Serum Paraoxonase-1 Activity in Dairy Cattle and its Association with Dystocia

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Abstract: The aim of this study was to investigate possible changes in the serum paraoxonase-1 (PON1) activity at the prepartum and postpartum stages in Holstein cows and to examine the relation between PON1 activity and dystocia. The 2nd objective of this study was to describe prepartum and postpartum serum concentrations of other relevant blood parameters and their associations with dystocia risk. Initially, 200 Holstein and 65 Holstein x Black home crossbreed cows, aged 3-8 year, were selected for this study. Samples were taken both in the 7th month of pregnancy and within the first 15 min postpartum. At the end of pregnancy, the cows were divided into two groups according to the type of birth: Difficult labour and normal labour. PON1 activities measured in the prepartum samples were significantly higher than in postpartum samples from cows which had normal or difficult labour. Although, PON1 activity from cows with dystocia was lower both pre- and postpartum, this difference did not reach statistical significance. Furthermore, there was a tendency to decreased PON1 activities in the case of absolute birth weight and twins. Evaluation of cholesterol, HDL-C, LDL-C, albumin, globulin and alkaline phosphatase levels does not appear to be useful in the assessment of dystocia. However, increased serum creatine kinase and creatine kinase isoenzyme MB may be a parameter which could be used in diagnosing dystocia in pregnancy.

Key words: Paraoxonase, oxidative stress, dystocia, prepartum, postpartum, cattle

INTRODUCTION

Difficult birth is known as dystocia and it is the major cause of decline in overall performance and consequently economic loss. Dystocia is one of the most serious complications of pregnancy in cattle. Its incidence is 3-25% in all pregnancies (Oakes et al., 2001; Roberts, 1986). Numerous factors are believed to influence dystocia such as pelvic area, calf’s birth weight, age of dam, twin pregnancy, hormonal control, nutrition of dam and other unknown factors.

Peroxynase 1 (EC 3.1.8.1: PON1), a glycoprotein of 354 amino acids with a molecular mass of 44 kDa, is a calcium-dependent enzyme synthesized primarily by the liver. PON1 is tightly bound to apolipoprotein A1 (Apo A1) of High Density Lipoprotein-Cholesterol (HDL-C) and circulates as an HDL-C component in the blood of humans and other vertebrates (Juretic et al., 2001; Mackness and Durrington, 1995). Also, it has been shown that PON1 protects against the oxidation of Low Density Lipoprotein (LDL) (Mackness and Durrington, 1995).

Many studies have shown that PON activity in humans is significantly lowered in diseases which are characterized by increased oxidative stress such as coronary artery disease (McElveen et al., 1986), chronic renal failure (Ece et al., 2006), nephropathy (Juretic et al., 2006) and non insulin dependent diabetes mellitus (Ikeda et al., 1998; Mackness et al., 1991). Furthermore, PON activities have been found to be significantly lower in women with preeclampsia (Kumru et al., 2004; Uzan et al., 2005) when compared to healthy women. However, knowledge about serum PON activity in veterinary medicine is still scarce (Turk et al., 2004, 2005, 2007; Bioraz et al., 2007) and there is no information concerning the role of PON activity in pathological pregnancy in cattle.

The aim of this study was to examine possible changes in PON1 activity at both prepartum and
postpartum stages in healthy cows and to examine its relationship to dystocia and other relevant metabolic variables [cholesterol, High Density Lipoprotein-Cholesterol (HDL-C), Low Density Lipoprotein-Cholesterol (LDL-C), albumin and globulin]. The second objective of this study was to describe the changes in other metabolic parameters [creatinine kinase (CK), creatinine kinase isoenzyme MB (CK-MB) and alkaline phosphatase (ALP)], where elevated levels are expected for increased muscular work and metabolic requirements due to the increased foetal weight, to predict dystocia in the prepartum period.

