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Condition of Immune System of the Fruit and Possibility of Vaccinal Prevention of Infectious Diseases of Young Growth of the Early Postnatal Period.

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ABSTRACT

The purpose of the real researches was studying of a condition of immunocompetent bodies of a fruit (in particular pigs) and pigs in early post-natal ontogenesis, their ability to react specifically to an antigene and definition of a possibility of stimulation of immunogenesis of young growth of early age by means of use of immunoexcitants. Cyto-, patogistologicheskoy, immune-chemical and electronic-microscopic methods have studied cellular structure, ultrastructure of cellular elements and immunocompetent bodies of 90 day embryos of a pig and the newborn pigs received from vaccinated against a bacterial infection and intact sows, in different terms after the birth. It is experimentally confirmed that the fruit contains all cellular elements and their specific functioning of immunocompetent cages, necessary for the immune answer, in the last third of the embryonic period. It is established also that colostric antibodies even in high credits don't possess ability. 100% to protect young growth from infection with the virulent causative agent of the corresponding infection. Immunization of young growth at early age, especially in combination with immunoexcitants, promotes involvement in immunological process a network of lymph nodes, lymphoid educations in other bodies and to formation of the intense immunity providing 95-100% safety of the young growth of animals vaccinated at early age at experimental infection of the virulent causative agent of the corresponding infection. Therefore, against infectious diseases of young growth of the early postnatal period it is expedient to approach questions of carrying out preventive actions from the point of view of an immunological maturity of newborn animals and their ability to formation of specific immunity on the arriving antigen. Increase of immunological efficiency of means of specific prevention of infectious diseases of young growth of early age is possible their use in association or in combination with the immunostimulating preparations.

Keywords: fruits, young growth of early age, ellular structure, ultrastructure of immunocompetent bodies, vaccine-challenged immunity.

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INTRODUCTION

The modern technology of animal husbandry dictates need of an intensification of specific prevention of infectious diseases of the most dangerous to young growth of the early postnatal period which mortality at viral and bacterial infections reaches 70-90% [1, 9].

Certain researchers note that the solution of a task of prevention of infectious diseases of newborn young growth by means of maternal specific antibodies isn't rather effective as colostric antibodies don't provide full protection of young growth against infection, besides exert the inhibiting impact on formation at them vaccine-challenged protection against infectious diseases. Prevention of infectious diseases of young growth of early age by their vaccination is complicated by discrepancy of the available data on a condition of their immune system in postnatal ontogenesis [5, 10, 11].

It is known that the cellular elements characteristic of earlier stages of phylogenetic development are involved in formation of immunological reactivity and expansion of immune reactions at mammals: phagocytes, macrophages of marrow and macrophages of system of mononuclear phagocytes. The main functional cage is the lymphocyte as which ancestor serves the stem cell of lymphoid fabric. Lymphoid fabric is most fully presented in lymph nodes of an organism. A bookmark them at animals occurs in the second half of an embryonal development. The defining role of the central bodies of immunity (a timus and marrow) consists in giving of immune competence to all listed cellular elements. Thus, development of immune reaction requires cooperative interaction of cellular elements micro, macrophages and lymphocytes [2, 4, 7, 8].

The purpose of the real researches was studying of a condition of immunocompetent bodies of a fruit (in particular pigs) and pigs in early postnatal ontogenesis, their ability to react specifically to an antigene and definition of a possibility of stimulation of immunogenesis of young growth of early age by means of use of immunoexcitants.

MATERIALS AND METHODS

The cellular structure, ultrastructure of cellular elements and immunocompetent bodies of a fruit of a pig and newborn pigs were studied by cyto-patogistologicheskoy, immunochemical and electronic and microscopic methods. For this purpose tests of fabrics of lymph nodes, a timusa, spleens, marrow, a liver, kidneys, adrenal glands and blood - 90-day fruits from not vaccinated sow and at 32 newborn pigs took from 8 in the 14th hour and 1, 2, 3, 7, 14, 21 and 30 days after the birth. At the same time 16 pigs have been received from intact sows, 16 pigs from the sow vaccinated intramuscularly against listeriosis by a live vaccine from a strain of "AUF", from them 8 pigs were immunizirovana at 3-day age aerogenic introduction of a protivolisteriozny vaccine from a strain of "AUF".

