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RESEARCH ARTICLE

Changes in Oxidative Stress Status in Sows from Days 100 of Gestation to Post-Partum Estrus

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ABSTRACT

During the stages of sow reproduction, their physiological conditions suffer from heavy burden. These phenomena can lead to the elevation of their metabolism level, the increase of active-oxygen free radicals and ultimately trigger oxidative stress. In the present study, we detected oxidative damage and antioxidant status in blood samples collected from multiparous sows (n=24) during their gestation and lactation, including days 100 of gestation (G100); farrowing day (F); days 3, 13, and 23 of lactation (L3, L13, and L23); day 3 post-weaning (W3); and the day of post-partum estrus (E). Oxidative damage and antioxidant status were determined using the reactive oxygen metabolites (d-ROMs) test and the plasma antioxidant test (PAT), respectively. These results indicate that oxidative damage significantly increased ($P<0.05$) from G100 to E and reached a peak at L13. The antioxidant defense levels were highest at L23 and were significantly higher than those at other stages ($P<0.05$), and antioxidant defense levels at G100 were significantly higher ($P<0.05$) than those at farrowing, lactation, weaning and post-partum estrus. Moreover, the overall oxidative stress also increased from G100 to E, and reached a peak at L3, which was significantly higher than any other stages except L13 ($P<0.05$). Besides, a significant correlation was observed between litter size and oxidative stress level. These results indicate that oxidative stress (OS) occurs during reproduction in the sow, and increases significantly at lactation stages, suggesting that some preventive measure should be taken during the reproductive stages.

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INTRODUCTION

Oxidative stress (OS) occurs when the system is unable to neutralize and eliminate the free radicals and active intermediates at the cellular or organismal level (Murphy, 2009). Under such conditions, the excess free radicals can cause oxidative damage to proteins, lipids, and nucleic acids by interacting with other molecules within the cells (Rahal *et al*., 2014). Numerous studies show that the oxidative stress can induce DNA damage, thereby disrupting cell proliferation and differentiation, while increasing the activity of exogenous carcinogens, potentially leading to cancer (Klaunig *et al*., 2010). In addition, lipids are the primary targets of reactiveoxygen species: abnormal increase of free radicals and the simultaneous decrease in antioxidant defense mechanisms can promote lipid peroxidation, leading to damage to cellular organelles. Hydroperoxides have toxic effects on cells directly or through their degradation into highly toxic hydroxyl radicals, and these consequences of oxidative stress are shown to promote complications of diabetes mellitus (Halliwell and Chirico, 1993; Nebbioso *et al*., 2012). Moreover, oxidative stress may be responsible for hydrosalpinx fluid–mediated embryo-toxicity, as well as poor embryonic development (Agarwal *et al*., 2005). Elevated levels of oxidative stress during gestation are also associated with premature birth, fetal growth restriction, preeclampsia, and miscarriage (Agarwal and Gupta, 2005; Roberts *et al*., 2010). Thus, oxidative stress plays a major role in the pathogenesis of chronic diseases, increase the risk of stroke in fast growing pigs, loss of appetite, diarrhea and the risk of abortions in gestation sows (Gupta *et al*., 2007).

Free radicals are produced during normal cellular metabolism, especially during ATP synthesis by the mitochondrial electron chain, which is linked to numerous biological processes. Due to one or more "unpaired" electrons are present in the external orbit of the free

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radicals, thus they are highly reactive and have the potential to "attack" most types of organic molecules, thereby generating a class of compounds called reactive oxygen metabolites (ROMs). ROMs are more stable than free radicals, therefore it can be accurately detected and quantitated using the determination of reactive oxygen metabolites (d-ROMs) test (Pasquini *et al*., 2008). The plasma antioxidant test (PAT) measures the antioxidant capacity of plasma. Antioxidants represent the principal defensive barrier against damage from reactive species, in particular reactive oxygen species (Rizzo *et al*., 2012; Serena *et al*., 2013). In this study, we monitored oxidative damage using the d-ROMs test and evaluated the antioxidant barrier against hypochlorite-induced oxidation using the PAT test, and the degree of OS was assessed by the ratio of d-ROMs/PAT×1000 (U.Carr/U.Cor) because that the higher ratio of ROMs/PAT indicates the greater relationship between OS and pathology (Costantini *et al*., 2006; Costantini and Dell'Omo, 2006; Celi *et al*., 2010).

