Paraoxonase activity an indicator of complications at early stage of complicated pregnant cows

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ABSTRACT

In healthy and complicated pregnant cows, on the 2nd and 6th months of pregnancy in order to determine the levels of maternal serum Paraoxonase 1 (PON1) activity and the possibility of complications can occur during the pregnancy might be a premise indication. Serum samples were taken at 2nd and 6th months of 252 pregnant cows at the end of their pregnancies. The cows were classified into two groups such as complicated (Abortion, Dystocia) and non-complicated. Maternal serum PON1 activity in 6th months was lower at complicated group than normally pregnant group (P=0.004; P=0.01), no difference was discovered between these groups in their 2nd month of pregnancy (P>0.05). Among the concentration of HDL, TP and globulin no statistical difference was observed between complicated, subgroups and normal births (P>0.05). Levels of PON1 in 2 and 6 months were statistically different between the groups of dystocia and normal pregnancy (P=0.003), and abort and normal pregnancy (P=0.03; P=0.033). In this study, it was inferred that the evaluation of PON1 activity early indicator of complications for clinicians that might occur in further periods of pregnancy. These results showed the fact that PON1 activity can be used as a marker relatively at the early phases of pregnancy in complicated cows.

Key words: Abortion, Cow, Complicated pregnancy, Dystocia, Paraoxonase.

INTRODUCTION

Severe births, which are major maternity complications of cows, have incidence rate between 3-25% and cause decrease of total performance, milk productivity and infertility. This is why early diagnose is important at maternity complications which can lead to serious economic losses. It is considered that many factors such as pelvic size, birth weight of calf, mothers’ age, twin pregnancy, presentation defect, hormonal level, nourishment, stress and many other unknown factors affect severe births (Abeni et al., 2004; Oakes et al., 2001; Osumgwhu et al., 1980; Roberts 1986). Abortion is defined as removing the fetus, which does not have the chance to live outside, either dead or alive before the pregnancy period is completed. At cows the births that happen before 200 days which is the normal period, are considered as abortion. Preliminary causes of abortion cows are infectious, non-infectious diseases, toxic agents, hormonal instabilities, nutritional problems and genetic anomalies (Alaçam, 2010).

Paraoxonase-1 is a glycoprotein structured mammal enzyme involving 354 amino acids that is synthesized from liver and transmitted into the blood. Both in human and cow serams tightly bonded to apolipoprotein of PON-1 HDL-C it circulates as component of HDL-C. PON-1 composed of lipoproteins (HDL and LDL) is defined as a protective marker against development of lipid peroxidation and oxidative stress (Bademkiran 2008; Juretic et al 2001; Turk et al 2008).

It is reported that oxidative stress has increased at healthy pregnancies both in human and animals (Castillo et al., 2005; Castillo et al., 2006; Serdar et al., 2002). The reason for this is regarded as the increase in free radical production, insulin resistance and negative energy balance due to growing metabolic activity (Castillo et al., 2005; Castillo et al., 2006; Erel 2004; Miller and Brzezinska-Slebodzińska 1993; Serdar et al., 2002). Another reason for the oxidative stress to arise in pregnancy is depletion of antioxidant reserves and preparation for lactation (Miller and Brzezinska-Slebodzińska, 1993). All these changes are a result of physiologic adaptation caused by cows being in need of energy during pregnancy (Miller and Brzezinska-Slebodzińska 1993; Brzezinska-Slebodzińska et al., 1994; Stec et al., 2001). During the pregnancy and lactation periods of cows, many studies are conveyed about increasing PON-1 activities on retained fetal membrane (RFM), severe birth and liver hypertrophy (Turk et al., 2004; 2005; Bademkiran et al, 2008).

This study, by determining PON-1 levels at cows with complicated and healthy pregnancy aims to find out

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whether it can be used as an early period marker for
diagnosing complicated pregnancies, abortion and dystocia.
Also, it aims to determine the correlation analysis between
albumin, globulin, total protein, HDL and PON-1 and the
etiology of changes in PON-1 levels.

MATERIALS AND METHODS

This study was approved by the Animal Research
Ethics Committee of Dicle University (Diyarbakir, Turkey).
(Number 2013/11)

Serum samples were taken from 252 pregnant cows
at 2nd and 6th months of pregnancy period and at the end of
their pregnancies. The cows were classified into two groups
such as complicated (Abortion, Dystocia) and non-
complicated.

