Prospects for using post-mortem genetic materials on the example of sable to ensure the biodiversity in natural systems

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Abstract. The article presents a technique of taking a genetic material from a postmortem material of appendage and testes of sables, conducted research to determine the mobility of sperms, morphological assessment and survival of sperms outside the animal body. When introducing artificial insemination in the sable industry, the use of post-mortem genetic material obtained from male sables is a promising direction in the reproduction of sable as a particularly valuable commercial species, as well as for its introduction into the zoo culture in order to ensure the biodiversity of natural systems where these animals live. It can also be used in the development of programs for the reproduction and conservation of populations of other particularly valuable, rare, and endangered species of mammals.

1. Introduction

In accordance with Article 2 of the International Convention on Biological Diversity [4], biological diversity means the variability of living organisms from all sources, including, among others, terrestrial, marine, and other aquatic ecosystems and ecological complexes, of which they are part; this concept includes diversity within a species, between species, and a variety of systems.

Biological diversity is the result of almost four billion years of development. Many forms of life and ecological processes ensure the continuation of biological evolution, which is a prerequisite for human well-being. In the framework of the Strategy and Action Plan for the Conservation of Biological Diversity of the Russian Federation [12], the ecosystem service for the production of hunting products is of regional and local importance, primarily. Also, the recreational component of this service is great. Besides, it is important for preserving the traditional lifestyle of the indigenous peoples of the North, Siberia, and the Russian Far East.

According to the fifth national report, Biodiversity Conservation in the Russian Federation, prepared by the Ministry of Natural Resources and Ecology in accordance with the identified important problems and unresolved obstacles that have persisted in recent years in connection with Russia’s fulfillment of its obligations under the International Convention on Biological Diversity, one of the priority tasks is to ensure the sustainable use of biodiversity resources in natural resource industries - agriculture, hunting, fisheries, and forestry [7].

Currently, the development of a bioresource strategy is becoming increasingly important for animal reproduction biotechnology. This strategy could provide a high yield of useful products and, at the same time, the preservation of natural diversity and a balanced abundance of zoocomponents of...
natural ecosystems. This global problem can be solved by developing technologies of zooculture for those species not yet being involved in it, as well as improving the existing ones. In principle, the zooculture technology should be developed for all living animal species [1].

Sable is one of the most valuable in the fur production of animals. Sable pelts are widely known and in constant demand at various international fur auctions. Commercial sable pelts are more in demand [5]. Very often, wild sable pelts are exported from Russia illegally, suggesting an oversupply of demand before offering [6].

As an independent branch of fur farming, the history of sable breeding in Russia has more than 75 years. Despite this, significant problems are noted in the biotechnology of sable breeding. For example, in animal farms, sables mature at 2-3 years, and in their natural habitat at 1 year. Among scientists, there is no consensus on this issue, some scientists believe that sable males become sexually mature at the age of one year [3], others point to the puberty of sable males at a later period [2], [9], [10]. There are the most numerous populations of subspecies (ridges) of sable, such as the Barguzin, Yenisei, Sayan, Sakhalin, Tobolsk, Kamchatka, Yakut, Far Eastern and Tungus [11].

Based on the above, we believe that the results of our research presented in this article in the future can solve the problem of reproduction of the highly valuable commercial animal. And globally, it will help to more effectively address the problems of ensuring the biodiversity of other species in various natural systems.

2. Materials and Methods
Seed plants with appendages of annual males of sables of the Yenisei and Barguzin ridges obtained by shooting from 5 animals in February 2019 in the Irkutsk district of the Irkutsk region (Russia) served as materials for our research. The experimental material taken was placed in special containers with ice and delivered to the laboratory of the Department of Special Veterinary Disciplines of the Irkutsk State Agricultural University named after A. A. Ezhevsky within 10-12 hours. After that, the post-mortal genetic material was placed in a thermostat at 37°C for 2 hours, in order to prevent a “cold” shock in sperm.

