Plasma Haptoglobin concentrations after normal parturition and caesarean operation in ewes with dystocia (preliminary study)

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SUMMARY

The aim of the study was to investigate changes in plasma haptoglobin (Hp) concentrations during an 18 day long period after a caesarean operation or normal parturition in sheep. The control group included six clinical healthy Pleven Blackhead sheep, 2-5 years old, with eutocia. All of them had a normal labour and a total of 10 lambs were delivered. In sheep with dystocia (n = 6, 3 with uterine torsion, 2 with absolute foetal oversize and 1 with ring womb), 3 lambs were born live whereas 6 lambs died in utero 12 to 24 hours before caesarean surgery. The foetal death and delayed uterine involution were observed in all cases of uterine torsion. Plasma haptoglobin (Hp) concentrations were determined on 0 hour (first stage of labour in ewes with eutocia, just before caesarean surgery in ewes with dystocia) then 4 hours and 1, 2, 4, 8, 14 and 18 days after parturition. Whereas the plasma Hp concentrations slowly and gradually increased until the 14th day in ewes with eutocia, they dramatically increased since the 2nd day and reached maximal values on days 4 and 8 compared to initial values in ewes with dystocia. Furthermore, the plasma Hp concentrations in ewes with dystocia (preliminary study) then 4 hours and 1, 2, 4, 8, 14 and 18 days after parturition. Whereas the plasma Hp concentrations slowly and gradually increased until the 14th day in ewes with dystocia, they dramatically increased since the 2nd day and reached maximal values on days 4 and 8 compared to initial values in ewes with dystocia. Furthermore, the plasma Hp concentrations were significantly higher in diseased ewes, particularly in those with foetal death/uterine torsion, than in healthy females, since the first stages of parturition. These results showed that the plasma Hp can be used as a marker of inflammation in sheep with dystocia and can help to decide a surgical intervention.

Keywords: Ewe, parturition, dystocia, caesarean operation, haptoglobin, foetal death.

Introduction

Acute phase proteins (APP) are a group of liver-derived serum proteins, whose concentrations changes in response to body injury, trauma or infection, and they are involved in an immediate series of complex physiological and biochemical reactions, named the acute phase response (APR). The cell responsible for eliciting this response is normally the macrophage or blood monocyte. A local reaction activates macrophages to release various chemicals, such as interleukins (IL-1 and IL-6), tumour necrosis factor (TNF), interferons and other cytokines, which mediate the systemic reaction [2, 20, 27, 28]. During the critical stages of stress, the liver regulates the amount of essential metabolites provided to the organism. This organ also supplies the necessary components involved in protecting the site of tissue damage, limiting tissue destruction, clearing harmful agents and promoting tissue repair [2]. The APR cascade stimulates hepatocytes, via cell-surface receptors, to release acute phase proteins (APP).

The concentrations of these proteins normally maintain a steady state which reflects a balance between synthesis and catabolism. As stress occurs and homeostasis is disrupted, the plasma concentrations of various acute phase reactants may change in magnitude, duration and direction [7]. Collectively, these responses play a vital role in containing the tissue damage and enhancing the processes of repair and resolution [47]. APP proteins are categorized as either positive or negative acute phase proteins, depending on whether they decrease (negative) or increase (positive) in concentration during the acute phase response [17]. There are several known APPs with
distinct species differences. Ceruloplasmin increases about 50% during inflammation or infection [5]. Serum amyloid A (SAA) is a high density lipoprotein and the precursor for amyloid protein A [11]. The α1-anti protease and α2-macroglobulin are anti-proteases [5]. The C-reactive protein (CRP) is a constitutive serum protein in cattle [30], in which the blood concentration does not change in response to tissue damage, while the protein is considered as a major acute phase reactant in humans, dogs [8], pigs [26] and rabbits [11], in which the blood CRP concentrations can increase 100-fold after an injury [6]. By contrast, in normal ruminants, the circulating haptoglobin (Hp) concentrations are negligible, whereas the concentrations of this protein considered as a positive APP increase over 100-fold with immune stimulation [5]. Haptoglobin that binds free haemoglobin in the circulation [7] is the most widely studied acute phase protein in cattle. It has been found to be a useful marker of inflammation in both experimental [5] and clinical investigations [21, 29] in this species and it is considered as a major acute phase reactant in ruminants. In humans and other species [5, 9] there is only a 2 to 4% increase in circulating concentrations, leading to consider the protein in these species contrary to ruminants as a moderate reactant [9, 11]. Moreover, it is accepted that in cattle, Hp is also involved in the regulation of lipid metabolism [34] and act as an immuno-modulator [33].