MATERIALS AND METHODS

Animals and sample collection: Initially, 200 pregnant Holstein cows and 65 Holstein x Black Angus breed (Home Breed) crossbreed cows, aged 3-8 year, were selected for this study. None of the cattle had diseases and none had received medication. Samples were taken both in the 7th month of pregnancy and within the first 15 min after delivery. After parturition, animals were divided into two groups according to the type of birth: 21 cows were placed in group 1 (dystocia/difficult labour); of the others (normal labour/easy unassisted calving), we randomly selected only 21 cows as a control group (group 2). Cows that experienced difficulty in calving were considered a clinical case of dystocia as previously defined by Oakes et al. (2001) and Roberts et al. (1986). When the first, or the second, stage of parturition is marked prolonged and becomes difficult or impossible for the dam without artificial aid, the condition is termed dystocia (difficult birth). Eutocia is safe, easy, natural or physiological parturition. However, there is no clear line between normal parturition and dystocia. Obstetricians have usually regarded dystocia as being either foetal or maternal, but maternal causes are usually difficult to identify. Difficult birth will occur when the birth canal is of inadequate size and shape, or when the presenting diameter of the foetus is unable to pass through the normal birth canal because the foetus is too large (absolute birth weight) or because of its disposition (abnormality of foetus position), or because twins enter the birth canal together (Roberts et al., 1986).

Additionally, the dystocia group was divided into three subgroups, according to the reason for the dystocia (1-absolute birth weight (high birth weight of the calf), 2-twins and 3-abnormalities of the foetus position).

Blood samples were obtained by jugular venepuncture, using evacuated tubes (Vacutainer®) and immediately transported on ice to the laboratory, where the serum was separated by centrifugation for 1 h (at 1800 g for 10 min); aliquots were stored at -80°C until analysis.

Measurement of serum paraoxonase activity: PCN1 activity was determined by using paraoxon as a substrate and measured by increases in absorbance due to the formation of p-nitrophenol as described by Furlong et al. (1989). The activity was measured at 37°C by adding 200 μL of serum to 0.132 M Tris HCl buffer (pH 8.5) containing 1.32 mM CaCl2 and 2.63 M NaCl and adding 6 mM (200 μL) freshly prepared paraoxon (O,O-diethyl-O-p-nitrophenyl-phosphate; Sigma Chemical Co., St. Louis, Mo., USA). The rate of generation of 4-nitrophenol was determined at 405 nm (Jasco V 530). Enzymatic activity was calculated by using a molar extinction coefficient of 18.05×10³ M⁻¹ cm⁻¹. Parnoxonase activity was expressed as units per litre (U/L) (one unit is the number of micromoles of paraoxon hydrolysed per minute). The intra and interassay Coefficients of Variation (CV) for PON1 activity were <3%. The ratio of PON1 activity and HDL-C concentration was also calculated (U/mmol).

Measurement of the other relevant blood parameters: Serum cholesterol, HDL-C, LDL-C, albumin, globulin, CK, CK-MB and ALP activities were measured colorimetrically with an auto analyzer (Olympus, AU 2700, Germany) using commercially available kits. The coefficients of variation for all variables were <5%.

Statistical analysis: The following statistical procedures were used: Analysis of differences between prepartum and postpartum stages for each group and paired-samples t-test. The independent-samples t-test was performed in order to compare the 2 groups for each evaluated parameter. Kruskal-Wallis analysis was followed by Mann-Whitney U-test were used for testing the data of the dystocia subgroups, where variance homogeneity was not normally distributed. To determine the significance of interactions between variables in each group, Pearson’s and Spearman’s correlation analysis was performed. A difference with p<0.05 was considered to be significant. All statistical analyses were performed with statistics package SPSS version 10.0 (SFSS Inc., Illinois, USA).