For the purpose of studying of a possibility of stimulation of immunological process in an organism of pigs of early age application of immunostimulators 2-3 daily allowances of age received from an intact uterus have made experiment on 15 pigs. Pigs were immunized aerogenic introduction of a protivolisteriozny vaccine from a strain of "AUF" in combination with the gidrochloride ksimedon (GHK) synthesized by employees of IOFH of A.E. Arbuzov (Kazan). At the same time the dry protivolisteriozny vaccine was suspenzirovat solution of GHK [6, 7].

For cyto-gistomorfologicheskoy researches of test of bodies and fabrics fixed in 10% solution of neutral formalin, ethanol-formalin (9:1) and cold 96 °C ethanol. Consolidation was carried out by filling to paraffin, and also freezing. Gistosreza painted hematoxylin-eozinom, azur II eozin according to Van-Gizona, RNK revealed across Brashe, a glycogen and neutral mucopolysaccharides – Chic-reaction across Shabadash, sour mucopolysaccharides – tolluidinovy blue, lipids Sudan in black B, activity of sour phosphatase across Gomori and azocombination. Antiteloproduitsiryushchyy cages in bodies and fabrics determined by an immunofluorescent method of Kuns. The description of morphological changes in bodies and tissues of experimental animals was carried out according to the international histologic nomenclature.

For microscopic researches used the cages received by centrifugation of suspension of tests from these bodies and blood. The deposit was washed out and concluded in Difko agar. Pieces, with the cages concluded in an agar, fixed 1% solution of glutarovy aldehyde (SERVA, Germany) on 0,1 M the phosphatic ph

7,4 buffer within 12 hours in the refrigerator with the subsequent fixing in 2% four oxides of osmium on the same buffer 2 hours at the room temperature. After dehydration and impregnation by the filling environment samples were concluded in eponovy pitches (SERVA, Germany). Ultrathin cuts received on the LKB-3 ultramicrotome and looked through on an electronic microscope of JEM 100CX-2. The received negatives scanned on the EPSON PERFECTION 4990 FHOTO scanner with the resolution of 600 dpi.

Blood from pigs for serological researches was taken in 1, 3, 7, 14 and 21 days after vaccination. The caption of specific antibodies in serums of blood and colostrum of the vaccinated sows, and also the colostrum and vaccine-challenged antibodies in blood of newborn pigs was defined in RA, using a color listeriozny antigene [8]. Presence of colostrum protivolisteriozny immunity at the pigs received from the vaccinated sows and at the pigs vaccinated at 3-day age was determined by intravenous infection with the virulent causative agent of listeriosis [5].

Immunochemical, electronic and microscopic, serological and hematologic researches accompanied with statement corresponding kontroly.

RESULTS OF RESEARCHES

When studying ultrastructure of cellular elements of the fruits received from intact sows in lymph nodes the set of lymphocytes at various stage of division, along with the blood formation centers which are available in a spleen is noted. Existence of outgrowths microfibrils and a significant amount of the mitochondrions in cytoplasm localized near the kernel consisting of melkogranulyarny chromatin with existence of a kernel is characteristic of lymphocytes and especially of lymphoblast. On a membrane of tanks of an endoplasmic network there is a large number of ribosome, Golgi's complex is developed poorly. The rim of cytoplasm of average lymphocytes contains a significant amount of channels of an endoplasmic network. Plazmoblasta represent large cages with it is central the localized kernel. The kernel of a plazmoblast has diffusion chromatin and quite large kernel. Cytoplasm is filled with channels of strongly developed endoplasmic network. Channels and tanks of a network are located parallel ranks. Mitochondrions are collected in groups, closely adjoining to membranes of a cytoplasmatic network. At research of transitional forms from a plazmoblast to a plazmotsit reduction of the size of a kernel, kernel chromatin consolidation, disappearance of a kernel, some shift of a kernel to the periphery of a cage, increase in number of tanks of an endoplasmic network and reduction of number of mitochondrions is tracked. Many mature plazmotsita have a hypertrophied endoplasmic network which channels are filled with substance of moderate electronic density. Such morphological picture indicates an active functional condition of a cage.