In prolific sows, systematic oxidative stress causes miscarriage, premature birth, mummies, stillbirths, and other reproductive disorders (Poston *et al*., 2011; Rizzo *et al*., 2012). However, very few studies have been conducted for overall assessments of oxidative stress in sows during the periods of gestation, lactation and weaning. To address this issue, we conducted a systematic assessment of the changes in oxidative stress during these stages. Our results provide theoretical guidance for the prevention of sow reproductive dysfunction disease caused by oxidative stress.

MATERIALS AND METHODS

Animals, management and sampling: Animal care and use were carried out according to the guidelines of the Animal Research Institute Committee of Nanjing Agricultural University, and with the specific approval of the Committee. Pregnant sows (n=24 Large White) were reared in gestation stalls (2.1×0.7 m) and fed 2.5 kg feed daily (Table 1). On days 100 of gestation, sows were transferred to farrowing crates (2.2×1.5 m), where they completed birth and lactation. Blood samples (4 ml each sample) were collected from breeding sows (n=24) on days 100 of gestation (G100); farrowing day (F); days 3, 13, and 23 of lactation (L3, L13, and L23) day 3 post-weaning (W3); and post-partum estrus day (E). Blood samples were collected in lithium–heparin anticoagulant tubes, and plasma was isolated from the blood by centrifugation at 2400 rpm for 10 minutes within 1 hour after venipuncture. Plasma was kept at -20°C until analysis of oxidative damage and endogenous plasma antioxidant concentrations. The litter size at birth was recorded for each sow.

Plasma oxidative damage test (d-ROMs test) and antioxidant defense test (PAT test): Oxidative damage and antioxidant defense levels were determined using the d-ROMs and PAT test kits (Diacron International, s.r.l, Italy) according to the manufacture's instruction, respectively. The d-ROMs test was used to estimate the concentration of hydroperoxides (ROOH) in the plasma. 10 µl plasma was added to an acidic buffer, which can promote release of iron from plasma protein. Then, the hydroxyperoxyl (ROO•) and alkoxyl (RO•) were generated by the reaction of these metal ion and hydroperoxides (Fenton's reaction). The newly-created radicals are able to oxidize an alkyl-substituted aromatic amine, and change them into a pink derivative. The resultant color change can be recorded and analyzed by the free radical analysis system (FRAS) to yield the d-ROMs value. The results of d-ROMs test were described with arbitrary units called "Carratelli Units" (U. Carr).

Moreover, the antioxidant capacity of plasma was determined by PAT test, which is a measure of the organism's capacity to tolerate oxidative stress produced by endogenous and exogenous factors. Briefly, 10 µl plasma was diluted with a colored solution composed by a solution of ferric ions and a chromogen, and incubated at 37°C for 5min, the change of the solution color will happen. The intensity of decoloration was assessed by spectro-photometry, which was directly related to the amount of reduced ferric ions. The resultant values were expressed as "Cornelli Unit" (U. Cor) (Inchingolo *et al*., 2014).

Statistical analysis: All the statistical analysis was conducted with SPSS 20.0 (IBM Corporation, Chicago, IL, USA). Differences between different time points were analyzed using one-way ANOVA with Duncan's multiple range test (Duncan, 1955). Coefficient of determination was used to assess the associations between oxidative stress with litter size and number of nursing piglets. Data were presented as means and SE. The $P<0.05$ was considered as statistical significance.

