Appraisal of endogenous antioxidant stature in pregnant sows from arid tracts in India

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Abstract. An endeavor was conceded to appraise endogenous antioxidant stature in healthy female indigenous pig from arid tracts in India. Collection of plasma was made to determine vitamin A, E, C and glutathione as markers of endogenous antioxidant stature in pregnant and non pregnant sows and gilts. Upshot clearly revealed significant ($p \leq 0.05$) dwindles in the values of plasma vitamin A, E, C and glutathione in pregnant as compared to gilts reflecting exhaustion of endogenous antioxidants in pregnant animals. Magnitude of per cent depletions was higher in plurigravidae as 39.06, 42.85, 36.17 and 44.16 % respectively in vitamin A, C, E and glutathione. Greatest percent change was observed in the mean value of plasma glutathione. In pregnant sows plurigravidae were found to be more affected than primigravidae. In non pregnant sows, pluripara were more affected than primipara. Pattern of result obtained implied the progression of oxidative stress in pregnant sows. Maximum per cent transformation was found in the mean value of plasma glutathione. The result of present endeavor has attempted to appraise the association of endogenous antioxidants with the oxidative stress. It can be deduced that pregnancy largely contributes towards the generation of free radicals. This was obvious as endogenous antioxidants were depleted in the endeavor. Endogenous antioxidants are used in the process to avert the development of oxidative stress. Explanation of these changes will assist in imparting insight to diverse veiled reactions in the progression of oxidative stress. Upshot of endeavor undeniably validated the contribution of pregnancy in the expansion of oxidative stress. Pregnant sows must be supplemented with antioxidants to augment endogenous antioxidants for protection from the threat of oxidative stress.

Key words: swine, gilts, indigenous, oxidative stress, gestation, *Sus scrofa domesticus*.

Introduction. Propensity of swine physiological mechanisms to counterbalance the reactive oxygen species (ROS) is an important concern for welfare. Oxidative stress is dangerous to health of pigs affecting production and quality of meat. Fabrication of ROS is a regular physiological process. However, when the animals ability to neutralize them by the antioxidant system is crippled, oxidative stress occurs. An unrestrained inequity between the manufacture and elimination of ROS may also produce oxidative stress. Oxidative stress may head to grave outcome not only for the cell membrane permeability but also for the metabolic processes which can be changed due to peroxidative damage of proteins and the occurrence of the toxic effects of the peroxidative destruction to macromolecules (Sies 1993).

Pregnant sows experiences inexorable oxidative stress expectedly (Wisdom et al 1991). Alterations in the metabolic pathways are a key feature during pregnancy ensuing in greater oxygen utilization along with burning up of fuel molecules with a consequential revelation to oxidative stress (Vannucchi et al 2007). Parturition-linked variations in hormones in the dam together with intrauterine to extrauterine ambience and the commencement of lung respiration in the young one are vital components associated with pregnancy associated oxidative stress. Exposure of young ones to excessive free radicals is mainly due to prenatal stress and the linked transformations in oxygen partial pressure. Vital components like proteins and other macromolecules are protected from the peril of peroxidative harm produced by excessive reactive oxygen species by the endogenous antioxidants in cells and body fluids (Stadtman 1992).

ROS are known to affect various physiological processes starting from oocyte maturation to fertilization, embryo and foetus development and other aspects of pregnancy. Outcome of oxidative stress can be from abortion to ovarian cancers. Oxidative stress is important in pathophysiology of infertility and assisted fertility (Agarwal et al 2005). It is identified that anti-oxidative mechanisms are bestowed with enzymatic and non-enzymatic features that work in an integrated manner as first line of defense against ROS. Chief non-enzymatic antioxidants include vitamin A, C, E and glutathione. Vitamin C has strong reductive properties whereas vitamin A neutralizes lipid oxidation in cell membranes (Kartha & Krishnamurthy 1977; Agarwal et al 2016). Iron plays an important role in production of free radicals (Rahman 2007). Oxidative stress is known to cause foetal growth retardation in humans (Karowicz-Bilinska et al 2007). There is paucity to establish oxidative stress status during gestation periods in sows. Looking towards various paraphernalia of oxidative stress in the aspects linked with pregnancy and associated economical losses in swine husbandry, an endeavor was taken up to explore the existence of oxidative stress in pregnant sows and gilts. The study will assist in health programming of pigs during gestation period and neonatal care.