RESULTS

The measured variables in the dystocia group (group 1) and the normal parturition group (group 2) in the 7th month of pregnancy and after delivery are summarized in Table 1. In addition, a summary of all measured variables in the dystocia sub-groups and the normal parturition group are given in Table 2. The results of the analysis are given as mean±standard deviation. The differences determined are shown according to groups and stage of pregnancy in the same tables.
Table 1: The comparison of the measured variables in dystocia and normal parturition groups at 7th month of pregnancy and after delivery (mean ±SD)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Dystocia (Group 1) (n = 21)</th>
<th>Normal parturition (Group 2) (n = 21)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>7th month of pregnancy</td>
<td>After delivery</td>
</tr>
<tr>
<td>Cholesterol (mg/dL)</td>
<td>60.81±24.77</td>
<td>104.4±23.81²</td>
</tr>
<tr>
<td>HDL (mg/dL)</td>
<td>119.9±10.43³</td>
<td>49.83±20.45³</td>
</tr>
<tr>
<td>LDL (mg/dL)</td>
<td>30.6±11.58²</td>
<td>52.56±15.53²</td>
</tr>
<tr>
<td>Albumin (g/dL)</td>
<td>3.1±0.27</td>
<td>3.02±0.71</td>
</tr>
<tr>
<td>Globulin (mg/dL)</td>
<td>3.60±0.57²</td>
<td>4.71±0.51²</td>
</tr>
<tr>
<td>Alb/Glob. Ratio</td>
<td>0.67±0.18</td>
<td>0.65±0.18</td>
</tr>
<tr>
<td>CK (U/L)</td>
<td>11.7±6.47</td>
<td>33.8±35.95</td>
</tr>
<tr>
<td>CKMB (U/L)</td>
<td>49.94±52.46²</td>
<td>53.44±52.86</td>
</tr>
<tr>
<td>ALP (UL⁻¹)</td>
<td>48.7±29.54</td>
<td>84.12±66.49</td>
</tr>
<tr>
<td>Pten (UL⁻¹)</td>
<td>1907.3±603.25⁵</td>
<td>1350±435.2⁵</td>
</tr>
</tbody>
</table>

The measured variables were compared within and between groups. A, B, C: Differences between mean values having same number in each group is significant (p<0.05, p<0.01, p<0.001, respectively).

Table 2: The concentration of all variables according to dystocia sub-groups and normal parturition group in 7th month of pregnancy (mean ±SD)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Dystocia (Group 1) (n = 21)</th>
<th>Twins (n = 5)</th>
<th>Anomalies of the fetus position (n = 6)</th>
<th>Normal parturition (Group 2) (n = 21)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol (mg/dL)</td>
<td>57.3±31.26</td>
<td>69.25±11.87</td>
<td>59.25±22.71</td>
<td>53.95±17.72</td>
</tr>
<tr>
<td>HDL (mg/dL)</td>
<td>32.12±18.1</td>
<td>34.25±13.6</td>
<td>26.25±20.1</td>
<td>32.38±20.37</td>
</tr>
<tr>
<td>LDL (mg/dL)</td>
<td>25.6±13.86</td>
<td>35.25±8.65¹</td>
<td>33.75±6.4</td>
<td>26.6±2.7²</td>
</tr>
<tr>
<td>Globulin (mg/dL)</td>
<td>3.04±0.24</td>
<td>3.02±0.29</td>
<td>3.3±0.27</td>
<td>2.88±0.3</td>
</tr>
<tr>
<td>Globulin (mg/dL)</td>
<td>3.42±0.59</td>
<td>4.02±0.92</td>
<td>3.7±0.66</td>
<td>3±0.28</td>
</tr>
<tr>
<td>Alb/Glob. Ratio</td>
<td>0.89±0.13</td>
<td>0.79±0.22</td>
<td>0.89±0.17</td>
<td>0.79±0.15</td>
</tr>
<tr>
<td>CK (U/L)</td>
<td>34.7±35.5¹</td>
<td>12.25±10.18</td>
<td>43.75±53.67</td>
<td>13.9±15.75¹</td>
</tr>
<tr>
<td>CK-MB (U/L)</td>
<td>58.8±53.5³</td>
<td>18.25±14.17</td>
<td>63.75±71.2³</td>
<td>23.48±24.56³</td>
</tr>
<tr>
<td>ALP (UL⁻¹)</td>
<td>57.2±31.97</td>
<td>39.25±23.96</td>
<td>25.75±21.54</td>
<td>51.76±31.47</td>
</tr>
<tr>
<td>Pten (UL⁻¹)</td>
<td>1952±573.3</td>
<td>1651±288.2</td>
<td>2073.6±916.5</td>
<td>2021±630.13</td>
</tr>
</tbody>
</table>

The measured variables were compared with both among the sub-groups and the other group. A, B, C: The differences between mean values having same superscript in dystocia group is significant. When the sub-groups of dystocia were compared with normal parturition (group 2) this difference was significant (at least p<0.05).

Serum PON1 activities and PON-HDL-C ratio were significantly increased in the 7th month of pregnancy compared to postpartum in both groups (p<0.01 and p<0.05). Although, the serum PON1 activity of the dystocia group in both periods was lower than that of the normal parturition group, the variation was not statistically significant. It was seen that PON1 activities decreased in cattle pregnant with twins when compared with those with high calf birth weight and presentation anomalies; however, the differences were not statistically significant.