Macrophages of a lymph node and spleen represent larger cages with a large number of organelles in cytoplasm. The kernel of rounded shape contains diffusion chromatin. Short channels and tanks of an endoplasmic network meet in all sites of cytoplasm, but most of all about a cytolemma. Golgi's complex is developed, numerous diktiosoma occupy a significant area about a kernel. Lysosomes at various stages of development and separate fagosoma are noted.

Distinctive feature of T-lymphocytes of a timus is lack at them of the outgrowths or microfibrils which are available for B-lymphocytes. Timotsita have a kernel with the chromatin concentrated about a kariolemma and elektronnopltny cytoplasm. The high density of cytoplasm is caused substantially by a large number of free ribosomes. The granular cytoplasmatic network is presented by rare and small channels, agranulyarny – separate bubbles. Besides, in cytoplasm single mitochondrions are revealed. Except mature timotsit in a timus there are cages corresponding by the sizes to average and big lymphocytes and also Gassal's little bodies and, more rare, phagocytic cells that is an indicator of a maturity of immunocompetent bodies.

After 1 day after the birth of piglets from sows intact cellular composition of lymph nodes represented by all types of cellular elements, available in adult animals (Table 1). Characteristic is the large number of poorly differentiated reticular cells and young blast forms, transition to the plasma cells. The presence of different maturity stage of plasma cells characterized by the potential ability of lymphoid organs to an immune response.

Table 1: Cellular structure of lymph nodes of pigs

Types of cellular elements	Age of pigs (days)			
	1	7	14	30
The low-differentiated retikulyarny	81,5	83,8	48,3	34,8
macrophages	1,7	1,0	1,0	1,2
blasta	53,9	13,3	36,9	34,7
plazmoblasta	1,4	12,1	11,8	10,3
unripe plasmatic	6,3	3,5	6,3	18,7
mature plasmatic	3,2	1,7	3,3	15,3
average lymphocytes	607,5	653,8	631,8	603,8
small lymphocytes	243,0	223,0	254,6	275,5

With increase in age of pigs in cellular composition of lymphoid fabric of lymph nodes and spleens observed reduction of quantity of young cages of a lymphoid row at simultaneous increase in quantity of the cages with hyperactivity of sour phosphatase located mainly in the thymus-dependent areas. In the period of the highest expressiveness of lymphoid and hyper plastic reaction (on 7-14 days) increase of T-cellular and B-cellular populations of lymphocytes in a parakortikalny zone of lymph nodes and periarterial site of lymphoid follicles of a spleen is noted. In 30 days after the birth in lymph nodes of pigs the number low-differentiated and the blastnykh of cages decreases by 2-2,5 times, and the quantity of cellular elements of a plasmatic row increases by 3-5 times in comparison with similar data at pigs of daily age.

In serum of blood of newborn pigs before colostrum reception by method of an immunoelectrophoresis revealed traces of M-, G-immunoglobulins, sometimes A-classes, but not representing functionally full-fledged groups. Age changes of immunoglobulins in serum of blood of newborn pigs are presented in table 2.

Table 2: Amount of immunoglobulins in serum of blood of pigs

Age of pigs (days)	Immunoglobulins (mg/ml)		
	G	M	A
0,5	14,2	1,5	1,4
1	15,2	1,5	1,3
2	16,2	1,6	1,5
3	12,7	1,1	1,0
7	13,8	1,2	1,1
30	23,0	1,2	1,3
sows	28,5	1,9	1,3

It should be noted that the amount of immunoglobulins G, rather high in the first 2 days after colostrum reception, on 3-7 days goes down, and in 30 days considerably raises (to level that at adult animals). High content in the first 2 days of life with the subsequent decrease on 7 days and slight increase on 30 days after the birth is characteristic of immunoglobulins of the classes M and A.

