CHANGES IN ANTIOXIDANT COMPONENTS IN BLOOD OF MARES DURING PREGNANCY AND AFTER FOALING

RENATA GÓRECKA, MIROSŁAW KLECKOWSKI, WŁODZIMIERZ KLUCIŃSKI, RYSZARD KASZTELAN* AND EWA SITARSKA

Department of Clinical Sciences, Faculty of Veterinary Medicine, Warsaw Agricultural University, 03-849 Warsaw, Poland
e-mail: gorecka@amaltea.sggw.waw.pl
* Regional Veterinary Diagnostic Laboratory, 18-400 Lomza, Poland

The changes in antioxidant components in the blood of ten cold blood mares during pregnancy and after foaling were investigated. Mares' blood samples were collected three times: at the 6th month of pregnancy, two weeks before delivery and four weeks after foaling. The activity of superoxide dismutase, glutathione peroxidase, total antioxidant status (TAS), total bilirubin and uric acid were measured. Values of all antioxidants except for uric acid differed in three measurements. The presented study showed that the greatest changes in antioxidant system of mares occur around perinatal period.

Key words: mare, antioxidant, pregnancy, foaling.

Oxidative damage has been implicated in pathogenesis of many diseases and tissue injury in animals and humans. The presence of reactive oxygen species (ROS) in cells using oxygen as an energy source led to the formation of protective mechanisms. Recent evidence suggests that these act as a form of an integrated antioxidant system (17). The antioxidant system is composed of a number of components including enzymes, proteins and small molecules. The antioxidant system comprises: antioxidants inhibiting the formation of free radicals, free radical scavengers, and a series of mechanisms involved in the recovery of damage caused by free radicals (17). A healthy organism maintains equilibrium among production, use and neutralization of ROS. Disruption of this equilibrium leads to oxidative stress.

To date the range of destructive effects of ROS in horses is not known. Little is also known about antioxidant protection and adaptive capabilities to oxidative stress in this species. It is known that various kinds of stresses on organisms accelerate the production of reactive oxygen species. Pregnancy is a physiological state accompanied by a high-energy demand of many bodily functions and an increased oxygen requirement. Because of the increased intake and utilization of oxygen, increased levels of oxidative stress would be expected. Available literature shows very few studies
relating to antioxidant protection during pregnancy in horses. This study, therefore, aimed to investigate changes in antioxidant values in the blood of ten cold blood mares during pregnancy and after foaling.

**Material and Methods**

Investigations were carried out on 10 pregnant cold blood mares. They ranged in age from 6 to 10 years. Mares originated from small farms of central and eastern parts of Poland. Mares were used for light agricultural work. Housing conditions and diet were very similar. Mares were fed mainly grains (oats, rye and barley), hay and straw. Occasionally carrot, potatoes and white beets were added. Fodder was prepared locally by horse owners. All mares were in similar state of pregnancy (six months).

Mares' blood samples were collected three times: at the 6th month of pregnancy, 2 weeks before delivery and 4 weeks after foaling. Blood samples were taken from the jugular vein from each horse into clean test tubes and test tubes with heparin. The activity of superoxide dismutase (SOD) in erythrocytes was determined by spectrophotometry with the use of Ransod kit (Randox Laboratories Ltd., UK) and method described by McCord and Fridovich (11). The activity of blood glutathione peroxidase (GSH-px) was determined by spectrometry with the use of Ransel kits (Randox Laboratories Ltd., UK) and method described by Paglia and Valentine (14). Total antioxidant status (TAS) in serum was assessed by spectrometry with the trolox equivalent antioxidant capacity method - TEAC (12, 15). TAS was determined using Total Antioxidant Status kit (Randox Laboratories Ltd., UK). The quality of measurements of SOD, GSH-px and TAS was determined each time with respect to applicable controls: Ransod Control, Ransel Control and Total Antioxidant Status Control Serum (Randox Laboratories Ltd., UK). Serum total bilirubin was determined with spectrometry using a reaction with p-diazobenzenosulphonic acid (2, 10). Spectrometric analysis of serum uric acid is based on the use of uric oxidase modified by a horseradish peroxidase reaction (9). Serum bilirubin and uric acid levels were determined using reagents and analysers from Pointe Scientific (USA). Statistical processing was performed using Friedman's test, with a level of 5% or less defined as a significant level. Table 1 shows the average and standard deviation.