Haptoglobin functions to bind and transport free circulating haemoglobin to the liver, thus recycling the haemic iron [3]. As it is observed active saturated complexes between haptoglobins from adult or foetus sheep and haemoglobins from different species (sheep, cow, horse, mouse and human) or the foetal heteroproteins, it is suggested that the haptoglobin binding to haemoglobin does not depend on the species and that the binding sites are probably relatively well conserved during the evolution, because of physiological significance of the complex formation. The study of SKINNER and ROBERTS [42] revealed that haptoglobin was a useful marker for the presence of bacterial infection in sheep, and was more sensitive, specific and efficient and less likely gave false positive and negative results than the haematological examination.

In humans, haptoglobin is recognized as an immunosuppressive factor in serum [32] as well as an adiposity marker [39]. In cattle, haptoglobin is considered as a diagnostic marker of inflammation and infections since its concentration is markedly elevated in cases of mastitis, metritis and retained placenta while the Hp concentration remain low in cases of metabolic diseases [10, 41]. It is also reported that the physical stress does not affect haptoglobin concentrations in calves [1] but elevated haptoglobin concentrations were observed in calves transported for 2 days [1]. In addition, a correlation was observed between serum haptoglobin concentrations and lymphopenia. These data suggested that bovine haptoglobin may be involved as an immunomodulator in the suppression of lymphocyte blastogenesis [33]. At parturition, haptoglobin concentrations were elevated in 74% of the cows observed [45]. In calves, experimental injections of Pasteurella hemo-lytica, Ostertagia ostertagi and endotoxin raised serum concentrations of haptoglobin and other APPs [5]. In sheep, haptoglobin has also been shown to be useful as a diagnostic tool in the prognosis of dystocia [40] and it is a reliable indicator of infection [42]. Grass sickness [31] and laminitis [12] in horses induced a rise in the haptoglobin concentration. The highest concentration of serum haptoglobin was found in newborn foals (5.25 ± 2.36 g/L) and this value was maintained until about 12 months of age, and then gradually decreased (2.19 ± 1.54 g/L in adults). In the case of inflammation, the Hp concentrations are increased 1.5 to 9 fold in horses [44]. In dogs, the haptoglobin concentration reaches maximal values 4 to 6 days following surgical trauma [6]. Pigs naturally or experimentally infected with Actinobacillus pleuropneumoniae show an elevation in haptoglobin concentration [18]. EURELL et al. [13] documented a haptoglobin increase following experimentally induced atrophic rhinitis in swine.

This investigation was undertaken to ascertain whether determination of the serum Hp concentration prior to correction of dystocia in sheep provides valuable prognostic information to surgery. As the assay can be performed rapidly before the surgery, the determination of serum Hp may give valuable information in this situation.

**Material and Methods**

**ANIMALS AND PROTOCOL DESIGN**

The study was performed on 12 Pleven Blackhead sheep, 2-5 years old and weighing 45-60 kg fed and housed under uniform conditions and subjected to the standard immunoprophylactic and anthelmintic regimens.

The control group included six clinical healthy sheep with eutocia. All of them exhibited a normal labour and 10 (4x2 and 2x1) lambs were delivered. The puerperium was without complications. The second group was constituted by 6 females with dystocia presented for veterinary assistance: uterine torsion was diagnosed in 3 cases, leading to the death of 6 lambs (3x2) and absolute foetal oversize (2x1 live lambs) and ring womb (1x1 live lamb) in two and one cases, respectively. Immediately after the clinical examination, a caesarean section was performed, using intravenous administration of a xylazine hydrochloride (0.2 mg/kg, Xylazine 2%, Alfasan International B.V., The Netherlands) and a local infiltration of 20 mL novocaine (Novocain 1%, Vetprom, Radomir, Bulgaria) as anaesthesia. For analgesia, methamizol sodium (5 mL, Analgin 30%, Biovet, Peshtera, Bulgaria) was intravenously injected. Among the 9 lambs born from females with dystocia, six were died. The presence of an autolysis suggested that they were dead at least since 12 to 24 hours. The uterine involution was occurred in the most of the patients and only in the cases of a uterine torsion it was delayed. All sheep were hospitalized after surgery for three weeks.

**BIOCHEMICAL ANALYSIS**

Blood samples from each sheep were taken from v. jugularis prior to (0 hour when it was possible) and 4, 24, 48 hours after the part as well as on days 4, 8, 14 and 18. In animals with eutocia, the time-point “0 hour” was accepted as the first stage of labour. However, in animals with dystocia, this time-point was delayed and the first sample collection was performed immediately before the surgery. Blood samples were taken in...
heparinised sterile tubes and were centrifuged immediately (1500g, 15 minutes, 4°C) to obtain plasma. Thereafter, plasmas were decanted and stored at -20°C until assayed. All samples were free of haemolysis.

