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Influence of Periparturient Period on the Serum Concentrations of Inflammation Biomarkers in Goats

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Abstract: The objective of the present study was to evaluate the influence of the periparturient period on the inflammation biomarkers haptoglobin (Hp), serum amyloid A (SAA) and fibrinogen. For this purpose, during the periparturient period, blood samples from 15 goats were collected at 3 weeks before expected parturition (T0), within 12 hours of parturition (T1) and 3 weeks after parturition (T2). A control group, comprised of 10 non-pregnant non-lactating goats was sampled at parallel time points. The total white blood cell count did not differ significantly among the values at T0, T1 and T2 time points. Compared to a mean value of 0.87 ± 0.3 mg/dL at T0, the serum concentration of Hp increased significantly at T1 to reach a mean value of 6.1 ± 0.8 mg/dL. The Hp serum concentration was then decreased significantly at T2 to a value of 0.26 ± 0.12 mg/dL compared to T0 and T1.Compared to a mean value of 164 ± 31 ng/mL at T0, the serum concentration of SAA increased significantly at T1 to reach a mean value of 722 ± 44 ng/mL. The SAA serum concentration was then decreased significantly at T2 to a value of 40 ± 22 ng/mL compared to T0 and T1. Compared to a mean value of 254 ± 56 mg/L at T0, the plasma concentration of fibrinogen increased significantly at T1 to reach a mean value of 400 ± 52 mg/L. The plasma fibrinogen concentration then decreased at T2 to a value of 256 ± 43 mg/L that differed significantly from the value at T1. In the control group, none the measured inflammation biomarkers differed significantly among T0, T1 and T2 values. The results of this study showed that an acute-phase reaction was manifested by significant increases in Hp, SAA and fibrinogen, had occurred in the goats around the time of parturition.

Key words: Fibrinogen · Goat · Haptoglobin · Periparturient period · Serum amyloid A

INTRODUCTION

The periparturient period is a physiological state that is known to modify metabolism in the goat [1-6]. During this period, many organs as well as the nervous and endocrine systems are highly mobilized in order to provide optimal conditions for embryonic development [3]. This period is also associated with an increased incidence of metabolic and production-related diseases arising because of inadequate homeorhetic adaptation in metabolism [4-6].

Acute-phase proteins (APPs) are blood proteins that can be used to assess the innate immune systemic response to infection, inflammation or trauma [7,8]. Thus, APP levels during the course of inflammation in an organism reflect the state of immune system activation. They decrease or increase in animals subjected to external or internal challenges [9]. The APPs are produced in the liver during acute phase reaction (APR), the rapid, nonspecific, prominent response to any kind of injury [9,10]. Changes in serum concentrations of APPs closely reflect the onset of APR and the clinical condition of the patient [9].

In ruminants, the major APPs are haptoglobin (Hp) and serum amyloid A (SAA) [7]. In goats, Hp and SAA could be considered as major APPs, while fibrinogen is considered as a moderate one [11]. It is suggested also that APPs could be employed in the assessment of animal health and welfare [9]. In addition, the inclusion of APP measurements in health monitoring programs on a herd basis in livestock has been suggested, not only for the identification of diseased

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animals, but also as a means to identify animals with subclinical disease [11].

In cattle, the serum concentrations of APPs have been determined during the periparturient period [12-15]. It was found that disease cases in periparturient, high-yielding dairy cows were correlated with signs of the accentuated markers of inflammatory phenomena [14]. In goats, the APPs have been proposed as sensitive and rapid indicators of inflammatory disturbances [11, 16]. This study was carried out to investigate the influence of the periparturient period on the serum concentrations of the inflammation biomarkers Hp, SAA and fibrinogen.

