Hormonal regulation of prooxidant-antioxidant homeostasis in gilts

V. G. Stoyanovskyy\textsuperscript{1}, S. O. Usenko\textsuperscript{2}, A. M. Shostya\textsuperscript{2}, L. M. Kuzmenko\textsuperscript{2}, V. G. Slynko\textsuperscript{2}, V. S. Tenditnyk\textsuperscript{2}

\textsuperscript{1}Stepan Gzhyskyi National University of Veterinary Medicine and Biotechnologies, Pekarska Str., 50, Lviv, 79010, Ukraine
\textsuperscript{2}Poltava State Agrarian Academy, Skovorody Str., 1/3 Poltava, 36003, Ukraine

Abstract

The results of research on the features of cyclic lability of homeostasis in gilts are highlighted. In the experiments it has been used gilts of the Red White belted breed of meat pigs at different phases of the reproductive cycle: luteal phase, estrus, on the 15th, 20th, 30th, 60th, 90th, 104th, 113\textsuperscript{rd} day of pregnancy and in 12 hours after farrowing. The dynamics of the content of thyroid and steroid hormones, as well as the peculiarities of the formation of prooxidant-antioxidant homeostasis were determined in blood serum. It was found out that the amount of steroid hormones in blood of gilts is labile and is determined by the physiological state, namely the onset of estrus, relative to the luteal phase, it is increased the level of thyroxine (30.0 %), triiodothyronine (26.3 %), estradiol-17β (by 22.8 %, \(P < 0.01 \)) testosterone (15.2 %), as well as a decrease in progesterone (by 37.2 %). Such changes are accelerated by the acceleration of peroxidation processes, xanthine oxidase activity increases, it is increased the content of diene conjugates (\(P < 0.05 \)) and TBA-active compounds. This is accompanied by a probable increase in the level of antioxidant protection – the activity of superoxide dismutase (\(P < 0.05 \)), vitamin A (\(P < 0.05 \)) and vitamin E (\(P < 0.05 \)). During two months of pregnancy, the concentration of thyroxine (\(P < 0.05 \)), triiodothyronine, progesterone (\(P < 0.01 \)), testosterone (\(P < 0.05 \)) and estradiol-17β increases. Such metabolic shifts cause changes in the state of prooxidant-antioxidant homeostasis in the direction of reducing the intensity of peroxidation processes. In gilts before farrow there is a decrease in thyroxin, triiodothyronin and progesterone. The intensification of peroxidation was found: the increase in SOD activity (\(P < 0.05 \)), the dehydroascorbic acid concentration decreases on 1.8 and 8.7 times, respectively. It was revealed that the intensity of lipid peroxidation processes decreased: DC content by 16.3 % and TBA-active complexes by 13.3 %. It was determined that the concentration of ascorbic acid decreased by 32.8 % and vitamin E – 18.9 %.

Key words: gilts, reproduction, blood, homeostasis, hormones, antioxidants, pregnancy.

1. Introduction

The reproductive cycle of sows consists of a clear alternation of certain phases – proestrus, estrus, pregnancy and the period of sexual rest, due to changes in hormonal background. Among the most sensitive homeostatic constants to changes in the endocrine profile in animals are the processes of peroxidation, which occur with the participation of reactive oxygen forms (AFO) (Usenko et al., 2016; Stupar, 2018; Shostya et al., 2019; Usenko et al., 2020).

It has been found that AFO plays a leading role in ensuring motility, sperm survival and egg maturation (Purdey et al., 2015). With the onset of pregnancy, the mother's body is under the influence of oxidative stress, the prolonged action of which is accompanied by disruption of the development of the placenta, fetus and premature birth (Ogbono et al., 2014). This encourages the development of targeted antioxidant nutrition programs based on a deep understanding of the mechanism of their specific action (Duhig et al., 2014).

