Ischemia modified albumin as an early biomarker in diagnosing the late stages abortus in cows

Beran Yokuş¹, Nazlı Ercan²*, Ulvi Reha Fidancı³ and Mustafa Can Gün⁴

Department of Biochemistry, Faculty of Veterinary Medicine, Dicle University, Diyarbakır, Turkey.

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ABSTRACT

Nowadays, Ischemia Modified Albumin (IMA) has been regarded as an indicator for various maladies and complicated pregnancy in humans. Therefore, it is hypothesized that evaluation of the maternal serum IMA degrees can serve as an early detection of abortion in dairy cows. 252 Holstein cows' serum samples were taken both in the 2nd and 6th months of their pregnancy. Twenty-two cattle were selected as a positive control group among the non aborted ones. Furthermore, blood samples from 20 non-pregnant cows were taken to identify the effects of physiological changes of pregnancy. IMA and Ischemia Modified Albumin Rate (IMAR) were markedly higher (p<0.01); and albumin was markedly lower (p<0.01) in aborted cows and healthy-pregnant cows compared with non-pregnant cows in both months. In aborted cows, IMA, IMAR and Oxidative stress index (OSI) degrees were found to be more increased than healthy pregnancies both in 2nd and 6th months of pregnancy; however, there were statistically significant only at 6th month (p<0.01). Total Oxidative Stress (TOS) levels were higher than healthy pregnancies. So, as a result of IMA which was caused by oxidative stress rising from hypoxia/ischemia could serve as an early bioindicator for miscarriage in cattle.

Key words: Abortus, Cattle, Ischemia modified albumin (IMA), Oxidative Stress Index (OSI), Serum chemistry, Total Oxidative Stress (TOS), Total Antioxidant Status (TAS).

INTRODUCTION

Albumin plays a truly important role in eliminating free radicals since it binds the free transitional metals including Co, Ni and Cu at the N-terminal region and thus albumin is regarded as the most outstanding serum protein (Jauniaux et al., 2001). Serum albumin is responsible for no less than 70% of the activities regarding free radical-trapping (Kragh-Hansen 1990). An alteration occurs in N-terminal region because of ischemia, acidosis and hypoxia which also account for the decrease in the binding capacity of the albumin. Because of the reduced blood flow during ischemia, tissues suffer from loss of oxygen and pH decreases thus resulting in production of superoxide radicals and hydrogen peroxide (Dursun et al., 2012). Serum IMA levels were highly related to the increase in the reactive oxygen radicals which were formed due to ischemia related incidents (Ukinc et al., 2009).

FDA (Food and Drug Administration) has given licence to IMA to be used in regular implementations carried out in the clinics for diagnosis of myocardial infarction (MI) and measured by Albumin Cobalt Binding (ACB®) assay (Guven et al., 2009, Shen et al., 2011).

It has been proved that high levels of IMA are also a sign of mesenteric, nephritic, intrauterine ischemia, lung embolism, paralysis, diabetes, dyslipidemia and metabolic syndrome (Refaai et al., 2006, Ukinc et al., 2009). So, it is possible to conclude that IMA can be formed in extra cardiac sites as a result of chronic/acute oxidative stress (Gündüz et al., 2009, Gugliucci et al., 2005, Prefumo et al., 2007).

IMA levels are mostly more elevated than the concentration detected during myocardial ischemia in women whose pregnancy has just started (Prefumo et al., 2007, Guven et al., 2009, Van Rijn et al., 2008). Moreover, IMA levels become higher in complicated pregnancies than regular ones (Dogan et al., 2015, Karadeniz et al., 2015, Cengiz et al., 2015).

Miscarriage appears to be the biggest trouble in dairy farm and obstetric. In dairy cattle, the fetus becomes dead between 42 days and nearly 260 days (Hovingh, 2002). Approximately 3-5% of the cows can miscarry. On the other hand, successful diagnosis of the miscarriage is only around...
25-30% of the total. Most of the miscarriages are not understood at all as the embryo or the fetus is too small to be noticed in early pregnancy.

Abortion and loss of pregnancy in cattle can occur for a variety of reasons. Many times the reason for the loss is complex and difficult to diagnose. Both infectious and non-infectious agents may cause abortion. Even though infectious agents (bacteria, fungi, viruses and one-celled) are considered as the number one reason of the cattle miscarriage, various other factors play a role in this including poor nutrition or inadequate antioxidant status (vitamins A and E, selenium, and iron), genetic factors (King 1990), hormonal irregularities, trauma, toxic agents (nitrates, mycotoxins and aflatoxin), and heat stress which causes fetal hypotension, hypoxia, and acidosis (Al-Samarai et al., 2012). Preservation of the pregnancy is important for the lactation, maintenance of cattle fertility, replacement stock, and in turn the prevention of the economic loss to dairy farms.

For this reason, early detection of aborts, especially during the late stage of gestation, is highly important. Reliable biochemical indicator to predict and diagnose cattle miscarriage has not been demonstrated till now. The present study is the leading one in using the maternal serum IMA levels for the early diagnosis of abortion in dairy cows.

