Evaluation of histochemical and molecular markers in the placenta of sows during pregnancy complicated by isoimmunization

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Abstract. It is known that the pathological course of pregnancy is associated with a disturbance in the interdependent “mother-fetus” complex and leads to specific clinical consequences at the molecular-cellular, tissue, organ, organismal and population levels of organization. In case of violation of placentation (placental barrier), a state of immune conflict occurs, characterized by an antigen-antibody reaction, carried out through the placenta (in relation to the fetus), or through colostrum after birth (in relation to the newborn offspring). Therefore, immunological relations at the population level should be considered both the consequences of the action of the maternal organism's antigens on the fetus, and the effects of the allogeneic action of the fetus on the mother's body. According to the results of the experiment, the placentas of 10 sows of Large White breed were used as the material, which showed signs of isoimmunization to the resulting offspring. For histological examination, immediately after delivery, pieces of placenta up to 0.5 cm thick were selected, which were fixed in a 10% aqueous solution of neutral formalin. The fixed material after wiring through alcohols of increasing concentration, xylene, xylene-paraffin, was poured into paraffin. The obtained preparations were stained with conventional methods-hematoxylin and eosin. d (fibrinoid masses).

1 Introduction

Given the high intensity of growth and development processes in multiple farm animals, as well as the fact that fetal nutrition is carried out through the placenta, intrauterine development and the fullness of the placental barrier are important [4-13].

The barrier function of the placenta is manifested only in a normal pregnancy, and under the influence of pathogenic factors, it is disturbed and becomes permeable even to such substances that in normal physiological conditions pass through it in limited quantities [10-12].

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Considering the results of studies conducted by various authors [2-16], we can conclude that isoimmunization (sensitization) in some cases creates disturbances in placentation in the induction of immunological reactivity.

The effect of previous isoimmunization may be associated with the formation of circulating antibodies [1-17], which neutralize the antigen administered during the tolerance induction. This is confirmed by the results of a number of authors [6-8], which showed that the introduction of specific antibodies early after the injection of the antigen, when full tolerance has not yet occurred, has prevented its development.

The aim of research: to conduct a comparative morphofunctional assessment of pathological changes in the placenta of sows with the revealed effect of isoimmunization in the resulting offspring.

2 Research materials and methods

Experimental studies were conducted on sows of a Large White breed on the farms in the Kurskoy district of the Stavropol territory. Histological studies were performed in the histological laboratory of the Scientific Diagnostic and Therapeutic Veterinary Center of the Stavropol State Agrarian University.

Test group included animals with increased antigenic load (hyperimmunization). The control group included animals subjected to the traditional vaccination scheme adopted on the farm.

Placentas of 10 sows of a Large White breed were used as the material for the study. Material for the research was selected immediately after delivery. For histological examination pieces of placenta up to 0.5 cm thick were selected, which were fixed in a 10% aqueous solution of neutral formalin. Fixed material after wiring through alcohols of increasing concentration, xylene, xylene-paraffin, was poured into paraffin. From the obtained paraffin blocks, histological sections 4-6 microns thick were made, which, for the studying purpose, were stained with hematoxylin and eosin, according to the recommendations set out in the guide of V. V. Semchenko and co-authors in 2006 [5].

Section microscopy was performed using an Olympus BX45 light microscope with a built-in C 300 camera (Japan). Oculars $\times 10$ and lenses $\times 4$, $\times 10$, $\times 20$, $\times 40$, $\times 100$ were used for microscopy.

Morphometric studies were performed using the program "Videotestmaster Morphology 4.0" for Windows. The obtained digital data were analyzed using the statistical method of one-factor analysis of variance "Biostatistics 4.03" for Windows. Reliable differences were considered when $P \leq 0,05$.

3 Results

When studying the histological structure of the placenta in the experimental group compared with the control group, it was revealed: a decrease in vascularization, (figure 1.); more pronounced alterative processes (vacuole dystrophy and desquamation of epithelial cells (figure 1a.), focal necrosis, loosening of the stroma (atrophy of syncytiotrophoblasts).

Fig. 1. Placenta of sows from the test group. Reduction of vascularizati loosening of the stromal base process

Fig. 1a. Vacuole dystrophy and desquamation of epithelial cells (staining with hematoxylin and eosin, $\times 400$)

In a significant part of the placental area, there is a decrease in the diameter of blood arterioles and venules (focal obliteration of the vascular lumen). The process of thrombosis on the damaged vessel walls and significant lymphoid-macrophage infiltrates were revealed (figure 2.).

Fig. 2. Lymphoid-lymphocytic infiltrates of the placenta of sows from the test group (staining with hematoxylin and eosin, $\times 400$).

The above changes relate to pathological processes of an alterative nature, indicating signs of violation of the morphofunctional structure of the placenta (its immaturity) of sows with signs of isoimmunization in the fetoplacental system. Specific disorders cause damage to the integrity of the placental barrier and changes in the functional structure in the functional system “mother-fetus-newborn”.

In the placentas of sows from the test group, there was an overgrowth of connective tissue elements with sclerotization of the stroma, especially around the blood vessels. This fact was regarded by us as an involutional process (figure 3.).

Fig. 3. Involution process in the placenta of sows from the test group (staining with hematoxylin and eosin, $\times 100$).

In sows from the test group, a significant deposition of fibrinoid masses was found, indicating the involution of placental tissues. Their main location is near large arteries and in the chorial plate with a compaction of the vessel wall (figure 4).