The plasma concentrations of Hp were determined by the method developed in Reactive Lab, Glasgow, Faculty of Veterinary Medicine according to time in mothers. Briefly, Hp in a serum sample was incubated with haemoglobin (Hb) to form an Hp-Hb complex. After pH reduction and inhibition of the peroxidase activity of unbound Hb by a chromogene, the generated hydrogen peroxide caused a deep blue colour which was measured using a biochemical analyser (Pentra 400, Horiba ABX) and the Hb peroxidase activity was directly proportional to the amount of Hp present in the serum sample. By comparison to standards with known Hp concentrations the assay was calibrated and samples with unknown Hp concentration measured. Unfortunately, plasma Hp concentrations were not measured in calves.

STATISTICAL ANALYSIS

The statistical analysis of the data was performed using one way analysis of variance (ANOVA). The significance of differences of means between post surgery and base line values was evaluated by LSD test. Differences were considered as significant when P values were less than 0.05. All data were expressed as mean ± standard error of the mean (SEM).

Results

Plasma haptoglobin concentrations (g/L) in control sheep (with a normal parturition) and in females subjected to caesarean are presented in Table I.

In control females, the concentrations gradually increased according to time from 0.118 ± 0.019 g/L at the first hour of labour to 0.281 ± 0.038 g/L at the 14th day after the part corresponding to an increase of 138% but variations were not significant.

In females with dystocia, the mean haptoglobin concentrations at the 4th and 8th days after the part were significantly elevated compared to the control group (P < 0.001). The initial mean value recorded immediately before surgery was 0.330 ± 0.077 g/L. Then, the APP concentration gradually increased during the first 4 days after caesarean becoming significantly different from control values since the 2nd day (P < 0.05) for reaching a peak value on day 4 (increase percentage: 163%, 0 hour vs. day 4; P < 0.001). After a slight but not significant decline on day 8 (0 hour vs. day 8; P < 0.05), the Hp concentrations in the operated sheep decreased and reached 0.510 ± 0.090 g/L on day 18 and remained higher but not significantly than the basal values and the values of controls without any dystocia.

However, within the group of ewes with dystocia, the plasma Hp concentrations have greatly differed between ewes with uterine torsion and dead lambs and ewes with other dystocias (Table II). Indeed, it was observed that the mean concentrations of the positive APP at 0 hour and at the 4th and 8th days post surgery were dramatically higher in females with uterine torsion than in the others (P < 0.05).

Discussion

The plasma Hp concentrations before parturition were 0.118 ± 0.019 g/L in clinically healthy sheep and 0.330 ± 0.077 g/L in ewes with dystocia, these results being in agreement with previous findings [40]. Indeed, SCOTT [39] reported that the Hp concentrations in sheep with eutocia were around 0.250 ± 0.270 g/L and that they were roughly similar in sheep subjected to caesarean surgery with live lambs (0.240 ± 0.230 g/L) but they were lower in healthy non-pregnant sheep (0.060 ± 0.015 g/L).

In addition to inflammatory conditions, the acute phase proteins are also released in normal physiological conditions such as pregnancy. ECKERSALL et al. [8] established that blood CRP concentrations in dogs increased moderately but significantly during the second half of gestation. This increase may be related to embryonic implantation, placental growth and hormonal alterations and a delayed increase was also associated to the initiation of parturition [46]. However, available information

<table>
<thead>
<tr>
<th>Time</th>
<th>Haptoglobin (g/L)</th>
<th>P</th>
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<tbody>
<tr>
<td></td>
<td>Normal parturition</td>
<td>With dystocia</td>
</tr>
<tr>
<td>0 hour</td>
<td>0.118 ± 0.019</td>
<td>0.330 ± 0.077&lt;sup&gt;A&lt;/sup&gt;</td>
</tr>
<tr>
<td>4 hours</td>
<td>0.169 ± 0.039</td>
<td>0.301 ± 0.053&lt;sup&gt;A&lt;/sup&gt;</td>
</tr>
<tr>
<td>1 day</td>
<td>0.205 ± 0.032</td>
<td>0.493 ± 0.012&lt;sup&gt;AB&lt;/sup&gt;</td>
</tr>
<tr>
<td>2 days</td>
<td>0.193 ± 0.038&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.558 ± 0.119&lt;sup&gt;AB&lt;/sup&gt;</td>
</tr>
<tr>
<td>4 days</td>
<td>0.245 ± 0.064&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.868 ± 0.274&lt;sup&gt;B&lt;/sup&gt;</td>
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<tr>
<td>8 days</td>
<td>0.241 ± 0.030&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.811 ± 0.234&lt;sup&gt;B&lt;/sup&gt;</td>
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<tr>
<td>14 days</td>
<td>0.281 ± 0.038</td>
<td>0.568 ± 0.106&lt;sup&gt;AB&lt;/sup&gt;</td>
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<tr>
<td>18 days</td>
<td>0.258 ± 0.031&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.508 ± 0.094&lt;sup&gt;AB&lt;/sup&gt;</td>
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NS: not significant.
Different superscripts<sup>a,b</sup> indicate significant differences (P < 0.05 or more) between the 2 groups (normal part vs. caesarean).
Different superscripts<sup>A,B,C</sup> indicate significant differences (P < 0.05 or more) according to time within each group.