Blood for biochemical analysis was taken from each
calf by jugular vein puncture, allowed clotting and centrifuged
at 4,000 rpm for 10 min. Sera were stored at –20°C until
analyzed. Serum concentrations of total protein, albumin,
globulin, and HDL were determined via automated analyzer
(Mindray BS200, PRC) with commercially available kits.

Paraoxonase activity was evaluated according to a
method defined by Furlong et al. (1989). The contained
of assay buffer paraoxon (O,O-diethyl-O-p-nitrophenyl-
phosphate) were added to assay buffer (0.132 M Tris HCl
(pH 8.5), 1.32 mM CaCl2 and 2.63 M NaCl). The amount of
production at p-nitrophenol was determined
via spectrophotometer at 405 nm in 37°C. A molar of number
18.05x10^3 was used for calculation with paraoxon as substrate
and activity signified as units per liter (a unit is the number
of micromoles of paraoxon hydrolysed per minute).

Statistical analysis: The normal distributions of parameters
were evaluated via Kolmogrov Smirnov (KS). For the
comparison of dual parameters Student T test, for multiple
parameters variation analysis Duncan and Tamhane T2
(variations not homogenic); for the dual comparison of groups
which are not parametric or do not have normal distributions
Man WhitneyU and for multiple comparison Kruskal Wallis
tests were used. Statistic program SPSS 22.00 was used for
evaluation at P<0.05 level (SPSS, 2014).

RESULTS AND DISCUSSION

The comparison of healthy and complicated
pregnancies and sub groups biochemical values according to 2nd and 6th month were distributed at Table 1 and Table 2.

It observed that cows had abortion after 6th
months of their pregnancy. Despite the fact that, maternal
serum PON1 activity in 6th months was lower at
complicated group than normally pregnant group (P<0.01; P=0.004) (Figure1), no difference was discovered between
these groups in their 2nd month of pregnancy (P>0.05).
Among the levels of HDL, TP and globulin no statistical
difference was observed between complicated and normal
births (P>0.05).

While statistically significant difference was
found at PON1 levels between healthy pregnant and
dystocia groups (P<0.01; P=0.003), statistical difference
was found between healthy pregnant and abort groups
(P<0.05; P=0.033), and statistical differences between
dystocia and abort at levels of HDL, Total Protein (TP)
and Globulin were observed when compared to sub groups
(P>0.05).

Although there was limited information about
importance of oxidative stress at complicated pregnancies’
pathophysiology, normally due to increasing physiologic
activity during severe birth the oxidation is expected to be
increased. Also at dystocia due to the increase at production
of free radicals, because of the need for antioxidants and
metabolic activity rising, the oxidative stress can occur. The
oxidative stress which occurs during pregnancy might be
related to excessive production of free radicals originating
from the increase in metabolic activity (Erel, 2004), insulin
resistance (Kocyigit et al., 2004) and the balance of negative
energy (Roche et al., 2000). Also the oxidative damage which
is observed during pregnancy supports the occurrence of
oxidative stress caused by decrease in antioxidant level
during preparation to lactation (Miller et al., 1993). It is
enounced that the total antioxidant capacity is reduced in
preeclampsia at humans (Harma et al., 2005). But the balance
disorder between lipid peroxidation and antioxidants with

| Table 1: Biochemical values in healthy and complicated pregnancies at 2nd and 6th month in cows. |
|---|---|---|
| Month | Healthy Pregnancy(n=22) | Complicated Pregnancy(n=20) |
| PON(U/l) | | |
| 2nd | 166.4±52.81 | 170.48±57.75 |
| 6th | 149.4±56.90 | 90.56±54.19a |
| HDL(mg/dL) | | |
| 2nd | 111.5±37.31 | 129.5±37.31 |
| 6th | 112.86±39.23 | 118±59.70 |
| GLB(g/dL) | | |
| 2nd | 4.5±1.06 | 4.09±0.71 |
| 6th | 4.27±1.34 | 4.5±1.51 |
| TP(g/dL) | | |
| 2nd | 8.09±0.84 | 7.8±0.74 |
| 6th | 7.31±1.84 | 7.87±1.87 |

a, b: It is statistically different from the same row with different superscript (p<0.01).
oxidative stress has increased (Atamer et al., 2005; El-Salahy et al., 2001; Gupta et al., 2005). It is reported that the index of oxidative stress has increased significantly (Davidge et al., 1992).

There are many researches that show increases of PON-1 activities during pregnancy and lactation periods of cows (Turk et al., 2004; 2005; 2008; Bademkiran et al., 2008; Yildiz et al., 2011).

