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Original article

Changes in oxidative stress markers in plasma of sows during periparturient period

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Abstract

The aim of the present study was to determine changes in oxidative status in plasma of sows during periparturient period using markers of lipid and protein peroxidation intensity. Plasma from 12 pregnant gilts collected on days 14 and 1 before parturition and on days 1, 7 and 14 postpartum was used in the study. As indicators of alterations in the oxidative status, plasma concentrations of thiobarbituric acid reactive substances (TBARS) and sulphhydryl groups (SH groups) were measured spectrophotometrically, as well as plasma concentrations of bityrosine and formylkinurenine were determined by the spectrofluorimetric method. Compared to the 14th day antepartum, the concentration of TBARS increased significantly on day 1 postpartum and then decreased significantly on days 7 and 14 after parturition. The concentration of SH groups increased significantly on the last day before parturition and decreased significantly on day 14 postpartum. The concentration of bityrosine increased significantly on the last day before parturition and decreased significantly on days 7 and 14 postpartum. The concentration of formylkinurenine increased significantly on the last day before parturition, remained at this level on the first day after parturition and then decreased slightly on days 7 and 14 postpartum. These results indicate that during periparturient period in sows, especially around the parturition, the oxidative/antioxidative balance is disturbed, which leads to oxidative stress. Considering that oxidative stress is potentially harmful for mothers and neonates, this may be an important factor in the increased risk of diseases occurring during this period.

Key words: oxidative stress markers, lipid and protein peroxidation, periparturient period, plasma, sows

Introduction

Oxidative stress can be defined as an imbalance between the production of reactive oxygen species (ROS) in the organism and the ability of the antioxidant molecules to neutralize them (Yoshikawa and Naito

2002). Although at low levels ROS play an important role in the course of many physiological processes, including the mammalian reproductive functions (Agarwal et al. 2005, Al-Gubory et al. 2010, Kumar and Pandey 2015), their uncontrolled production is a threat to the body (Halliwell 2006, Pham-Huy et al. 2008).

The accumulation of excessive amounts of ROS results in oxidative damage to cellular molecules (such as lipids, proteins and DNA) and consequently tissue injury, immune suppression and disturbances in the metabolism and physiology (Halliwell 2006, Lykkesfeldt and Svendsen 2007). These conditions can contribute and/or lead to the development of health disorders (Halliwell 2006, Lykkesfeldt and Svendsen 2007, Pham-Huy et al. 2008).

Periparturient period is especially critical for health and subsequent performance of farm animals (Sordillo and Aitken 2009, Sharma et al. 2011). The results of studies in humans and cows indicate that an imbalance between generation and neutralization of ROS in this period is likely to impair its course and may be involved in etiopathogenesis of peripartum diseases (Agarwal et al. 2005, Al-Gubory et al. 2010, Kankofer et al. 2010, Sharma et al. 2011, Solberg et al. 2017). An increased oxidative stress is also responsible for impaired milk production and reproductive performance, as well as influences neonate vitality and development (Saugstad 1998, Zhao et al. 2013, Kim and Kim 2017).

The intensity of oxidative stress can be monitored with several biomarkers including antioxidative enzymes and non-enzymatic antioxidants, as well as end products or intermediates of peroxidative processes of macromolecules (Abuja and Albertini 2001, Passi 2001). Lipids and proteins are the main biological targets of ROS (de Zwart et al. 1999). Therefore, oxidative damage products of these macromolecules are often used to study the intensity of oxidative stress in humans and animals (Halliwell and Whiteman 2004). The most commonly used method for determining the intensity of lipid peroxidation is reaction with thiobarbituric acid (TBA), which is based on the formation of a colorful adduct in reaction between thiobarbituric acid and some products of lipid peroxidation, mainly malonic dialdehyde (Abuja and Albertini 2001).

Proper function of protein molecules requires the right number of sulphhydryl groups (SH groups) on cysteine residues (Marnet et al. 2003). Any decrease in concentration of SH groups may indicate peroxidative damage to proteins. On the other hand, the organism may also react to protein damage by compensatory increase of SH groups. The content of SH groups, as well as the concentration of products of peroxidative damage to tyrosine and tryptophane-bityrosine and formylkoinurenine, respectively, may be used as markers of peroxidative damage of proteins (Halliwell 2006).