It has been proven that in critical periods of embryonic development, peroxidation processes are accelerated, as a result, the sensitivity of the embryo to AFO damage increases, and its protective capabilities are reduced (Patil et al., 2006). Taking into account that different species of agricultural animals have critical periods, and pigs in particular, some of their features are common. The revealed patterns in pigs can be the basis for creating effective methods to reduce embryonic mortality. Among the important characteristics of pregnancy in animals and humans there is the intensification of metabolism before birth, which indicates the importance of studying the processes of peroxidation and their correction during this period (Atiba et al., 2013).

In this regard, it is promising to clarify the peculiarities of the formation of homeostasis, where the leading role belongs to hormones and prooxidant-antioxidant balance in different periods of the reproductive cycle. This will open the possibility to develop effective methods of regulating the
reproductive cycle, fertilization, growth and development of fetuses, which will increase the intensity of use of sows.

The purpose of research is to determine the features of hormonal regulation of the formation of prooxidant-antioxidant homeostasis in gilts depending on the phases of the reproductive cycle.

To achieve this goal the following tasks are solved:
- the dynamics of the content of steroid and thyroid hormones in blood of gilts during the sexual cycle and pregnancy was determined;
- features of the formation of prooxidant-antioxidant homeostasis in gilts depending on their physiological condition were found out.

### 2. Materials and methods

In the experiments on the principle of analogues it has been used 5 clinically healthy gilts of the Red White-belted breed of meat pigs aged 8 months and with weight of 125–130 kg. The pigs were bled on an empty stomach during different periods of the reproductive cycle: luteal phase, estrus, on the 15th, 20th, 30th, 60th, 90th, 104th, 113th day of pregnancy and in 12 hours after farrowing. The content of thyroxine, triiodothyronine, estradiol-17β and progesterone in blood serum was determined by radioimmunological method, and testosterone – by enzyme-linked immunosorbent assay. The state of prooxidant-antioxidant homeostasis (PAG) in blood was studied by the activity of xanthine oxidase (CSO) (Kyseliova et al., 2005), the concentration of diene conjugates (DC) (Havrilov & Melkorudnaya, 1983), the content of TB compounds (Kaidashev, 1996). The level of antioxidant protection was assessed by the activity of superoxide dismutase (SOD) (Brusov et al., 1976), catalase activity (CT) (Velichko et al., 2009), the content of reduced glutathione (Shabunin, 2010), ascorbic and dehydroascorbic acids(ADAA) (Kaidashev, 1996), the content of vitamin A and vitamin E (Rybalko, 2005).

### 3. Results and discussion

#### Results

Experimental data show the fact that in blood of cycling gilts in the estrus phase, compared with luteal one, there was a significant restructuring of metabolic processes, especially hormonal background (Table 1). Thus, during sexual arousal, it has been determined an increase in the amount of thyroxine and triiodothyronine by 33.0 and 26.3 %, respectively. The most pronounced changes in this period were characteristic for sex hormones: the concentration of progesterone decreased by 37.2 % and testosterone by 15.2 %, and estradiol 17β increased by 22.8 % (P < 0.01).