Table I: Variations of the plasma haptoglobin concentrations (g/L) according to time after the part in sheep control females (with eutocia, n = 6) and in females with dystocia (n = 6). Results are expressed as mean ± standard error of the mean (SEM).
about the circulating APP concentrations during the different periods of pregnancy or according to the stage of the oestrous cycle was limited. UCHIDA et al. [45] reported increased serum Hp concentrations during pregnancy in cows and especially near parturition, probably because of up-regulation of its expression by cortisol and non-steroid fatty acids [45]. NAZIFI et al. [35] also reported that the Hp concentrations (0.300 ± 0.090 g/L) in dry cows near parturition were slightly higher than in dairy cows (0.120 ± 0.050 g/L) and pregnant cows (0.220 ± 0.030 g/L) and that the APP concentrations measured during pregnancy or lactation were markedly higher than in non-pregnant females (0.080 ± 0.060 g/L). In the non-pregnant females, the circulating Hp concentrations are often bordering the minimal detectable value [35]. In apparently healthy Iranian fat-tailed sheep, the serum Hp concentrations were comprised between 0.050 and 0.180 g/L [35]; in healthy cows, it varied from 0.022 to 0.047 g/L [38]. EURELL et al. [14] reported that the usual values for Hp concentrations were 0.250 - 0.600 g/L in ponies. According to NOWROOZI et al. [36] no significant effect of the age or the gender was evidenced in sheep. GHUMAN et al. [15] found a slight physiologically increase in Hp concentrations in buffalos after normal delivery at the day of birth and several days after and that buffalos exhibiting distorsion during delivery showed significantly higher values of Hp concentrations at 24 and 48 hours after birth (P < 0.05). The concentration of Hp in sheep with normal parturition was 0.118 ± 0.019 g/L, which is in agreement with other findings [34, 35], and 48 hours after the parturition the mean Hp concentration was 64% higher than the initial value. BERTONI et al. [4] reported that serum Hp concentration on the 15th day of lactation was more than 1g/L. According to Taira et al. [44] who investigated the Hp concentration in horses, the serum Hp concentrations were increased 4 months prior to and during parturition and decreased 12 weeks postpartum. In the present experiment, the Hp concentrations remained still elevated compared to initial values until 18 days after parturition.

The results of the present study revealed that Hp was a sensitive marker of inflammatory conditions in sheep subjected to a caesarean operation and could be useful for the decision of a surgical intervention. Indeed, the higher Hp concentrations were observed in ewes with dystocia (uterine torsion) and with dead lambs and this measurement could be serve as a marker of foetal or embryonic loses [46] and to probably differentiate dystocia situations in which dead lambs were present in utero to those in which live lambs were delivered. A serum Hp concentration above 1.0 g/L may represent a poor surgical risk prior to surgery indicating an inflammatory reaction [40]. KOSTRO et al. [25] recommended to regularly determine the circulating Hp concentrations in herd at different breeding or production stages in order to monitor the herd health status and to detect some infectious situations. In the present preliminary study, Hp has appeared as a major APP in ewes, which the concentration markedly increased in inflammatory reaction as reported by HORADAGOJA et al. [22], presenting some diagnostic advantages, notably because of its stability in plasma and its easy analysis and because of its virtual absence in the blood of healthy animals [10]. As the Hp determination in blood is adapted to semiautomatic and robotic analysers and is independent from the species, it may be performed for routine estimation in clinical practice. However, the plasma or serum should not be haemolysed. SKINNER et al. [42] concluded that an Hp concentration above 0.400 g/L indicated significant infection and an Hp concentration around 0.200 g/L would indicate early or mild infection. According to HUZZEY et al. [24], the Hp concentrations peaked on day 3 in cows with mild metritis (1.060 ± 0.150 g/L) and on day 6 in cows with severe metritis (1.620 ± 0.470 g/L) whereas in healthy cows they were 0.580 ± 0.120 g/L and 0.310 ± 0.080 g/L on days 3 and 6, respectively. On the other hand, these authors also considered that dairy cows exhibiting a circulating Hp concentration above 0.400 g/L required an improved clinical surveillance (by monitoring the rectal body temperature, for example) in order to detect the signs of illness [40]. In the case of dairy herds, early detection of disease is critical for improving health management programs.

