Serum antioxidant status and adenosine deaminase activity during the gestational period of sheep

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SUMMARY
The aim of this study was to evaluate the serum oxidative status and adenosine deaminase activity during the gestational period in sheep. A total of 16 sheep fed with rations formulated according to the period of gestation were included in this study, and serum samples were obtained at pre-conception (PC), on the 1, 2, 3, and 4 months of pregnancy and on the 5th month just after the postpartum (JP) for determining the variations of the malondialdehyde (MDA), glutathione (GSH), nitric oxide (NO), β-carotene and vitamin A concentrations as well as the enzyme superoxide dismutase (SOD) and adenosine deaminase (ADA) activities with adequate enzyme-linked immunosorbent assays. The MDA and β-carotene concentrations were significantly depressed by contrast, vitamin A and NO concentrations were significantly depressed pregnancy but reached significantly elevated values just after the postpartum. These results demonstrate the occurrence of an oxidative stress during pregnancy in sheep and that the tissue adenosine contents were sequentially controlled by the important ADA activity fluctuations.

Keywords: Sheep, pregnancy, malonedialdéhyde, antioxidans, β-carotene, adenosine deaminase.

RÉSUMÉ
L’objectif de cette étude était d’évaluer l’équilibre oxydants / antioxydants dans le sérum ainsi que l’activité de l’adénosine désaminase (ADA) pendant la gestation chez la brebis. Au total, 16 brebis nourries avec des rations adaptées à la période de gestation ont été utilisées et des prélèvements sérémiques ont été réalisés avant la conception, lors du 1er, 2ème, 3ème et 4ème mois de gestation et au 5ème mois juste après l’agnelage afin de déterminer les concentrations de MDA (malonedialdéhyde), de GSH (glutathion), de NO (oxyde nitreux), de β-carotène et de vitamine A ainsi que les activités sérémiques de la SOD (superoxyde dismutase) et de l’ADA par des tests immunoenzymatiques adaptés. Les concentrations de MDA et de β-carotènes ont progressivement et fortement augmenté pendant la gestation alors que l’augmentation des concentrations de GSH s’est avérée plus tardive et plus progressive et que l’activité de la SOD a très faiblement varié mais a atteint des valeurs significativement élevées juste après le post-partum. En revanche, les concentrations de vitamine A et de NO ont été significativement diminuées et l’activité de l’ADA a varié de façon bimodale avec un maximum le 1er mois et un minimum le 4ème mois. Ces résultats démontrent l’existence d’un stress oxydatif durant la gestation chez la brebis et que les concentrations tissulaires en adénosine sont séquentiellement contrôlées par d’importantes variations de l’activité ADA.

Mots clés : Brebis, gestation, malonedialdéhyde, antioxydants, β-carotène, adénosine désaminase.

Introduction
Pregnancy involves changes in physiological parameters, including hormones and enzymes, and functions of the gastrointestinal and cardiovascular systems. These progressive changes are vital for the developing foetus and for preparing the mother for parturition [10].

Cells need oxygen to maintain life [36], but oxygen can produce toxic derivatives called reactive oxygen species [16]. The superoxide anion, hydroxyl radicals, hydrogen peroxide, nitric oxide (NO), and peroxynitrite are well known reactive oxygen species (ROS) produced from oxygen [31]. NO is a free radical generated by NO synthase (NOS) and expression of inducible NOS causes the sustained production of large quantities of NO, and the excessive NO formation produces peroxynitrite [41]. Increased NO synthesis has been reported in placental veins and arteries [32], and it may play a significant role in implantation, regulation of blood flow in the placenta, and relaxation of the uterine muscle during pregnancy [7, 24].

