NON-ENZYMATIC ANTIOXIDATIVE DEFENCE MECHANISM IN PLASMA OF PIGS DURING PERIPARTURIENT PERIOD: VITAMIN C AND GLUTATHIONE

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Abstract

The aim of the study was to determine plasma levels of vitamin C and reduced glutathione (GSH) in sows between the day 14 prepartum and day 14 postpartum. The study involved twenty-four sows of three breeds - Polish Large White (PLW), Polish Landrace (PL) and PLWxPL aged 1-3 years. All the animals were from one closed-cycle production farm. The mean vitamin C level on days 13-14 prepartum reached 0.49 ±0.19 mmol/g of protein and decreased significantly (P<0.05) at 24-48 h postpartum to 0.33 ±0.19 mmol/g of protein. On days 6-7 and 13-14 postpartum, the vitamin C level further decreased to 0.17 ±0.006 and 0.15 ±0.007 mmol/g of protein, respectively. The mean GSH level on days 13-14 before delivery was 0.071 ±0.009 mmol/g of protein and decreased significantly (P<0.05) at 24-48 h before delivery to 0.062 ±0.018 mmol/g of protein. In this period, the mean GSH level was similar to that observed during the first 24-48 h postpartum. On day 6-7 after delivery, the level of GSH reached the values observed on days 13-14 and 6-7 prepartum. On days 13-14 postpartum, the level of GSH was found to be 0.115 ±0.029 mmol/g of protein and was significantly higher (P<0.001) compared to that on days 13-14 prepartum. The findings suggest that porcine levels of vitamin C and glutathione decrease during the periparturient period, which may lead to a decreased antioxidative defence system and an imbalance in redox homeostasis.

Key words: pigs, periparturient period, vitamin C, glutathione.

The regulation of reactive oxygen species (ROS) generation is essential for good health of humans and animals. Ineffective neutralisation of ROS leads to oxidative stress, which may deteriorate health status (24). An uncontrolled increase in ROS production leads to oxidative damage to cell membranes, degradation of cell structures, lysis of cells and tissue damage (6). Oxidative stress is even likely to result in impaired steroidogenesis and metabolism of arachidonic acid (24), and dysfunction of phagocytes (1).

Numerous studies have demonstrated that oxidative stress develops during pregnancy and delivery (4, 14, 21, 31). An imbalance between generation and neutralisation of ROS in the periparturient period is likely to impair its course and may be involved in aetiology and pathogenesis of periparturient diseases (13, 24, 32).

The oxidative defence system, consisting of enzymatic and non-enzymatic mechanisms, is responsible for maintaining appropriate levels of ROS (12). The key non-enzymatic antioxidants include vitamin C and glutathione.

Vitamin C (ascorbic acid) is considered the most important antioxidant in extracellular fluids (8, 9). Together with water-soluble antioxidants such as uric acid, bilirubin, albumins, and thiol groups of proteins, it protects plasma lipids against oxidative damage (8, 9). Moreover, vitamin C is involved in cellular antioxidative defence (9). At low levels and in the presence of transition metals, vitamin C can also act as a pro-oxidant (27).

Glutathione (GSH) is one of the key water-soluble antioxidants (2). It is a tripeptide synthesised in the cells from glutamate, cysteine, and glycine. Glutathione is the main source of thiol groups (-SH) in mammalian cells (12). It has important antioxidative functions and is involved in xenobiotics detoxication (23). Moreover, it is responsible for proper oxidation-reduction potential of thiol groups in the cell, maintaining the SH groups on proteins in their reduced form (7). Antioxidative effects of glutathione include ROS scavenging and participation in catalytic activities of such antioxidative enzymes as glutathione peroxidase (GSH-Px), glutathione transferase (GSH-Tr), and glutathione reductase (GSR) (2).

Since the available literature does not provide many data on vitamin C and glutathione levels in periparturient pigs, the study was undertaken to determine plasma levels of vitamin C and reduced glutathione in pigs between the day 14 prepartum and day 14 postpartum.
Material and Methods

Animals and material collection. The study was carried out on 24 pregnant sows of the Polish Large White (11 sows), Polish Landrace (7 sows), and Polish Large White x Polish Landrace (six sows) breeds, aged 1-3 years. All the animals were from one closed-cycle production farm, in which the main herd consisted of 1,200 sows. From the moment of piglet weaning, sows were kept individually in pigsties with grate floors. Artificial insemination was performed for reproduction. Once the pregnancy was diagnosed, sows were kept in pigsties, six-eight individuals each. During pregnancy, sows received the full-portion feed according to changing demands for nutrients and energy. The feed was prepared in the farm’s mixer. The feed for sows in farrow contained: barley, oats, wheat-rye, wheat bran, rape cake, soybean meal, mineral lick, and premix for pregnant sows. The feed for lactating sows contained wheat, barley, maize, wheat-rye, wheat bran, soybean meal, mineral lick, and premix for lactating sows. Eight to ten days before delivery, sows were transferred into the farrowing facilities and kept in single pigsties. All deliveries were uneventful. The sows farrowed alive, 9-14 piglets (11 on average). In the period between day 13-14 prepartum and day 13-14 postpartum, animals were clinically assessed daily, in the morning and at night, in order to rule out early manifestations of any disease. Blood samples were collected from the jugular vein, 9 ml, to the heparinised (14 U/ml blood) vacuette tubes (Greiner Labortechnik GmbH, Austria). Blood was sampled 6 times: on days 13-14 and 6-7 prepartum, at 24-48 h prepartum, at 24-48 h postpartum, and on days 6-7 and 13-14 postpartum. After blood centrifugation, the plasma was obtained. Plasma levels of vitamin C and reduced glutathione (GSH) were determined.