**Results**

A comparison of antioxidant values measured in the 6th month of pregnancy, 2 weeks before foaling and a month after foaling shows differences in all values except for uric acid (Table 1). The activity of superoxide dismutase was unchanged during the pregnancy but after foaling dropped significantly (by about 40%). Changes were seen in glutathione peroxidase level, which dropped 2 weeks before foaling to become extremely low a month after foaling. TAS was at the same level during pregnancy and increased slightly after foaling and this increase was statistically significant. Bilirubin concentration was at the highest level 2 weeks before foaling and lowest one 4 weeks after foaling. There was no evident difference in uric acid levels.
Table 1
Antioxidant values from blood samples of pregnant mares

<table>
<thead>
<tr>
<th></th>
<th>6th month of pregnancy</th>
<th>2 weeks before delivery</th>
<th>4 weeks after foaling</th>
</tr>
</thead>
<tbody>
<tr>
<td>SOD (U.I./g HGB)</td>
<td>1705.7 ± 224.1 *</td>
<td>1744.4 ± 347.8 *</td>
<td>1096.4 ± 205.0 *</td>
</tr>
<tr>
<td>GSH-px (U.I./g HGB)</td>
<td>35.4 ± 11.7 *</td>
<td>27.0 ± 5.0 *</td>
<td>19.0 ± 4.0 *</td>
</tr>
<tr>
<td>TAS (mmol/l)</td>
<td>1.19 ± 0.05 *</td>
<td>1.19 ± 0.04 *</td>
<td>1.25 ± 0.08 *</td>
</tr>
<tr>
<td>Bilirubin (μmol/l)</td>
<td>15.4 ± 2.3 *</td>
<td>23.3 ± 8.6 *</td>
<td>12.3 ± 2.1 *</td>
</tr>
<tr>
<td>Uric acid (μmol/l)</td>
<td>31.2 ± 8.8</td>
<td>30.2 ± 13.9</td>
<td>46.8 ± 23.2</td>
</tr>
</tbody>
</table>

* Values on the same line are significantly different, P< 0.05.

Discussion

Several studies have indicated that the antioxidative defence system is modified during pregnancy (6, 8, 16, 18). In the first measurement (the 6th month of pregnancy) antioxidant levels were very approximated to reference values in horses (3). This confirms that changes in antioxidant system of mares occur in later pregnancy during the period of biggest development of placenta and foetus. The same was observed in pregnant woman and rats (6, 8, 18). The greatest changes were observed 2 weeks before and 4 weeks after foaling. The opposite findings in mares were reported by Ishida et al. (7). Our research showed an increased superoxide dismutase activity (by about 20%) in comparison to previous findings (3), which is contrary to what was found in humans (21). SOD activity decreased significantly only during lactation (4 weeks after foaling), which would suggest that the largest oxidative stress rate occurs in perinatal period.

In the 6th month of pregnancy glutathione peroxidase activity was similar to that found in cold blood horses in earlier research (3) and comparable with values described by Caple et al. (1). The next measurements showed a decrease in GSH-px activity, which is in accordance to works done in humans and rats (13, 19). Values measured 4 weeks after foaling were low and could cause muscular dystrophy (1). In earlier research equivalent low glutathione peroxidase activity in horses originating from the same area was found (3, 4). This could indicate selenium deficiency because the enzyme activity is regarded as a good index of selenium bioavailability (5). Total antioxidant status (TAS) levels were lower compared to reference values in cold blood horses (3), and raised slightly after foaling. These results are concurrent with those reported in women, where total antioxidant activity increased from the 24th week of
pregnancy (8). Both concentrations of bilirubin and uric acid were at comparable levels throughout the experiment and within normal limits (3, 20). The presented research shows that the biggest changes in antioxidant defence occur around perinatal period.

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References