

**ФИЗИОЛОГИЯ ЧЕЛОВЕКА И ЖИВОТНЫХ****HUMAN AND ANIMAL PHYSIOLOGY**

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Original article

**IDENTIFICATION OF DE-EPITHELIZED  
STRUCTURES OF PIG PLACENTA**

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***Abstract***

The article presents the structural features of de-epithelized structures in the placenta of sows. A systematic histological examination of placentas in pregnant animals allowed us to identify new methods for assessing their condition, taking into account the embryonic periods of fetal development, substantiate critical periods of newborn development, and put forward a scientific position on maintaining the rhythm of labor activity in animals. In addition, this paper discusses some issues of neonatal development of newborn offspring.

The study of sow placentas revealed clear differences in the structure of the epithelial layer. Foci of diepithelialization of the main chorionic villi have been identified. According to the signs of the difference, two groups of sows were identified. The main criterion for the correlation of animals by contact groups was the identification of de-epithelized structures in the placenta of pigs. Individuals with pronounced de-epithelized structures of placental tissue were identified in the first group, while the second group acted as a control without obvious changes in the epitheliochoric layer.

When organizing therapeutic and preventive measures in pig farms, it is necessary to take into account this change in placental tissue in pigs as a predictor of a later (indirect) pathological condition of the offspring obtained with a high infectious risk group.

We consider it necessary to continue research in this area to study the effect of the identified destructive changes in the placental barrier of the fetoplacental

complex in pigs on changes in immunobiological reactivity, natural resistance and adaptive characteristics of animals.

**Keywords:** de-epithelized structures of the placenta; intrauterine period of development; placental insufficiency; sows; placental barrier

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Научная статья

## ВЫЯВЛЕНИЕ ДИЭПИТЕЛИЗИРОВАННЫХ СТРУКТУР ПЛАЦЕНТЫ СВИНЕЙ

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### *Аннотация*

В статье описано исследование по выявлению особенности строения диэпителизированных структур в плаценте свиней. Системное проведение гистологических исследований плацент у беременных животных, результаты которых представлены в данной работе, позволило выявить новые методы оценки состояния беременных животных с учётом эмбриональных периодов развития плода, обосновать критические периоды развития новорождённого и выдвинут научное положение о сохранении ритма родовой деятельности животных, а также вскрыть некоторые вопросы неонатального развития новорождённого потомства.

При исследовании плацент свиноматок определены явные различия в структуре строения эпителиального слоя. Установлены очаги диэпителизации основных ворсин хориона. По признакам различия определены две группы свиноматок. Основным критерием соотношения животных по интактным группам стало выявление диэпителизированных структур в плаценте свиней. В первую группу определены особи с выраженными диэпителизированными структурами плацентарной ткани, а вторая выступала контролем – без явных изменений эпителиохориального слоя.

При организации профилактических и превентивных лечебных мероприятий свиноводческих хозяйствах необходимо учитывать выявленные изменения плацентарной ткани у свиней как предиктор более позднего (опосредованного)

патологического состояния полученного потомства с соотнесением в группу высокого инфекционного риска.

Считаем необходимым продолжение исследований в данном направлении по изучению влияния обозначенных деструктивных изменений плацентарного барьера фетоплацентарного комплекса у свиней на изменения становления иммунобиологической реактивности, естественной резистентности и адаптивных особенностей животных.

**Ключевые слова:** диэпителизированные структуры плаценты; внутриутробный период развития; плацентарная недостаточность; свиноматки; плацентарный барьер

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## Introduction

The main task of modern productive animal husbandry is to produce healthy offspring. Recent studies have established that the assessment of the immune status of an animal's body in normal and pathological conditions is necessary for immunoprophylaxis and epizootological forecasting [1; 5; 9; 11].

However, the formation of immunobiological parameters in newborn animals is determined by the time interval during which it is possible to assess the dynamic change in the studied parameters [8; 10; 15; 18].

In this regard, the search for the earliest and most informative criteria for the diagnosis of abnormalities in the immunological relationship in the functional system "mother-fetus-newborn", as well as the expansion of knowledge about the conditions of fetoplacental development are of critical importance [3; 4; 12; 14; 17].