MATERIALS AND METHODS

Animals: The experimental protocol was approved by the Animal Ethical Committee, Deanship for Scientific Research, Qassim University, Saudi Arabia. Fifteen non-pregnant does (age, 22.6 ± 8.7 months; weight 42.3 ± 6 kg), reared at Qassim University Farm, were synchronized by inserting the controlled-release internal drug EAZI-BREED CIDR (Pfizer, Auckland, New Zealand), containing 0.3 g progesterone, into the vagina for 12 d. At the time of CIDR removal, each doe received an injection of 600 IU equine chorionic gonadotropin. Two mature fertile bucks were used for breeding the does. A control group, age-matched, comprised of 10 non-pregnant, non-lactating goats (age, 26.2 ± 10.5 months; weight 46.7 ± 10.9 kg) and reared at Qassim University Farm was sampled at parallel time points (3 times, 3 weeks apart). All goats were maintained in a free-stall barn and kept under the *Laboratory Animal Control Guidelines* of Qassim University, which basically conform to the *Guide for the Care and Use of Laboratory Animals* of the National Institutes of Health in the USA (NIH publications No. 86 to 23, revised 1996).

Blood Sampling: Three jugular blood samples were collected from each goat 3 wk before expected parturition (T0), within 12 h of parturition (T1) and 3 wk after parturition (T2). Parallel, 3 blood samples were collected from the control group, 3 wk apart. The first blood sample was collected in EDTA tubes for the determination of white blood cell counts. The second blood sample was collected in citrated tubes for plasma fibrinogen measurement and the third blood sample was collected in plain tubes. Both the second and third blood samples were centrifuged at 1200 × g for 15 min and the citrated plasma and serum samples obtained were aliquotted in tubes and immediately stored at -20°C pending the clinical chemistry analyses.

White Blood Cell, Cortisol, Estrogen and Progesterone Assays: White blood cell counts were carried out using an automated analyzer (VetScan HM5, Abaxis, CA, USA). Estrogen, progesterone and cortisol were determined in the serum samples using electrochemiluminescent immunoassay kits (Roche Diagnostics, Indianapolis, IN, USA), with measuring ranges of 5.00-4300 pg/mL, 0.030-60.00 ng/mL and 0.018-63.4 µg/dL, respectively. The intra- and inter-assay coefficients of variance for estrogen, progesterone and cortisol were 3.7 and 3.8%, 2.2 and 5.0% and 1.22 and 1.54%, respectively.

Acute-phase Proteins Assays: The APPs Hp and SAA were measured in the serum samples with validated methods for goats as previously recorded [11, 16]. Serum concentrations of Hp were quantified by spectrophotometric method using a commercial kit based on the peroxidase activity of the haptoglobin-hemoglobin complex (Phase Haptoglobin Assay, Tridelta Development Ltd., Ireland). The analytical sensitivity of the assay was 0.0005 mg/mL and the intra- and inter-assay CVs were 5-6% and 4-6%, respectively. Serum concentrations of SAA were determined using a commercially available enzyme-linked immunosorbant assay (ELISA) kit (Phase SAA Kit, Tridelta Development Ltd., Ireland) according to the manufacturer's instructions. The analytical sensitivity of the assay was 0.15 µg/mL, with intra- and inter-assay CVs of 4.5% and 6%, respectively. Fibrinogen concentrations were measured with a commercially available ELISA kit validated for use in goats (Life Sciences Advanced Technologies Inc., FL, USA).

Statistical Analysis: Data are presented as means ± standard deviation and were analysed for period effects using a repeated measures ANOVA, with Dunnett's test as the post-hoc test. The Kolmogorov–Smirnov test was used to evaluate data normality. The level of significance among T0, T1 and T2 values in each group was tested at $P<0.05$. Statistical analysis was done with the SPSS statistical package (version 18.0; SPSS, Chicago, IL) [17].

RESULTS

The WBC did not differ significantly among T0, T1 and T2 time points ($P = 0.414$). The serum concentration of progesterone decreased sharply at T1 and at T2 ($P<0.0001$), while the serum concentration of estrogen reached its peak at T1 and declined at T2 ($P<0.0001$).