| Table 1 |
|---|---|---|---|---|
| **Hormones** | **Phases of the reproductive cycle** | **Pregnancy days** | **12 hours after farrowing** |
| | Luteal | Estrus | 15th | 30th | 60th | 90th | 104th | 113th |
| Thyroxine, nmol/l | 36.34 ± 4.82 ± 50.43 ± 61.20 ± 55.52 ± 36.98 ± 34.46 ± 29.07 ± 36.52 ± | | | | | | | |
| Triiodothyronine, nmol/l | 5.46 | 5.11 | 7.39 | 5.74* | 6.47* | 4.92 | 3.38 | 2.76 | 3.97 |
| Progesterone, nmol/l | 0.95 ± 1.14 ± 1.28 ± 1.43 ± 1.35 ± 1.59 ± 1.45 ± 0.67 ± 1.84 ± | | | | | | | | |
| Testosterone, nmol/l | 0.10 | 0.12 | 0.23 | 0.21 | 0.19 | 0.16* | 0.27 | 0.08 | 0.22** |
| Estradiol-17β, | 32.10 ± 20.15 ± 39.53 ± 52.21 ± 70.74 ± 82.33 ± 85.41 ± 90.97 ± 50.69 ± | | | | | | | | |
| Estradiol-17β, | 4.66 | 3.71 | 3.73 | 3.13** | 10.99** | 8.19*** | 5.62*** | 8.54*** | 7.05 |
| Estradiol-17β, | 5.48 ± 4.65 ± 6.09 ± 5.83± 10.28± 10.77± 8.15± 6.80± 5.82± | | | | | | | | |
| Estradiol-17β, | 0.61 | 0.74 | 0.97 | 0.79 | 1.57* | 1.09** | 0.74* | 0.79 | 0.63 |
| Estradiol-17β, | 0.14 ± 0.32 ± 0.42 ± 0.33 ± 0.52 ± 1.62 ± 3.75 ± 5.41± 0.83 ± | | | | | | | | |
| Estradiol-17β, | 0.02 | 0.05** | 0.06*** | 0.06** | 0.17 | 0.33** | 0.69*** | 0.60*** | 0.11*** |

Note: * – P < 0.05; ** – P < 0.01; *** – P < 0.001 compared with the luteal phase

These changes in the hormonal background were accompanied by a significant restructuring of metabolic processes in the direction of accelerating the course of peroxidation. This is confirmed by a slight increase in the activity of the prooxidant enzyme – CSO, which significantly accelerated the hemolysis of erythrocytes by 10.8 % (Table 2). Such changes are accompanied by an increase in the content of DC in 2.3 (P < 0.05) and TBA-active complexes in 1.4 times. At the same time, there was an acceleration of the functional activity of antioxidant enzymes: SOD by 36.8 % (P < 0.05) and a decrease in CT – 51.3 % (P < 0.05). During this period it was found out the fact that there was a significant use of reduced glutathione by 25.7 %. It was found that the significant accumulation of vitamin A in blood on 1.6 times (P < 0.05), vitamin E – on 1.7 (P < 0.05), ascorbic and dehydroascorbic acids, respectively, on 1.5 times and 1.4 times (P < 0.05).

The first 15 days of the pregnancy development were characterized by a further intense course of peroxide oxidation processes, which was manifested in the activation of CT – 39.0 %, and a decrease in the functional activity of SOD – 25.3 % (P < 0.05) compared with the estrous phase. This occurred against the background of reducing the concentration of DC – 10.7 %, TBA-active complexes by 16.5 %, as well as accelerating the use of low molecular weight antioxidants – reducing the content of reduced glutathione and ascorbic acid, respectively, by 15.1 % (P < 0.05) and 45.3 % compared to the estrous phase. Such changes are obviously due to an increase in the amount of steroid hormones: progesterone in 2.01 and estradiol-17β in 1.3 times compared to the estrous phase. An increase in thyroxine and triiodothyronine concentrations was observed during this period.
The obtained research materials indicate that in blood of gilts during the reproductive cycle the change in hormonal background significantly affects the state of PAG. During estrus, in parallel with the increase in the concentration of estradiol-17β and thyroid hormones, the level of CSO, SOD, the amount of DC and TBA-active complexes increased, but the content of reduced glutathione decreased, which indicators of peroxidation, due to the increased activity of SOD by 81.4% (P < 0.05) and diene conjugates by 35.9% and TBA-active complexes by 42.0%, as well as a decrease in the concentration of low molecular weight antioxidants: reduced glutathione by 22.9%, vitamin A by 46.6% and vitamin E by 57.6%. Such metabolic changes were accompanied by a decrease in the level of functional activity of the thyroid gland to the secretion of thyroxine by 21.4% and triiodothyronine by 57.9%, as well as a rapid increase in the concentration of antioxidant – estradiol-17β, in 3.3 times (P < 0.05).

