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Serum Haptoglobin Levels in Complicated Pregnancies in Cows

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ABSTRACT

This study was carried out to determine whether the serum haptoglobin levels of pregnant cows at the 6th month can be a marker in determining whether complications may develop during pregnancy or during the birth process. A total of 28 animals were included in the study, 14 cows with complicated pregnancy (abortion = 8, dystocia = 6), and 14 cows with healthy birth by following the pregnancy and birth processes in cows from the same farm. Blood samples were collected during the sixth month of their pregnancy. Serum haptoglobin, total protein, albumin, and globulin analyses were performed. Serum haptoglobin, total protein, globulin, and alb/glb values were statistically significant in the complicated pregnancy (abortion, dystocia) group compared to the control group ($p<0.05$). Both the abortion and dystocia groups had significantly higher haptoglobin levels than the control groups ($p = 0.000$). There is a statistical difference in total protein ($p = 0.026$), globulin levels ($p = 0.01$), and alb/glb levels ($p = 0.022$) between the control and dystocia groups. As a result, it was concluded that haptoglobin may be useful in the follow-up of pregnancies with complications, which cause significant physiological effects in cows.

Keywords: Complicated pregnancy, Cow, Haptoglobin

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Introduction

The acute phase is the defense system that is activated by response, trauma, infection, stress, neoplasia, and inflammation. It plays a role as a part of the immune system in protection from infections, inflammation, clearance of pathogens, and healing processes. The increase in plasma proteins such as haptoglobin, serum amyloid A, and fibrinogen, which are part of the acute phase response, is used in the diagnosis and prognosis of diseases (Taşçene, 2017). Haptoglobin (Hp) is a hemoglobin-binding serum protein that is produced by the liver. While its elevation is not observed in healthy bovine serum, its course is evident in the case of inflammation as an acute phase protein (Morimatsu et al., 1991).

Offspring death and abortion can be seen at any stage of intrauterine development during pregnancy. Births that occur before 200 days of gestation in cows are defined as abortions. Nutritional problems, hormonal imbalances, poisoning, traumas, heredity, and non-infectious or infectious factors such as bacteria, fungi, and viruses are among the causes. Dystocia refers to cases where the cow's or calf's life is endangered if the birth does not occur at a certain time or cannot be performed without human intervention. The rates of dystocia in cows vary from 1–10% compared to normal births. Dystocia may develop depending on nutrition, heredity, traumas, infections, the environment, and climatic conditions. In addition to maternal-origin dystocia, which includes problems related to the birth canal, fetal causes, including the size, position, and number of twins, can cause dystocia (Alaçam, 2010).

Pregnancies can have complications such as twin pregnancy, dystocia, stillbirth, abortion, retained fetal membrane, and metritis, which influence yield and reproductive performance in dairy cows. These complications can cause deterioration in dairy cows' performance and can significantly affect reproductive efficiency (Hossein-Zadeh, 2013). It is extremely important to detect and follow the processes of complicated pregnancies that cause physiological effects such as calf deaths, infertility, and decreases in milk yield

in dairy cows (Dematawena and Berger, 1997; Oakes et al., 2001).

Haptoglobin is an acute-phase protein with diagnostic value in dairy cows (Bhat et al., 2020). However, many studies in cows (Aziz and Mohammed, 2016; Bayyit and Merhan, 2020; Bhat et al., 2020; Macmillan et al., 2020; Gädicke et al., 2021) have associated haptoglobin levels with various complications such as dystocia and abortion in pregnancy. While the variability in haptoglobin levels is observed in studies close to birth or in the postnatal processes, in this study, the measurement of serum haptoglobin levels during pregnancy was carried out to have information about the determination of cows at risk of pregnancy with complications.

This study was carried out to determine whether the serum haptoglobin levels of pregnant cows can be used as a marker for the determination of complicated pregnancies that may be encountered during pregnancy or during the birth process.

Material and Methods

This study was approved by the Sivas Cumhuriyet University Animal Research Ethics Committee on 03.12.2021 and was numbered 492.

A total of 28 Montofon, Simental cows that are between 3 and 5 years old were included in the study: 14 cows with healthy pregnancies and 14 cows with complicated pregnancies (dystocia, abortion) whose birth processes were followed on the same farm in Sivas. At the 6th month of their pregnancy, blood samples were taken from the vena jugularis into yellow-capped biochemistry tubes. After the samples were taken, they were centrifuged at 3000 rpm for 10 minutes. The obtained sera were transferred to eppendorf tubes and stored at -20 °C and then at -80 °C until analysis.

Serum albumin, globulin, and total protein measurements were made with an autoanalyzer (Mindray BS200). The levels of haptoglobin in serum samples were determined by a competitive ELISA (enzyme-linked immunosorbent assay) method using the bovine haptoglobin Phase TM enzyme immunoassay kit from Tridelta Company Ireland (Cat. No. TP-801) on a microplate reader (Tecan Sunrise) according to kit procedures. The verification of elisa kit method for serum haptoglobin determination, the intra-assay CVs were 5.3 % and 6.3 % (n=32) and the inter-assay CVs were 5.7 % and 4.1 % (n=64) respectively.