The technique of sperm aspiration from the testis and its appendage is as follows: a 2 ml disposable syringe needle is inserted through the testis head for its entire length along the axis of the testis network. This needle position allows aspiration from the tubule network (rete testis), which is a complex system of tubules connecting the appendage with the testis, and which is functionally justified because it contains the largest number of sperms. After insertion of the needle, the latter slowly retracts back, at this moment, a drop of unclear liquid appears in the needle cannula. A 2 ml syringe containing 0.3 ml of a solution of neutralized 2.9% sodium citrate is attached to the needle, after which, at the same time, the aspiration of the contents of the testis network is carried out and the needle is removed. Additionally, the genetic material was taken from the anterior portion of the testicle. For this, the puncture of the anterior portion of the testis was carried out using a butterfly needle, the tube of which was connected to a 2 ml disposable syringe, creating a vacuum in it using a piston, additionally pressing fingers on the testicle, changing the position of the needle tip at 15 different points inside the testis tissue.

For the evaluation of the obtained seminal fluid from the postmortal material of the testis and the appendage of sable males, the method for evaluating the freshly obtained sperm from farm animals was used [8], since in the literature available to us, we have not found methods for assessing sperm in sables and other commercial animals, as well as methods for evaluating sperm obtained from the post-mortal material.

3. Results
Evaluation of the results was carried out according to three criteria: determination of sperm motility, morphological assessment of sperm and its survival outside the body [8].

First, determination of sperm motility was conducted. The visual microscopic evaluation was performed in a darkened field of view with an increase in the obtained material 140 times in dilution.
with 2.9% sodium citrate (1:3). According to the results of the analysis carried out on all five samples with a generally recognized assessment of the sample, the results obtained from the epididymis averaged 4.3±0.3 points, where each point corresponds to 10% of sperm with translational motion. According to the results of the analysis carried out on five samples taken from the testis tissues, the results averaged 2.7±0.3 points. Here, a large number of immature sperms at the stage of round spermatids marked.

Second, we conduct a sperm morphology assessment. For morphological evaluation of sperm, a drop of the obtained material in dilution with 2.9% sodium citrate (1:3) was placed on the edge of a defatted slide, a thin smear, prepared, dried, then fixed in 96% ethanol for 3 min and stained with eosin. Evaluation of sperm in a stained smear was performed under a microscope at a magnification of 400 times. On average, the percentage of viable sperm taken from the epididymis was 47.5 ± 3.3% and 24.3 ± 3.0%, respectively.

Third, the survival of sperm outside the body is one of the objective indicators of the fertilizing ability of sperm in the biotechnology of animal reproduction. According to the results of our research, in samples taken from the epididymis of the testis, it averaged 6.9 ± 0.6 hours and 4.1 ± 0.4 hours, respectively.

4. Discussion
Our studies of postmortem genetic material taken from sable males and the original methods tested by us for collecting seminal fluid from the tissues of the epididymis and testis allowed us to obtain the following results.

According to the results of our studies on the generally accepted methods for assessing sperm in animals with respect to determining the ability of sperm to progressive movement on a 10-point scale, we can say that the sperm of male sables obtained from the post-mortal material is quite suitable for artificial insemination. Its goal is to ensure the unique biodiversity of sable in various natural systems and to preserve its gene pool as a highly valuable commercial species. We believe that the results obtained (4.3 ± 0.3 and 2.7 ± 0.3) on a 10-point scale, taking into account the high level of modern biotechnologies, are quite acceptable results, considering the fact that the material was post-mortal, and 12-14 hours had passed since it was delivered to the laboratory (on average), taken, and placed in an optimal biological environment.

In our opinion, while conducting a morphological assessment of sperm, the percentage of viable sperm is relatively high, given the fact that the material was obtained after the death of animals. The survival rate of sperm outside the body was at a low level, but one should take into account the fact that we used the technique adopted to evaluate the freshly obtained sperm from live animals. When performing artificial insemination in order to preserve the biodiversity of valuable and endangered species, we consider that this assessment methodology is unacceptable for the above purposes.

5. Conclusion
Based on our research and the results obtained, we can say that when artificial insemination is introduced into sable breeding, the use of post-mortal genetic material obtained from sable males is a promising direction in the reproduction of sable as a particularly valuable commercial species. Also, the use of post-mortal genetic material is a promising direction for its introduction into the zooculture in order to ensure the biodiversity of the natural systems where this animal lives. Research in this direction should be continued, improving the methods of obtaining post-mortal material from male sables, increasing the methods of preservation, survival and fertilized ability of sperm cells using advanced technologies in biotechnology and animal reproduction, as the most optimal methods of rational use and management of biological resources. The results of our research can be used in the development of programs for the reproduction and preservation of a population of other especially valuable, rare, and endangered mammalian species.
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