Under normal physiological conditions, a balance exists between the ROS production and the efficiency of antioxidants, including both enzymatic (superoxide dismutase, glutathione peroxidase, catalase, etc.) and non enzymatic (glutathione, β-carotene, vitamin A, etc.) systems [23]. Superoxide dismutase (SOD) catalyzes the dismutation of two superoxide radicals to molecular oxygen and water [31]. Glutathione (GSH) neutralizes ROS and lipid peroxides to maintain intracellular homeostasis and redox balance [16]. While β-carotene, a precursor of vitamin A and vitamin A have antioxidant effects [23, 35], β-carotene may also play a significant role in reproduction [17]. In addition, the vitamin A plays a very
important role in the healthy development of the foetus [35]. Oxidative stress occurs when antioxidant balance is disrupted by the excessive production of ROS and/or an inadequate antioxidant defence system [23] and ROS in excess may cause damage to DNA and proteins, and lipid peroxidation [36]. Malondialdehyde (MDA), a global and very crude indicator of lipid peroxidation occurring under oxidative stress, remains the most useful marker in clinical settings [5, 36]. Increased MDA concentrations have been reported in normal and complicated pregnancies [7, 33]. Oxidative stress may develop in a normal pregnancy [2, 7] because the developing embryo may produce ROS in intracellular and extracellular fluids [16].

Adenosine deaminase (ADA) catalyzes the deamination of adenosine and deoxyadenosine to inosine and deoxyinosine. It is present in all tissues of the body, but lymphoid tissues have a higher ADA activity than other tissues [42]. The enzyme appears fundamental for the differentiation of lymphocytes [15]. Indeed, adenosine has a regulatory effect on the immune system: it depresses production of tumour necrosis factor (TNF) and stimulates the production of NO, playing hence a significant role in maintaining tissue perfusion [1]. ADA is considered to be a marker of cell-mediated immunity in pregnancy [4, 22]. ADA regulates the cellular mechanisms associated with blood flow, vasodilatation, angiogenesis, proliferation, etc. under physiological conditions [32, 40] and a high enzyme activity in placenta plays an important role during gestation. Its main role may be to defend the developing embryo from the accumulation of toxic substrates [8, 9]. It is firmly established within the tissues that form the maternal/foetal interface during the early post-implantation period [19]. Placental and serum activities of ADA have been researched in normal and/or pathologic pregnancies. However, conflicting results have been reported when ADA activity has been evaluated during pregnancy: increased [26], decreased [46], and unchanged [39] serum ADA activities have been reported in normal pregnancy. In many studies, serum markers of oxidative stress and ADA activity have been evaluated in one or only in few time points during the gestational period [20, 46]. However, the values of these markers were found to be different between non pregnant control subjects and pregnant subjects.

The aim of this study was to evaluate variations in the serum antioxidant status (MDA, NO, SOD, GSH, β-carotene and vitamin A) and ADA activity during the healthy gestational period in sheep. For that, serum markers of oxidative stress and ADA activity were evaluated six times from pre-conception to parturition in the same subjects.

Materials and Methods

ANIMALS AND PROTOCOL DESIGN

Sixteen Anatolian Merinos and cross-bred sheep (2-3 years old, 60-75 kg) from the Animal Research Farm of the Konya Veterinary Faculty were fed with rations formulated according to the nutrient recommendations provided by NRC during the gestational period. The rations consisted in forage as the main diet and a supplementary concentrate mix. The diet, which was formulated at the farm, was offered twice daily and fresh water was provided ad libitum. No flushing period was applied to the sheep because the condition scores of the animals at mating were sufficiently high. During the trial, no mineral/vitamin supplementation was provided orally or parenterally. Study protocol was approved by the Veterinary Faculty Ethic Committee.

Blood samples were collected by puncture of the jugular vein into sterile polypolyrene tubes without anticoagulant and after clotting for 1.5 hours at room temperature and centrifugation (2,000 g, 15 minutes, 4°C), sera were carefully harvested and stored at -20°C until analysis. Serum samples were obtained at pre-conception (PC), at 1, 2, 3, and 4 months after conception and on the 5th month, just after parturition (JP).

