UDC 619:612.017.1:618.63:636.4

DOI: 10.29326/2304-196X-2019-3-30-15-18

CELLULAR IMMUNITY AND CYTOKINE PROFILE IN PRE-FARROW AND LACTATING SOWS

A. G. Shakhov[1], S. V. Shabunin[2], L. Yu. Sashnina[3], M. I. Adodina[4], M. Yu. Zhejnes[5], K. V. Tarakanova[6], K. O. Kopytina[7]

[1] Chief Researcher, Doctor of Science (Veterinary Medicine), Professor, RAS Associate Member, FGBRI "VNIVIPAT", Voronezh, Russia, *e-mail: A.G.Shakhov@mail.ru*

[2] Director, Doctor of Science (Veterinary Medicine), Professor, RAS Member, FGBRI "VNIVIPAT", Voronezh, Russia, *e-mail: vnivipat@mail.ru; ORCID ID 0000-0002-2689-6998*

[3] Chief Researcher, Doctor of Science (Veterinary Medicine), FGBRI "VNIVIPAT", Voronezh, Russia, *e-mail: L.Yu.Sashnina@mail.ru*

[4] Chief Researcher, FGBRI "VNIVIPAT", Voronezh, Russia, *e-mail: vnivipat@mail.ru*

[5] Post-Graduate Student, FGBRI "VNIVIPAT", Voronezh, Russia, *e-mail: vnivipat@mail.ru*

[6] Junior Researcher, FGBRI "VNIVIPAT", Voronezh, Russia, *e-mail: vnivipat@mail.ru*

[7] Junior Researcher, FGBRI "VNIVIPAT", Voronezh, Russia, *e-mail: vnivipat@mail.ru*

SUMMARY

Study results of cellular immunity and cytokine profile of commercial pre-farrow and lactating sows are shown. Before farrow, the animals demonstrated physiological immunodeficiency characterized by relative leukopenia and lymphopenia, decreased T-cell number, higher helper/suppressor ratio providing immunological tolerance in dam/fetus system. Their cytokine profile was specified by relatively deficient interleukin-1β and tumor necrosis factor-α indicative of immune system suppression and high level of γ-interferon involved in parturition and activation of suppressor cells. Post-farrow sows demonstrated higher cellular immunity involving higher levels of leukocytes, lymphocytes and T-cells as well as tendency of T-lymphocyte helper/suppressor ratio reduction being indicative of T-cells' suppressor activity. The cytokine profile of the sows was specified by the recovered numbers of interleukin-1β, tumor necrosis factor-α and γ-interferon, decreased level of interleukin-2 and interleukin-4 that regulate cellular and humoral immunity, respectively as well as their subsequent increase (in particular, interleukin-2) following animal immunization against parvoviral infection and erysipelas on day 7 post farrowing (Parvoruvax vaccine, Merial, France) and against classical swine fever on day 14 post farrowing (culture dry virus-vaccine LK-VNIVIPFIT). This is due to the formation of the cellular and humoral immunity.

Key words: sows, leukocytes, lymphocytes, T- and B-cells, cytokines, immunodeficiency, cellular and humoral immunity.

INTRODUCTION

Diseases of parent herd and young animals are widespread at the large commercial livestock breeding farms in spite of intensive use of biologicals and chemotherapeutics for their prevention and therapy [1, 11, 13].

Multiple researches demonstrated that immunodeficiency disorders are the basis of the majority of pathological processes [3, 6, 7, 15].

One of the key reasons of the secondary immunodeficiency is poor ecology of the environment associated with the following factors: contamination with nitrogen and sulfur dioxides, carbon oxides, heavy metal salts, nitrates and other xenobiotics; feed contamination with toxins of biological origin (mycotoxins); feed deficiency in biologically active substances (microelements, vitamins); high concentration of potentially pathogenic microorganisms in the animal keeping facilities [2, 9, 12, 17].

The pig industry transfer to the commercial basis involving indoor keeping of large groups of animals, early weaning, regrouping, multiple vaccinations, use of chemotherapeutics and other agents significantly affected the immune status of the animals, their susceptibility to bacterial and viral infections [13, 16].

Within the current scenario assessment and control of food-producing animal immunity become increasingly relevant for the purpose of the recovery of general and immune homeostasis, improvement of the efficacy of preventive and therapeutic measures being taken.