It is known, that many diseases in sows are linked with oxidative stress (Brambilla et al. 2013, Štukelj et al. 2013, Dimri et al. 2014). It has been also found that

oxidative stress effects sows performance (Kim et al. 2013, Kim and Kim 2017). However, there is few information in the literature about oxidative stress during periparturient period in sows. Therefore, the aim of the present study was to determine changes in oxidative status in plasma of sows during periparturient period using markers of lipid and protein peroxidation intensity.

Materials and Methods

The study was approved by the local ethics committee convened by the University of Life Sciences in Lublin and was performed in accordance with animal protection regulations.

Animals and material collection

The study was performed on 12 pregnant gilts (7 Polish Large-White gilts and 5 Large-White x Landrace gilts). All the animals were kept on one closed-cycle production farm. Once the pregnancy was diagnosed, the gilts were kept in pigsties, six individuals per sty. The sows were moved from the gestation to the farrowing unit 8-10 days before the expected farrowing date and kept in individual farrowing pens. During pregnancy, the sows were fed according to the changing demands for nutrients and energy. The feed was prepared in the farm's mixer and contained: barley, oats, wheat barn, rape cake, soybean meal, mineral lick, and premix for pregnant sows. Between day 14 antepartum and day 14 postpartum, all the animals were clinically daily examined, in order rule out any diseases. All the gilts qualified for the study were clinically healthy, the farrowings occurred spontaneously at 114-116 days of pregnancy, and no drug was administered during any stage of farrowing. Furthermore, no obstetrical assistance had been given. To exclude any influence of the duration of the expulsive stage of farrowing, litter size and fetal death, only gilts in which the duration of the expulsive stage was below 3 h and which had litters with 9-12 live born piglets in total were used in the study.

Blood samples were collected from the vena cava cranialis into plastic tubes containing heparin. Blood was sampled 5 times: on days 14 and 1 antepartum, and on days 1, 7 and 14 postpartum. After blood centrifugation, the plasma was obtained. The plasma was frozen immediately after collection and stored at -76°C until analysis. Plasma levels of thiobarbituric acid reactive substances (TBARS), SH groups, bityrosine and formylkoinurenine were determined.

Table 1. Mean concentrations of markers of lipid peroxidation (TBARS) and protein peroxidation (SH groups, bityrosine and formylkinurenine) in the plasma of the examined sows

Parameter	Time sampling				
	14 days antepartum	1 day antepartum	1 day postpartum	7 days postpartum	14 days postpartum
TBARS (mmol/g protein)	0.115 ^a ±0.023	0.129 ^b ±0.026	0.135 ^{acd} ±0.021	0.110 ^c ±0.016	0.090 ^{abd} ±0.012
SH groups (μmol/g protein)	2.69 ^{abcd} ±0.53	3.78 ^{ac} ±0.41	3.87 ^{bf} ±0.43	3.87 ^c ±0.54	3.34 ^{def} ±0.36
Bityrosine (μg/mg protein)	0.160 ^{ab} ±0.044	0.205 ^{acde} ±0.022	0.232 ^{bctg} ±0.016	0.156 ^{df} ±0.020	0.142 ^{eg} ±0.013
Formylkinurenine (μg/mg protein)	0.065 ^{ab} ±0.010	0.079 ^{acd} ±0.006	0.075 ^b ±0.009	0.070 ^c ±0.010	0.065 ^d ±0.019

a, b, c, d, e, f, g – values within a row with the some letters differ at $p < 0.05$.

TBARS determination

The concentration of TBARS was determined according to the method described by Ledwozyw et al. (1986). The reaction products were measured spectrophotometrically at 532 nm (Ultrospec 2000, Pharmacia, Uppsala, Sweden). The calculation was based on a standard curve prepared with different dilutions of malondialdehyde (MDA). The results were expressed in μmol/L/g protein.

SH groups determination

The content of SH groups was determined as described by Rice-Evans et al. (1991). The reaction mixture absorbance was measured at 412 nm (Ultrospec 2000, Pharmacia, Uppsala, Sweden). The concentration of SH groups was calculated using a standard curve prepared with different dilutions of glutathione (GSH, Sigma, Poznan, Poland) ranging from 0 to 1 mmol/L in sodium phosphate buffer (10 mmol/L, pH 8.0) and expressed in μmol/g protein.