BIOCHEMICAL ANALYSES

Serum concentrations of MDA [12], NO [25], GSH [6], vitamin A and β-carotene [38] and serum activities of SOD [37] and ADA [14] were measured using methods described previously, with an enzyme-linked immunosorbent assay/spectrophotometric reader (MWGt Lambda Scan 200, Bio-Tek Instruments, VT, USA).

STATISTICAL ANALYSIS

Data were compared at the various time points using ANOVA and the Tukey test (SPSS release 10.0). Results are expressed as means ± standard errors (SE). The significance of correlations between the various biochemical parameters was established using the Person test. Significance was accepted when $P$ value was less than 0.05.

Results

Serum MDA and antioxidant concentrations and enzyme SOD and ADA activities during pregnancy were reported in Table I. Compared to pre-conception values, MDA, β-carotene and GSH concentrations have significantly increased during pregnancy ($P < 0.05$). MDA and β-carotene concentrations were significantly increased since the first month of pregnancy and reached maximal values on the 2nd month, then gradually declined, but whereas the MDA concentrations on the 4th month of pregnancy and just after parturition were similar to initial values, β-carotene concentrations have increased more slowly and remained significantly elevated compared to pre-conception values. Nevertheless, the 2 parameters were highly positively correlated ($r = 0.620$, $P < 0.001$) (Table II). In addition, GSH concentrations have increased during pregnancy more progressively than MDA and β-carotene concentrations, reaching maximal values on the 3rd - 4th months (PC values vs. 3rd month or 4th month values: $P < 0.05$) and abruptly decreased after parturition. The GSH concentrations were significantly and positively
Associated with β-carotene concentrations \((r = 0.463, P < 0.001)\) but not with the MDA concentrations (Table II). Albeit differences with pre-conception values were not statistically significant, the SOD activity also tended to increase during pregnancy and highest values in the enzyme activity were observed on the 5th month, just after parturition \((JP \text{ vs. } PC: P < 0.05)\). However, no significant correlation was obtained between SOD activity and MDA, β-carotene or GSH concentrations (Table II).

Vitamin A and NO concentrations have significantly declined during pregnancy compared to initial values \((P < 0.05)\) but only a moderate positive correlation was observed between these 2 parameters \((r = 0.291, P < 0.01)\). Vitamin A concentrations have early significantly decreased since the first month of pregnancy, reached minimal values on the 2nd month and remained low for the whole pregnancy period but, they have suddenly increased and were similar to initial values just after parturition. This variation profile was strictly opposite to that of MDA or β-carotene and vitamin A concentrations were correlated highly negatively with MDA concentrations \((r = -0.390, P < 0.001)\) and with β-carotene concentrations \((r = -0.635, P < 0.001)\) and moderately with the GSH concentrations \((r = -0.326, P < 0.01)\) (Table II). The decline in NO concentrations during gestation appeared delayed: the NO concentrations have significantly differed with pre-conception values only since the 2nd month, were low until the 4th month \((P < 0.05)\) and finally slightly increased at the end of the period but remained significantly depressed compared to initial values \((P < 0.05)\). Consequently, a negative and highly significant correlation was evidenced between NO and GSH concentrations \((r = -0.470, P < 0.001)\) whereas NO concentrations were moderately associated with the β-carotene concentrations \((r = -0.316, P < 0.01)\) (Table II). No significant correlation was observed between SOD activities and vitamin A or NO concentrations.

Finally, the variations of the ADA activities during pregnancy appeared biphasic (Table I): the enzyme activity highly and rapidly increased on the 1st month of pregnancy \((1\text{st month values vs. } PC: P < 0.05)\) and thereafter, they strongly decreased, were minimal on the 4th month \((P < 0.05)\) and remained significantly

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**Table I**: Serum anti-oxidant status and adenosine deaminase activity in sheep \((n = 16)\) during pregnancy. Results are expressed as means ± standard errors.