In the postpartum period, in 12 hours after farrowing the content of thyroid hormones increased: thyroxine by 1.3 and triiodothyronine by 2.7 times, which is the evidence of their leading role in ensuring adaptation processes in this period for sows and piglets. Exactly in this phase of the reproductive cycle it has been determined a deep hormonal adjustment – a decrease in the concentration of progesterone by 1.8 and estradiol-17β is by 8.7 times. There was a decrease in the indicative indexes of the intensity of lipid peroxidation: DC by 16.3% and TBA-active complexes by 13.3%. This decrease was accompanied by depletion of the antioxidant defense system: functional activity of CT by 8.8% and SOD is by 36.5%. Such changes occurred against the background of a decrease in the concentration of ascorbic acid by 32.8%, vitamin A is by 6.9% and vitamin E is by 18.9%.

### Table 2

| Indexes of PAG | Luteal | Estrus | Phases of the reproductive cycle | 12 hours after farrowing |
|----------------|--------|--------|----------------------------------|-------------------------|
| Peroxide resistance of erythrocytes, % | 16.36 ± 0.06 | 18.50 ± 0.06 | 15.78 ± 0.06 | 15.17 ± 0.06 | 12.35 ± 0.06 | 12.92 ± 0.06 | 10.71 ± 0.06 | 10.44 ± 0.06 | 18.26 ± 0.06 |
| Xanthine oxidase, mkkat/sec | 40.63 ± 0.06 | 42.27 ± 0.06 | 44.13 ± 0.06 | 45.98 ± 0.06 | 48.31 ± 0.06 | 39.64 ± 0.06 | 42.41 ± 0.06 | 40.37 ± 0.06 | 39.91 ± 0.06 |
| Superoxide dismutase, unit/ml | 0.95 ± 0.06 | 1.38 ± 0.06 | 1.03 ± 0.06 | 1.57 ± 0.06 | 0.99 ± 0.06 | 0.86 ± 0.06 | 1.34 ± 0.06 | 1.56 ± 0.06 | 0.99 ± 0.06 |
| Catalase, μkat/l | 0.12 ± 0.06 | 0.14* ± 0.06 | 0.09 ± 0.06 | 1.12 ± 0.06 | 0.10 ± 0.06 | 0.12 ± 0.06 | 0.16 ± 0.06 | 0.11 ± 0.06 | 0.08 |
| H2O2/min - l | 1.85 ± 0.06 | 1.46 ± 0.06 | 2.03 ± 0.06 | 1.66 ± 0.06 | 1.74 ± 0.06 | 1.20 ± 0.06 | 1.14 ± 0.06 | 1.04 ± 0.06 | 1.04 ± 0.06 |
| Reduced glutathione, μmol/l | 0.71 ± 0.06 | 0.53 ± 0.06 | 0.45 ± 0.06 | 0.41 ± 0.06 | 0.32 ± 0.06 | 0.35 ± 0.06 | 0.29 ± 0.06 | 0.27 ± 0.06 | 0.31 ± 0.06 |
| Vitamin A, μmol/l | 1.94 ± 0.06 | 2.14* ± 0.06 | 1.77 ± 0.06 | 2.43 ± 0.06 | 2.20 ± 0.06 | 2.43 ± 0.06 | 1.71* ± 0.06 | 0.94* ± 0.06 | 1.10* |
| Vitamin E, μmol/l | 0.18 ± 0.06 | 0.29* ± 0.06 | 0.35* ± 0.06 | 0.34 ± 0.06 | 0.10 ± 0.06 | 0.09 ± 0.06 | 0.11* ± 0.06 | 0.17* |
| Diene conjugates, mmol/l | 1.38 ± 0.06 | 3.17* ± 0.06 | 2.83 ± 0.06 | 2.21 ± 0.06 | 1.86 ± 0.06 | 1.55 ± 0.06 | 2.16 ± 0.06 | 2.12 ± 0.06 | 1.76 ± 0.06 |
| TBA-active complexes, μmol/l | 15.7 ± 0.06 | 22.31* ± 0.06 | 18.63 ± 0.06 | 12.56 ± 0.06 | 12.41 ± 0.06 | 15.79 ± 0.06 | 12.41 ± 0.06 | 20.6 ± 0.06 | 17.86 ± 0.06 |
| after incubation, μmol/l | 20.13 ± 0.06 | 23.64* ± 0.06 | 22.28 ± 0.06 | 19.37 ± 0.06 | 14.88 ± 0.06 | 20.32 ± 0.06 | 19.28 ± 0.06 | 17.71 ± 0.06 | 15.63 ± 0.06 |