Cholesterol, LDL-C and HDL-C values were increased postpartum in both groups (p<0.001, p<0.01 and p<0.001, respectively) and there were no differences between the two groups (p>0.05). It was seen that PON1 activities increased while HDL-C concentrations decreased; however, correlations were not statistically significant in any group. Pearson's correlation analysis and linear regression analysis gave no significant correlation between studied variables.

Albumin and ALP activities were not different between groups or subgroups. There were increased globulin levels postpartum in both groups (p<0.001). Also, globulin concentration postpartum in the dystocia group was significantly decreased compared with the corresponding period of the normal parturition group (p<0.05). Alb/Glob ratios were decreased postpartum in both groups (p<0.01 and p<0.001). Activities of CK and CK-MB were significantly increased postpartum in the normal parturition group (p<0.01), but not in the dystocia group (group 1). We found significantly higher CK and CK-MB activities in the dystocia group compared to the normal parturition group in the 7th month of pregnancy (p<0.01). Also, increased CK-MB activities were observed in the cases of high calf birth weight and presentation anomalies when compared with the twin pregnancies in the 7th month of pregnancy (p<0.05). Furthermore, when these dystocia sub-groups (absolute birth weight and presentation anomalies) were compared with the normal parturition group, both CK and CK-MB activities were found to be significantly increased (p<0.01 and p<0.001, respectively).

**DISCUSSION**

Many studies confirm that PON activity increases in normal pregnancy in humans (Kumru et al., 2004; Uzun et al., 2005). Furthermore, it is reported that serum PON activity is decreased in humans with preeclampsia. It is also thought that the reason for the decreased PON
activity in preeclampsia may depend on the over production of free radicals (Kumru et al., 2004; Uzun et al., 2005).

To date, there are no reliable values for PON activities in the veterinary literature, except for a series of studies by Turk et al. (2004, 2005, 2007) and Bionaz et al. (2007). They reported that PON activities were decreased in healthy cattle during late pregnancy (Turk et al., 2004) and early lactation (Turk et al., 2005) as compared with the late lactation and dry periods, respectively. A recent study by Turk et al. (2007) found decreased PON1 activity in late pregnancy and early postpartum compared to the first and second trimester of pregnancy and the mid-lactation period. Bionaz et al. (2007) also found lower PON1 activity in transition dairy cows. These findings are similar to our statement that decreased PON1 activities in the early postpartum period (early lactation) can depend on increased oxidative stress (Turk et al., 2007) and the influence of inflammatory conditions (Bionaz et al., 2007).

Furthermore, increased PON1 activity in pregnancy may be the result of elevated lipid and lipoprotein metabolism (Kumru et al., 2004; Turk et al., 2004, 2005; Uzun et al., 2005) and increased Apo A1 synthesis caused by estradiol (Marcos et al., 1990; Miyamoto et al., 2005). These observations suggest that physiological variations have to be taken into consideration for the correct interpretation of serum PON1 activities.

Normally, increased oxidative stress is expected in dystocia, due to the physical effort of the delivery. Also, oxidative stress may be elevated because of the increased requirements for the antioxidants and increased free radical production, as a result of the increased metabolic activity caused by higher foetal weight in dystocia (Yokus et al., 2007). The majority of human studies recognise increased oxidative stress in pathological delivery (Atamer et al., 2005; Davidge et al., 1992; Gupta et al., 2005a; Kumru et al., 2004; Uzun et al., 2005). However, there is lack of information concerning the role of oxidative stress in the aetiology of pathological parturition in cattle (Yokus et al., 2007).

In these studies, serum antioxidant enzyme activities and lipid peroxidation were investigated in postpartum disorders such as Retained Foetal Membranes (RFM). These investigators observed higher MDA concentrations in RFM cases than in control animals (Gupta et al., 2005b; Kankofer, 2001). Moreover, Miller and Brzezinska-Skleboodzinska (1993) reported that plasma antioxidant capacity was lower in RFM.

However, there is a tendency to a decrease of PON1 activity in dystocia; our findings can be interpreted to mean that PON1 activities are a part of an antioxidant system that did not change statistically as compared with the other group. These data confirm our previous observation that antioxidant capacity did not alter in dystocia (Yokus et al., 2007).