<table>
<thead>
<tr>
<th>Protein</th>
<th>PC (0 month)</th>
<th>1 month</th>
<th>2 month</th>
<th>3 month</th>
<th>4 month</th>
<th>5 month</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDA ((\text{mmol/L}))</td>
<td>3.43 ± 0.13^b</td>
<td>4.74 ± 0.23^a</td>
<td>5.02 ± 0.21^a</td>
<td>4.83 ± 0.20^a</td>
<td>3.65 ± 0.17^b</td>
<td>3.35 ± 0.16^b</td>
</tr>
<tr>
<td>NO ((\text{μmol/L}))</td>
<td>15.4 ± 0.91^a</td>
<td>15.2 ± 0.99^a</td>
<td>8.22 ± 0.38^b</td>
<td>5.12 ± 0.36^b</td>
<td>5.30 ± 0.47^b</td>
<td>8.13 ± 0.93^b</td>
</tr>
<tr>
<td>GSH ((\text{μmol/L}))</td>
<td>18.4 ± 0.59^c</td>
<td>20.7 ± 0.65^abc</td>
<td>21.3 ± 0.57^bc</td>
<td>22.1 ± 0.76^ab</td>
<td>23.2 ± 0.67^a</td>
<td>19.9 ± 0.54^bc</td>
</tr>
<tr>
<td>β-carotene ((\text{μg/L}))</td>
<td>38.0 ± 1.16^d</td>
<td>82.1 ± 2.59^abc</td>
<td>91.4 ± 3.60^a</td>
<td>84.6 ± 2.86^ab</td>
<td>78.9 ± 2.8^b</td>
<td>50.4 ± 2.69^c</td>
</tr>
<tr>
<td>Vitamin A ((\text{μg/L}))</td>
<td>23.7 ± 1.22^a</td>
<td>15.5 ± 0.87^b</td>
<td>13.2 ± 0.72^b</td>
<td>15.2 ± 0.98^b</td>
<td>14.1 ± 0.74^a</td>
<td>22.5 ± 1.13^a</td>
</tr>
<tr>
<td>SOD ((\text{μg/L}))</td>
<td>87.8 ± 2.34^b</td>
<td>99.7 ± 3.29^abc</td>
<td>100 ± 2.98^abc</td>
<td>94.1 ± 2.57^ab</td>
<td>99.2 ± 4.09^ab</td>
<td>103 ± 3.26^a</td>
</tr>
<tr>
<td>ADA ((\text{U/L}))</td>
<td>18.6 ± 0.54^b</td>
<td>30.4 ± 1.83^a</td>
<td>21.3 ± 0.88^b</td>
<td>9.32 ± 0.89^d</td>
<td>7.85 ± 0.33^d</td>
<td>13.8 ± 0.65^c</td>
</tr>
</tbody>
</table>

**Table II**: Correlations between biochemical parameters during pregnancy. Significant correlations were indicated in bold.