Multiple studies are devoted to the immune status of pigs, in particular of sows, whose immunity status highly influence the offspring quality and viability; however, among the domestic publications there are only singular reports on its separate issues including cytokine profile, which is one of the most significant performance indicators of the immunity.

The study is aimed at the examination of the cellular immunity and cytokine dynamics in sows before farrowing and during lactation period.

MATERIALS AND METHODS

The study was performed in third-parity cross-bread sows (large white + landrace + Duroc) at the commercial pig farm OOO "Vishnevskoye", Verkhnekhavsky Raion, Voronezh Oblast.

After sanitary treatment on day 108 of the pregnancy, the sows were transferred to cleaned and disinfected room and placed in individual pens. The animals were kept under the optimal environment conditions with due regard of their physiological status. The average temperature in the room amounted to 20–22 °C, relative humidity – to 65–70%. During the experiment, the sows were fed with SK-2 feed that according to the manufacturer's guarantees was well-balanced in metabolic (mJ/kg) and net energy (kCal/kg), protein, amino acids, vitamins, macro- and microelements.

The animals were clinically examined before farrowing, during parturition and for 26 days post farrowing.

On day 1, 2 and 3 post farrowing Uteroton was intramuscularly administered to the sows for prevention of postpartum disorders (acute postpartum endometritis, mastitis-metritis-agalactia). On day 7 post farrowing the animals were vaccinated against parvoviral infection and erysipelas with inactivated vaccine Parvoruvax (Merial, France) and on day 14 – against classical swine fever using

Table 1
Cellular immunity in sows

Parameters	Day 5–7 before farrowing	Day post farrowing				
		1	7	14	22	26
Leukocytes, 10^9/l	9.8 ± 0.73	10.3 ± 0.95	12.2 ± 1.26	14.2 ± 0.69*	14.0 ± 0.77*	13.4 ± 0.77
Lymphocytes,%	38.6 ± 2.8	33.3 ± 1.10	36.0 ± 2.73	41.3 ± 2.60	59.3 ± 2.15*	48.7 ± 1.36
abs, 10^9/l	3.6 ± 0.34	3.42 ± 0.49	4.2 ± 0.83	5.1 ± 0.66	7.42 ± 0.75*	6.2 ± 0.08*
T-cells,%	50.3 ± 1.54	61.8 ± 1.32*	63.0 ± 0.91*	59.3 ± 1.11*	53.0 ± 1.11	45.7 ± 0.65
abs, 10^9/l	4.9 ± 0.35	6.3 ± 0.53	8.6 ± 0.78*	8.4 ± 0.75	6.7 ± 0.91	6.2 ± 0.49
Suppressor T-cells, %	16.0 ± 0.45	21.5 ± 0.64*	21.5 ± 1.04*	17.5 ± 0.96	11.3 ± 1.11*	8.5 ± 1.04*
abs, 10^9/l	0.79 ± 0.07	1.32 ± 0.14	1.69 ± 0.39	1.5 ± 0.12*	0.81 ± 0.09	0.55 ± 0.009
Helper T-cells, %	39.3 ± 1.05	43.8 ± 1.11	42.8 ± 0.85	39.5 ± 1.41	21.3 ± 1.93*	18.5 ± 1.41*
abs, 10^9/l	2.0 ± 0.16	2.7 ± 0.32	4.1 ± 0.39*	3.4 ± 0.37	1.5 ± 0.31	1.2 ± 0.14*
Suppressor T-cells/ Helper T-cells	2.4:1 ± 0.07	2:1 ± 0.001*	2:1 ± 0.09*	2.3:1 ± 0.55	1.9:1 ± 0.41	2.2:1 ± 0.15
B-cells, %	23.7 ± 1.28	21.8 ± 0.85	23.0 ± 0.91	20.3 ± 0.63	18.0 ± 0.91	14.8 ± 1.11*
abs, 10^9/l	2.3 ± 0.24	2.3 ± 0.29	2.8 ± 0.63	2.9 ± 0.27	2.3 ± 0.32	2.0 ± 0.27

* $p < 0.001$ against the pre-farrow value.

cultural dry virus vaccine LK-VNIIVVIM (OAO Pokrov Biofactory). 5–7 days before parturition and on days 1, 7, 14, 22 and 26 post farrowing six sows were bled for cell immunity and cytokine profile examination.

