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Досвід використання кріотехнологій у тваринництві для забезпечення продовольчої безпеки України

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The Experience of Using Cryotechnology in Animal Breeding to Ensure Food Security in Ukraine

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According to the official report of the Ministry of Agrarian Policy and Food of Ukraine issued in October 2023, more than 300 livestock farms were damaged or destroyed as a result of the full-scale war. In particular, about 7,000 cattle were killed in the war, which raises the problem of food security in the country. Most of these animals are highly productive or unique Ukrainian breeds that have been bred by researchers and farmers for decades. It can take a long time for a herd to recover naturally, although assisted reproductive technologies (ART) in animal breeding can speed up the process. Moreover, breeding highly productive animals allows for more efficient use of resources. Cryotechnologies, namely cryopreservation of sperm, oocytes and embryos, have an important place in ART. The aim of our study was to analyse the effect of cryopreservation of buck semen on embryo development, pregnancy and kidding rates. Ejaculates of 5 sexually mature Saanen bucks were obtained every two weeks. Sperm motility, viability, and DNA fragmentation were determined. Spermatozoa were cryopreserved in a solution of 10% glycerol and 20% egg yolk. The samples were thawed for 30 seconds in a water bath (37°C). After removing the cryoprotectant, sperm motility, viability and DNA fragmentation were assessed and *in vitro* fertilisation of Saanen goat oocytes was performed. The development of embryos was assessed on day 7 of culturing, then embryos were transferred to recipient goats of local breed and the pregnancy rate on day 50 and the kidding rate were determined.

Cryopreservation caused a significant ($p \leq 0.05$) decrease in sperm motility by 30% and sperm viability by 20%, led to a significant ($p \leq 0.05$) increase in sperm DNA fragmentation by 5%. The analysis of the embryo development rate on day 7 showed a significantly ($p \leq 0.05$) lower blastulation rate after fertilisation with cryopreserved spermatozoa (55.7 ± 8.1%) compared to fresh spermatozoa (76.1 ± 6.5%). The pregnancy rate after embryo transfer was insignificantly ($p > 0.05$) lower for the group of cryopreserved spermatozoa (26.3%) compared to fresh spermatozoa (32.1%). A similar trend was observed for the kidding rate, which was 23.5% for the cryopreserved sperm group and 32.1% for the fresh sperm group. However, in the group of fresh spermatozoa, all diagnosed pregnancies resulted in the birth of goats, and in the group of cryopreserved spermatozoa, 3% of pregnancies were aborted. In conclusion, the use of cryotechnology allows to obtain high pregnancy and kidding rates when used in goat breeding, which can enable rapid reproduction of a highly productive herd on farms to ensure food security in Ukraine.

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Різні підходи до виділення мезенхімальних стромальних клітин із Вартонових драглів

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Different Approaches for Mesenchymal Stromal Cells Isolation from Wharton's Jelly Tissue

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Wharton's jelly (WJ)-derived mesenchymal stromal/stem cells (WJ-MSCs) are more ontogenetically earlier compared to the cells isolated from adult tissues. WJ-MSCs possess high proliferative potential, are able to suppress inflammation following tissue damage and to secrete growth factors that contribute to tissue repair. WJ-MSCs can differentiate into various cell types, including nerve cells, bone and cartilage cells, adipocytes, and other. WJ-MSCs are used in regenerative medicine for the treatment of heart and kidney diseases, autoimmune diseases, multiple sclerosis, and wound healing. An important advantage of WJ tissue is the painless and safe sample collection. Typically, WJ-MSCs are isolated from fresh tissue samples, expanded under culture conditions, and then cryopreserved for long-term banking. However, MSCs may undergo modifications during subsequent *in vitro* passages. Cryopreservation of WJ tissue samples for isolation of WJ-MSCs is an alternative method to obtain WJ-MSCs with a lower degree of genomic and epigenetic changes.

The aim of this study was to compare the efficacy of the tissue explant method with a modified method for isolating WJ-MSCs from both fresh and cryopreserved WJ tissue samples.

Fresh human umbilical cords were collected after cesarean section delivery, following mothers' informed consent, and processed within 3–6 hours of harvesting. For the tissue explant method, small pieces of WJ were placed on the surface of a T25 culture flask (0.5 g per flask) for 3 hours, then MEM alpha-modification (PAA, Austria) supplemented with 20% fetal bovine serum was added, the medium was replaced with fresh medium after 1 week and then twice a week. The modified method of WJ-MSCs isolation has the following differences from the method described above: small pieces of WJ were placed in culture medium on a gelatin-coated surface of a T-25 culture flask, after 1 week, tissue pieces and migrated cells were collected by incubating them with trypsin solution for 5–10 minutes, centrifuged, and then seeded onto a culture flask again. Such modification results in significantly rapid MSC migration, with up to a 10-fold increase in WJ-MSC yield in pilot experiments. WJ tissue samples were cryopreserved with 10% DMSO only or together with 90% autologous umbilical cord blood serum by slow cooling in Mr. Frosty (Nalgen) to –80°C, and subsequently transferred to liquid nitrogen for storage. Cryopreserved and stored samples will be used for WJ-MSC isolation in future studies.