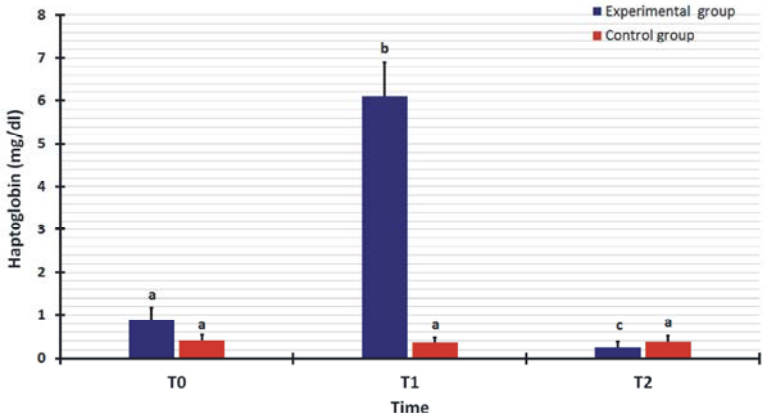

Fig. 1: Serum concentrations of haptoglobin in goats (n=15) during the periparturient period. T0, 3 wk before expected parturition; T1, within 12h of parturition; T2, 3 wk after parturition. Small letters indicate a significant difference among the same group (P<0.5).

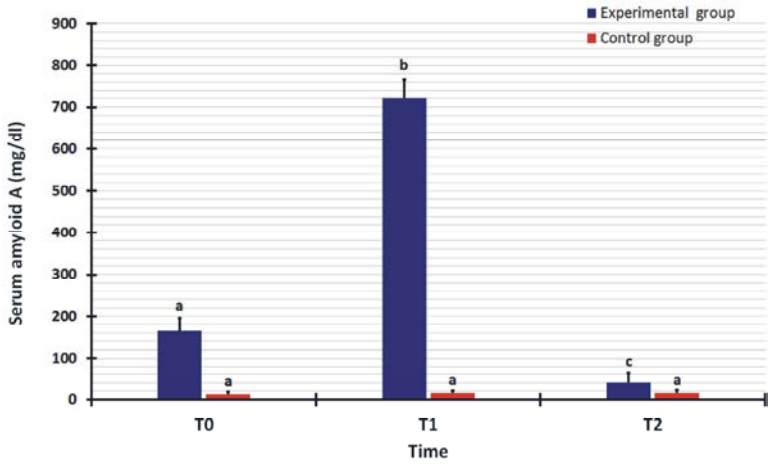

Fig. 2: Serum concentrations of amyloid A in goats (n=15) during the periparturient period. T0, 3 wk before expected parturition; T1, within 12h of parturition; T2, 3 wk after parturition. Small letters indicate a significant difference among the same group (P<0.5).

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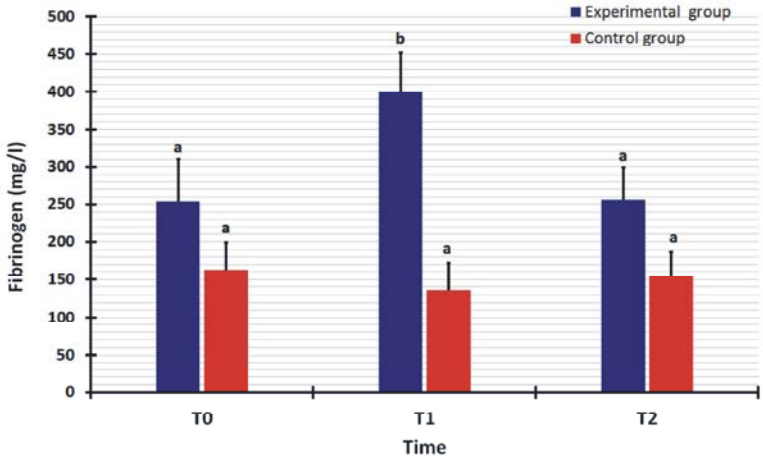

Fig. 3: Serum concentrations of fibrinogen in goats (n=15) during the periparturient period. T0, 3 wk before expected parturition; T1, within 12h of parturition; T2, 3 wk after parturition. Small letters indicate a significant difference among the same group (P<0.5).

Compared to a value of 0.9 ± 0.4µg/dL at T0, the serum concentration of cortisol increased significantly at T1 to 1.47 ± 0.9µg/dL (P = 0.004) then decreased to 0.1 ± 0.1µg/dL at T2. In the control group, the WBC count and the serum concentrations of progesterone, estrogen and cortisol did not differ significantly among T0, T1 and T2 values.

Figure 1 summarizes the serum concentrations of Hp 3 wk before expected parturition (T0), within 12 h of parturition (T1) and 3 wk after parturition (T2). Compared to a mean value of 0.87 ± 0.3 mg/dL at T0, the serum concentration of Hp increased significantly at T1 to reach a mean value of 6.1 ± 0.8 mg/dL (P < 0.0001). The serum concentration of Hp was then decreased significantly at T2 to a value of 0.26 ± 0.12 mg/dL (P< 0.0001) compared to T0 and T1 values.

