Influence of technological manipulations on the parameters of equine semen quality

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Abstract. The aim of the work was to determine the influence of traditional technological manipulation (vaccination of stallions) performed when housing animals, on the quality indicators of spermatozoa. As a result of the experiment, it was discovered that vaccination did not affect the potency of stallions. The time and the number of jumps on the mare were the same before and after vaccination. There were no changes in the number of spermatozoas in the ejaculate in the first and subsequent weeks after vaccination. The study of sperm indicators showed a decrease in mobility (P>0.95) and survival (P>0.99) of freshly obtained and cooled sperm. Control of frozen-thawed sperm showed an even more dramatic decrease in the quality of sperm after vaccination. So in the first two weeks after vaccination, sperm motility after thawing fell by an average of 40 % (P>0.99), survival – by 44–49 % (P>0.99). The number of whole membranes decreased (unreliably) only by the end of the second week after vaccination within 15 %. Changes in the morphological composition of germ cells were observed in the second half of observations as result of influence on spermatogenesis processes. An identical picture was observed after revaccination, carried out 4 months later. A very characteristic drop in sperm motility and survival occurred in the first two weeks after vaccination. By the end of the third week, these indicators began to recover, but before the end of observations (day 55), recovery occurred in the range of 80–92 %, which is important to consider when conducting cryopreservation of sperm.

1. Introduction

A large number of stallions have low sperm quality, which negatively affects the possibility of its cryopreservation. Most often this applies to horses that participate in various competitions and constantly move from one area to another. Such stallions must have constantly high immunity with a full set of vaccinations against a number of dangerous diseases. These are mandatory vaccinations against anthrax, dermatomycosis, influenza, rhinopneumonia, tetanus, as well as very often leptospirosis, rabies and some other diseases.

The main immunological restructuring of the body after vaccination occurs in the first 2 weeks and affects both cellular and humoral mechanisms of protection. It is accompanied by an increase of the total number of leukocytes due to an increase in lymphocytes, eosinophils, monocytes and a decrease in their phagocytic activity against other microorganisms, increasing the quantity of total protein, total immunoglobulin, gamma-globulins, while reducing the number of beta-globulin and albumin, and hemoglobin in blood [1]. In 3–4 weeks after vaccination, all these indicators come to normal.
During the development of the immune system during vaccination, changes of a non-specific nature occur, which relate to the 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 [2].

The quality of semen of producers is influenced by many factors, but the impact of preventive vaccination has not been studied, although there are a number of studies that show the impact of vaccination on the reproduction processes in cows [3, 4].

In the years 2018-2019, we started research on the impact of vaccination on the quality of stallions' sperm. The limited number of stallions forced us to conduct the experiment, dividing it into stages, or periods. At the initial stages, the effect of bacterial and fungal vaccines—anthrax and dermatomycosis—were tested [5, 6]. At the same time, the damaging effect of these treatments was revealed and a decrease in the quality of stallions' sperm was noted, which was especially strongly reflected in the indicators after freezing-thawing, which is important to take into account when carrying out cryopreservation of genetic material. At the final stage, we set a task: to identify the impact of vaccination of stallions from a viral disease-rhinopneumonia on sexual potency, sperm quality indicators and its cryopreservability, as well as to determine the recovery time to the initial level.

2. Methods

In the experiments, 6 stallions of the stable of the Institute of horse breeding were used. The diet consisted of hay, oats, wheat bran, without special feed additives or premixes. For vaccination of stallions, a dry culture virus vaccine from the SV/69 strain was used against equine rhinopneumonia (production: Shchelkovsky bio combinat, Russia) in the amount of 2 ml/m in the neck area. Repeated revaccination was performed in 4 months.