Free hemoglobin shows peroxidase activity that is inhibited at low pH. Haptoglobin combines with hemoglobin in the sample, maintaining peroxidase activity at a low pH. The peroxidase activity of hemoglobin is proportional to the amount of haptoglobin present in the sample.

After adding 7.5 µl of samples and standards to the wells, 100 µl of reagent-1 and 140 µl of reagent-2 were added. It was incubated for 5 minutes at room temperature. Following incubation, the absorbances of standards and samples were measured in an ELISA device at 600 nm. A calibration curve was created, and haptoglobin levels were calculated as µg/ml against the absorbance of the samples.

Statistical Analysis

The SPSS 22.00 program was used for analyzing data (SPSS, 2014). The Shapiro-Wilks test was used to determine the normality of data distributions. The Man Whitney U test was used to compare nonparametric variables. The One-way Anova test was used to compare parametric variables. The difference had a significance value of $p<0.05$.

Results

The serum haptoglobin, total protein, albumin, globulin, and alb/glb results of the complicated and control pregnant groups are given in Table 1. Serum haptoglobin, total protein, globulin, and alb/glb values were found to be statistically significant in the complicated pregnancy group compared to the control group ($p<0.05$).

The serum haptoglobin, total protein, albumin, globulin, and alb/glb results of the abort, dystocia, and control pregnant groups are given in Table 2. Both the abort and dystocia groups had significantly higher haptoglobin levels than the control groups ($p=0.000$). There is a statistical difference in total protein ($p=0.026$), globulin levels ($p=0.01$), and alb/glb levels ($p=0.022$) between the control and dystocia groups.

Table 1. Analysis results of complicated and healthy pregnant groups

	Groups	N	Mean±SE	p
Haptoglobin (µg/ml)	Control	14	46.71±2.98	0.000*
	CP	14	226.64±24.09	
Total Protein (g/dl)	Control	14	7.58±0.12	0.006*
	CP	14	8.52±0.28	
Albumin (g/dl)	Control	14	3.34±0.04	0.581
	CP	14	3.31±0.08	
Globulin (g/dl)	Control	14	4.25±0.15	0.002*
	CP	14	5.21±0.24	
Alb/Glob	Control	14	0.80±0.04	0.008*
	CP	14	0.65±0.03	

CP: Complicated Pregnant; P<0.05*

Table 2. Analysis results of abort, dystocia, and healthy pregnant groups

	Groups	N	Mean±SE	p
Haptoglobin (µg/ml)	Control	14	46.71±2.98[b]	0.000*
	Abortion	8	253.37±37.71[a]	
	Dystocia	6	191.00±20.51[a]	
Total Protein (g/dl)	Control	14	7.58±0.12[b]	0.026*
	Abortion	8	8.56±0.42[a]	
	Dystocia	6	8.47±0.40[a]	
Albumin (g/dl)	Control	14	3.34±0.04	0.809
	Abortion	8	3.28±0.15	
	Dystocia	6	3.36±0.05	
Globulin (g/dl)	Control	14	4.25±0.15[b]	0.01*
	Abortion	8	5.28±0.31[a]	
	Dystocia	6	5.11±0.40[a]	
Alb/Glob	Control	14	0.80±0.04[b]	0.022*
	Abortion	8	0.63±0.03[ab]	
	Dystocia	6	0.68±0.05[a]	

[a, b]: Those with different superscripts in the same row are statistically different.

Discussion

Abortion and dystocia are important pregnancy complications for both cow and calf health and production activities. Stillbirth risks may occur in cows developing dystocia, and mortality rates that can develop within 30 days can be observed in neonatal calves (Lombard et al., 2007). In addition, it can negatively affect production, fertility, morbidity, and mortality rates in both cows and calves (Dematawena and Berger, 1997). Abortions may also result in reduced milk production and reproductive performance (Keshavarzi et al., 2020). Haptoglobin, an acute-phase protein, has diagnostic value in dairy cows. Haptoglobin levels have been found to increase 5.51 times in cows (Pohl et al., 2015). It is evaluated not only in inflammatory conditions but also in conditions that can be seen in the prepartum and postpartum periods (Chan et al., 2004; Pohl et al., 2015; Aziz and Mohammed, 2016; Bayyit and Merhan, 2020; Bhat et al., 2020; Macmillan et al., 2020).

Chan et al. (2004) analyzed basal haptoglobin levels in Taiwan and took blood from 10 cows every week for one year in their study. Basal haptoglobin levels were calculated to be less than 73.6 mg/L. They observed that the results were not affected by pregnancy or seasonal differences. Haptoglobin levels were found to be 1133.5±627.1 mg/L in patients with postpartum reproductive diseases and 104.6±61.0 mg/L in healthy postpartum heifers. They concluded that haptoglobin could be a useful indicator of postpartum reproductive diseases.