RESULTS

Oxidative damage (d-ROMs), antioxidant defense capacity (PAT) and oxidative stress (OS) in multiparous sows during gestation, farrowing, lactation, weaning, and estrus periods: The results of the ANOVA test contrasting d-ROMs, PAT and OS change for sows at different stages (G100, F, L3, L13, L23, W3 and E) are shown in Table 2. On d-ROMs, the results of Duncan's multiple range tests indicated that oxidative damage levels gradually increased from G100 to L13 stage, and then gradually decreased from L13 to E stage. Notably, the oxidative damage level was significantly higher ($P<0.05$) at L3, L13, L23, W3 and E stages than that at G100. While, no statistical significant differences were observed among different lactation periods (L3, L13 and L23).

Antioxidant defense levels were first decreased gradually from G100 to L3, and then gradually recovered to level of G100 from L3 to L23 and declined again from L23 to E stage. Moreover, the antioxidant defense levels were highest at L23 and were significantly higher ($P<0.05$) than that at G100, farrowing, lactation, weaning, and post-partum estrus (G100, F, L3, L13, W3 and E), and they were lowest at F stage. No differences in levels were detected among the G100, F, L3, L13, W3, and E stages.

In addition, an overall assessment of the oxidative stress level was conducted based on the ratio of the d-ROMs and PAT scores (d-ROMs/PAT×1000) at each stage. The oxidative stress level at G100 was significantly lower than that at other stages ($P<0.05$). By contrast, the oxidative stress levels were relative high at lactation stages,

and reached a peak at L3 which was significantly higher ($P<0.05$) than any other stages, except L13 and W3 stages.

Correlation of oxidative stress with litter size and number of nursing piglets: In this study, the correlations between litter size and the overall oxidative stress level (d-ROMs/PAT×1000) and the correlations between nursing size and overall oxidative stress level were assessed by linear regression analysis (litter size is the total number of the piglets that each sow reproduced and the number of nursing piglets is the actual number of the piglets that be nursed by each sow). The results showed that significant correlation existed between litter size at birth and oxidative stress level ($R^2=0.84$, $P=0.01$) and larger litter size at birth was accompanied with higher oxidative stress (Fig. 1a). However, no significant correlation was observed between the number of offspring and oxidative stress at various stages of lactation (Fig. 1b), ($R^2_{L3}=0.08$, $P=0.41$; $R^2_{L13}=0.07$, $P=0.49$; $R^2_{L23}=0.01$, $P=0.58$).

DISCUSSION

In humans and farm animals, oxidative stress has been demonstrated to play an important role in early aging and in many diseases (Lykkesfeldt and Svendsen, 2007; Epel, 2009). However, the knowledge of oxidative stress effect throughout the reproductive stages of sow is still very limited. In this study, we investigated oxidative stress status by determining oxidative damage (d-ROM) and antioxidant defense (PAT) levels during different stages of reproduction in multiparous sows and analyzed the association of oxidative stress levels with reproductive performance. Our results indicated that sows were suffering from severe oxidative stress during the process of reproduction, and a consistent result was reported by a previous study (Berchieri-Ronchi *et al*., 2011).

Reproduction consumes energy, energy expenditure increases the metabolic rate and the accumulation of metabolic production of toxic and harmful substances, especially reactive-oxygen free radicals (Losdat *et al*., 2011). During the gestation stage, sows may not only need energy for its own metabolism but also need extra energy for the piglets that is still developing, thus result in the production of higher levels of ROS. On farrowing day (F), the sows were suffering from the painful farrowing process and consumed more energy. Therefore, we observed that d-ROMs levels increased at F stage and reached a peak at L13 stage (Table 2). During lactation stage, the reproductive demands of the sow gradually increased with the growth of piglets, because of the secretion of milk; secretion of milk requires a great deal of protein metabolism, resulting in a sudden increase in energy requirements (Sadowska *et al*., 2013). So, the dramatic increase of energy need, further enhance the basal metabolic rate, leading to the production of higher concentrations of ROS (Bertrand *et al*., 2006). Therefore, the higher d-ROMs levels were observed at lactation stages (L3, L13 and L23) (Table 2).