Bityrosine determination

Bityrosine was determined by the spectrofluorimetric method according to Rice-Evans et al. (1991). The plasma sample was excited by light at 325 nm and emissions were measured at a wavelength of 420 nm. The standardization of the spectrofluorimeter (Jasco, Tokyo, Japan) to 100 deflection was performed with quinine sulphate (0.1 μg/ml in 0.1 mol/l H₂SO₄) at an excitation of 350 nm and an emission of 445 nm. Samples were diluted with sodium phosphate buffer (10 nmol/l, pH 8.0). The results were expressed as μg/mg protein.

Formylkinurenine determination

Formylkinurenine was determined by the spectrofluorimetric method according to Rice-Evans et al. (1991). The plasma sample was excited by light at 360 nm, and emissions were measured at a wave-

length of 454 nm. The spectrofluorimeter (Jasco) was standardized as described above. The results were expressed as μg/mg protein.

Protein determination

The protein contents in the plasma samples were measured by the biuret method using commercial assay kits (Cormay, Lublin, Poland) based on spectrophotometric measurement (Ultrospec 2000, Pharmacia, Uppsala, Sweden). These values were only used for recalculations of the examined parameters for more objective comparisons between the different sample sources.

Statistical analysis

Statistical analysis was performed using the computer program STATISTICA version 10.0 (Statsoft, USA). The one-way ANOVA test with the HSD Tukey's test was used to determine significant differences in the concentrations of the examined biomarkers between the successive sampling periods. All values are expressed as the mean ± standard deviation (SD). The level of significance was set at $p < 0.05$.

Results

The results of the study are presented in Table 1. The mean plasma concentration of TBARS increased markedly on day 1 antepartum and significantly ($p < 0.05$) on day 1 postpartum compared to the values obtained on day 14 antepartum. Then it decreased significantly ($p < 0.05$) on days 7 and 14 after parturition.

The mean concentration of SH groups was the lowest on day 14 before parturition. Then it increased significantly on the last day before parturition. On the first day postpartum the concentration of SH groups increased further and remained stable on the day 7 postpartum. On day 14 postpartum the concentration of SH groups decreased significantly ($p < 0.05$).

The mean concentration of bityrosine increased significantly ($p < 0.05$) on the last day before parturition, compared to the value obtained on day 14 antepartum. On the first day after parturition there was a further marked increase in bityrosine concentration. Then the concentration of bityrosine decreased significantly ($p < 0.05$) on days 7 and 14 postpartum.

The mean concentration of formylkinurenine increased significantly ($p < 0.05$) on the last day before parturition, compared to the value obtained on day 14 antepartum. On the first day after parturition the concentration of formylkinurenine remained at a similar level as on the last day before parturition. Then the concentration of formylkinurenine decreased slightly on days 7 and 14 postpartum.

Discussion

In the present study the changes in oxidative status in plasma of sows during periparturient period was assessed using markers of lipid and protein peroxidation intensity.

The results obtained in our study demonstrate a dynamic and time-dependent pattern of analysed parameters. We found that the TBARS concentration increased markedly but not significantly on the last day before parturition and significantly on the first day after parturition, compared to the values obtained on day 14 before parturition. Then the TBARS concentration dropped significantly on day 7 postpartum. In term of protein peroxidation markers, our study showed an increase in the concentration of SH groups before parturition, which was observed up to 14 days postpartum. We suppose this may suggest compensatory increase of SH groups in response to protein damage by ROS. The pattern of the formylkinurenine was similar to the SH groups pattern. In turn, the changes in bityrosine concentration were similar to TBARS. These results indicate that the intensity of lipid and protein peroxidation in sows with uncomplicated periparturient period increases significantly around parturition and may persist up to day 14 postpartum. This suggests that the amount of ROS produced at the end of pregnancy and immediately after parturition in sows may exceed the capacity of antioxidant defenses for their neutralization. This leads to oxidative stress and, consequently, to increased lipid and protein peroxidation intensity. If comparing oxidative markers between the first day after parturition and days 7 and 14 postpartum, our study showed progressive decrease in the concentration of these markers, which could mean restoring balance between oxidative and antioxidative processes.