Considering the marked reduction of colostrum immunoglobulin value after five days, increasing the amount of immunoglobulins in the blood serum of pigs can be explained only by the synthesis of immunoglobulins in body pigs themselves. This demonstrates the synthesis of specific antibodies, blood examination confirmed that vaccinated piglets early age.

In serum of blood of the pigs received from the sows vaccinated against listeriosis, specific antibodies in the first days after reception of colostrum come to light in credits 1:140-1:180 and represent colostrum immunoglobulins, got to the blood course of pigs in not changed look. Level of specific antibodies goes down by second week of life of pigs and by the end of month in serum of their blood specific antibodies revealed in isolated cases in low credits (1:20-1:40). After experimental infection of pigs at 15-18 day age it is established that the antibodies received with colostrum of the sows vaccinated against listeriosis only for 37,5% protect pigs from the subsequent infection with the virulent causative agent of listeriosis.

Following immunization of pigs are inhaled in 3 day old was observed post-vaccination complications. Histomorphological studies found that the lungs of pigs from the first days of birth are sufficient formed body capable to perceive the impact of aerosol particles with no significant adverse reactions.

The analysis of lymph node cell responses of vaccinated piglets (Table. 3) indicates an increase in the amount of 1.5 times undifferentiated reticulum cells, macrophages, and basophil lymphocyte blasts. It should be noted that the processes of differentiation of cellular elements takes place in parallel with developing macrophage reaction, indicating that accelerated and actively occurring cell transformations.

Table 3: Cellular reactions of lymph nodes of the vaccinated animals

Виды клеточных элементов	Term of research (days)			
	hum noise	7	14	21
the low-differentiated retikulyarny cages	19,6	47,5	51,0	62,6
macrophages	1,1	4,2	2,9	9,7
blasta	23,7	60,2	112,2	128,3
plazmoblasta	5,3	17,7	18,3	17,3
unripe plasmatic	8,5	14,0	10,3	11,3
mature plasmatic	3,2	7,0	5,5	9,3
average lymphocytes	635,0	552,3	566,0	517,0
small lymphocytes	204,5	263,8	180,2	185,6
basophiles	11,4	25,2	37,8	46,3
neutrophils	3,1	4,2	6,3	8,3
eosinophils	4,4	6,0	7,5	4,0
basophiles	-	-	4,5	-

The expressed active proliferation of cellular elements in lymph nodes of the vaccinated pigs, their increased functional activity were followed by change of amount of immunoglobulins in serum of blood (tab. 4). So, in serum of blood of the vaccinated pigs reduction in the amount of immunoglobulins of all classes in three- and seven day age isn't observed.

Table 4: Immunoglobulins in serum of blood of the vaccinated pigs

Term of research (days)	Immunoglobulins (mg/ml)		
	G	M	A
before vaccination	13,3	2,2	2,2
7	15,2	2,2	2,2
14	17,3	1,8	1,7
21	17,1	1,5	1,4
30	23,2	1,3	1,2

Moreover, natural increase in amount of immunoglobulin G in 7, 14, 21 and 30 days after vaccination is noted. In parallel with increase of level of immunoglobulin G in serum of blood of the vaccinated pigs specific antibodies which caption in 7 days 1:80 – 1:120, in 14 days – 1:180, and in 21 days – 1:240-1:320 come to light. And, in the first week of vaccine-challenged process specific antibodies are presented by macroglobulins, and in 14 and 21 days they treat mainly immunoglobulin G.

Therefore, aerogenic immunization of pigs at 3-day age was followed by active proliferation of cellular elements of lymphoid fabric. Aerogenic immunization of pigs on a background of colostric immunity was followed by lowering of the level the colostric of antibodies on 1-2 log in the first 8-12 days in the subsequent terms registered their increase to the size of credits of the specific antibodies characteristic of the vaccinated pigs from intact sows.

The insignificant inhibiting influence of existence in an organism of pigs the colostric antibodies is reflected also in expansion of cellular reactions in an organism of the imparted pigs. Level of cellular changes in lymphoid bodies of pigs in this case held average position between not vaccinated and vaccinated pigs from intact sows.

However, aerogenic vaccination of pigs at early postnatal age and on a background of colostric immunity doesn't exert negative impact on formation of the intense specific immunity protecting for 95-98% of pigs of infection with the virulent causative agent of listeriosis.

