A PRELIMINARY STUDY ON PLASMA HAPTOGLOBIN CONCENTRATIONS IN BUFFALOES FOLLOWING SPONTANEOUS OR ASSISTED DELIVERY

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ABSTRACT

This study was designed to assess the impact of spontaneous or assisted delivery on plasma haptoglobin concentrations. The study consisted of five spontaneously calving and 15 dystocia affected buffaloes. In the latter, fetal delivery was achieved following application of fetal mutation technique (n=10) or following correction of uterine torsion (n=5). Compared to dystocia affected buffaloes, the buffaloes exhibiting spontaneous delivery had significantly (p<0.05) low plasma haptoglobin on the day of delivery and during the post-delivery period. The slight increase (p<0.05) in normally delivering buffaloes at 24 h post-delivery was seen as physiologic. Buffaloes which had to go through uterine detorsion process for delivery of fetus exhibited significantly (p<0.05) higher plasma haptoglobin at 24 and 48 h post-delivery. However, no such rise was observed in buffaloes in which fetal delivery was achieved with fetal mutations. In conclusion, plasma haptoglobin concentrations may serve as an indicator of degree of impairment of the uterus during spontaneous or assisted delivery.

Keywords: acute phase protein, buffalo, fetal mutation, haptoglobin, uterine torsion

INTRODUCTION

Haptoglobin is an exclusive acute phase proteins in bovines (Rink, 1997). It is synthesized in liver in response to tissue injury or inflammation (Saini et al., 1996). In healthy cattle, plasma haptoglobin exists in range of 0.02-0.04 mg/ml (Saini et al., 1996) and a concentration of 0.1 mg/ml is considered raised (Makimura und Usui, 1990). Elevated plasma haptoglobin was found in cattle diagnosed with acute mastitis and retained placenta, but not in the cattle with chronic endometritis (Eckersall, 1995; Carter et al., 2002). Subsequent to normal parturition, there was a slight increase (0.05 mg/ml) in plasma haptoglobin (Schönfelder et al., 2005). These haptoglobin concentrations may indicate the degree of injury to the uterus during parturition as well as being a predictor for regenerative potential (Schönfelder et al., 2005). In ovine dystocia cases, plasma haptoglobin is a useful prognostic indicator (Scott et al., 1992). Also, a post-operative increase in plasma haptoglobin was observed in uterine torsion affected cattle subjected to caesarean operation (Schönfelder et al., 2006). However, the assessment of uterine injury in buffaloes undergoing spontaneous or assisted delivery has never been evaluated through changes in plasma haptoglobin. Therefore, the objectives of this study were to assess the impact of spontaneous or assisted delivery on plasma haptoglobin concentrations and to further investigate the impact of obstetrical maneuvers applied during assisted fetal delivery on plasma haptoglobin.

MATERIALS AND METHODS

Animals and treatment: Fifteen buffaloes presented for the treatment of dystocia at the University Hospital were included in this study. Following obstetrical examination, appropriate maneuvers were applied for the delivery of fetus. In 10 buffaloes, fetal delivery was achieved with
mutation (technique of bringing the fetus into normal presentation, position and posture), whereas the remaining five buffaloes with uterine torsion were subjected to Sharma’s modified Schaffer’s method (Singh and Nanda, 1996) for detorsion of uterus followed by assisted delivery of fetus. During the post-delivery period, supportive treatment administered to the dystocia affected buffaloes included intravenous fluid, antibiotic, anti-inflammatory and liver tonic. Furthermore, in the present study, five buffaloes exhibiting spontaneous delivery of fetus were also included.

Blood sampling: Blood samples in heparinised vials were collected from jugular vein of dystocia affected buffaloes upon the arrival of the animal at the hospital, and subsequently at 0, 6, 15, 24 and 48 h post-delivery. Normally calving buffaloes were blood sampled on the day of fetal delivery and subsequently at 24 and 48 h post-delivery. Plasma was separated following centrifugation (1500 x g, 10 min) and stored at -20°C.

Haptoglobin assay: Haptoglobin in the blood plasma was measured by means of Enzyme-Immunoassay (Eckersall et al., 1999). Reagents: Haemoglobin stock solution (0.6 mg/ml, Nice chemicals, Cochin) was diluted in 0.9% NaCl to prepare the working solution (0.06 mg/ml). Haptoglobin standards used in the standard curve were prepared by dilution (in 2% bovine serum albumin, Sigma) of a bovine acute phase serum in which haptoglobin concentration had been measured with the manual haptoglobin-haemoglobin binding method, using purified haptoglobin as calibrator (Makimura and Susuki, 1982, Horadagoda et al., 1999). Substrate was prepared by addition of 100 μl hydrogen peroxide (30%) to 25 ml distilled water. The working chromogen buffer (SB-7) was a solution of peroxidase chromogen, detergent and protein binding inhibitors in a citrate buffer at pH 3.8.

