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## THE GENE POOL PRESERVATION OF WHITE HEADED UKRAINIAN BREED OF CATTLE BY BIOTECHNOLOGICAL APPROACHES

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*As a result of applied biotechnological complex approaches, we received and laid 30 viable embryos of biloholova ukrainian breeds of cattle to the Bank of Animal Genetic Resources of Institute of Animal Breeding and Genetics nd. a. M.V.Zubets National Academy of Agrarian Science of Ukraine. We analyzed national and foreign literature and our own experimental researches. We found out, that receiving viable preimplantation embryos in vivo and its cryopreservation is the most effective way to save the national gene pool object. These embryos may be saved indefinitely*

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*with appropriate conditions. In order to obtain animals of endanger or already extinct species and can frozen-thawed and transplanted embryos to recipient of embryos any cattle breeds at any time. We applied effective removal of embryos from donor cows of biloholova ukrainian breed. An effective way to preserve the gene pool of local breeds of cattle is realized on the basis of genetic-biotechnological approaches.*

**Key words: White Headed Ukrainian Breed of cattle, gene pool preservation, embryos produced *in vivo*, cryopreservation of embryos, biotechnological methods, cytogenetic analysis**

## **БИОТЕХНОЛОГИЧНІ ПІДХОДИ ДО ЗБЕРЕЖЕННЯ ГЕНОФОНДУ БІЛОГОЛОВОЇ УКРАЇНСЬКОЇ ПОРОДИ ВЕЛИКОЇ РОГАТОЇ ХУДОБИ**

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*В результаті застосованих комплексних біотехнологічних підходів нами отримано і закладено на довготривалі зберігання до банку генетичних ресурсів тварин ІРГТ ім. М.В.Зубця НААН 30 життєздатних ембріонів білоголової української породи великої рогатої худоби. Аналізом вітчизняних та іноземних літературних джерел і результатів власних експериментальних досліджень аргументовано, що в зв'язку із складним економічним становищем в країні, одним з ефективних способів збереження вітчизняного генофондового об'єкту є отримання *in vivo* та кріоконсервація життєздатних доімплантаційних ембріонів. При відповідних технологічних умовах такі ембріони можливо зберігати необмежено довго, і в разі необхідності вони можуть бути деконсервовані та трансплантовані реципієнту будь-якої породи великої рогатої худоби, в любий час з метою отримання тварин зникаючої або вже зниклої породи. Нами застосовано ефективно вилучення ембріонів від корів-донорів білоголової української породи. На основі застосування генетико-біотехнологічних підходів реалізовано дієвий спосіб збереження генофонду локальних порід великої рогатої худоби.*

**Ключові слова: білоголова українська порода великої рогатої худоби, збереження генофонду, ембріони одержані *in vivo*, кріоконсервація ембріонів, біотехнологічні методи, цитогенетичний аналіз**

## **БИОТЕХНОЛОГИЧЕСКИЕ ПОДХОДЫ К СОХРАНЕНИЮ ГЕНОФОНДА БЕЛОГОЛОВОЙ УКРАИНСКОЙ ПОРОДЫ КРУПНОГО РОГАТОГО СКОТА**

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*В результате примененных комплексных биотехнологических подходов нами получено и заложено на длительное хранение в банк генетических ресурсов животных ИРГТ им. М.В.Зубця НААН 30 жизнеспособных эмбрионов белоголовой украинской породы крупного рогатого скота. Анализом отечественных и иностранных литературных источников и результатов собственных экспериментальных исследований аргументировано, что в связи со сложным экономическим положением в стране, одним из эффективных способов сохранения отечественного генофондового объекта является получение *in vivo* и кріоконсервація жизнеспособных доімплантационных эмбрионов. При соответствующих технологических условиях такие эмбрионы можно хранить неограниченно долго, и в случае необходимости они могут быть деконсервированы и трансплантированы реципиенту любой породы крупного рогатого скота, в любое время с целью получения животных исчезающей или уже исчезнувшей породы. Нами применено эффективное получение эмбрионов от коров-доноров белоголовой украинской породы. На основе применения*

генетико-биотехнологических подходов реализовано эффективный способ сохранения генофонда локальных пород крупного рогатого скота.

**Ключевые слова:** белоголовая украинская порода крупного рогатого скота, сохранение генофонда, эмбрионы полученные *in vivo*, криоконсервация эмбрионов, биотехнологические методы, цитогенетический анализ

**Introduction.** As a result of changes in the ecosystem and increase usage of natural and economic resources faced the problem of survival small quantity breeds farm animals. It is not always possible to keep the gene pool such unique species play traditional methods. Saving in small closed populations leads to uncontrolled growth of inbreeding in each generation [2, 3]. Prevent loss of biodiversity of farm animal's case of complex application of modern biotechnological methods. Methods of obtaining *in vivo* embryos and their cryopreservation provide an opportunity in the future of the reproduction species or breeds by transplantation of female recipients. In addition, use of applied biotechnology approaches ensures implementation of tasks in the accumulation of genetic material with a view to maintaining or commercial use when improving existing or create new breeds [7, 8]. Saving embryos and gametes known genesis by cryopreservation is aimed at reducing the negative effects a limited number breeding material.