Material and Method. The present endeavor was undertaken in fifty healthy adult female indigenous pigs kept by private pig raisers with almost similar type of management conditions. Indigenous female pigs were categorized as gilts, pregnant sows and non pregnant sows. Pregnant sows were further classified as primigravidae and plurigravidae. Non pregnant sows were also grouped as primipara and pluripara. Each category comprised of 10 animals. Blood sample were collected from tail vein in sterile tubes with anticoagulant (EDTA dipotassium) to harvest plasma. Sampling was carried out in morning hours. Pregnant sows were in mid gestation phase. Plasma vitamin A, vitamin C, vitamin E and glutathione were determined as markers of endogenous antioxidant stature.

Plasma vitamin A was determined by the methods as described by Varley (1988) with little modification (Joshi 2012). Alcohol was used for protein precipitation and light petroleum was used for retinol extraction. After evaporation of light petroleum, residue was dissolved in chloroform before carrying out color reaction. Plasma vitamin C was determined by the method as described by Varley (1988). This method is based upon the titration of plasma ascorbate by 2,6-dichlorophenolindophenol dye. Plasma vitamin E was determined by the spectrophotometric method of Nair & Magar (1955) with slight modification (Joshi 2012). The method is based upon the color reaction between phosphomolybdic acid and vitamin E. Plasma glutathione was determined by the rapid colorimetric micro method of Owens & Belcher (1965) with modifications for plasma samples (Joshi 2012). Mean value of healthy gilts for each parameter was considered as control.

Results. The mean ± SEM values of plasma vitamin A, vitamin E, vitamin C and glutathione along with per cent variations in the values in gilts and pregnant sows are presented in Table 1. The mean values of antioxidants obtained from gilts were considered as control and all the comparisons for each parameter in pregnant and non pregnant sows have been made from respective value in gilts. Findings revealed significant ($p \leq 0.05$) decline in the echelon of plasma vitamin A, vitamin E, vitamin C and glutathione in pregnant and non pregnant sows as compared to respective value in gilts. Magnitude of per cent depletions was higher in plurigravidae as 39.06, 42.85, 36.17 and 44.16% respectively in vitamin A, C, E and glutathione. Greatest percent change was observed in the mean value of plasma glutathione. In pregnant sows plurigravidae were found to be more affected than primigravidae. In non pregnant sows, pluripara were more affected than primipara.

Table 1

Plasma levels of antioxidants in indigenous female pigs (n=10, mean±SEM)

Indigenous sows		*Plasma endogenous antioxidants, µmol L^{-1}*			
		Vitamin A	*Vitamin E*	*Vitamin C*	*Glutathione*
Gilts		1.94±0.005	6.9±0.06	28.90±0.02	6.90±0.04
Pregnant sows	Primigravidae	1.18±0.003^{b}	4.71±0.05^{b}	20.00±0.02^{b}	4.20±0.02^{b}
	Plurigravidae	1.17±0.006^{bc}	4.34±0.08^{bc}	16.00±0.01^{bc}	3.35±0.07^{bc}
Non pregnant sows	Primipara	1.70±0.004^{bd}	5.90±0.03^{bd}	26.00±0.03^{bd}	5.21±0.02^{bd}
	Pluripara	1.60±0.005^{bce}	5.00±0.04^{bce}	24.00±0.02^{bce}	4.89±0.03^{bce}

'b' =Significant (p≤0.05) difference from respective gilt mean value for a parameter;
'c' = Significant (p≤0.05) difference from respective primigravidae mean value for a parameter;
'd' = Significant (p≤0.05) difference from respective pregnant primigravidae mean value for a parameter;
'e' = Significant (p≤0.05) difference from respective pregnant plurigravidae mean value for a parameter.

Discussion. There is rareness of information on plasma antioxidant level in the indigenous pigs during pregnancy in research writings. Highest percent change was observed in the mean value of plasma glutathione indicating the development of oxidative stress. Elevated oxidative stress is testified to be related with pregnancy impediments in highly prolific sows (Berchieri-Ronchi et al 2011). Drop down in plasma concentrations of endogenous antioxidants were observed in the pregnant and non pregnant sows. However, scale of diminution was utmost in pregnant animals. This genuinely validated the presence of oxidative stress in the pregnant sows. Plurigravidae were pretentiously showed greatest blow. Instance of changes in plasma antioxidants made the scaffold for the possible physiological variations under the bang of probable escalation in ROS during pregnancy. It can be conjectured that pregnancy is the cause of oxidative stress in the sows. It can be stated that metabolic changes during pregnancy interposes the happening of oxidative stress. Sows must be supplied with antioxidants repeatedly to protect them from the danger of oxidative stress largely throughout pregnancy.