After infection, inflammation or trauma, increased Hp concentrations may contribute to differentiate healthy cows from diseased ones [1, 9], except in the cases of babesiosis and theileriosis in which the circulating Hp concentrations may become nearly undetectable during a haemolytic crisis. REGASSA and NOAKES [37] found greater Hp concentrations

<table>
<thead>
<tr>
<th>Time</th>
<th>With uterus torsion</th>
<th>With other dystocia</th>
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<tbody>
<tr>
<td></td>
<td>Ewe 1</td>
<td>Ewe 2</td>
</tr>
<tr>
<td>0 hour</td>
<td>0.12</td>
<td>0.37</td>
</tr>
<tr>
<td>4 hours</td>
<td>0.13</td>
<td>0.40</td>
</tr>
<tr>
<td>1 day</td>
<td>0.15</td>
<td>0.80</td>
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<tr>
<td>2 days</td>
<td>0.15</td>
<td>0.90</td>
</tr>
<tr>
<td>4 days</td>
<td>0.60</td>
<td>2.13</td>
</tr>
<tr>
<td>8 days</td>
<td>0.70</td>
<td>0.92</td>
</tr>
<tr>
<td>14 days</td>
<td>0.40</td>
<td>0.85</td>
</tr>
<tr>
<td>18 days</td>
<td>0.41</td>
<td>0.78</td>
</tr>
</tbody>
</table>

Maximal increase (%) 483% 476% 185% 158% 189% 125%

TABLE II: Plasma haptoglobin concentrations (g/L) according to time after caesarian in ewes with uterine torsion (n = 3) or with other dystocia (n = 3).
in postpartum ewes with uterine bacterial contamination than in comparable ewes with normal uterine involution. As haptoglobin binds to haemoglobin and reduces the iron availability in this way, the bacterial proliferation is limited [24]. On the other hand, the serum Hp concentration appears reduced in haemolytic anaemia such as babesiosis, anaplasmosis and theileriosis despite increases in circulating free haemoglobin concentrations during a haemolytic crisis [1]. Haptoglobin is considered as a potent acute phase protein in cattle, having a low constitutive concentration and exhibiting a high relative increase during the acute phase reaction [19]. HUMBERT et al. [23] demonstrated that both Hp and SAA had a low sensitivity, but higher specificity for determining disease status, compared to clinical examinations in cattle. By contrast, SORENSEN et al. [43] recommended to couple the determination of the concentrations of two or three acute phase proteins for improving the infection / inflammation diagnosis. However, a high circulating Hp concentration was observed in ewes after caesarean operation in the current study but it was impossible to conclude if this result was due to the inflammatory process after operation or to an infection process. GONZALEZ et al. [16] reported that a subcutaneous injection of turpentine oil induced an increase in serum Hp, SAA, ASG and fibrinogen concentrations and a decrease in albumin concentrations in goats. However, they concluded that further investigations are required to validate this test as marker of inflammatory diseases in cases encountered in clinical practice.

As a conclusion, this study confirms that parturition was coupled to a moderate increase in plasma haptoglobin concentrations in ewes and also provides evidence that there is an inflammatory response that precedes clinical signs of dystocia and/or foetal death and that this response can be detected by evaluating plasma Hp concentration. Moreover, the response was not associated with inflammation of the udder in the present study because sheep were without clinical mastitis. Consequently, despite the low number of ewes investigated here, it was concluded that the measurement of circulating haptoglobin concentrations in sheep may help to the dystocia diagnosis but further studies conducted on large commercial herds are necessary to explore the APP expression according to the different dystocia causes.

References


An elevated postpartum uterine disease incidence contributes to reduced fertility interfering with the main goal of efficient reproductive management: to have cows pregnant at a biologically optimal and economically profitable time after parturition (Sheldon et al., 2006; Gilbert, 2016). An inflammatory milieu at inappropriate stages of the estrus cycle interferes with fertility by creating suboptimal conditions for sperm transportation and storage, oocyte maturation and ovulation, zygote development, implantation, and embryonic and fetal growth (Gilbert, 2011).