

Stabilization of stallion sperm parameters in the post-vaccination period due to a complex of vitamins

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Abstract. The aim of our research was to diminish the damaging effect of vaccination on the quality of stallion sperm and its cryostability. The task was to study the effect of the complex vitamin of tetrahydrovit on sperm parameters under conditions of vaccination against leptospirosis. Stallions that were vaccinated against leptospirosis were divided into 3 groups: the first – control, without vitamin supplements, the second – with the addition of 2 ml of tetrahydrovit vitamin daily, the third group – intramuscular injection of tetrahydrovit 1 time in 7 days. The reaction of the body to the introduction of the vaccine in the group without vitamins proceeded with a significant decrease in the quality of sperm in the first two weeks. Later, the motility and the number of whole membranes of fresh sperm gradually improved, but the survival rate of chilled and frozen sperm remained low until the end of the observations. Among stallions in the experimental groups, which were given tetrahydrovit vitamin (orally or intramuscularly), the decrease in sperm parameters was insignificant, within the usual fluctuations, which allowed one to avoid culling sperm doses. Tetrahydrovit vitamin promotes a stabilizing effect on the quality indicators of sperm of stallions-producers after vaccination.

1 Introduction

The quality of sperm producers is influenced by many factors of the external and internal environment. One of the important conditions for a good quality of sperm production is a good state of health of the body, proper feeding and conditions for the maintenance of producers. Age [1], origin [2], seasonality [3], ecology [4], level of physical and sexual activity [5], as well as individual characteristics [6] have a great influence on sperm parameters [7].

One of the factors that affect the body as a whole, as well as its various systems, is vaccination. This procedure, being a powerful stress factor, has a significant impact on the morphological and biochemical composition of the blood, on the homeostasis system, on the functional activity of organs and tissues [8].

It was found that the main immunological restructuring of the body after vaccination occurs in the first 2 weeks and affects both cellular and humoral defense mechanisms. It is accompanied by an increase in the total number of white blood cells in the blood due to an increase in eosinophils, monocytes and basophils, a decrease in their phagocytic activity in relation to other microorganisms, an increase in the amount of total protein, total immunoglobulins, gamma-globulins, while simultaneously reducing the amount of beta-globulins and albumins, as well as hemoglobin [8]. After 3-4 weeks after vaccination, all these indicators come back to normal.

Along with the development of immunity during vaccination, changes of a non-specific nature occur, which relate to the morphology and protein composition of the blood, enzyme activity, the blood clotting system, the function of the adrenal glands and other endocrine organs. These changes, as a rule, are not pathological in nature, they last 1-2 weeks, in rare cases up to two months [9].

The influence of vaccine antigens on the reproductive functions of cows by the example of preventive vaccination against anthrax and rabies was studied by Manuilov A.V., Nezhdanov A. G. [10]. In the process of actively developing specific immunity, a negative impact on the reproductive abilities of cows was revealed in certain periods - this is the period of fertilization, early embryogenesis, the period of formation of the fetoplacental complex and the final stage of pregnancy. Therefore, it is recommended not to vaccinate against anthrax during critical periods, as well as within two weeks after delivery [11].

The question of the effect of preventive drugs on the reproductive function of manufacturers remains practically open. At the same time, there is very little research on this topic. The reproductive system of producers, although protected by a hemotesticular barrier, cannot be completely isolated. How the introduction of antimicrobial drugs affects the sperm production and cryostability of stallion sperm, we have not found in the scientific literature.

In 2018-20, we conducted studies to study the effect of vaccination on the quality indicators of stallion sperm.