<table>
<thead>
<tr>
<th></th>
<th>NO</th>
<th>GSH</th>
<th>β-carotene</th>
<th>Vitamin A</th>
<th>SOD</th>
<th>ADA</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDA</td>
<td>(r = -0.38, P &lt; 0.05)</td>
<td>(r = 0.05, P &lt; 0.05)</td>
<td>(r = 0.62, P &lt; 0.05)</td>
<td>(r = -0.39, P &lt; 0.05)</td>
<td>(r = 0.079, P &lt; 0.05)</td>
<td>(r = 0.287, P &lt; 0.05)</td>
</tr>
<tr>
<td>NO</td>
<td>(r = -0.47, P &lt; 0.05)</td>
<td>(r = -0.316, P &lt; 0.05)</td>
<td>(r = 0.291, P &lt; 0.05)</td>
<td>(r = -0.160, P &lt; 0.05)</td>
<td>(r = 0.658, P &lt; 0.05)</td>
<td>(r = 0.01, P &lt; 0.05)</td>
</tr>
<tr>
<td>GSH</td>
<td>(r = 0.463, P &lt; 0.05)</td>
<td>(r = -0.326, P &lt; 0.05)</td>
<td>(r = 0.038, P &lt; 0.05)</td>
<td>(r = -0.228, P &lt; 0.05)</td>
<td>(r = 0.01, P &lt; 0.05)</td>
<td>(r = 0.01, P &lt; 0.05)</td>
</tr>
<tr>
<td>β-carotene</td>
<td>(r = -0.635, P &lt; 0.05)</td>
<td>(r = 0.165, P &lt; 0.05)</td>
<td>(r = 0.074, P &lt; 0.05)</td>
<td>(r = 0.01, P &lt; 0.05)</td>
<td>(r = 0.01, P &lt; 0.05)</td>
<td>(r = 0.01, P &lt; 0.05)</td>
</tr>
<tr>
<td>vitamin A</td>
<td>(r = -0.176, P &lt; 0.05)</td>
<td>(r = 0.002, P &lt; 0.05)</td>
<td>(r = 0.01, P &lt; 0.05)</td>
<td>(r = 0.01, P &lt; 0.05)</td>
<td>(r = 0.01, P &lt; 0.05)</td>
<td>(r = 0.01, P &lt; 0.05)</td>
</tr>
<tr>
<td>SOD</td>
<td>(r = -0.031, P &lt; 0.05)</td>
<td>(r = 0.01, P &lt; 0.05)</td>
<td>(r = 0.01, P &lt; 0.05)</td>
<td>(r = 0.01, P &lt; 0.05)</td>
<td>(r = 0.01, P &lt; 0.05)</td>
<td>(r = 0.01, P &lt; 0.05)</td>
</tr>
</tbody>
</table>

MDA: Malondialdehyde; NO: nitric oxide; GSH: Glutathione (reduced form); SOD: superoxide dismutase; ADA: adenosine deaminase; NS: Not significant.
Depressed at the end of the gestation compared to initial values (JP values vs. PC values; \( P < 0.05 \)). For the whole period, ADA activities were positively associated with MDA concentrations \((r = 0.287, p < 0.01)\) and with NO concentrations \((r = 0.658, P < 0.001)\) and negatively with the GSH concentrations \((r = -0.228, P < 0.05)\) (Table II).

**Discussion**

In the normal pregnancy, many physiological changes occur, including those related to oxidative stress [2, 7, 10].

Firstly, serum concentrations of MDA, a marker of lipid peroxidation [5, 20], early significantly increased in the pregnancy and remained elevated since the 3rd month in the present study. It has been reported that the developing embryo may produce ROS [16], and that high MDA concentrations were observed in the second trimester of pregnancy in woman [7]. As consequence of excessive ROS production, maternal antioxidant systems are mobilized. Serum GSH concentrations gradually increased during sheep pregnancy and just after parturition suddenly declined. In parallel, the activity of SOD, an antioxidant enzyme, has slowly increased for reaching values just after parturition more elevated than the pre-conception values. However, conflicting results about antioxidant systems in pregnancy have been reported. ADEMUYIWA et al. [2] did not find any significant difference in the GSH concentrations between pregnant and non-pregnant women whereas BIONDI et al. [7] have observed significant increases during pregnancy. In the same way, increased [7, 33] or unchanged [2] SOD activity have been reported in pregnancy. Nevertheless, as the removal of prostaglandin at the end of pregnancy causes the production of superoxide radicals [36], increased activity of SOD may be required for the detoxification of excessive amounts of superoxide radicals.