Cellular immunity parameters included leukocytes, T- and B-cells, suppressor T-cells, helper T-cells, ratio of suppressor T-cells and helper T-cells, and they were examined according to the Methodical guidelines on animal immune status assessment and correction [8].

Interleukin-1β (IL-1β), interleukin-2 (IL-2), interleukin-4 (IL-4), interleukin-10 (IL-10), tumor necrosis factor-α (TNF-α), γ-interferon (IFN-γ) were determined in blood sera using enzyme-linked immunosorbent assay (ELISA) followed by result reading with Uniplan™ according to the test-kit manufacturer's instructions.

The obtained data were statistically processed using Statistica v6.1 software, confidence was estimated using Student's t-test.

RESULTS AND DISCUSSION

During the immunological studies, all sows demonstrated normal clinical parameters.

Examination of the cellular response demonstrated that before farrowing the sows had relatively low level of leukocytes playing the indispensable role in protection against infections – $(9.8 \pm 0.73) \times 10^9$/l, and after farrowing this level increased against the pre-farrow level: by 5.1% on day 1, by 24.5% on day 7, by 44.9% on day 14, by 42.6% on day 22 and by 36.7% on day 26 (Table 1).

Similar dynamics (excluding day 1 post farrowing) was also demonstrated for absolute lymphocyte count (ALC), which are key immune cells in charge of all immune responses. ALC was not high in sows before the parturition $(3.6 \pm 0.34) \times 10^9$/l, after farrowing ALC increase was reported on day 7 and amounted to 16.7%, on day 14 it increased by 41.7%, on day 22 – 2.1-fold and on day 26 – by 72.2%. Relative WBC count decreased by 13.7 and 6.7% on day 1 and 7 post farrowing and on day 14, 22 and 26 it increased by 7.0, 53.6 and 26.2%, respectively.

Before farrowing absolute T-cell count in sows amounted to $(4.9 \pm 0.35) \times 10^9$/l, and after parturition at all test time points it increased by 28.6, 75.5, 71.4, 36.7 and 26.5% as well as relative T-cell count: on day 1 – by 21.8%, on day 7 – by 25.2%, on day 14 – by 17.9% and on day 22 – by 5.4%. Herewith, the tendency of relative T-cell count decrease was reported starting from day 14 of lactation and on day 26 this parameter was 9.1% lower as compared to pre-farrow sows.

Absolute and relative counts of suppressor T-cells inhibiting immune response and responsible for microorganism-associated immunosuppression and helper T-cells being pivotal for the development of humoral (antibody synthesis) and cellular immunity and macrophage activation changed after farrowing. Thus, absolute count of suppressor T-cells in sows was by 67.1%, 2.1 times, by 89.9% and 2.5 higher on day 1, 7, 14 and 22 after farrowing, respectively, but was lower by 30.4% on day 26 after farrowing as compared that one before farrowing. Their relative count was higher by 34.3% on day 1 and day 7 after farrowing, by 9.4% on day 14 after farrowing, by 29.4% on day 22 after farrowing and 1.9 times higher on day 26 after farrowing as compared to that one before farrowing.

Absolute count of helper T-cells was higher by 35%, 2.1 and 1.7 times higher on day 1, 7 and 14-after farrowing and decreased by 25.0% and 40.0% on day 21 and 26 after farrowing as compared to that one before farrowing. Relative count of helper T-cells was higher by 11.5% and 8.4% on day 1 and 7 after farrowing and similar on day 14 after but was lower by 45.8 and 52.9% on day 22 and 26 after farrowing, respectively, as compared to that one before farrowing.

T-lymphocyte helper/suppressor ratio was the highest (2.4:1 ± 0.07) in sows before farrowing that was indicative of relatively low suppressor activity of T-lymphocytes providing immunological tolerance in dam-fetus system.