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The limited number of stallions forced us to conduct the experiment, breaking it into stages. At the initial stages, the effect of vaccines of bacterial and fungal nature - anthrax and dermatomycosis (12) was tested, at the next stage, the effect of a vaccine against a viral infection - rhinopneumonia (13) was studied. At the same time, the damaging effect of these treatments was revealed and a decrease in the quality of stallion sperm was noted, which was especially strongly reflected in the indicators after freezing-thawing. A characteristic feature was a sharp drop in the quality indicators of sperm, observed in the first two to three weeks after all vaccinations; in the future, there was a gradual recovery of indicators. These features should be taken into account when performing cryopreservation of genetic material.

At the final stage, the goal of our research was to reduce the damaging effect of vaccination on the quality of sperm and its cryostability. To do this, the task was set: to study the effect of the complex vitamin preparation tetrahydrovit on the sperm parameters of stallions after vaccinations.

2 Material and methodology

12 stallions of the experimental stable of the Institute of Horse Breeding were used in the experiments. The main feeding diet consisted of hay, oats, wheat bran, without the addition of special compound feeds or premixes in order to establish the net effect of vaccination on the characteristics of sperm.

All stallions were vaccinated against leptospirosis using a concentrated vaccine produced by Vetbiochim LLC, in the amount of 2 ml per muscle in the neck area. The animals were divided into three groups: the first – control, without vitamin supplements, the second – with the addition of 2 ml of tetrahydrovit vitamin daily for 50 days, and the third group – the introduction of tetrahydrovit intramuscularly (2 ml per 100 kg of weight) once every 7-8 days. The influence of the tetrahydrovit is explained by the effects of its constituent vitamins that contribute to increase in the adaptive capacity of the organism and enhance resistance to infections due to antioxidant, immunomodulating, anti-inflammatory effects [14]. The tetrahydrovit composition in 1 ml includes:

- retinol palmitate (vitamin a) – 25 000 ME,
- cholecalciferol (vitamin D3) – 5 000 ME;
- tocopherol acetate (vitamin E) 25 mg;
- ascorbic acid (vitamin C) - 50mg

The first days after the introduction of the antigen, the stallions were not used in mating, they were given rest, the general condition of the animals was monitored, as recommended by the instructions for vaccinations. Starting with day 5-6, the stallions were used to take sperm on an artificial vagina according to the generally accepted method. After receiving the sperm, it was evaluated by volume, concentration, motility (points), membrane integrity (%), and cell pathology (%).

Dilution of sperm was performed by LCCG medium. One part of the diluted sperm was stored in a refrigerator, the other part was frozen in accordance with

the recommendations [15]. Thawed sperm was evaluated by mobility (points), membrane integrity (%), cell pathology (%). Chilled and thawed sperm was monitored by survival time in hours when stored in a refrigerator (at a temperature of 2-4°C). The integrity of the membranes was determined by a hypoosmotic test [16].

Sperm collection was performed before and after vaccination for 50 days 1-2 times a week. For statistical processing of the results, we used the program Microsoft Excel 2010, Statistica 8.

3 Results and discussion

The study of the qualitative characteristics of freshly diluted and cryopreserved sperm after the introduction of the vaccine showed a decrease in motility, survival, and The integrity of cell membranes is in all 3 groups. Upon detailed examination, it turned out that the most significant decrease in indicators was observed in the control group without vitamin supplements. In this group, the motility and the number of whole membranes of freshly diluted sperm (Table 1) decreased in the first 2 weeks by 20-28% ($P \geq 0.95$), but in frozen-thawed samples (Table 2), a significant decrease in motility by 45% ($P \geq 0.99$) was revealed, especially sharply manifested on the 14th and 33rd days of the experiment.

Sperm survival is a very important indicator that reflects the lifetime of cells in the external environment, it has the most significant fluctuations. This indicator was also the most reduced in the control group of stallions. Its decrease in freshly diluted sperm ranged from 25-43 % ($P \geq 0.99$), and in frozen-thawed sperm it was 25-52 % ($P \geq 0.99$).