The first days after the introduction of the antigen, stallions were not used in mating, they were given rest and monitored for the general condition of the animals, as recommended by the instructions for vaccination. From day 5–6, stallions began to be used for taking sperm on an artificial vagina according to the generally accepted method. At the same time, the severity of sexual reflexes was monitored – the total time for a successful jump, the number of jumps on the mare. After receiving the sperm, it was evaluated by volume, concentration, mobility (%), membrane integrity (%), and cell pathology (%). Sperm was diluted with a special medium for storage (lactose-chelate-citrate-yolk) in accordance with the recommendations [7]. One part of the diluted sperm was stored in a refrigerator, the other part was frozen in accordance with the instructions [8]. Thawed sperm was evaluated by motility (%), membrane integrity (%), and cell pathology (%).

The cooled and thawed sperm was monitored for survival in hours at a temperature of 2–4 °C.

3. Results and discussion

As a result of the experiment, it was found that vaccination did not affect the potency of stallions. The total time to the successful jump and the number of jumps on the mare was the same both before and after vaccination. There were also no significant changes in the number of isolated sperms in the ejaculate in the first and subsequent weeks after vaccination.

The study of qualitative characteristics of sperm showed a decrease in mobility (P> 0.95) and survival (P>0.99) of freshly obtained and stored sperm. In the first two weeks after vaccination, the decrease in these indicators was the most significant and reliable compared to the pre-vaccination level. The number of preserved membranes decreased slightly (not significantly) by the end of the 2nd week, the remaining periods remained close to the level before the introduction of the vaccine.

Improved mobility and survival rates started at week 3. The most complete recovery of mobility was observed on the 26-th day of the experiment. The survival rate by day 26 has recovered by 80 % from the original level. On the 55-th day of observations, this indicator has recovered to 93 %.

The control of frozen-thawed sperm (table 2) showed an even sharper decrease in sperm quality indicators after vaccination of producers compared to the decrease in cooled sperm. So in the first two weeks after vaccination, sperm motility after thawing fell by an average of 40 % (P>0.99), survival –
by 44-49 % (P>0.99). By the end of the third week, these indicators began to recover, but before the end of the observations (day 55), the recovery occurred in the range of 80–92 %.

As table 1 and 2 show, the number of preserved membranes decreased (unreliably) only on day 12 after vaccination within 15 %, and recovered to the initial level by the next week – by day 20.

**Table 1. Indicators of fresh and chilled sperm before and after vaccination from equine rhinopneumonitis**

| Days of observation | Sexual behavior | Number of sperm in the ejaculate, ml | Mobility, % | whole membranes, % | pat. sperm cell, % | Survival clock, min |
|---------------------|-----------------|--------------------------------------|-------------|--------------------|-------------------|-------------------|
| Before vaccination  | 3.2±0.7         | 1.3±0.2                              | 9 000±446   | 56±4.5             | 52±6             | 242±18            |
| 5                   | 3.0±1.2         | 1.4±0.2                              | 9 200±425   | 54±3.8             | 51±6             | 20±3              |
| 12                  | 2.9±1.1         | 1.5±0.3                              | 10 500±465  | 58±4.4             | 44±5             | 19±3              |
| 20                  | 3.5±1.0         | 1.2±0.3                              | 8500±394    | 53±4.7             | 52±5             | 18±2              |
| 26                  | 2.5±1.1         | 1.2±0.3                              | 11 000±553  | 56±4.6             | 67±7             | 21±3              |
| 34                  | 2.9±0.9         | 1.2±0.1                              | 9 000±769   | 55±5.6             | 60±7             | 23±4              |
| 42                  | 3.2±1.0         | 1.5±0.2                              | 8 500±667   | 55±5.3             | 71±8             | 27±3              |
| 55                  | 3.5±0.8         | 1.3±0.2                              | 10500±549   | 54±5.2             | 69±7             | 30±4              |

There were no significant differences in the number of pathogenic cells before and after vaccination, although there was an increase in their number from the second half of the experiment after 30 days, i.e. as a result of exposure to spermatogenesis processes.