In this study, the hypothesis anticipates that it is possible to utilize maternal serum IMA levels for detecting miscarriage in cattle. In order to understand the possible reason of increases in IMA, the degrees of Total Antioxidant Status (TAS) and Total Oxidative Stress (TOS) and Oxidative Stress Index (OSI) were also evaluated.

**MATERIALS AND METHODS**

**Animals and sample collection:** Serum samples were collected from 252 Holstein cows (age 3-8 years and 2-6 months pregnant) by jugular venipuncture. The samples were coagulated for 30 minutes and then centrifuged for 10 minutes at 3500 rpm. The sera were kept at -20°C till the examination. Frozen samples were blended when they are melted and then centrifuged again before being examined. The hemolyzed samples were excluded. Then the sera of the cows developing abortus throughout pregnancy were separated from those developing none (n=13). In this study, abortus are taken into account at last trimester and before insemination. Throughout the study same conditions were noted for quantification. mL).

**Measurement of the Ischemia Modified Albumin Rate (IMAR):** IMAR value/individual serum albumin formula was utilized to sustain the IMAR rate (IMAR) so that the undesired effects of the distinctions between the albumin concentrations can be avoided.

**Measuring albumin concentrations:** Albumin levels measured colorimetrically with an auto analyzer with the kits (Cobas 8000, Roche Diagnostics, USA). All variables’ variation coefficient were <5%.

**Measuring Total Antioxidant Capacity (TAS):** For measuring the total antioxidant capacity, a new automated colorimetric method of Erel (2005). The principle of this measurement method is based on the fact that the ABTS molecule is oxidized to the ABTS + molecule in the presence of hydrogen peroxide. The reaction rate is calibrated with the standard method Trolox. Unit is Trolox equivalent / L. (μmol H2O2 Equiv./L). Each serum samples’ variation coefficient was lower than 5%.

**Oxidative stress index:** Oxidative stress index (OSI) is obtained by the ratio of TOS to TAS in percentage (Erel 2005). For quantification, the units of TAS outcomes, mmol Trolox equivalent/l, were changed into μmol equivalent/l, and the OSI was measured using the formula;

\[
OSI = \left( \frac{\text{TOS, mmol l}}{\text{TAS, mmol Trolox equivalent/l}} \right) \times 100.
\]

**Statistical analysis:** A paired sample t test was carried out so that the variables can be compared in line with the periods (between 2nd and 6th months) in the groups. Student’s t-test was employed for evaluating the significance of the
difference among the groups’ mean values and to make a comparison between them.

The parameters were demonstrated as mean ± SD values. Statistics package SPSS version 10.0 was employed in performing every statistical analysis (SPSS Inc., Chicago, IL).

RESULTS AND DISCUSSION

Serum IMA, Albumin, TAS and TOS levels of cows with abortus, healthy pregnant, non pregnant groups were shown in Table 1.

In aborted cows, IMA and derived IMAR levels were higher than healthy pregnancies both in 2nd and 6th months of pregnancy; however, it was statistically significant only at 6th month (p<0.01) (Figure 1) and (Figure 2).

There wasn’t found any statistical distinction in terms of IMA, IMAR and TOS degrees between 2nd and 6th months in both healthy pregnant and aborted groups.

Serum levels of IMA as well as IMAR were markedly higher (p<0.01); and serum level of albumin was markedly lower (p<0.01) in aborted cows and healthy pregnant cows compared with non-pregnant cows in both months.

The serum IMA level was increased in cows developing abortion throughout pregnancy.

Serum TOS level was higher in aborted group than healthy pregnant; however these differences was not statistically significant.

The TOS levels were elevated in aborted and healthy pregnant groups relatively to Non-Pregnant group, however this distinction was statistically significant only in aborted cows (p<0.01).

It was observed that serum TAS levels in 6th months were more decreased than the 2nd months in both aborted and healthy pregnant cows. Also, in aborted cows TAS levels in 6th month was markedly decreased (P < 0.01), compared to non-pregnant cows.

In aborted cows, serum OSI level was higher than healthy pregnancies at 6th months of pregnancy (p<0.01) (Figure 3).

In this study population, the incidence of abortion was within the normal ranges of 5.31 % reported in the literature.

IMA has taken the approval of the Food and Drug Administration (FDA) as to be a serum bioindicator to detect the cardiac ischemia at its beginning phase (Shen et al.,

Table 1: Serum IMA IMAR, Albumin, TAS and TOS values (mean±SD) in healthy pregnant, abortus and non pregnant groups.