In the second and third groups, there was also a slight decrease in the quality characteristics of sperm. The motility of fresh and frozen-thawed sperm had a decrease in the range of 10-13 %, a decrease in whole membranes – no more than 12 %. The decrease in the survival rate of both fresh and frozen-thawed sperm was also low in the range of 24-26 % ($P \geq 0.95$), which is not reliable and is within the usual fluctuations. The content of pathological sperms in the first half of this experiment (up to 33 days) changed very little in all groups. In the second part of the observations, there was a tendency to increase the number of pathological sperm species in all groups, although it was not very reliable ($P \geq 0.95$), which shows the effect of the antigen on spermatogenesis.

The experiment has shown that the reaction to the injection of the leptospirosis vaccine was similar to the previously tested vaccines for anthrax, dermatomycosis and rhinopneumonia [12, 13], i.e., there was a characteristic decrease in the quality of sperm in the first two weeks. In the future, the motility and the number of whole membranes of fresh sperm were close to the initial level. But the survival rate of chilled and frozen sperm was kept at a low level until the end of the observations.

The injection of tetrahydrovit vitamin intramuscularly, or inside with food, had a positive effect on the state of the sexual sphere of breeding stallions. According to the working hypothesis, we expected

different results from the method of adding the drug. The equally rapid effect of different methods of adding the drug suggests that the quality of sperm in this case depends on the general condition of the body. The support of the animal during and after vaccination due to

the introduction of vitamins makes it possible to maintain the internal balance of biochemical processes (homeostasis). The reaction of the testes is detected later - by the end of the concomitant cycle of spermatogenesis, after 40-45 days [17].

Table 1. Quality indicators of freshly diluted and chilled sperm after vaccination in different groups

days	Mobility, points	Survival, clock	intact membranes' %	pathology sperm cell %
Without vitamins (Group 1 - control)				
Before Vaccines	6.0±0.5	160±14	85±10	17±1.4
6	4.7±0.5	120±11	67±7	16±1.2
14	4.5±0.4	110±10*	61±8	19±1.6
20	5.5±0.4	110±11*	70±8	16±1.5
33	5.0±0.3	92±8*	75±6	19±1.8
40	5.0±0.4	100±9*	72±6	24±1.9*
50	4.9±0.4	112±9	64±5	23±2.0*
Vitamins in feed (Group 2)				
Before Vaccines	5.5±0.5	190±15	80±11	22±1.8
6	5.0±0.5	170±13	72±8	20±1.8
14	4.8±0.4	160±15	76±8	17±1.5
20	5.5±0.5	160±14	70±9	20±1.7
33	5.0±0.5	142±12	72±7	23±1.9
40	5.2±0.5	158±14	77±7	29±2.2*
50	5.1±0.4	165±17	75±6	30±2.5*
Vitamins in injections (Group 3)				
Before Vaccines	5.7±0.6	165±13	82±9	27±2.0
6	6.0±0.6	156±12	80±9	24±2.1
14	5.4±0.5	145±13	70±6	23±2.0
20	5.4±0.5	145±14	75±7	22±1.7
33	6.0±0.5	125±10	79±6	29±2.3
40	5.4±0.4	130±11	70±5	32±2.5
50	6.0±0.5	145±12	74±6	35±2.6*

* (P > 0,99)

Table 2. Quality indicators of frozen-thawed sperm after vaccination in different groups