Note: * – P < 0.05; ** – P < 0.01; *** – P < 0.001 compared with the luteal phase.
cates a tense metabolic process and regulatory function of reactive oxygen form (Villamor et al., 2019). With increasing growth of pregnancy, the concentrations of progesterone and estradiol-17β significantly increased, which caused a change in the balance in the functional activity of prooxidant and antioxidant enzymes, which is consistent with the statements of K. Duhig (Duhig et al., 2016), S. O. Ogbodo (Ogbodo et al., 2014), M. S. Purdey (Purdey et al., 2015). With the formation of individual organs and systems of the fetuses and the emergence of its own synthesis of certain hormones in the mother's body there was a slowdown in peroxidation processes – reducing functional activity of SOD, CT and the content of DC and TBA-active complexes. However, the prenatal period was characterized by a significant maximum level of sex hormones and an intensive course of lipid peroxidation, which is confirmed by V. N. Romanenko and I. A. Boiko (Romanenko & Boiko, 2015), D. N. Mitarev (Mitarev, 2009). At the same time with the change of the phase of the reproductive cycle in the postpartum period there is a shift of homeostatic constants, primarily the content of thyroid and steroid hormones at the level of sexual rest, accompanied by a change in prooxidant antioxidant homeostasis in the direction of slowing peroxidation. The generalization of the obtained data testifies to one of the features of the reproductive function of sows – the cyclic lability of homeostasis in female pigs, which is characterized by certain periodic fluctuations due to changes in their physiological state, aimed at maintaining the physiological norm of metabolic processes (Kovalenko, 2012). In cycling pigs, the significant lability of prooxidant-antioxidant homeostasis is aimed at creating the necessary conditions for fertilization. At the onset of pregnancy, the shift of homeostatic constants helps to meet the needs of growing and developing embryos. In the changes of condition of peroxide homeostatic constants in blood of females it is possible to judge a morphofunctional state of genitals and fetoplacental system.

4. Conclusions

1. The amount of steroid hormones in blood of gilts is labile and is determined by the physiological state, namely at the onset of estrus, relative to the luteal phase, it is increased the level of thyroxine (30.0 %), triiodothyronine (26.3 %), estradiol-17β (by 22.8 %, P < 0.01) testosterone (by 15.2 %), and it is decreased progesterone (by 37.2 %). Such changes are accompanied by the acceleration of peroxidation processes, there is the increase of xanthine oxidase activity, there is the increase of the content of diene conjugates (P < 0.05) and TBA-active compounds, too. This is accompanied by a probable increase in the level of antioxidant protection – the activity of superoxide dismutase (P < 0.05), vitamin A (P < 0.05) and vitamin E (P < 0.05).

2. During two months of pregnancy, the concentration of thyroxine (P < 0.05), triiodothyronine, progesterone (P < 0.01), testosterone (P < 0.05) and estradiol-17β is increased. Such metabolic shifts cause changes in the state of prooxidant-antioxidant homeostasis in the direction of reducing the intensity of peroxidation processes.

3. In gilts before farrowing there is a decrease in the level of thyrocoxin, triiodothyronine and progesterone. It has been determined the intensification of peroxidation: the increase in SOD activity (P < 0.05), the dehydroascorbic acid content (P < 0.05), diene conjugates and TBA-active complexes, as well as the decrease in the concentration of reduced glutathione and vitamin A (P < 0.05) and vitamin E (P < 0.05).