Assay: In the wells of a microtitre plate, 1.75 μl of standards and plasma samples were dispensed to 50 μl of haemoglobin. After 50 seconds, 22.5 μl of chromogen was added. Thereafter, 25 seconds later, 12.5 μl of substrate was added and the plate was read immediately at 600 nm. Haptoglobin concentrations in unknown samples were derived by comparison with the standard curve. The reaction was maintained at 37°C throughout.

Statistics: Differences within (*; Table 1) and between (a vs b; Table 1) groups were examined using two sample t-test (Dytham, 1999) with the help of Minitab release 14.4 statistical software (Minitab Inc., State College, PA, USA).

RESULTS AND DISCUSSION

In naturally calving buffaloes, haptoglobin concentrations exhibited a slight but significant (p<0.05, Table 1) increase 24 h after fetal delivery. In these buffaloes during the course of investigations, haptoglobin crossed merely the value of 0.5 mg/ml, which is tolerable in the early puerperium (Schönfelder et al., 2005). Parturition is generally an inflammatory event, it is, therefore, logical that haptoglobin concentrations would be higher around delivery on account of natural rebuilding in the uterus. Previous studies in normal births in cattle have also reported significant increase in haptoglobin (Fürll et al., 1998; Schönfelder et al., 2005).

In the dystocia affected buffaloes of the present study, 3000-4000 fold increases (p<0.05) in plasma haptoglobin were observed on the day of delivery compared to the normally calving buffaloes (Table 1). This suggested the degree of impairment of uterus during difficult parturition (Schönfelder et al., 2005). Evidence for the fact that these huge increases in plasma haptoglobin were due to uterine damage was that in a previous study, there was an immediate drop in plasma haptoglobin after ovariohysterectomy in cattle suffering from uterine torsion (Schönfelder et al., 2005). In addition, in bovines, manifold fold increase in plasma haptoglobin in response to infection and tissue injury has been reported (Eckersall and Conner, 1988).

The alterations in plasma haptoglobin during the post-delivery period may depend upon the obstetrical maneuvers applied for the delivery of fetus. Dystocia affected buffaloes in which mutation technique were applied for the delivery of fetus showed no further significant increase in plasma haptoglobin (Table 1). However, buffaloes which had to undergo uterine detorsion process before the successful delivery of fetus exhibited significant (p<0.05) increase in plasma haptoglobin at 24 and
48 h post-delivery (Table 1). A 24 h response time for detection of alteration in plasma haptoglobin has been reported previously (Richter, 1975). This revealed that the process of uterine detorsion might have caused severe uterine damage (Schönfelder et al., 2005). In fact, haptoglobin concentration correlates directly with the gravity of the illness (Heegaard et al., 2000).

Nevertheless, an increase in plasma haptoglobin during post-delivery period (till 48 h) in uterine torsion affected buffaloes may suggest the start of “healing processes” by endogenous regeneration in the uterus (Schönfelder et al., 2006). In fact, the acute phase response prevents further injury, promotes convalescence and is a predictor for regenerative potential (Krüger et al., 1995). Interestingly, in a previous study, the correlation of plasma haptoglobin concentrations in uterine torsion affected cattle with future fertility revealed significantly higher haptoglobin concentrations shortly after surgery in later fertile cattle but not in later infertile cattle (Schönfelder et al., 2005).

Unfortunately, due to short clinical stay of the buffaloes in the present study, blood samples after 48 h of fetal delivery were not available to reveal whether haptoglobin started to fall in subsequent days or not. A fall might be interpreted as a quick “normalization” whereas a persistent elevation might have suggested constant inflammation and future breeding suitability (Scott et al., 1992; Schönfelder et al., 2005).

To sum up, this preliminary study on plasma haptoglobin concentrations in buffaloes indicator that the degree of damage in uterus is in direct relation to spontaneous or assisted delivery and in particular obstetrical maneuvers applied to assist the delivery of fetus.

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Table 1. Plasma concentrations of haptoglobin (mg/ml, mean±SEM) in buffaloes with spontaneous and assisted delivery.

<table>
<thead>
<tr>
<th>Groups</th>
<th>On day of spontaneous delivery / at arrival of dystocia</th>
<th>Hours after delivery</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>6</td>
</tr>
<tr>
<td>Spontaneous delivery (n=5)</td>
<td>0.04 ± 0.03a</td>
<td>-</td>
</tr>
<tr>
<td>Delivery after fetal mutation (n=10)</td>
<td>1.46 ± 0.21b</td>
<td>1.44 ± 0.32</td>
</tr>
<tr>
<td>Delivery after uterine detorsion (n=5)</td>
<td>1.24 ± 0.34b</td>
<td>1.66 ± 0.12</td>
</tr>
</tbody>
</table>

*p<0.05: different within group from values on day of spontaneous delivery / at arrival of dystocia, a vs bp<0.05: different between groups.
REFERENCES