It is known that the fetoplacental barrier is the main link in the transmission chain of the causative agent of an infectious disease [2; 13; 16; 19]. The direct effect of changes in the fetoplacental barrier on the formation of immune tension from the first days of life has also been proven [6; 7; 20].

One of the important conditions for physiological pregnancy is the principle of complete structural and functional differentiation of the fetoplacental complex. Therefore, the study of the placental barrier can be used as the earliest method of assessing the functional maturity of the offspring obtained in animals. Taking into account the pathological changes in the future, it is possible to predict epizootics and plan preventive measures.

*Purpose*

Study of pathomorphological features of de-epithelized structures of sow placenta based on histological research methods

**Materials and methods***Materials:*

Placentas of sows with normal immunological reactivity (control group – 10 heads) and with a change in the immunological profile of the blood (experimental group – 10 heads).

*Equipment:*

- Binocular with double layer stage, B-159, Optica;
- Cabinet Exhaust Fan IIIB-1,0 «Laminar-C»;
- AB Veriti Thermal Cycler;
- Software VideoTest 4.0 (Build 353);
- Software «Biostatistics 4.03»;
- Ocular micrometer (Leica DM 2500);
- Fluorescence microscope Olympus BX41;
- Histology sample preparation system Tissue-Tek;
- Microtome Microm HM 340E (Microm GmbH, Germany).

*Reagents and medications:*

Histological stains: Hematoxylin (150 ml) – to overview staining of placental sections; eosin hydroalcoholic solution (500 ml).

Melted paraffin Histomix.

Buffered formalin 10% – to fixate of placental sections.

Electrolytic decalcifying solution (150 ml).

IzoPrep reagents – to increase the penetrating power of histological stains in tissues.

*The object of research:*

The object of research was the placentas of sows with different receptive profiles.

*Methods:*

The prepared sections were stained hematoxylin and eosin. Changes in placenta thickness was measured by ocular micrometer (Leica DM 2500). The relative area, diameter, perimeter, length and width of the placenta tissues were determined by VideoTest 4.0 software (Build 353). The morphological characteristics of the arterial and venous vascular bed were also studied.

All histological material was fixed in a 10% solution of neutral formalin. Histological sections were formed using a microtome Microm HM 340E (Mi-

crom GmbH, Germany). Fixed sections were stained using the method of increasing alcohol concentration and the Mallory's staining method [11].

Microscopic analysis was performed by Olympus BX41 microscope under magnification  $\times 4$ ,  $\times 10$ ,  $\times 20$ ,  $\times 40$ ,  $\times 100$ .

*Research procedure:*

The experimental part of the study was performed on two groups of sows (15 heads each) in the conditions of the Glushchenko's agricultural production cooperative (Krasnodar Krai).

In order to study the histological structure of placental tissue, the placentas of sows with normal immunological reactivity (control group – 10 heads) and with a change in the immunological profile of blood (experimental group – 10 heads) were analyzed.

Placentas were taken immediately after the end of labor. The subject of the study was the epitheliochoric layer of the placenta. Immediately after taking the material, the placenta was dissected into segments with a thickness of at least 5 mm with a total area of up to 3 cm<sup>2</sup> and fixed in neutral formalin. The fixed material was embedded in paraffin wax. A series of sections were made to cover the entire thickness of the placental wall, which made it possible to evaluate the entire surface and internal structural parts of the placenta.

*Data analysis:*

The analysis of microdensitometric data allowed us to identify clear differences in the structure of sow placentas. Foci of diepithelialization of the main chorionic villi were identified and, according to the signs of difference, the sows were divided into two groups. Individuals with clear de-epithelized structures of placental tissue were identified in the first group (experimental), while the second group (control) showed no obvious changes in the epitheliochoric layer.

The obtained digital data were analyzed using the statistical method of single-factor analysis of variance with the program "Biostatistics 4.03" for Windows. The differences were considered significant at  $P \leq 0,05$ .