Table 2
Cytokine profiles in sows

Content, pg/ml	5–7 days before farrowing	Days after farrowing				
		1	7	14	22	26
IL-1β	10.9 ± 0.30	11.2 ± 0.19	11.0 ± 0.29	11.3 ± 0.1	12.0 ± 0.83	11.4 ± 0.15
IL-2	11.0 ± 0.94	7.6 ± 0.23	10.6 ± 0.94	12.2 ± 1.53	49.7 ± 3.92*#	15.5 ± 0.87#
IL-4	10.7 ± 1.69	6.0 ± 0.85	6.3 ± 0.66	4.9 ± 0.34	10.6 ± 0.92#	9.6 ± 0.85
IL-10	21.0 ± 0,32	20.7 ± 0.59	22.8 ± 1.59	22.4 ± 1.08	21.5 ± 0.86	21.4 ± 0.35
TNF-α	3.6 ± 0.07	3.9 ± 0.37	4.0 ± 0.23	3.5 ± 0,12	3.7 ± 0.12	4.3 ± 0.17*
IFN-γ	96.6 ± 15.29	70.3 ± 10.49	15.3 ± 1.26*#	18.4 ± 3.21*	17.3 ± 1.02*	11.1 ± 1.73*

* $p < 0.001$ as compared to pre-farrow values;
# $p < 0.001$ as compared to value on previous day of testing.

After farrowing T-lymphocyte helper/suppressor ratio decreased in sows on all days of testing that evidenced the increase in suppressor activity of T-lymphocytes mediated by microorganisms circulating in the sows' environment.

Absolute count of B-lymphocytes responsible for inducing immune response including specific antibodies development changed significantly in sows before and after farrowing. B cell level increased by 21.7 and 26.1% on day 7 and 14, respectively, and decreased by 13.0% on day 26 after farrowing as compared to that one before farrowing. Relative B-cell count decreased by 8.0%, 3.0%,14.3%, 24.1% and 37.6% on day 1, 7, 14, 22 and 26 after farrowing respectively, as compared to that one before farrowing.

Results of tests of animals for their immunity before and after farrowing showed the physiological immunodeficiency in pregnant sows required for continued pregnancy and successful pregnancy outcome. It was characterized with decreased leucocyte count and absolute and relative count of lymphocytes, T lymphocytes and increased helper/suppressor cell ratio as compared to that ones after farrowing that was indicative of reduced suppressor activity of T-lymphocytes, required for inevitable dam-fetus conflict prevention.

Insignificant changes in numbers of two out of four tested pro-inflammatory cytokines, IL-1β and TNF-α, produced by phagocytes and dendritic cells and involved in immune response induction, inflammation and cell regeneration were detected when sows were tested for their cytokine profiles (Table 2).

IL-1β levels in sows on all days of testing after farrowing were slightly higher (1.0 to 10.0%) that that ones in sows before farrowing.

Similar positive dynamics in TNF-α level (2.8 to 19.4%) were detected in the sows during lactating period. Immune suppression was observed in the sows demonstrating relatively insufficient IL-1β, TNF-α cytokine levels at the end of their pregnancy period [10].

Levels of pro-inflammatory interleukin, IL-10, synthesized by type 2 helper T cells (Th-2) and cytotoxic T-lymphocytes involved in humoral immunity induction and having cytotoxic effect [4] were almost similar in sows before and after farrowing.

Levels of IL-2 produced by type 1 helper T-lymphocytes (Th-1) and cytotoxic T-lymphocytes and responsible for cell immunity development significantly changed [4].

In sows IL-2 counts decreased by 30.9 and 3.6% on day 1 and day 7 days after farrowing, respectively, but became by 10.9%, 4.5 times and 40.9% higher on day 14, 22 and 26 after farrowing, respectively, as compared to that ones before farrowing.

Level of IL-4, secreted by Th-2 and responsible for humoral immunity development was by 45.9%, 41.4% and 2.2 times lower on day 1, 7 and 14 after farrowing but demonstrated 2.2 times increase on day 22 as compared to the previous level and then decreased by 10.3% on day 26 after farrowing.

Decrease in IL-2 and IL-4 counts in sows after farrowing appeared to be associated with termination of fetal antigen effect on sow immune competent cells synthesizing the said interleukins.

Significant increase in IL-2 and IL-4 levels on day 22 after farrowing was indicative of cellular and humoral immunity development after vaccination of sows against parvovirus infection, erysipelas and classical swine fever on day 7 and 14 after farrowing. Therewith, the increase was the highest in IL-2 count, cytokine regulating cell immunity developed by animals after administration of live virus vaccine against classical swine fever.