PON-1 activity might change depending on different physiological or pathological cases. In another study of Turk et al., (2008) has conveyed that milking cows were vulnerable to oxidative stress at late pregnancy and early lactation periods and during these transition periods depending on low energy balance, the production of metabolic adaptation sourced reactive oxygen types increase. PON-1 levels, compared to first and second trimester of pregnancy and midterm lactation, were observed significantly low during late pregnancy and early postpartum periods. An important degree of correlation was determined between PON-1 and HDL-C.

In a study that Bademkiran et al. (2008) has conveyed, the comparison between prepartum and postpartum phases at dystocia, despite noticing increase in values at PON-1 activity at 7th month of pregnancy, downward tendency was reported at PON-1 activity during pre and postpartum phases, at cows having dystocia and twin birth. They have reached the conclusion that it was useless to evaluate HDL and globulin values at cows which have dystocia.

In a study made at retained fetal membrane (RFM), it was reported that oxidative stress caused by lipid peroxidation product increased, was higher as compared to animals in control group, but in return for this the lower total level at RFM (Gupta et al., 2005b; Kankofer, 2001).

In a study conveyed by Yildiz et al. (2011), plasma PON-1 activity was lower at cows with normal delivery than cows which have dystocia. They have associated PON-1 activity decreased at dystocia with oxidative stress which was caused by increasing lipid peroxidation products that damage the balance between oxidant and antioxidant systems (Yildiz et al., 2011).

The studies which examine low PON-1 activities at complicated pregnancies have reached the conclusion that; decrease in PON-1 activities might be related to increasing free radical production (Kumru et al., 2004; Uzun et al., 2005).

Yokus, et al. (2007) have reached the conclusion that compared to control group the reason why there was not a difference at oxidative stress at cows with severe birth was that these animals externally issued to antioxidant reinforcement. Again in this study when they evaluate dystocia under subgroups, TAC values of mothers with a big offspring were found to be lower compared to cows with presentation anomaly. This emphasizes mother need of antioxidant depending on offspring’s size (Yokus, et al., 2007).

Variable reports showed that as a result of carrier systems nature, serum concentration of PON-1 at first discovered by Uriel (1961) and after HDL and PON-1’s collaboration related with HDL-C (Juretic, 2006). In this study,

### Table 2: Biochemical values in groups of healthy, abortion and dystocia at 2nd and 6th month in cows.

<table>
<thead>
<tr>
<th>Month</th>
<th>Healthy(n=22)</th>
<th>Abortus(n=15)</th>
<th>Dystocia(n=5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PON(U/l) 2nd</td>
<td>166.41±52.81b</td>
<td>158.77±49.1c</td>
<td>193.4±81.32a</td>
</tr>
<tr>
<td>6th</td>
<td>149.41±56.9a</td>
<td>103±57.26a</td>
<td>68.2±12.72c</td>
</tr>
<tr>
<td>HDL(mg/dL) 2nd</td>
<td>111.5±37.31</td>
<td>124.69±52.06</td>
<td>165.6±50.16</td>
</tr>
<tr>
<td>6th</td>
<td>112.86±39.23</td>
<td>131±63.45</td>
<td>113.2±45.30</td>
</tr>
<tr>
<td>GLB(g/dL) 2nd</td>
<td>4.56±1.06</td>
<td>4.1±0.84</td>
<td>4.06±0.46</td>
</tr>
<tr>
<td>6th</td>
<td>4.27±1.34</td>
<td>6.09±4.52</td>
<td>4.33±0.56</td>
</tr>
<tr>
<td>TP(g/dL) 2nd</td>
<td>8.09±0.84</td>
<td>7.81±0.87</td>
<td>7.69±0.50</td>
</tr>
<tr>
<td>6th</td>
<td>7.31±1.84</td>
<td>9.37±8.10</td>
<td>7.98±7.69</td>
</tr>
</tbody>
</table>

a, b, c : It is statistically different from the same row with different superscript (p<0.01; p<0.05).

**Figure 1:** Serum PON1 levels in groups during 6th month of pregnancy in cows.
any correlation between PON-1 activities and HDL levels was not observed. This finding coincides with the result of the study that Türk et al. (2005) have conveyed.

In conclusion it inferred that the evaluation of PON1 activity an early indicator of complications for clinicians that might occur in further periods of pregnancy. These results showed the fact that PON1 activity could be used as a marker relatively at the early phases of pregnancy in complicated pregnant cows.

CONFLICT OF INTEREST

We certify that there is no conflict of interest.

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