higher levels of fibrinogen found in Bulgarian native goats during the first pregnancy trimester are explained by the synthesis of cytokines due to an inflammatory reaction from embryonic implantation and placental formation.

The highest values of plasma ceruloplasmin in goats were found at the end of the first trimester of pregnancy and immediately before parturition. The ceruloplasmin plays an important role in the transport and metabolism of copper (Jain, 1989; Gruys *et al.*, 1994). In humans, a link between copper and 17-β estradiol concentrations was demonstrated. This hormone was also thought to stimulate ceruloplasmin synthesis, therefore this acute phase protein can be used as an indicator of placental function (Haram *et al.*, 1983). Vannucchi *et al.* (2002) have found significantly higher plasma levels of ceruloplasmin in pregnant than in non-pregnant animals during the second week of gestation. According to the authors, this acute phase protein may be an indicator of embryo implantation and placental development. The authors found a gradual in-

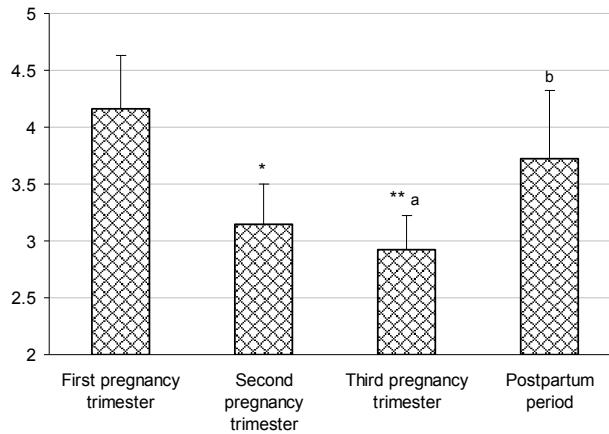


Fig. 1. Average plasma fibrinogen concentrations (g/L) in goats during the pregnancy and the postpartum period (mean±SD; n=6); *P<0.05 between first and second pregnancy trimester; **P<0.01 between second and third pregnancy trimester; ^aP<0.001 between third and first pregnancy trimester; ^bP<0.05 between third pregnancy trimester and postpartum period.

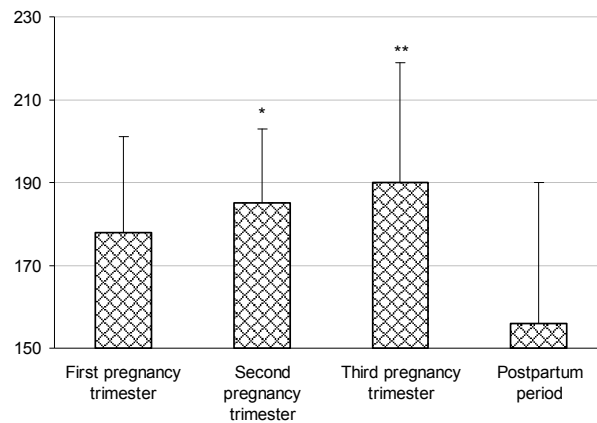


Fig. 2. Average plasma ceruloplasmin concentrations (g/L) in goats during the pregnancy and the postpartum period (mean±SD; n=6); *P<0.05 between second pregnancy trimester and postpartum period; **P<0.05 between third pregnancy trimester and postpartum period.

crease in ceruloplasmin levels until the 5th week of gestation in bitches, followed by a decrease around the 8th week of pregnancy, followed by a second peak a few days before parturition, which is once again evidence that the increased concentration of 17-β estradiol in late pregnancy stimulates the synthesis of ceruloplasmin.

Ulutas *et al.* (2009) also found a significant increase in ceruloplasmin levels in dogs and supported the theory that this condition may be an indicator of the body's adaptation to pregnancy. Studies by Mas & Sarkar (1992) showed that increased ceruloplasmin in late pregnancy is

associated with the need to transport copper to the foetus.

Gursel *et al.* (2010) investigated and compared plasma ceruloplasmin levels between non-pregnant, pregnant sheep fed a balanced ration and pregnant sheep fed an energy-deficient ration. Thus, the authors found significant differences in ceruloplasmin levels between groups only in the last week of pregnancy. The probable cause, according to them, was also in the increased level of 17- β estradiol at the end of pregnancy and immediately before parturition. In addition, under normal conditions, oxidative stress in pregnant animals is increased (Wand *et al.*, 1991). Ceruloplasmin has an antioxidant activity and acts as a protective mechanism against oxidative cell damage (Agroyannis *et al.*, 1993). Gursel *et al.* (2010) believe that ceruloplasmin can be used as a sensitive indicator of energy deficiency in the last days of pregnancy and thus changes in its levels in sheep with pregnancy toxemia may have significant clinical significance. This statement is supported by the studies of Czopowicz *et al.* (2017) in pregnant goats who also demonstrated the highest levels of acute-phase protein serum amyloid A in the last week of pregnancy.

Our results indicate that during the postpartum period the highest values of fibrinogen and ceruloplasmin were established in the first week after parturition. Similar results in Saanen goats have been reported by Samimi *et al.* (2020), who studied the levels of haptoglobin, serum amyloid A, ceruloplasmin and fibrinogen. The high acute-phase protein response after parturition is explained by the significant physiological stress that usually subsides in the second week of the postpartum period. The results demonstrating high levels of acute-phase proteins in the first week of the postpartum period (Al-

semgeest *et al.*, 1993; Koets *et al.*, 1998; Coutinho da Silva *et al.*, 2013) are in line with this statement.

In conclusion, the analysis of the results showed that plasma concentrations of fibrinogen and ceruloplasmin increased during the first pregnancy trimester and immediately before parturition. During the postpartum period there was a significant decrease in their average values after the 20th day. The obtained values of the studied acute-phase proteins could be used for comparison in cases of pathological changes during pregnancy and the postpartum period in goats.

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