In the present study, the \( \beta \)-carotene concentrations appeared rapidly and dramatically elevated for the whole gestational period, including the end of pregnancy (just after the parturition), whereas the vitamin A concentrations were rapidly and durably depressed until the 4th month. High positive correlations were obtained between the \( \beta \)-carotene concentrations and the MDA or GSH concentrations but, a strong and negative correlation was determined between the vitamin A and \( \beta \)-carotene concentrations. Although low plasma vitamin A and high \( \beta \)-carotene concentrations have been previously reported to occur during pregnancy [17, 18], these 2 parameters were not found significantly associated [17]. As the vitamin A plays a significant role in the healthy development of the foetus [29, 35], HERRERA et al. [18] and VANNUCCHI et al. [44] have suggested that the decreasing profile of plasma vitamin A during pregnancy may result from the greater utilization by the foetus. In addition, the excessive ROS production during pregnancy may also neutralize vitamin A [16]. In this way, negative correlations were observed between vitamin A and MDA (direct marker of the oxidative stress) or GSH (indirect marker) concentrations. Consequently, a 40% increase in vitamin A intake is recommended for pregnant women because of its importance especially in middle and late pregnancy [35]. In addition, prostaglandin E2, an inactive form of prostaglandin F2\( \alpha \), is an antagonist of the retinoid function and it has a negative modulator effect on retinoic acid synthesis [27]. It is well known that the corpus luteum secretes prostaglandins to maintain pregnancy during the gestational period, but the increased production of prostaglandin E2 during the gestational period may cause indirect reduction of the vitamin A concentrations [44]. Furthermore, the corpus luteum contains large amounts of \( \beta \)-carotene [3, 13] which are involved in the positive regulation of the progesterone synthesis [11, 28]. As a vitamin A deficiency induces low progesterone secretion [13], it would be probable that liver released high amount of \( \beta \)-carotene in the blood flow, then the provitamin A compounds were accumulated into the corpus luteum where they were gradually converted into retinol for controlling progesterone synthesis, allowing spare of the circulating pool of the vitamin A, preferentially used for the foetus growth.

In the present study, NO concentrations have progressively declined during pregnancy and remained lower than pre-conception values just after the parturition. In agreement with these results, decreased NO concentrations have been previously reported at the end of pregnancy [7]. Moreover, NO concentrations were positively correlated with the vitamin A concentrations but negatively with \( \beta \)-carotene and GSH concentrations. Because \( \beta \)-carotene are known to down regulate the induction of NOS [43], and/or because of antioxidant properties of \( \beta \)-carotene and GSH [23], low NO concentrations could be related to high concentrations of these antioxidants.

ADA, which is produced by the immune system and by the placenta [15], is considered to be a marker of cell-mediated immunity in pregnancy [4, 22]. It catalyzes the deamination of increased amount of adenosine [22] and plays an important role in the protection of the foetus from intoxication by adenosine in the critical period of pregnancy [8, 9, 30, 34]. It has been reported that a low ADA activity in the early pregnancy may cause pregnancy loss [21]. In the present study, the serum ADA activity rapidly increased in the first month then gradually declined and reached minimal values on the 4th month. In agreement, it has been reported that placental ADA activity increased rapidly in the early part of pregnancy and then fell gradually in cattle, guinea pigs and cats [34]. A significant decrease in ADA activity has been shown to occur in pregnant women in the third trimester, in comparison with non pregnant women [45, 46]. Because of this biphasic profile, the ADA activities moderately correlated positively and negatively with the MDA and the GSH concentrations, respectively. In the late gestational period, an elevated amount of adenosine coupled to a decreased ADA activity may represent a compensatory mechanism that tends to maintain vascular integrity and blood flow to the uterus and placenta [22]. In fact, the body needs to maintain a correct content of adenosine, which acts as a local mediator, in order to maintain the regulation of the immune system and tissue perfusion throughout vasodilatation [1, 32, 40]. In addition, a strong positive correlation was evidenced between the ADA activities and the NO concentrations, in this study. It has been reported that adenosine stimulates the production of NO [1].

As a conclusion, the occurrence of an oxidative stress during pregnancy in sheep was evidenced especially in the mid-pregnancy, and biphasic fluctuations of the ADA activities were encountered, suggesting the importance of a strict control of the adenosine tissue contents in the foetus protection and in the placenta perfusion. Prevention of the oxidative stress, the use of antioxidants (vitamin/mineral supplements), and oral and/or parenteral administration of anti-inflammatory drugs may be acceptable in pregnant women with particular nutritional deficits. These results may also indicate that some parameters investigated in pregnancy should be measured several times and at least in the middle of each of the three trimesters.

References


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