Level of IFN-γ synthesized by Th-1, cytotoxic T lymphocytes and natural killer cells and being the key cytokine for cellular immunity and an inhibitor for humoral adaptive immunity was the highest in sows before and on day 1 after farrowing.

Researchers [14] showed that count of IFN-γ involved in partition initiation by activation of synthesis of E2 и F prostaglandins stimulating uterine muscle contractions and enhancing oxytocin receptor activity in uterine muscles increased before parturition. Furthermore, high IFN-γ levels inhibited antigen-dependent T- and B-lymphocyte proliferation and activated suppressor cells [5]. Relatively high IFN-γ level in sows after farrowing promoted involution.

IFN-γ number became 6.3 times lower on day 7; 5.3 times lower on day 14; 5.6 times lower on day 22 and 8.7 times lower on day 26 after farrowing.

Tests of sows for their cytokine profiles before farrowing showed relative deficiency of IL-1β and TNF-α, pro-inflammatory cytokines, resulting in immune suppression, high level of IFN-γ involved in partition initiation and suppressor cell activation as well during lactation period decrease in IL-2 and IL-4 levels within the first seven days

and on day 14 after farrowing, respectively, followed by their subsequent significant increase associated with cell and humoral immunity development after vaccination of sows against parvovirus infection erysipelas and classical swine fever.

CONCLUSION

Physiological immunodeficiency characterized with relative leukocyte- and lymphocytopenia, low T-lymphocyte numbers, higher helper/suppressor cell ratio providing immunological tolerance in dam-fetus system was detected in sows before farrowing. Sows' cytokine profiles demonstrated relative IL-1β and TNF-α deficiency resulting in immunosuppression and high levels of IFN-γ involved in partition and activating suppressor cells.

Increased cellular immunity level in sows after farrowing was associated with increase in leukocyte, lymphocyte, T-lymphocyte counts and with decreasing trend in helper/suppressor cell ratio that was indicative of enhanced suppression T-lymphocyte activity mediated by microorganisms circulating in the sows' environment. After vaccination of sows against parvovirus infection erysipelas and classical swine fever their cytokine profiles demonstrated restoration of IL-1β, TNF-α and IFN-γ production and decrease in IL-2 and IL-4 levels, regulating cellular and humoral immunity, respectively, followed by their significant increase (especially IL-2).

Identified features of cellular immunity and cytokine profile in sows before farrowing and within lactation period allow assessment of immune system state in normal sows and in sows with disorders as well as control of taken preventive and curative measures for their effectiveness.

Conflict of interests. The authors claim no conflict of interests.

REFERENCES

1. Baborenko Ye. P., Dolganova Ye. K., Gruzdev K. N. Testing of combined vaccines against Aujeszky's disease, porcine reproductive and respiratory syndrome and porcine parvovirus infection for their antigenicity [Izuchenie antigennoj aktivnosti associirovannyh vakcin protiv bolezni Aueski, reproduktivno-respiratornogo sindroma i parvovirusnoj infekcii svinej]. *Veterinary Science Today*. 2018; 2: 13–17; DOI: 10.29326/2304-196X-2018-2-25-13-17 (in Russian).

2. Donnik I. M., Smirnov P. N. Ecology and animal health [Ekologiya i zdorov'e zhivotnyh]. Yekaterinburg: UTK, 2001 (in Russian).

3. Immune status of pigs in industrial pig establishments [Immunnyj status porosyat v hozyajstvah promyshlennogo tipa]. Yu. N. Fyodorov, O. A. Verkhovsky, B. G. Orlyankin [et al.]. *Veterinariya*. 2006; 6: 18–21 (in Russian).

4. Examination of cytokine gene expression during cultivation of leukocytes from healthy donors [Issledovanie ekspressii genov citokinov v processe kul'tivirovaniya lejkocitov zdorovyh donorov]. L. V. Kovalchuk, L. V. Gankovskaya, M. V. Mezentseva [et al.]. *Journal of Microbiology, Epidemiology and Immunobiology* [Žurnal mikrobiologii, èpidemiologii i immunobiologii]. 2012; 2: 60–63 (in Russian).

5. Clinical immunology and allergy [Klinicheskaya immunologiya i allergologiya]. ed. by L. Jäger, translated from German, ed. by R. V. Petrov. M.: Meditsina, 1990 (in Russian).