Few studies have concerned oxidative stress markers in sows during periparturient period (Berchieri-

-Ronchi et al. 2011, Zao et al. 2013, Tan et al. 2015, Rubio et al. 2019). Our findings are supported by the results of Tan et al. (2015), who have found the higher serum levels of TBARS during late gestation (days 90 and 109) and early lactation (days 1 and 3) than in early gestation (day 10). In turn, Rubio et al. (2019) have shown a significantly higher concentration of advanced oxidation protein products (AOPP) in saliva of sows on the first day than on day 9 and 20 of lactation. The results of study by Berchieri-Ronchi et al. (2011) have demonstrated elevated oxidative DNA damage throughout the late gestational and lactational periods compared to early gestation. On the contrary, the studies by Zao et al. (2013) have shown no differences in plasma concentrations of oxidative protein damage marker (protein carbonyl) among measurements on days 60 and 109 of gestation and day 18 of lactation. Similarly, plasma concentrations of malonic dialdehyde (MDA) – end product of lipid peroxidation - have not differed among measurements on different days of gestation (days 60, 90, and 109) and lactation (days 3 and 18).

The results of our study correspond to the results of studies carried out on cows. It has been found an increase in plasma TBARS concentration at parturition and during the first week postpartum (Bernabucci et al. 2005, Castillo et al. 2005). Castillo et al. (2006) have demonstrated a significant increase in lipid peroxidation during the first week after parturition comparing to the following 8 weeks postpartum. In turn, Sharma et al. (2011) have shown a significantly higher lipid peroxidation (plasma MDA concentration) in early lactating cows as compared to advanced pregnant cows. It has been also shown that the intensity of protein peroxidation in cows was significantly higher in the early postpartum period compared with prepartum measurements (Bernabucci et al. 2005, Albera and Kankofer 2010, Kankofer et al. 2010).

Also data from human medicine indicate an increase in plasma markers of oxidative stress around parturition in women (Nakai et al. 2000, Kobe et al. 2002, Mocatta et al. 2004, Cindrova-Davies et al. 2007).

Oxidative stress results from an increased production of ROS and/or not sufficient antioxidant defense. This may occur when ROS are produced faster than they can be safely neutralized by antioxidant mechanisms. Many studies, in both humans and animals, including sows, have demonstrated a reduction in the body's antioxidant potential in late pregnancy and after parturition (Albera and Kankofer 2011, Szczubiał 2010, Berchieri-Ronchi et al. 2011, Tanaka et al. 2011). Another explanation for the occurrence of increased oxidative stress intensity around parturition may be increased metabolism during this period, associated

with the rapid fetal growth, the development of mammary gland and the start of lactation (Gitto et al. 2002). It has been also found an increased ROS production during parturition as a result of transitory hypoxia/reoxygenation of maternal and fetal tissues during uterine contractions (Cindrova-Davies et al. 2007). Some authors point to a pain during parturition as a factor increasing ROS production (Mehmetoglu et al. 2002). It is also believed that the increased ROS generation during parturition is probably due to long-term participation of the mother's skeletal and uterine muscle (Schulpis et al. 2007). Moreover, social stresses and stress connected to parturition may be responsible for increased ROS production (Kashif et al. 2003, Zhao et al. 2013).

Lipids of cell membranes and plasma lipoproteins are most vulnerable to an attack by ROS (de Zwart et al. 1999). The process of lipid peroxidation results in the production of lipid peroxides and aldehydes, which are highly toxic and can damage cells (Aw 1998). As a result of lipid peroxidation cell membrane function is impaired and the receptors and enzymes associated with it are inactivated (de Zwart et al. 1999). Lipid peroxidation also leads to an increase in cell membrane permeability, which can lead to release of cell content and its death (de Zwart et al. 1999). Toxic lipid peroxidation products can change enzyme activity, react with DNA and inhibit the synthesis of enzymes involved in DNA and RNA repair processes (Halliwell and Whiteman 2004).

Oxidative damage to proteins can affect their functions as receptors, enzymes, transport or structural proteins (Marnett et al. 2003). Proteins that are altered during peroxidation may secondarily damage other biomolecules (Wiseman and Halliwell 1996). Moreover, oxidized proteins can generate new antigens and provoke immune response (Halliwell and Whiteman 2004). It was also found that protein peroxidation leads to release of various inflammatory signals molecules (Salzano et al. 2014).

Conclusions

Our results showed dynamic changes in plasma parameters of oxidative status during periparturient period in sows with uncomplicated pregnancy and parturition. The changes in concentrations of lipid and protein peroxidation products indicate, that during periparturient period, especially around the parturition, the oxidative/antioxidative balance is disturbed, which leads to oxidative stress. Considering that oxidative stress is potentially harmful for mothers and neonates, this may be an important factor in the increased risk of diseases occurring during periparturient period.

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