days	mobility points	Survival clock	intact membranes' %	pathology sperm cell %
Without vitamins (Group 1- control)				
Before Vaccines	2.75±0.2	90±7	41±3.5	27±2.0
6	2.0±0.2	60±5*	28±1.9*	22±1.8
14	1.5±0.1*	40±3*	24±2.0*	21±1.7
20	2.0±0.1	65±5	29±2.3*	20±1.7
33	1.5±0.1*	50±3*	24±2.2*	22±1.8
40	1.8±0.1*	55±5*	26±2.3*	22±1.7
50	1.8±0.1*	68±5	30±2.7	32±2.7
Vitamins in feed (Group 2)				
Before Vaccines	2.6±0.2	85±8	36±2.5	25±2.1
6	2.5±0.1	95±8	31±2.7	27±2.3
14	2.35±0.1	95±9	30±2.2	26±2.2
20	2.8±0.2	72±6	35±2.4	25±2.3
33	2.65±0.1	70±6	33±2.4	21±2.0
40	2.8±0.2	70±7	32±2.1	32±2.2
50	2.3±0.1	79±5	34±2.3	37±2.7*
Vitamins in injections (Group 3)				
Before Vaccines	2.0±0.1	95±10	42±2.9	32±2.5
6	1.8±0.1	91±9	37±2.2	25±2.2
14	1.7±0.1	90±8	34±2.0	20±1.6
20	1.8±0.1	72±6	36±2.5	28±2.0
33	1.8±0.1	80±6	36±2.3	22±1.6
40	1.7±0.1	70±5	35±2.7	32±2.2
50	1.8±0.1	73±5	33±2.4	42±2.7*

* (P > 0,99)

4 Conclusion

The reaction to the administration of the leptospirosis vaccine occurred with a significant deterioration in the quality of sperm. Among stallions in the experimental groups that were given tetrahydrovit vitamin after vaccination, the decrease in sperm parameters was insignificant, within the usual fluctuations. Tetrahydrovit vitamin can be recommended for the corrective effect of qualitative indicators of sperm production of stallions-producers after vaccination.

References

1. M.M. Atroschenko, E.E. Bragina, A.M. Zaitcev, V.V. Kalashnikov, E.V. Nikitkina, A.A. Krutikova, J. Reproduction in Domestic Animals, **54(S3)**, 127 (2019)
2. L.A. Khrabrov, V.A. Naumenkova, M.M. Atroschenko, J. Genetics and animal breeding, **2**, 3-9 (2020)
3. O.A. Fedosova, A.A. Terekhina, O.V. Bakovetskaya, J. Horse breeding and equestrian sport, **2**, 29–30 (2010)
4. Yu.P. Fomichev, *Mat. of conf: Actual problems of animal reproduction biology*, pp. 90–102 (2007)
5. M.M. Atroschenko, E.Y. Borodkina, J. Horse breeding and equestrian sport, **4**, 5-6 (2008)
6. E.V. Nikitkina, I.Sh. Shapiey, N.P. Platonova, V.A. Naumenkova, *Mat. of conf: Actual problems of animal reproduction biology*, pp. 476–478 (2007)
7. J. Morrell, T. Nongbua, S. Valeanu, I. Lima-Verde, *Animal Reproduction Science*, **185** (2017)
8. V.N. Kislenko, N.M. Kolychev, R.G. Gosmanov, *Veterinary Microbiology and Immunology* (2012)
9. N.V. Anokhina, *General and Clinical Immunology* (2008)

10. A.V. Manuilov, A.G. Nezhdanov, *Mat. of conf.: Actual problems of animal reproduction biology*, pp. 238–241 (2007)
11. A.S. Moskvina, V.I. Maksimov, O.A. Verkhovskikh, *Bulletin of Oryol State Agrarian University*, **6**, 65–67 (2011)
12. V.A. Naumenkova, A.V. Kalinova, *J. Russian Agricultural Sciences*, **45(6)**, 589–592 (2019)
13. V.A. Naumenkova, *J. Veterinary medicine*, **7**, 45–48 (2019)
14. E.A. Tyapugin, V.P. Zhirokhov, V.I. Netecha, A.A. Ivanovsky, R.V. Rusako, *J. Zootechnics*, **8**, 29–31 (2001)
15. *Recommendations for taking, diluting and freezing* (2006)
16. G.C. England, J.M. Plummer, *J. of Reproduction and Fertility*, **47**, 261–270 (1993)
17. A. McKinnon, E. Squires, W. Vaala, D. Varner-John, *Equine Reproduction. 2nd edition* (2011)