The reason for the tendency to decreased PON1 activity in dystocia and twin parturition is probably increased free radical production due to a Negative Energy Balance (NEB) caused by increased energy requirements (Miller and Brzezinska-Skleboodzinska, 1993). Moreover, these data may support the hypothesis that there is a higher production of free radicals and a higher demand for antioxidants as a result of increased metabolic activity caused by increased foetal weight in dystocia (Yokus et al., 2007). Furthermore, the tendency to decreased PON1 activity in the case of absolute birth weight and twins may support this hypothesis.

The association of PON1 with HDL was first described by Uriel (1961). Later reports suggested that as a result of the nature of its carrier system, serum concentration of PON1 is usually correlated with HDL-C (Karayaka et al., 1999; La-Du, 1992; Uriel, 1961; Turk et al., 2007). On the other hand, some studies showed, similar to our results that PON1 activities were independent of HDL-C concentrations (Turk et al., 2004, 2005; Bionaz et al., 2007). The reasons for these conflicting results probably stems from differences in lipid and lipoprotein metabolism in various diseases, the breed and physiological condition. Most importantly, the reasons for the differences could also be a result of the degree of NEB which commonly occurs in the transition period due to the increased energy requirements during the early postpartum period (Turk et al., 2007).

In our study, there were increased serum HDL-C, LDL-C and cholesterol concentrations postpartum in both groups (dystocia and normal parturition groups); however, there was no statistical difference between these groups. These data suggest that there is no imbalance of lipid metabolism and lipoprotein composition between the two groups investigated.

Increased CK activity after parturition in normal pregnancies is compatible with other findings (West, 1989; Yildiz et al., 2005; Yokus et al., 2006). Higher CK-MB activity was also found after normal parturition; however, there was no difference in the dystocia group because of increased CK and CK-MB activity in the 7th month of pregnancy in this group. Furthermore, increased CK-MB activities were observed in the cases of the absolute to birth weight of calves and presentation disposition. The reason for the higher activity of CK and CK-MB in the 7th month of pregnancy in the dystocia group compared with the normal pregnancy group is presumably due to higher muscular
activation and increased myometrial compulsion of the dam bearing the relatively higher foetus and to myometrial compulsion. CK-MB is an isoenzyme specific to heart muscles. It is well known that the artery carrying blood to the uterus (arteria uterina media) is observed to increase in diameter in parallel with the size of the foetus and the volume of blood flow (Oakes et al., 2001; Roberts, 1986). As the weight and size of the foetus or foetuses increase in pregnancy, the load on the mother’s circulation and thus on her heart increases. This may be the reason for the increase in serum CK-MB levels in the prepartum period. These results indicate that serum CK and CK-MB activities might be a parameter which could be used in the diagnosis of dystocia in pregnancy.

In this study, albumin concentrations did not vary according to periods in both group as reported by Yokus and Cakir (2006) and Yildiz et al. (2005). Unchanged serum albumin levels were also observed by Turk et al. (2007) and Bionaz et al. (2007) between early postpartum and late pregnancy in dairy cows. ALP activities did not show a significant difference before and after parturition in either group. In this respect, prepartal and postpartal changes in albumin and alkaline phosphatase enzyme activities are not predictive for dystocia.

It was seen that globulin concentrations increased while alb/glob ratios decreased after delivery in both groups. Lower levels of globulin after delivery in dystocia compared to normal parturition may be a consequence of a larger incidence of acute haemorrhagic anaemia at parturition.

**CONCLUSION**

It can be concluded that the tendency to decreased PON1 activity cannot related to the occurrence of dystocia. However, the tendency to decreased PON1 activity in dystocia may be a consequence of the higher production of free radicals and a higher demand for antioxidants as a result of increased metabolic activity caused by increased foetal weight in dystocia. Furthermore, the tendency to decreased PON1 activity in the case of normal to high birth weight and twins may support this hypothesis.

Evaluation of measured parameters other than CK and CK-MB in the 7th month of pregnancy does not appear to be useful in the assessment of dystocia. However, more information is needed to determine whether PON1 activities and serum chemistry may be used to identify cows at high risk for developing dystocia.

**REFERENCES**