Immunological reorganization in an organism of the pigs vaccinated against listeriosis in combination with a hydrochloride of a ksimedon is characterized by increase 2-2,5 times of total of leukocytes at simultaneous increase in number T - and B-lymphocytes, triple increase in number of the activated lymphocytes, ninefold – macrophages and quadruple increase the blastnykh of cages in comparison with similar data of pigs of the same age group with vaccine-challenged (without stimulator) immunity. Active cellular reaction in lymphoid fabric was followed, as a rule, and more expressed development specific the protivolisterioznykh of antibodies to 1:320-1:640.

In 3 days in lymph nodes along with the large centers of follicles also again formed secondary small knots which most often settled down directly under a knot capsule were found. Sinus macrophages with high activity of sour phosphatase contained intermediate and central sine.

Lymphoid and hyper plastic reaction on activity conceded that in a spleen in the regionarnykh lymph nodes. Periarternialny sleeves were moderately giperplazirovana, in them the germinativny centers were formed the small sizes. The red pulp has been moderately deposited by blood.

On 7 days of experience lymphoid and hyper plastic reaction in the regionarnykh and the remote lymph nodes remained expressed. A follicle the germinativny centers in which along with blastny and transitional retikulyarny cages the quantity of average lymphocytes has increased had the majority. In subcapsular and brain sine makrofagalny reaction in the form of a congestion of sinus macrophages was noted, in pulpy fabrics of brain substance the plazmokletochny reaction presented by mainly unripe plasmatic cages with the expressed cytoplasm pironinofiliya was accurately shown.

Germinal centers of the follicles of the spleen were enlarged. The red pulp containing a significant number of small and medium-sized clusters of lymphocytes and plasma cells.

In the spleen there was an increase the size of the follicle germinal centers, which contained a large number of blast cells with mitotic figures. The red pulp occurred increased number of plasma cells.

At 14 days of experience in regional, distant lymph nodes and spleen were recorded weak hyperplastic lymphoid and macrophage reaction.

100% safety of the animals vaccinated in combination with an immunoexcitant (GHK) after infection with the virulent causative agent of an infectious disease demonstrated presence at them of intense specific immunity. Therefore, vaccination of young growth of early age in combination with immunoexcitants, perhaps to increase formation of intense specific immunity. It is also established that immunity against listeriosis, is provided, generally cellular and humoral reactions of an organism.

CONCLUSION

In experimental conditions it is confirmed that bodies of immune system of a fruit contain all cellular components, necessary for the immune answer, in the last third of the suporosny period. Moreover, during this period also specific functioning of immunocompetent cages is possible. Protective ability the colostric of antibodies, also their ability to inhibit immunological processes in an organism of animals of early age are in direct dependence on level their colostrum of the vaccinated uterus and in serum of blood of newborn pigs.

However colostric antibodies even in high credits don't possess ability of 100% to protect young growth from infection. Immunization of young growth in the early postnatal period, especially in combination with immunoexcitants, promotes involvement in immunological process a network of lymph nodes and lymphoid educations, thereby accelerating processes of their maturing without negative influence on a physiological condition of an organism of the imparted animals. Specific protection of young growth of early age at vaccination is characterized by active makrofagalny reaction, synthesis of immunoglobulins and specific

antibodies. Immunological reorganization comes to the end with formation at the imparted young growth of animals of intense specific immunity from the corresponding infection.

The colostric antibodies received from the vaccinated mothers exert the short-term inhibiting impact on development of vaccine-challenged reactions of young growth of early age. Their inhibiting influence was shown by lowering of the level of specific antibodies in young growth blood serum within one week after vaccination.

Thus, for the purpose of elimination of infectious diseases of young growth of the early postnatal period it is expedient to approach questions of carrying out preventive actions from the point of view of an immunological maturity of newborn animals and their ability to formation of specific immunity on the arriving anti-genes. For the purpose of increase of immunological efficiency of the used means of specific prevention it is necessary to apply them in association or a combination to the immunostimulating preparations [3, 6].

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