*Ethical aspects:*

The experimental procedures were conducted in accordance with European Union Directive 2010/63/EU.

There is no conflict of interest between the authors in the field of related scientific research.

## **Research results**

Significant differences in immunological reactivity were observed in the studied animals during pregnancy. This was expressed over the entire research period in a

significant decrease in the main subpopulations of Th and Tm (table 1). Dynamics of IFN- $\gamma$  and TNF changes indicates a decrease in TLR7/8 at 46%, TLR1/2 at 36,4%, TLR1/2 + 9 at 34,7%, TLR1/2+ 7/8 + 9 at 19,0% in experimental group of sows. This trend causes a significant immunosuppressive state in this group of animals.

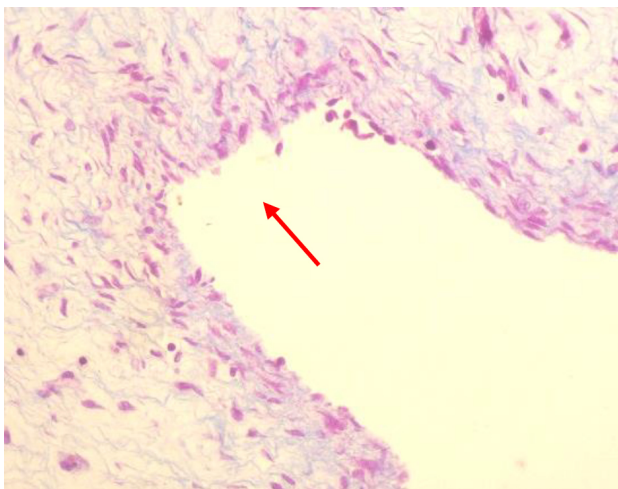
Table 1.

**Changes in the main subpopulations of Th and Tm in sows during pregnancy**

Groups of animals	Immunological parameters				
	TLR1/2	TLR7/8	TLR9	TLR1/2 + 7/8 + 9	TLR1/2 + 9
<b>Control group</b>	1,98 $\pm$ 0,14*	2042,0 $\pm$ 2,03*	1125,3 $\pm$ 0,98	904,6 $\pm$ 0,22	1258,0 $\pm$ 0,25
<b>Experimental group</b>	0,72 $\pm$ 0,11*	940,8* $\pm$ 1,66	851,2 $\pm$ 0,47	725,5 $\pm$ 0,97	823,0 $\pm$ 0,01

Note: \* significant difference  $p \leq 0.05$  with the control group

The illustrative material was obtained using a digital camera of Olympus BX41 microscope.



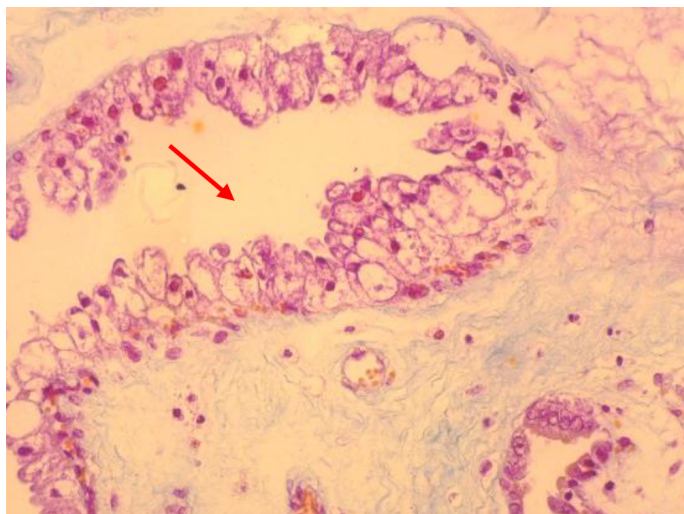
**Fig. 1.** Diepithelialization of the main villi of the chorion of the sow placenta

Figure 1a shows the characteristic absence of epithelial cells. The epithelial layer is cubic in shape, with explicit nuclear polymorphism at this stage of pregnancy. The basal part of individual epithelial cells is characterized by the presence of a spherical cluster of homogeneous material. In the material of the experimental group of sows, dystrophic increment of the epithelium of the main and terminal villi of the chorion of the placenta is common.