Aziz and Mohammed (2016) compared serum haptoglobin levels in cows with dystocia and cows with normal calving. Blood samples were taken from cows with normal calving once every three days for the last two weeks before calving and at 3, 7, and 10 days after calving. In the cows with dystocia, blood was taken at 3, 7, and 10 days after calving. Haptoglobin levels were found to be 300±140 mg/L in normal parturition and 360±240 mg/L in dystocia cows on day 0; 330±150 mg/L, 660±220 mg/L on day 3; 230±90 mg/L, 510 ± 300 mg/L on day 7; and 220±160 mg/L, 400±110 mg/L on day 10. Haptoglobin levels were 250±90 mg/L at the time of delivery and 670±230 mg/L on the third postpartum day in single offspring, 520±180 mg/L in twin offspring, and 320±80 mg/L in twin offspring. They observed that haptoglobin levels increased in cows with dystocia, while they decreased between 7 and 10 days after calving. They found that haptoglobin levels on postpartum days 3 and 7 could be an indicator of complications.

Bayyit and Merhan (2020) evaluated the levels of oxidative stress and acute phase proteins in normal parturition and in cows with dystocia in their study. In blood samples taken after birth (3–48 hours), haptoglobin and albumin levels were determined as 0.176±0.007 g/L and 3.15±0.08 g/dL in cows with normal calving; 0.842±0.013 g/L and 2.91±0.08 g/dL in cows with dystocia. They found a decrease in reduced glutathione and albumin levels, while malondialdehyde, ceruloplasmin, and haptoglobin levels were increased in cows with dystocia.

Aziz and Mohammed (2016) evaluated haptoglobin levels in the group with dystocia in terms of complications that may occur after birth in their study; Bayyit and Merhan (2020) evaluated serum haptoglobin levels in dystocia cows by taking blood samples within 48 hours after birth and found that it increased in the dystocia group. In this study, serum haptoglobin levels were measured in the sixth month of their pregnancy, and the increased haptoglobin levels in the dystocia group are similar to those in these studies (Aziz and Mohammed, 2016; Bayyit and Merhan, 2020).

Bhat et al. (2020) evaluated the haptoglobin levels in the periparturient period in 15 clinically healthy pregnant cows in the prenatal period and took blood samples four weeks before and four weeks later. Haptoglobin levels were determined to be 55.60±0.54 mg/dL and 4.80±0.59 mg/dL, respectively. They concluded that increased haptoglobin levels may be a response to transient inflammation and negative energy. Macmillan et al. (2020) evaluated the

nutritional and metabolic profiles, haptoglobin, non-esterified fatty acids (NEFA), magnesium, and liver enzyme levels in cows with early postpartum lactation and pregnancy loss in the first lactation following artificial insemination. In pregnant cows, serum aspartate aminotransferase (AST) levels were 92.3±1.6 vs. 84.6±2.0 U/L, NEFA levels were 0.73±0.02 vs. 0.54±0.02 mmol/L, haptoglobin levels were 0.77±0.04, etc., 0.60±0.05 g/L, Mg levels were 0.86±0.02 vs. 0.89±0.02 mmol/L, and cholesterol levels were 2.1±0.03 vs 2.4±0.04 mmol/L. They found levels of magnesium 0.86±0.02 vs. 0.88±0.02 mmol/L and haptoglobin 0.82±0.1 vs. 0.63±0.09 g/L lower in pregnant cows compared to 150 DIM non-pregnant cows. They found that the serum haptoglobin concentration in cows who experienced pregnancy loss was higher (1.1±0.09 vs. 0.5±0.05 g/L; $P < 0.01$) than that of those who did not experience pregnancy loss. They found that serum AST, NEFA, and haptoglobin concentrations decreased fertility in the early postpartum period, and serum haptoglobin levels had a positive effect on pregnancy loss. Gädicke et al. (2021) took blood samples every month from the 42nd day of their pregnancy until the time of abortion. They analyzed haptoglobin, serum amyloid A, and biochemistry parameters in 18 cows with abortion. Levels of plasma haptoglobin before abortion were lower in cows with a history of the diseases analyzed as a control group (average 0.17 mg/ml) than in cows without positivity or seroconversion to these diseases (average 0.38 mg/ml). In this study, increased haptoglobin results in the prenatal period are similar to the results of Bhat et al. (2020). The higher haptoglobin levels in the abortion group compared to the other groups are also consistent with the results of Macmillan et al. (2020) and Gädicke et al. (2021).

Conclusion

As a result, in this study, serum haptoglobin, globulin, and total protein levels were found to be higher and statistically significant in cows with dystocia, abortion, and complicated pregnancy compared to healthy pregnant cows. Therefore, it has been concluded that haptoglobin may be useful in the follow-up of complicated pregnancies, which are characterized by significant physiological effects in cows.

Conflict of Interest

There is no conflict of interest between the authors.

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