Fig. 4. Fibrinoid masses in the subepithelial base of the placenta of sows from the experimental group (staining with hematoxylin and eosin, $\times 400$).

The animals in the control group did not have the same characteristics as the animals in the test group. This state of the morphofunctional structure of the placenta is the fact of the absence of the damaging effect of high antigenic load in the maternal body during pregnancy.

Summarizing the results, it can be stated that of the total number of newborns, neonatal losses amounted to 10.2%. Conditionally healthy, without specific signs of isoimmunization and other developmental disorders, 77.2% of piglets were born (part of piglets of the second group and the third group). With clinical signs of isoimmunization, 9% were born, as confirmed by luminescent microscopy (newborns of the first group). Part of the offspring of the second group - 13.8%, subject to negative results of luminescent microscopy, had different signs of developmental impairment.

The peculiarities of neonatal development of piglets belonging to different experimental groups are compared with the level of isoimmunized mothers. The results are shown in Table 1.

The presented results show that the maximum number of piglets of experimental groups was born from sheep with titers of 1:40, which was 53% of all newborns. Offspring from sheep with titers 1:20 are 32.9% of the total number of piglets obtained, and in sheep with titers 1:10, offspring respectively amounted to 14.1%.

4 Discussion

Table 1. Peculiarities of neonatal development of piglets belonging to different experimental groups are compared with the level of isoimmunized mothers

Piglet groups		Maternal antibody titers before childbirth		
		1:10	1:20	1:40
Group 1	newborns	-	8	49
	postnatal losses	-	6	18
Group 2	newborns	3	16	85
	postnatal losses	-	7	14
Group 3	newborns	56	114	89
	postnatal losses	2	3	2

An important addition to the studies conducted was the analysis of the distribution of piglets in groups depending on the seropositivity of mothers. So, in the first group, 86% were born from sows with titers 1:40, 14% - from sows with titers 1:20.

In 81.7% of piglets of the second group of mothers had isoantibodies titers 1:40, in 15.4% - 1:20, and piglets whose mothers had titers 1:10 were 2.9%.

In the third group, the distribution of newborns by the degree of isoimmunization of sows is presented as follows: piglets born from sows with titers 1:40 amounted to 34.4%, newborns. Newborns, whose mothers had 1:20 titers before the eye, accounted for 44%. The rest were born from sows with titers of 1:10, which corresponded to 21.6%.

Therefore, the nature of the detected changes depends on the level of antigenic load of the mother's body during pregnancy; these changes are the reason for the high risk of isoimmunization in the offspring. The established pathological signs in the placental structure reveal the mechanism of isoimmunization effect in newborn piglets and underlie the further level of their viability (see table 2).

It was found that most aborted fetuses during this period of pregnancy were long-term assigned to the first group. This is 24 fetuses - 37.4% of the total number of abortions for the entire period of prenatal development for all experimental groups. The registration of

abortions in this group in the pre-birth period took place on the 34-37 day - 15 abortions, on the 41- 43 day - 9 abortions.

Part of the aborted fruits made up the second group. Their number is lower than in the first group - 8 fetuses (12.1% of the total number of abortions for the entire period of prenatal development for all experimental groups). Abortions in this group were observed for 30-46 days, 3 of which occurred on 30-35 days of embryogenesis and 5-43-46 days.

No abortions were observed in the third group. In the control group during embryogenesis (group 4), 5 fetuses were aborted (41.6% of the total number of abortions observed in isoimmunized sows) for 30-35 days and two fetuses (16.8%) - 41-43 days.

Table 2. The established pathological signs in the placental structure reveal the mechanism of isoimmunization effect in newborn piglets

Aborted fruit	Prenatal age, days	Sets:Lidina (cm), massa (g)	
Pre-pregnancy period			
Group 1	34-37	massa	1,0±0,16*
		lidina	1,9±0,24*
	41-43	massa	5,4±0,35*
		lidina	3,6±0,15*
Group 2	30-35	massa	1,1±0,12*
		lidina	2,1±0,31*
	43-46	massa	7,8±0,28*
		lidina	4,2±0,15*
Group3 (control)	30-35	massa	1,8±0,21
		lidina	2,8±0,18
	41-43	massa	9,8±0,08
		lidina	5,1±0,31
The fetal period			
Group 1	95-100	massa	* 540,0±0,24
		lidina	23,5±0,54*
	117-120	massa	3* 1100,0±0,2
		lidina	27,9±1,17*
Group 2	90-94	massa	* 540,0±0,35
		lidina	24,5±0,64*
	117-120	massa	1* 1600,0±0,2
		lidina	32,8±0,42*
Group3 (control)	90-95	massa	800,0±0,10
		lidina	25,7±0,30
	114	massa	5 2500,0±0,1
		lidina	35,4±0,23

5 Conclusions

As the study showed, morphofunctional changes in the placenta of sows during pregnancy complicated by isoimmunization were characterized by the following processes:

1. The decrease in cellular elements with the loosening of the stromal framework and focal atrophy.
2. Post-inflammatory lymphoid-lymphocytic infiltrates of the placenta.
3. Involution or post-inflammatory process in the placenta of sows from the test group.
4. Fibrinoid masses deposition in the subepithelial base of the placenta of sows from the test group.

Thus, the revealed changes in the histological structure in the placenta of sows showed that the phenomenon of isoimmunization can be a trigger mechanism for the pathological course of pregnancy in the studied animals.

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