Morphological analysis showed clear changes in epithelial structures. However, there were no obvious changes in the chorial part of the fetoplacental complex in the placental tissue of pigs from the experimental group.

Figure 2 shows the complete formation of the epithelium of the main chorionic villi of the placenta of pigs from the control group. Microscopic examination shows extensive development of the main symplast, with the proliferation of multiple round (oval) shaped nuclei in the oxyphilic cytoplasm.

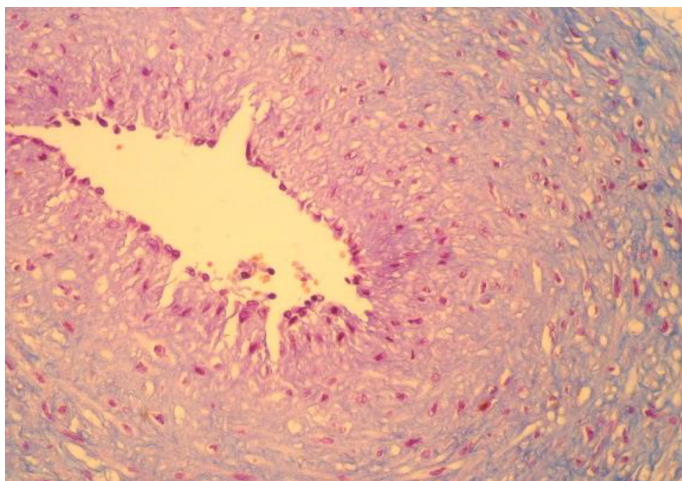
We have identified an extensive expansion of placental septa, where the main distribution of adjacent chorial complexes of a single fetoplacental complex occurs.



**Fig. 2.** Formation of a complete epithelium of the main villi of the chorion of the sow placenta

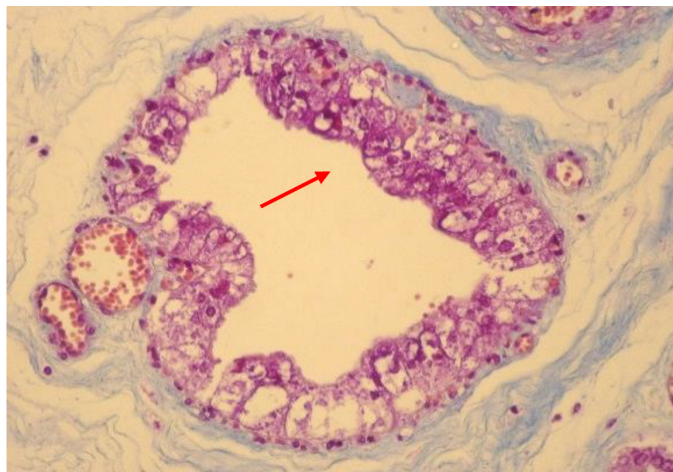
In the placentas of sows with signs of chorionic diepithelialization, reactive changes in vascular wall elements (Figure 3), erythrocyte aggregation, active proliferation of endothelium and adventitia cells with signs of accumulation of polymorphocellular infiltrates in the perivascular zone were also found.

The main unfavorable feature of the structural organization of placental tissue is the predominance of the substitutive nature of epithelial elements by interstitial fragments. This phenomenon affects the intramembrane transport of nutrients, oxygen, and metabolites, leading to a focal dystrophic (necrobiotic) process of the chorial epithelial layer.



**Fig. 3.** Destructive formation of intravascular endothelial plate of sow placenta

The above changes were not detected in animals in the experimental group (Figure 4). The epithelial layer is not thickened, without significant densification of the nuclear apparatus. There is no pattern of discharged chromatin. There is no obvious distribution of dystrophic impregnated material in all areas of the placental tissue.