By contrast, the PAT levels successively experienced a decrease, increase, and then decrease, and reached the lowest level at F stage (Table 2). During the reproductive stages, to maintain the oxidation–reduction balance of the body, antioxidants are consumed to counterbalance the increase in ROS. Therefore, the elevation of the d-ROMs value accompanied by a corresponding reduction in the PAT value compared to G100 was observed in our study (Table 2). Moreover, the reproductive demands of the sow gradually increased with the growth of piglets, which results in energy insufficiency and antioxidant decrease, oxidation and anti-oxidation imbalance in sows. Therefore, in order to rebalance the oxidation and anti-oxidation, more antioxidants may be being produced to meet the need of the growth of the piglets. Actually, our results also showed that the antioxidant level at 23 days of lactation was higher than that in other stages, suggesting the potential compensatory mechanism as increase in antioxidant may happen in sow to prioritize self-maintenance at the lactation stages. The similar scenario was supported by data in Adelie penguins (Beaulieu *et al*., 2011). In addition, we observed that the oxidative stress level was positively correlated with litter size in our study, while the number of nursing piglets was not associated with the oxidative stress level during the lactation period (Fig. 1). These results suggested that close relationship may be present between oxidative stress and animal births and a similar phenomenon was also observed in a study of

Table 1: Ingredient and nutrient composition of experimental feed during sow gestation and lactation stage

	Gestation (%)	Lactation (%)
Ingredient		
Corn yellow	53.6	55.0
Soybean meal	16.0	17.0
Fish meal	3.0	4.0
Food salt	0.5	0.5
Wheat bran	8.0	5.7
Rice bran	3.0	0.0
Soya bean oil	13.8	15.3
Calcium hydrogen phosphate	1.6	1.8
Lysine	0.0	0.2
Vitamin mineral premix[1]	0.5	0.5
Total	100.0	100.0
Calculated composition		
Dry matter	86.3	86.1
Metabolizable energy (MCal/kg)	3.7	3.8
Cp	14.7	15.1
Lysine	0.3	0.3
Calcium	0.4	0.4
Total phosphorus	0.7	0.8

[1]The vitamin mineral premix provided per kilogram of complete diet: 0.65mg of Cu as copper sulfate; 0.3mg of I as ethylenediamine dihydroiodide; 3.96mg of Mn as manganous oxide; 12.69mg of Fe as ferrous sulfate; 11.17 mg of Zn as zinc sulfate; 0.30mg of Se as sodium selenite; 6614IU of vitamin A as vitamin A acetate; 994IU of vitamin D3; 19.81 IU of vitamin E; 2.34mg of vitamin K as menadione sodium bisulfate; 4.63 mg of riboflavin; 0.03mg of vitamin B12; and 0.07mg of biotin; 18.52mg of D-pantothenic acid as calcium panthonate; 25.01mg of niacin.

Table 2: Oxidative damage (d-ROMs), antioxidant defense capacity (PAT) and oxidative stress (OS) in multiparous sows during gestation, reproduction, lactation, weaning and estrus periods

	Time point						
	G100	F	L3	L13	L23	W3	E
d-ROMs	598^{a}	664^{ab}	781^{c}	786^{c}	783^{c}	714^{bc}	703^{bc}
SE	124	143	164	161	162	121	107
PAT	1551^{a}	1431^{a}	1498^{a}	1508^{a}	1680^{b}	1435^{a}	1516^{a}
SE	93	113	307	163	156	199	88
OS	393^{a}	466^{ab}	545^{c}	523^{bc}	465^{ab}	506^{bc}	464^{ab}
SE	94	106	163	105	76	111	77

G100=days 100 of gestation; F=farrowing day; L3=days 3 of lactation; L13=days 13 of lactation; L23=days 23 of lactation; W3=day 3 post-weaning; E=post-partum estrus. d-ROMs=Reactive Oxygen Metabolites Test. PAT=Plasma antioxidant capacity. SE=Standard error. In the same row, means with the different superscripts indicate significant differences ($P<0.05$), with same superscripts are not significant difference ($P>0.05$).