Vitamin C determination. The procedure was performed as outlined by Omaye et al. (25). Briefly, 1 ml of plasma was mixed with 1 ml of cold 5% HPO3 and centrifuged for 20 min at 3,500 x g at 4 °C; 600 µl of supernatant were mixed with 300 µl of citrate acetate buffer (22 g of trisodium citrate dihydrate/100ml of distilled H2O, pH adjusted to 4.15 by glacial acetic acid) containing p-chloro mercuribenzoate (200 mg/100 mL of distilled H2O ) and 300 µl of 2.6 dichlorophenyl-indofenol (100 mg/L of distilled H2O). Exactly after 30s, the absorbance was measured at 520 nm and a few crystals of ascorbic acid were added to get rid of the colour. Then, the absorbance was re-measured. Controls were prepared accordingly but instead of supernatant, an equal volume of 5% HPO3 was added. Two measurements of sample, one before addition of ascorbic acid, and one after, were subtracted. Two measurements of control, one before addition of ascorbic acid, and one after, were subtracted. The sample results were subtracted from the control results. The level of vitamin C was calculated from the standard curves prepared with ascorbic acid solution in 5% HPO3 and concentrations of 0-100 µmol/L. The results were expressed as mmol/g of protein.

GSH determination. The concentration of GSH in blood plasma was determined using the ready kit (Glutathione Assay Kit, Cayman Chemical Company, USA). The method is based on the reaction of SH groups of glutathione with DTNB [5,5’-dithiobis (2-nitrobenzoic acid)] and the synthesis of yellow TNB (5-thio-2-nitrobenzoate anion). The amount of TNB correlates positively with the concentration of GSH in the sample. The absorbance was measured at 405 nm and recalculated into GSH content using the standard curves prepared with different dilutions of GSH. The results were expressed as mmol/g of protein.

Protein determination. The protein content in plasma was determined using the Lowry’s et al. method (17).

Statistical analysis. The results were statistically analysed calculating a mean, standard deviation, and significance of differences. Mean values in a given group were compared to baseline values (days 13-14 prepartum) using the Statistica 5.0 software. Significance of differences was defined at P<0.05, P<0.01, and P<0.001.

Ethics of the study. The study design was approved by the Local Ethical Committee.

Results

The mean vitamin C levels before delivery were similar ranging from 0.49±0.19 to 0.57±0.24 mmol/g of protein (Table 1). During the first 24-48 h postpartum, the mean vitamin C level reached 0.33 ±0.19 mmol/g of protein and was significantly lower (P<0.05) compared to values on days 13-14 prepartum. On days 6-7 and 13-14 postpartum, the level of vitamin C further decreased: 0.17 ±0.06 and 0.15 ±0.07 mmol/g of protein, respectively.

The mean level of GSH at 2 weeks prepartum was 0.07 ±0.009 mmol/g of protein and significantly decreased (P<0.05) at 24-48 h prepartum - 0.062 ±0.018 mmol/g of protein. This level maintained during the first 24-48 h postpartum. On about day 7 postpartum, the level of GSH reached the values observed in the first two measurement periods. On days 13-14 postpartum, the GSH level was 0.115 ±0.029 mmol/g of protein and was significantly higher (P<0.001) compared to its value on days 13-14 prepartum.

Discussion

The literature data concerning the prooxidative-antioxidative status of pigs during the periparturient period are fragmentary (15, 18, 22, 30). Moreover, reference data regarding parameters of antioxidative status during the proper periparturient period in pigs are lacking. Our study enabled to follow the changes in plasma vitamin C and glutathione levels in healthy pigs between the day 14 prepartum and day 14 postpartum.
Vitamin C and glutathione are the most important, water-soluble non-enzymatic antioxidants. Low-molecular, non-enzymatic antioxidants, both endogenous and exogenous, are the second line of defence against ROS (12). They neutralise those ROS, which have not been neutralised by antioxidative enzymes and prevent the chain reaction of lipid peroxidation.