6. Makarov V. V. Factorial diseases [Faktornye bolezni]. *Russian veterinary journal. S.-kh. zhivotnye*. 2017; 4: 22–27 (in Russian).

7. Masyanov Yu. N., Shakhov A. G. Humoral immunity development in normal calves and in calved with disorders [Formirovanie gumoral'nogo immuniteta u telyat v norme i pri patologi]. *Reports to the Russian Academy of Agricultural Sciences* [Doklady rossijskoj akademii sel'skohozyajstvennyh nauk]. 2012; 5: 40–43 (in Russian).

8. Methodical guidelines for animal immune status assessment and correction [Metodicheskie rekomendacii po ocenke i korrekcii immunnogo statusa zhivotnyh]. A. G. Shakhov, Yu. N. Masyanov, M. I. Retsky [et al.]. *New test methods in veterinary field. P. III. Methods of testing for non-contagious pathologies in food-producing animals. M.: Russian Academy of Agricultural Sciences*, 2007 (in Russian).

9. Molev A. I., Blokhin A. A. Mixed infections in newly born calves and basic principles for their prevention [Mikst-infekcii novorozhdennyh telyat i osnovy ih profilaktiki]. *Main epidemiological characteristics of animal population: Proceedings of the International Scientific Practical Conference*. N. Novgorod: BIKAR, 2015 (in Russian).

10. Nagoyev B. S., Nagoyeva M. Kh. Clinico-pathogenetic assessment of pro-inflammatory cytokine dynamics in patients with bacterial tonsillitis [Kliniko-patogeneticheskaya ocenka dinamiki provospalitel'nyh citokinov u bol'nyh bakterial'noj anginoj]. *Infectious Diseases*. 2008; 6 (2): 42–45 (in Russian).

11. Guidelines for maintaining cattle performance: textbook [Prakticheskoe rukovodstvo po obespecheniyu produktivnogo zdorov'ya krupnogo rogatogo skota: uchebnoe posobie]. S. V. Shabunin, F. I. Vasilyev, A.G. Nezhdanov [et al.]; ed. by S. V. Shabunin, F. I. Vasilevich. Voronezh: Antares, 2011. (in Russian).

12. Samokhin V. T. Microelements in agricultural lands – a key ecological factor for high efficiency of arable lands and animal and human health [Mikroelementy na sel'skohozyajstvennyh ugod'yah – vazhnejshij ekologicheskij faktor obespecheniya vysokoj produktivnosti polej i zdorov'ya zhivotnyh i cheloveka]. *Current aspect of metabolic disorders in livestock animals. International Scientific and Practical Conference devoted to the 40th anniversary of SSI VNIVIPFiT*. Voronezh: Istoki, 2010: 11–34 (in Russian).

13. Sashina L. Yu. Epidemic situation on porcine respiratory diseases at the industrial pig establishments, etiology and clinical-experimental justification for application of new preventive and therapeutic tools [Epizooticheskaya situaciya po respiratornym boleznyam svinej v hozyajstvah promyshlennogo tipa, etiologiya i kliniko-eksperimental'noe obosnovanie primeneniya novyh sredstv ih profilaktiki i terapii]: author's abstract. PhD theses (Veterinary medicine). M., 2013 (in Russian).

14. Simbirtsev A. S. Role of cytokines in human disease pathogenesis and therapy [Citokiny v patogeneze i lechenii zabolevanij cheloveka]. S-Pb: Foliant, 2018 (in Russian).

15. Fyodorov Yu. N. Immunodeficiency in cattle [Immunodeficity krupnogo rogatogo skota]. *Veterinariya*. 2006; 1: 3–6 (in Russian).

16. Shubina T. P., Choporova N. V. Morphology of some lymphoid organs of pigs in postnatal ontogenesis [Morfologiya nekotoryh limfoidnyh organov u svinej v postnatal'nom ontogeneze]. *Veterinarnaya patologiya*. 2015; 1 (51): 64–68 (in Russian).

17. Immunotoxicological risk of mycotoxins for domestic animals. I. P. Oswald, D. E. Marin, S. Bouhet [et al.]. *Food Addit. Contam*. 2005; 22 (4): 354–360; DOI: 10.1080/02652030500058320.

Submitted on 27.06.19
Approved for publication on 12.08.19