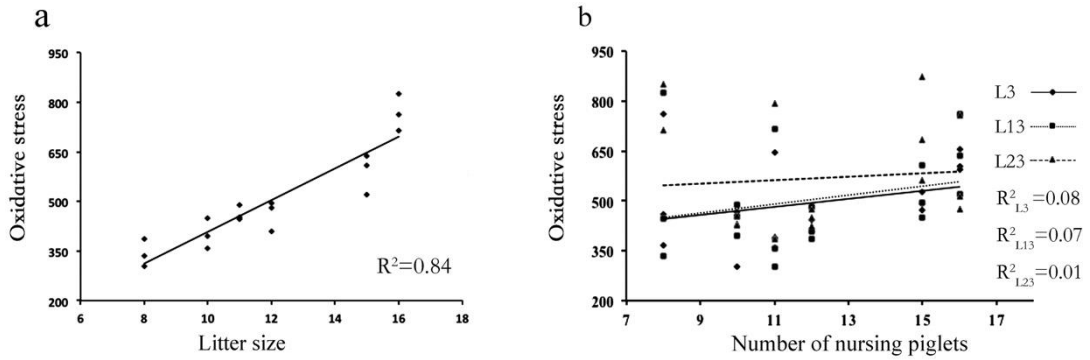

Fig. 1: Relationship between the level of oxidative stress (d-ROMs/PAT×1000) and litter size or the number of nursing piglets. (a) A linear regression analysis between the level of oxidative stress and litter size at birth: coefficient of determination R^2=0.84, P = 0.01. (b) A linear regression analysis between the level of oxidative stress and the number of nursing piglets, in different periods of lactation (L3, L13, L23). Coefficient of determination R^2_{L3}=0.08, P=0.41; R^2_{L13}=0.07, P=0.49; R^2_{L23}=0.01, P=0.58, respectively.

study of mice (Stier *et al*., 2012). However, the relationship between pre- and post-reproductive oxidative balance and reproductive investment in sows, the mechanisms of this underlying association remain unknown.

It is worth noting that sow is subjected to oxidative damage during the gestation and lactation stages of reproduction in mammals, and such damage is thought to contribute to lower the reproductive capacity (Finkel and Holbrook, 2000). Therefore, it may be necessary to add antioxidant substances (Such as vitamin A and E) to the diet during gestation and lactation period to compensate or alleviate oxidative stress, thereby improving reproductive performance.

Conclusions: Our data indicated that oxidative stress were happened during the whole reproduction stages of sow, and the level of oxidative stress reached peak at lactation period and showed significant correlation with litter size. Therefore, the appropriate antioxidant substances were suggested to add to the diet during gestation and lactation period of sow.

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Authors contribution: HLL conceived the idea and designed research; KL performed experiments; KL and WW analyzed data; KL, WW interpreted the results of experiments; KL drafted manuscript; All authors approved final version of manuscript.

REFERENCES

Agarwal A and Gupta S, 2005. Role of reactive oxygen species in female reproduction. Part I. Oxidative stress: A general overview. Agro Food Ind Hi-Tech 16:21-5.

Agarwal A, Gupta S and Sharma RK, 2005. Role of oxidative stress in female reproduction. Reprod Biol Endocrin 3.

Beaulieu M, Reichert S, Maho YL, *et al*., 2011. Oxidative status and telomere length in a long-lived bird facing a costly reproductive event. Funct Ecol 25:577-85.

Berchieri-Ronchi CB, Kim SW, Zhao Y, *et al*., 2011. Oxidative stress status of highly prolific sows during gestation and lactation. Animal 5:1774-9.