Analysis of plasma levels of vitamin C in pigs showed its statistically significant decrease after delivery. This is likely to indicate its use during neutralisation of excessive ROS, as it is known that under oxidative stress conditions vitamin C is the antioxidant used first (8). Increased production of ROS during pregnancy results from an increased metabolism and subsequent higher oxygen demands of tissues (29). Moreover, the potential source of ROS during pregnancy is metabolism of arachidonic acid (32) and activation of circulating leukocytes (10). Another possible cause of increased levels of ROS is reduced activity of antioxidative defence of the organism. Many authors demonstrated decreased periparturient serum levels of such antioxidants as vitamin E (22) or selenium (30) and decreased activity of glutathione peroxidase (18). The lowest levels of these antioxidants were found during delivery. Increased production of ROS during delivery may be associated with delivery stress and efforts related to pushing of the foetus (14).

The literature data concerning plasma levels of vitamin C in periparturient pigs are sparse. Our results correspond with those reported by Yen and Pond (33), who demonstrated decreased levels of vitamin C between the pregnancy day 108 and day 7 postpartum. According to Pinelli-Saavedra and Scaife (26), the concentration of vitamin C in sow serum decreased between the pregnancy days 60 and 103. Malinowska (19) observed decreased vitamin C levels in porcine blood serum and tissues during the last period of pregnancy, which she explained by a passage of vitamin C from the sow to foetuses. The occurrence of placental transfer of vitamin C in sows was demonstrated by Yen and Pond (33). According to some other studies published by Malinowska et al. (20), the levels of vitamin C decreased by the end of pregnancy and decreased further in the first period of lactation. Decreased vitamin C levels observed after delivery might have also resulted, at least partially, from its passage to milk. The passage of vitamin C to milk of sows was confirmed by Ching et al. (5).

The role of vitamin C in the antioxidative defence is to scavange ROS and regenerate vitamin E (28). Vitamin C effectively eliminates superoxide anion radical, hydrogen peroxide, peroxyl radical, and singlet oxygen and can readily react with lipid peroxides (28). Moreover, vitamin C can scavange hypochlorous acid and tyrosol radicals and prevent lipid oxidation by reactive nitrogen forms (3). Vitamin C is the main antioxidant of extracellular fluids and plays an important role in the antioxidative defence of cells (28). Its levels in leukocytes are high; it is also essential for normal function of neutrophils (1).

Our findings demonstrated that the plasma GSH level in pigs significantly decreased in the last 48 h before delivery and remained low during the first 48 h after delivery. This suggests that the production of ROS during this period is the highest and thus higher amounts of glutathione are used for their neutralisation. Glutathione plays an important role in the defence against ROS, particularly in the defence of enzymatic proteins containing SH groups (2). This results from the fact that glutathione thiol groups are more available for oxygen than protein thiol groups (7). Glutathione reacts directly with such ROS as superoxide anion radical, hydroxyl radical, peroxyl radical, anion radical of peroxynitrous acid, hydrogen peroxide, and lipid peroxides (12). Moreover, glutathione is the donor of electrons in peroxide reduction reactions catalysed by glutathione peroxidase (GSH-Px) (12). The final product of glutathione oxidation is glutathione disulfide (GSSG). An important function of glutathione is its involvement in the repair of DNA damage caused by peroxidation and in protection against apoptosis induced by ROS (11). In its reduced form, glutathione is necessary for function-

### Table 1

Mean plasma levels of vitamin C and GSH in pigs during the periparturient period

<table>
<thead>
<tr>
<th>Parameter</th>
<th>13-14 d prepartum</th>
<th>6-7 d prepartum</th>
<th>24-48 h prepartum</th>
<th>24-48 h postpartum</th>
<th>6-7 d postpartum</th>
<th>13-14 d postpartum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin C (mmol/g of protein)</td>
<td>0.49 ±0.19</td>
<td>0.55 ±0.18</td>
<td>0.57 ±0.24</td>
<td>0.33*</td>
<td>0.17**</td>
<td>0.15**</td>
</tr>
<tr>
<td>GSH (mmol/g of protein)</td>
<td>0.071 ±0.009</td>
<td>0.075 ±0.036</td>
<td>0.062* ±0.018</td>
<td>0.061*</td>
<td>0.075 ±0.02</td>
<td>0.115*** ±0.019</td>
</tr>
</tbody>
</table>

* - P<0.05 compared to baseline (days 13-14 prepartum);
** - P<0.01 compared to baseline (days 13-14 prepartum);
*** - P<0.001 compared to baseline (days 13-14 prepartum); ± SD.
ing of glutathione peroxidase (GSH-Px) and glutathione transferase (GSH-Tr) (12). The levels of glutathione may be reduced by stress (16). The literature data concerning plasma levels of glutathione in periparturient pigs are lacking. The only data about the levels of glutathione in pregnant pigs were published by Kovalenko and Anisikina-Levchuk (15). The authors observed decreased glutathione levels on gestation day 90 compared to its value on gestation day 60. Furthermore, they found increased glutathione levels in foetal membranes and its decreased levels in the foetal liver on gestation day 90.

In conclusion, our findings demonstrate that the levels of vitamin C and glutathione in periparturient pigs are decreased, which may lead to a decreased antioxidant defence system and an imbalance in redox homeostasis.

References