Vitamin A. Decrease in vitamin A level in the present study in pregnant sows may perhaps be due to its increased utilization for metabolic reasons. Vitamin A is considered as a strong tool in protecting the body from the peril of oxidant threats. As a potent antioxidant, it neutralizes free radicals, hence supplementation becomes mandatory. Vitamin A is elemental for normal cellular function, including reproduction and development. It is one the immune-nutrients. Function of vitamin A starts in the embryo quickly after conception and maintained all over the life (Ross et al 2000). The role of vitamin A is imperative in retaining normal growth, controlling proliferation and differentiation of epithelial tissues and sustaining visual and reproductive functions. Need of vitamin A as an immune-nutrient is higher than for other functions like growth and reproduction. Scientists have reported reduced plasma retinol concentration at the end of gestation in sows resulting in an enhanced systemic oxidative stress during late gestation in sows. A substantial reduction in antioxidant nutrients in circulation is observed even after parturition in lactating sows (Berchieri-Ronchi et al 2011).

Vitamin E. Vitamin E is a potent primary antioxidant required to lower down the number of excessive free radicals. Pregnancy linked oxidative stress in association with vitamin E has been observed. Researchers have recommended higher dietary level of vitamin E for both gestating and lactating sows (Mahan 1994). Vitamin E helps to protect cell membranes. It is one of the important immunonutrients to include in pregnant animals diet. Although vitamin E deficiency is rarely seen in healthy adults, however, for pregnant, insufficient dietary vitamin E may lead to complications such as pre-eclampsia and the foetus being born small. Scientists (Berchieri-Ronchi et al 2011) have reported reduced plasma α-tocopherol concentrations at the end of gestation in sows. During the last month of pregnancy the foetuses grow fast, which needs a high amount of nutrients. At this time supplementation with immune-nutrients is required. Vitamin E is also known to boost the immune system. A decrease muscle mass and increased sensitivity to pain is observed in vitamin E deficient cases. Vitamin E is vital in maintaining and sustaining immune system because it suppresses the ROS formed and averts much of the harm that could direct to grim health predicament. Vitamin E is an acceptably renowned neuroprotectant, potent antioxidant and an important agent for tumbling the effect of oxidative stress on foetal brain tissue damage (Erdemli et al 2016). Lowered plasma vitamin E concentration in pregnant sows divulged its exhaustion to fight the ROS and marked the existence of oxidative stress in pregnant sows.

Vitamin C. Synthesis of collagen is an important function of vitamin C which is important for growth and development. Immune system for its appropriate function requires vitamin C. Major role of vitamin C is that of water-soluble antioxidant, where its function is linked to that of the antioxidant enzymes, such as glutathione peroxidase and to vitamin E. Vitamin C is mandatory for the regular functioning of various vital organs, endocrine gland and immune system. Different cells of the body need vitamin C to sustain veracity and for detoxification functions. Vitamin C is primary antioxidant. The function of vitamin C is well noted as an anti-oxidant protecting the body against oxidative stress (Padayatty et al 2003). Vitamin C can act directly by scavenging ROS generated by stressors, prevent ROS mediated cell damage by maneuvering gene expression, regulate keratinocyte differentiation maintaining a balanced redox state, promote cell cycle arrest and apoptosis in response to DNA damage. Lowered concentration of vitamin C affirmed the existence of oxidative stress. Scientists (Kataria et al 2010) have recommended the use of antioxidants in the disease conditions causing oxidative stress.

Glutathione. There is scarce data available in literature on antioxidant parameters in sows especially during pregnancy. Many pregnancy impediments and birth shortcoming have been associated to oxidative stress. Glutathione is a well known master antioxidant of the body. Dam and foetus both are protected by glutathione. Pregnancy produces free radicals in large amounts. Glutathione not only counterbalance free radicals but also work in checking birth defects. It is known to perk up antioxidant power of blood. In the placenta, GSH neutralizes free radicals so that they cannot approach to developing foetus. Effect of physiological states on blood glutathione activity was has been reported in animals (Joshi et al 2013). Lower plasma levels of glutathione in pregnant and non pregnant sows implied the existence of oxidative stress with greater magnitude in pregnant sows.

Conclusions. Decreased plasma concentrations of endogenous antioxidants were found in the pregnant and non pregnant sows. However, magnitude of depletion was greatest in pregnant animals. This authenticated the existence of oxidative stress in the pregnant sows. Plurigravidae were affected with maximum impact. Precedent of variations in plasma antioxidants made the stanchion for the potential physiological changes under the impact of credible intensification in ROS during pregnancy. It can be speculated that pregnancy fabricated the oxidative stress in the sows. An affirmation can be made that metabolic alterations during pregnancy put in the occurrence of oxidative stress. Sows ought to be provided with antioxidants regularly to safeguard them from the peril of oxidative stress predominantly throughout pregnancy.

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