4. In blood of sows after farrowing, the concentration of thyroxine increases in 1.3 times, triiodothyronine is in 2.7 times, and progesterone and estradiol-17β decreases in 1.8 and 8.7 times, respectively. It was revealed that the intensity of lipid peroxidation processes decreased: DC content by 16.3 % and TBA-active complexes by 13.3 %. It has been determined that the concentration of ascorbic acid decreased by 32.8 % and vitamin E by 18.9 %.

References

Atiba, A. S., Niran-Atiba, T. A., Akindele, R. A., Jimoh, A.K., Oparinde, D. P., Dudcumi, B. M., & Ghazali, M. S. (2013). Effect of Weight Gained In Pregnancy on Lipid Peroxidation Product. *Journal of Asian Scientific Research*, 3(2), 122–127. URL: http://www.aessweb.com/pdf-files/Jasr-3(2)-122-127.pdf.

Duhig, K., Chappell, L. C., & Shennan, A. H. (2016). Oxidative stress in pregnancy and reproduction. *Obstet Med*, 9(3), 113–116. doi: 10.1177/1753495X16648495.

Purdey, M. S., Connaughton, H., & Whiting, S. (2015). Boronate probes for the detection of hydrogen peroxide release from human spermatozoa. *Free Radic Biol Med*, 81, 69–76. doi: 10.1016/j.freeradbiomed.2015.01.015.

Romanenko, V. N., & Boyko, Y. A. (2015) Hormono-korrigiuriushchie svoystva syntehtescheskogo tymgena pri stimulyatsiy vosproizvoditelnui funktsiy u svinomatok. *Vestnik Krasnodarskogo HAU*, 4, 144–149 (in Russian).

Rybalko, V. P. (2005) Suchasni metodky doslidzhun u svarnosti. *Poltava*, 114–123 (in Ukrainian).

Shabanin, S. V. (2010). Metodicheskie polozeniya po izucheniyu protsessov svobodnoradikalnogo okisleniya v sisteme antioksydatnnyi zaschity organizma. Voronezh, 36– 37; 51–52 (in Russian).

Shostya, A. M., Stupar, I. L., Usenko, S. O., Bondarenko, O. M., Tsybenko, V. G., Chukhlub, Ye. V., & Slynko, V. G. (2019). Reproduktivnyi yakosti svinok riznyh porid. *Veterynariia, tekhnolohii tvarynyntva ta pryrodokorystuvannia*, 3, 230–236. doi: 10.31890/vttp.2019.03.31 (in Ukrainian).
Stupar, I. I. (2018) Prooksydantno-antyoksydantnyi homeostaz u svynok u rizni fazy statevoho tsyku. Visnyk Poltavskoi derzhavnoi ahrarnoi akademii, 4, 178–184. doi: 10.31210/visnyk2018.04.28 (in Ukrainian).

Usenko, S. O., Shostya, A. M., & Tsybenko, V. G. (2016). Osoblyvosti dynamiki estradiolu-17β v krovi svynei riznoi stati, viku ta fiziologichnoho stanu. Tavriiskyi naukovyi visnyk, 96, 165–169 (in Ukrainian).

Usenko, S. O., Shostya, A. M., Stoyanovskyy, V. G., Tenditnyk, V. S., Birta, G. O., Kravchenko, O. I., & Kuzmenko, L. M. (2020). Influence of vitamins on the prooxidant-antioxidant homeostasis in boars under the conditions of heat stress. Ukrainian Journal of Veterinary and Agricultural Sciences, 3(2), 30–35. doi: 10.32718/ujvas3-2.05.

Velichko, A. K., Solovyev, V. B., & Henhyn, T. (2009). Metody laboratormogo opredeleniya obschey razrushashchey aktyvnosti fermentov rasteniy. Yzv. Penzenskoho hos. ped. un-ta, 14(18), 44–48 (in Russian).

Villamor, E., Moreno, L., & Mohammed, R. (2019) Reactive oxygen species as mediators of oxygen signaling during fetal-to-neonatal circulatory transition. Free Radical Biology and Medicine, 142, 82–96. doi: 10.1016/j.freeradbiomed.2019.04.008.