An absolutely identical picture was observed after revaccination, carried out 4 months later. A very characteristic drop in sperm motility and survival occurred in the first two weeks after vaccination. From day 20, the indicators began to improve gradually.

**Table 2. Indicators frozen-thawing sperm before and after vaccination against equine rhinopneumonitis**

| Days of observation | Mobility, % | Survival, clock | Whole membranes, % | pat. Sperm cell, % |
|---------------------|-------------|-----------------|--------------------|-------------------|
| Before vaccination  | 25±0.2      | 13±12           | 24±4               | 27±3              |
| 5                   | 15±0.1      | 75±10           | 22±3               | 30±4              |
| 12                  | 19±0.1      | 66±7            | 20±3               | 28±4              |
| 20                  | 20±0.1      | 110±9           | 27±3               | 24±3              |
| 26                  | 21±0.1      | 98±8            | 27±3               | 28±3              |
| 34                  | 22±0.2      | 104±9           | 30±4               | 30±5              |
| 42                  | 20±0.1      | 124±11          | 29±3               | 32±5              |
| 55                  | 21±0.2      | 120±10          | 32±5               | 33±6              |

To control the general state of the body before and after vaccination, a morphological analysis of the blood of vaccinated stallions was performed. Indicators that can change during the development of immunity were determined [1]: the total number of red blood cells, white blood cells, leukocyte formula, ESR. As table 3 shows, on day 14 after vaccination, the total number of red blood cells and white blood cells did not actually change. But the leukocyte formula has changed due to an increase in the percentage of segmentonuclear neutrophils (P >0.95) and eosinophils. There was also a sharp increase in ESR (P 0.99). By day 30, these indicators had returned to their initial level.

It is in the first 2 weeks after the introduction of the antigen that the main restructuring of the body occurs. The period of immunity formation is accompanied by a change in the overall blood indicators, which affects the state of the body without visible changes, but the resulting tension leads to a decrease in the quality indicators of stallions' sperm.
Table 3. Morphological indicators of blood before and after vaccination

| Period                  | Erythrocytes $10^{12}$/l | Leukocytes $10^9$/l | Lymphocytes % | Neutrophiles-segment % | Eosinophils % | ESR |
|-------------------------|--------------------------|---------------------|---------------|------------------------|--------------|-----|
| Before vaccination      | 7.8±1.0                  | 8.0±1.1             | 56.1±6        | 42.8±0.3               | 2±0.1        | 37.7±6 |
| After vaccination -14th day | 7.7±1.0                  | 8.1±1.2             | 38.5±3        | 58.1±0.5               | 2.7±0.6      | 52±8 |
| After vaccination -30th day | 7.0±0.7                  | 78±1.5              | 54.2±5        | 43.8±0.2               | 1.6±0.4      | 33±5 |

4. Conclusion

Comparison of the results of this study with previous observations on the quality of stallions' sperm after vaccination against anthrax and dermatomycosis showed virtually the same functional response of the sexual sphere of producers to the introduction of an antigen of different nature-bacterial, fungal or viral.

It is believed that changes in the quality of sperm do not occur immediately, but 4-7 weeks after a certain exposure [9]. This is due to the duration of spermatogenesis and the time of cell movement along the channel of the appendage of the testis. But in this case, the effect on the motility, survival and stability of cell membranes appeared very quickly and, apparently, got into the sperm through the secrets of the additional sex glands [10], which affected the quality of sperm without directly affecting the testis. But the change in the morphological composition of germ cells, which appeared in the second half of observations – is the result of influence on the processes of spermatogenesis. As a result, we see a double impact on the quality of sperm after vaccination-rapid and remote.

Thus, vaccination of stallions led to a decrease in the quality of sperm. Sperm indicators fell especially sharply after freezing-thawing. In the case of cryopreservation of the seed, it is necessary to take into account the recovery period of antiepizootic measures.

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