

**Кореляційний зв'язок біохімічних показників сім'яної плазми та кріорезистентності сперматозоїдів цапів зааненської породи**

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**Correlation Between Biochemical Parameters of Seminal Plasma and Cryoresistance of Spermatozoa of Saanen Bucks**

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Cryobanks in animal breeding allow to store the reproductive material of valuable animals with the best genetic traits and use it to inseminate more dams regardless of the mating season. The effectiveness of cryopreservation depends on the cryoresistance of spermatozoa, so the criteria for assessing this parameter is an essential step in selecting material for the cryobank and needs to be developed. Seminal plasma creates an environment for spermatozoa, and thus can directly affect their functional characteristics. Therefore, the aim of the study was to investigate the correlation between biochemical parameters of seminal plasma and cryoresistance of spermatozoa of Saanen bucks.

Ejaculates were obtained from three sexually mature Saanen bucks once a week during the breeding season (September-December). The concentration, motility, viability, morphological characteristics, and DNA fragmentation rate of spermatozoa were determined. The level of total protein, glucose, and cholesterol was determined in the seminal plasma. Cryopreservation of semen was performed in a medium with 10% glycerol and 20% egg yolk in 0.25 ml cryostraws. The ejaculate was equilibrated with the cryoprotective medium for 15 min at room temperature (23°C), 2.5 h at 5°C, then placed at a distance of 4 cm from liquid nitrogen for 15 min and plunged into liquid nitrogen. The straws were thawed in a water bath at 37°C for 30 sec. The spermatozoa were then washed from the cryoprotective medium and further evaluated for the indicated above parameters.

It was found that the level of total protein in seminal plasma positively correlated with sperm concentration ( $r = 0.636$ ,  $P < 0.05$ ) and the number of morphologically normal sperm after cryopreservation ( $r = 0.619$ ,  $P < 0.05$ ) while negatively correlated with the DNA fragmentation rate ( $r = -0.607$ ,  $P < 0.05$ ). It is known that seminal plasma proteins are able to bind to the sperm membrane and, possibly, thereby reduce the rate of morphological defects caused by cryopreservation. Glucose level positively correlated with sperm motility after cryopreservation ( $r = 0.674$ ,  $P < 0.05$ ). This may be due to the fact that glucose is an energy substrate, so its level has an effect on cell motility. Cholesterol level had a positive correlation with sperm viability after cryopreservation ( $r = 0.758$ ,  $P < 0.05$ ), and a negative correlation with the DNA fragmentation rate ( $r = -0.571$ ,  $P < 0.05$ ). This is probably because cholesterol is able to stabilize cell membranes.

In conclusion, biochemical parameters of seminal plasma such as protein, glucose and cholesterol levels have a correlation with the cryoresistance of spermatozoa of Saanen bucks.

**Потенційні шляхи збереження біорізноманіття риб у басейні Сіверського Дінця на території України з використанням методів кріобіології та кріобанкінгу**

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**Potential Ways for Fish Biodiversity Conservation Within Siversky Donets Basin in Ukraine Using Cryobiology and Cryobanking Techniques**

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Biodiversity is an essential element of environmental sustainability, and its loss is believed to be one of the factors driving ecosystem degradation (Biodiversity and the 2030 agenda for sustainable development, 2016). Currently, there are many measures to preserve the wild diversity of organisms, but there are destructive events that cannot be stopped by conservation efforts. In Ukraine, the Russian military invasion since 2014 has caused many environmental problems, and with the beginning of full-scale aggression from 24.02.2022, we have huge devastation for natural communities, which may be impossible to compensate for by a self regulatory process, losses to natural ecological systems. Water bodies are affected particularly negatively, as typical fish spawning and feeding habitats are destroyed. Methods that can preserve biodiversity separately from the habitat are the most requested at the moment. Long-term preservation methods for fish gametes are the most optimal due to the lack of maintenance facilities for adult animals as well as full maturation of the gametes. Reproduced juveniles require follow-up action, *i. e.* the introduction, which is problematic at the moment. Methods for long-term storage of fish gametes under cryobank conditions are the most perspective due to the unavailability of alternatives. Safety of the performers during field sampling is the main condition here for the accumulation of gametes.

It is important to emphasize that the problem of preserving local fish fauna as a consequence of the war is only being discussed, and it is important to understand that the circumstances are dynamic [Onysko 2022, 2023].

In the last review, modern fauna of the whole Siverskyi Donets basin includes 58 species, and 54 species are found in the waters of the North-East of Ukraine (represented by 44 genera, 15 families and 9 orders) [Shandikov, 2008]. A total 44 species are representatives of the autochthonous fauna, 6 species are included in the regional red list (Kharkiv region) and 13 are listed in the Red Data Book of Ukraine [Tokarsky, 2013].

According to our preliminary assessment, the large number of military operations around the Siverskyi Donets River, including unsuccessful attempts to force it by Russian troops, has led to significant habitat disturbance and chemical pollution. In summary, even species that were considered "common" before 24.02.2022 may significantly decrease in number and genetic diversity over time.

In our opinion, cryopreservation technologies can become a very important part in conservation of local fish diversity. Cryopreserved gametes can be used for the reintroduction of all fish species, in favourable circumstances, especially since the need to restore our diversity is in great demand [Honcharov 2020].