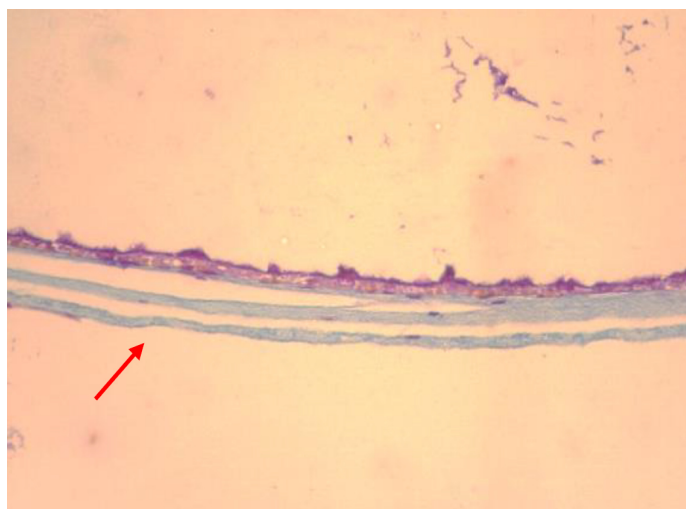


**Fig. 4.** Formed intravascular endothelial plate of sow placenta

The revealed morphological characteristics indicate the lowest connective tissue proliferative activity of epithelial cells in the control group in relation to the material obtained from the experimental group of animals.



**Fig. 5.** Transformation of the terminal epitheliochoric layer of sow placenta



**Fig. 6.** Formed terminal epitheliochoric layer of sow placenta

In the experimental group, specific changes in the vascular bed (Figure 5) were caused by significant invaginations of the terminal epitheliochoric layer of the placenta. The main feature of specific epithelialization of placental tissue is the transformation and invagination of elements of the terminal epitheliochoric layer when correlating the integral terminal epitheliochoric layer of the placenta.

Signs of destruction are also observed in the deep layers of the placenta. The accumulation of collagen in the vascular bed of the terminal chorionic villi is associated with a predictor transformation of the perivascular atherotic process.

Figure 6 shows the formation of an integral terminal epitheliochoric layer of the placenta of pigs in the control group. An explicit pattern of chorial layer vascularization in the initial, middle and distal sections of the placental surface is identified.

### **Discussion**

The study made it possible to identify the structural features of de-epithelized structures in the pig placenta. When organizing therapeutic and preventive measures in pig farms, it is necessary to take into account this change in placental tissue in pigs as a predictor of a later (indirect) pathological condition of the offspring obtained with a high infectious risk group.

Based on practical experience, we conclude that the morphological state of the placenta can show how the fetus developed in placental conditions, and whether there were pathological changes that could affect the viability of newborns.

We consider it necessary to continue research on the effect of destructive changes in the placental barrier of the fetoplacental complex in pigs on changes in the development of immunobiological reactivity, natural resistance and adaptive characteristics of animals.

### **Conclusion**

The use of established changes in the fetoplacental complex (the presence of de-epithelized structures) based on the principles of histomorphometric analysis of the placenta in animals can be the main focus in the study of the pathogenesis of a number of diseases and will allow to form groups of the most healthy individuals.

The morphological method of predicting the degree of physiological maturity of the offspring of productive animals obtained, in relation to their further growth and development, makes it possible to identify among newborn animals the most suitable for breeding purposes and identify examples with low adaptive potential or reduced viability.

The integrity parameters of the placental wall structure also make it possible to determine productive longevity. At the same time, it becomes possible to prevent the identified changes in the fetoplacental complex during pregnancy, which makes it possible to reduce the mortality of young animals at an early age, improve the quality and increase the quantitative composition of the breeding and production stock.

The use of the proposed criteria for evaluating placental tissue in combination with veterinary and sanitary measures can provide significant economic benefits in pig farming.

**Ethics committee conclusion.** The study was conducted in accordance with the principles of the Helsinki Declaration of the World Medical Association (Declaration of Helsinki, and approved by the Institutional Review Board).

**Conflict of interest information.** There was no conflict of interest in the field of related scientific research between the teams of authors.

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