<table>
<thead>
<tr>
<th></th>
<th>Healthy Pregnant(n=22)</th>
<th>Abortus(n=13)</th>
<th>Non Pregnant (n=20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IMA (ng/ml)</td>
<td>490.1 ± 19.25 a</td>
<td>440.6 ± 26.85 hv</td>
<td>531.9 ± 48 c</td>
</tr>
<tr>
<td>Alb (g/dl)</td>
<td>3.531 ± 0.08 ab</td>
<td>3.236 ± 0.08 bc</td>
<td>3.565 ± 0.07 d</td>
</tr>
<tr>
<td>IMAR</td>
<td>139.6 ± 5.33 a</td>
<td>136.1 ± 7.79 bc</td>
<td>149.7 ± 13.75 c</td>
</tr>
<tr>
<td>TAS (mmol/l)</td>
<td>0.82 ± 0.03 a</td>
<td>0.68 ± 0.04 abc</td>
<td>0.87 ± 0.05 c</td>
</tr>
<tr>
<td>TOS (µmol/l)</td>
<td>3.70 ± 0.54 a</td>
<td>3.79± 0.38</td>
<td>5.11 ± 0.68 a</td>
</tr>
<tr>
<td>OSI (Arb.U)</td>
<td>4.58 ± 0.68 a</td>
<td>4.99 ± 0.35 ab</td>
<td>5.62 ± 0.95 a</td>
</tr>
</tbody>
</table>

Measured variables were compared both according to the months in same group (paired sample t- test) and between groups (independent-samples t-test). a,b,c,d,e,f The distinctions among the mean values which have the same superscripts in every row is significant (at least, p<0.05).
Moreover, IMA can be present for various conditions which have a likelihood of posing a threat to the life and which can result in local or general hypoxic conditions (Lippi et al., 2006), including progressed nephritic disease, skeletal muscle ischemia, lung embolism, paralysis, acute mesenteric ischemia and intrauterine ischemia (Kotani et al., 2011, Refaai et al., 2006, Ukinc et al., 2009).

Recent studies show that serum IMA levels are physiologically elevated during normal pregnancies in humans, and that maternal IMA levels are lower than the cutoff which was applied in detecting cardiac ischemia (Prefomu et al., 2007, Guven et al., 2009, Van Rijn et al., 2008).

As far as it is concerned, this is the leading study regarding serum IMA levels in dairy cows. However, no information about circulating IMA in cattle is presently available, it is seen the increasing IMA levels in healthy pregnant cows compared with non-pregnant ones, and these results were consistent with previous studies reported in humans as given above.

The fact that IMA degrees are increasing supraphysiologically is associated with the intrauterine environment with less oxygen than is needed and leads to an increase in the physiologic oxidative stress during pregnancy (Gündüz et al., 2008). However, as an already acknowledged fact, early fetal organogenesis occurs in a low-oxygen environment, probably because of the limitation of maternal-fetal oxygen transfer due to the gestational sac (Jauniaux et al., 2001).

It was not until the year 2005 that IMA became the subject in question to be examined in umbilical cord blood in complicated pregnancies by Gugliucci and other researchers who set out with the hypothesis that decreased blood flow in complicated births lead to anaerobic metabolism, localized acidosis, inadequate oxygenation and possibly increased IMA.

Several researches have been conducted to study the maternal IMA levels in preeclampsia (Ustun et al., 2011, Bahnipati et al., 2015, Dogan et al., 2015). In contrast to results of Van Rijn and others (2008) other groups have shown that IMA degrees in pre-eclamptic women were significantly more when compared to regular controls and were associated with the severity of pre-eclampsia. The discrepancy in these studies could be accounted for by the number of patients and the distinctions between the severeness of preeclampsia. Moreover, the other studies report that first trimester serum IMA might be a potential biological indicator for defective placental development (Papageorghiou et al., 2008) and intrauterine growth restriction (Karadeniz et al., 2015).

Increased maternal IMA levels were also seen in recurrent pregnancy loss (Ozdemir et al., 2011) and early pregnancy loss (Cengiz et al., 2015). It is put forward that an intrauterine environment with abnormally reduced amount of blood might be related to abnormal placental development which has a share in the early abortion (Ozdemir et al., 2011).

All of these previous reports have clearly demonstrated that complicated pregnancies which are correlated with placental hypoxia and high oxidative stress emerges simultaneously with ischemia.

This study has been suggested that in cattle with miscarriage, a significant elevation was found in the serum IMA levels than regular pregnant cattle. This observation was in accordance with the studies done in humans with complicated pregnancies especially in pregnancy losses (Ozdemir et al., 2011, Cengiz et al., 2015). Even if they are different species, the underlying causes of these complicated pregnancies are the similar.

Thus, it is conclude that, the rise in serum IMA during pregnancy possibly originates from decreased clearance of the free radicals, and increased hypoxia due to ischemia, which causes muscular excitability and consequent abortion. The findings of higher OSI and TOS level observed in aborted cows also supports the hypothesis of this study. Additionally, it is known that elevated oxidative stress and hypoxia are among the reason of abortion.
As a result of monitoring increased serum IMA levels enables early detection of aborted pregnancies in cattle, as well as being a beneficial tool for the clinician. However, further researches need to be conducted so that the relation between IMA and cattle miscarriage can be built.

CONFLICT OF INTEREST
We certify that there is no conflict of interest.

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REFERENCES