Figure 2 summarizes the serum concentrations of SAA at T0, T1 and T2 time points. Compared to a mean value of 164 ± 31 ng/mL at T0, the serum concentration of SAA increased significantly at T1 to reach a mean value of 722 ± 44 ng/mL (P < 0.0001). The SAA serum concentration was then decreased significantly at T2 to a value of 40 ± 22 ng/mL (P< 0.0001) compared to T0 and T1 values.

Figure 3 summarizes the plasma concentrations of fibrinogen at T0, T1 and T2 time points. Compared to a mean value of 254 ± 56 mg/L at T0, the plasma concentration of fibrinogen increased significantly at T1 to reach a mean value of 400 ± 52 mg/L (P < 0.0001). The plasma fibrinogen concentration then decreased at T2 to a value of 256 ± 43 mg/L, that did not differ significantly from value at T0 (P = 0.94), but differed significantly from the value at T1 (P < 0.0001). In the control group, none the measured inflammation biomarkers Hp, SAA and fibrinogen differed significantly among T0, T1 and T2 values (Figures 1-3).

DISCUSSION

To the authors' knowledge, this is the first study to investigate the effect of the periparturient period on the serum concentrations of the inflammation biomarkers Hp, SAA and fibrinogen in goats. Compared to values 3 wk before and after parturition, all of these biomarkers increased significantly at parturition.

The acute-phase response (APR) is a rapid, nonspecific, systemic response occurring secondary to many types of tissue injury and might be a physiological protective mechanism during inflammatory events [18]. The origin of APR can be attributable to infection, inflammation, surgical trauma, or other causes [8,19]. The purpose of APR is to restore homeostasis and to remove the cause of its disturbance [19]. The changes in APPs due to various inflammatory and non-inflammatory conditions have been studied intensively in many animal species [7]. The APPs have received attention as biomarkers for APR due to their facilitating properties that include low physiological levels, the fast incline and marked rise in concentration during APR that eases detection and the fast decline after a ceased stimulus [19].

In small ruminants, the APPs concentration has been assessed in several different diseases. Of them, pneumonic pasteurellosis in sheep [16], pregnancy toxemia in goats [20] and infectious and parasitic conditions such as ovine caseous lymphadenitis [21], mixed helminth infection [22] and sarcoptic mange [23]. Whereas APPs are proven to rise considerably in acute conditions and remain elevated for up to 2 weeks [24-26].

In dairy cows as well as in the goat [4-6], the periparturient period is a critical period because of the higher probability of developing pathologic disorders related to parturition. The APPs have been used in dairy cows as markers in the periparturient period [12-15,27] and it was increased in diseased cows during the periparturient [14]. In this study, the significant increases of Hp, SAA and fibrinogen at parturition (T1) cannot be associated with pathological conditions as the WBC did not change significantly among T0, T1 and T2 values, thus confirming the absence of pathological conditions. These elevations could be due to a number of factors including cortisol release, stress and hormone changes at T1. Parturition, often considered as a physical stress, represents a variety of physical and psychological stimuli that alter the homeostasis and metabolism of the goat [1]. The mechanism behind the stress-induced APPs release at parturition is not known, but a hypothesis based on a neuroendocrine-immune network concept has recently been put forward, indicating that non-inflammatory and psychophysical stressors activate the combined action of the sympatho-adrenal axis and the hypothalamic–pituitary–adrenal axis. This would affect both the immunity-related cells and the release of glucocorticoids and would ultimately lead to the production and release of APPs [7]. Therefore, the significant increases of the Hp, SAA and fibrinogen at parturition and its decline 3 wk after parturition suggested that parturition is associated with a physiologic APR.

The results of this study showed that APR takes place in goats around the time of parturition. This was manifested by significant increases in Hp, SAA and

fibrinogen. The elevations in the inflammation biomarkers clearly differentiated the physiological response to parturition versus inflammation related to pathologies during the periparturient period. Another study is therefore required to verify the influence of the periparturient period in goats with reproductive and metabolic disturbances.

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