Bertrand S, Alonso-Alvarez C, Devevey G, *et al*., 2006. Carotenoids modulate the trade-off between egg production and resistance to oxidative stress in zebra finches. Oecologia 147: 576-84.

Celi P, Sullivan M and Evans D, 2010. The stability of the reactive oxygen metabolites (d-roms) and biological antioxidant potential (bap) tests on stored horse blood. Vet J 183:217-8.

Costantini D, Casagrande S, Filippis SD, *et al*., 2006. Correlates of oxidative stress in wild kestrel nestlings (falco tinnunculus). J Comp Physiol B 176:329-37.

Costantini D and Dell'Omo G, 2006. Environmental and genetic components of oxidative stress in wild kestrel nestlings (falco tinnunculus). J Comp Physiol B 176:575-9.

Epel ES, 2009. Psychological and metabolic stress: A recipe for accelerated cellular aging? Horm-Int J Endocrino 8:7-22.

Finkel T and Holbrook NJ, 2000. Oxidants, oxidative stress and the biology of ageing. Nature 408:239-47.

Gupta S, Agarwal A, Banerjee J, *et al*., 2007. The role of oxidative stress in spontaneous abortion and recurrent pregnancy loss: A systematic review. Obstet Gynecol Surv 62:335-47.

Halliwell B and Chirico S, 1993. Lipid-peroxidation - its mechanism, measurement, and significance. Am J Clin Nutr 57:715-25.

Inchingolo F, Marrelli M, Annibali S, *et al*., 2014. Influence of endodontic treatment on systemic oxidative stress. Int J Med Sci 11:1-6.

Klaunig JE, Kamendulis LM and Hocevar BA, 2010. Oxidative stress and oxidative damage in carcinogenesis. Toxicol Pathol 38:96-109.

Losdat S, Helfenstein F, Gaude B, *et al*., 2011. Reproductive effort transiently reduces antioxidant capacity in a wild bird. Behav Ecol 22:1218-26.

Lykkesfeldt J and Svendsen O, 2007. Oxidants and antioxidants in disease: Oxidative stress in farm animals. Vet J 173:502-11.

Murphy MP, 2009. How mitochondria produce reactive oxygen species. Biochem J 417:1-13.

Nebbioso M, Federici M, Rusciano D, *et al*., 2012. Oxidative stress in preretinopathic diabetes subjects and antioxidants. Diabetes Technol The 14:257-63.

Pasquini A, Luchetti E, Marchetti V, *et al*., 2008. Analytical performances of d-roms test and bap test in canine plasma. Definition of the normal range in healthy labrador dogs. Vet Res Commun 32:137-43.

Poston L, Igosheva N, Mistry HD, *et al*., 2011. Role of oxidative stress and antioxidant supplementation in pregnancy disorders. Am J Clin Nutr 94:1980s-5s.

Rahal A, Kumar A, Singh V, *et al*., 2014. Oxidative stress, prooxidants, and antioxidants: The interplay. Biomed Res Int http://dx.doi.org/10.1155/2014/761264

Rizzo A, Roscino MT, Binetti F, *et al*., 2012. Roles of reactive oxygen species in female reproduction. Reprod Domest Anim 47: 344-52.

Roberts JM, Myatt L, Spong CY, *et al*., 2010. Vitamins c and e to prevent complications of pregnancy-associated hypertension. New Engl J Med 362:1282-91.

Sadowska J, Gebczynski AK and Konarzewski M, 2013. Basal metabolic rate is positively correlated with parental investment in laboratory mice. P Roy Soc B-Bio 280.

Serena B, Primiterra M, Catalani S, *et al*., 2013. Performance evaluation of the innovative pat test, comparison with the common bap test and influence of interferences on the evaluation of the plasma antioxidant capacity. Clin Lab 59:1091-7.

Stier A, Reichert S, Massemin S, *et al*., 2012. Constraint and cost of oxidative stress on reproduction: Correlative evidence in laboratory mice and review of the literature. Front Zool 9.