The first embryo transplantation complied English explorer Walter Hip (Walter Heap, 1855–1929 gg.), in April 1890 transplanted two 4-cell embryos from angora rabbit in uterus females Belgian champion breed rabbit before fertilized with a male of the same breed. A month later the rabbit-recipient birth first transplant in world, two long-haired Angora rabbits with four Belgian champion breed rabbit [9, 10]. However, interest in embryo transplantation in cattle was only in the late 1960-s XX century, when the U.S.A. and Australian scientists set about improving Charolais beef breed, which prevailed over traditional meat breeds. The first commercial center of the embryo was created in Canada [5, 6], where the first calf by embryo transplantation received in 1973. In subsequent years, the number of transplants calf increased ten years and reached 5413 calves. Currently, Canada is a leader in obtaining embryos *in vivo*. Thus, in 2013, received 84 940 suitable for embryo transfer [www.ceta.ca]. Simultaneously, business associations created in other countries. In Europe the first-calf received transplants in 1974 in Denmark. In EU is widely used such this biotechnological approach. According to the European Embryo Transfer Association in 2013 received the leading European countries 128 055 embryos. The leader among European nations on the volume of receipt and embryo transfer in cattle in 2013 was France (38 244 high-grade embryos) [www.aete.eu].

The gene pool preserve breeds of farm animals should be used biotechnological approach that includes: obtaining embryos of cattle by stimulating superovulation donor cows, their artificial insemination, no surgical technique receiving embryos from genetically valuable cow's evaluation derived embryos and their cryopreservation for the purpose of long-term preservation.

White Headed Ukrainian Breed of cattle is one of the oldest breeds in Ukraine. According to the "Program preserve the gene pool of the main types of livestock in Ukraine until 2015" this breed is attributed to domestic gene pool object that is on the verge of extinction. Animals of this breed are well adapted to local conditions, characterized by a high ability to use nutrients coarse and succulent fodder, unique resistance to diseases is of great interest for breeding in the future. The population of White Headed Ukrainian Breed of cattle in Ukraine is 596 as of 01.01.2014 of them 300 cows («Agency of Animal Identification and Registration»). This breed is bred in only Khmelnytsky region Private Corporation «Podilsky gospodar».

Modern biotechnology and molecular genetic techniques have radically changed the traditional approach to the conservation of species diversity of animals. Given the lack of economic support for the economy, which maintains herd of White Headed Ukrainian Breed of cattle, one of the most effective ways of saving this species is cryopreservation of viable embryos. When appropriate technological conditions such embryos may maintain indefinitely and, if necessary, they will frozen-thawed and transplanted recipient of any breed of cattle to obtain animals endangered or already extinct species. Therefore, the aim of our study was to evaluate the efficacy of the method

of obtaining *in vivo* and cryopreservation of embryos of bilogolova ukrainian breed of cattle and extension approaches to preserve the gene pool.

**Materials and methods.** Studies on cryopreservation of embryos and getting carried out on the stock Private Corporation «Podilskyy gospodar» (Khmelnysky region) in cows that were hormone treatment follicle stimulating hormone (FSG-Super) Cows donor preparing receiving and cryopreservation embryos performed by known techniques [1, 4]. Donor's embryos were clinically healthy bark without violating the sexual cycle. Donor cows stimulated from the 8-th to the 12-th day of the manifestation of signs desire. Processing steps hormones took 5 days and 6 day hunt recorded. Animal insemination 2–3 times a double dose of white bull sperm bilogolova ukrainian breed of cattle at intervals of 12 hours. After 7–8 days spent nonsurgical receiving embryos. Obtained *in vivo* bovine embryos were washed in medium Dulbecco's (PBS, Sigma, D-5652) with the addition of 0.075 mg / ml kanamycinum with 20 % fetal calf serum (FST, Sigma, F 9665).

For immobilization animals injected the drug «Ksilazin-50» (Interchemie, Netherlands) at dose of 0.1 ml per 100 kg weight body. For washout embryos using a modified two-way catheter Folleya («IMV», France). For cryopreservation embryos used single-stage saturation method using 1.4 M glycerol (Sigma, G2025). Freezing was carried out in 0.25 ml straw [11, 12]. Production cytogenetic preparations unsuitable for cryopreservation of embryos and oocytes was performed in the laboratory of biotechnology Institute of Animal Breeding and Genetics nd. a. M.V.Zubets National Academy of Agrarian Science of Ukraine. Cytogenetic preparations of embryos and oocytes were prepared by a modified method of Tarkovsky (S. I. Kovtun, 2009). Preparations were analyzed with a microscope Jenaval Carl Zeiss OK  $\times 10 \times 100$  OB.

**Results.** Flushed embryos of White Headed Ukrainian Breed of cattle on the farm Private Corporation «Podilskyy gospodar» of nine cows-donors who were hormone treatment (fig. 1, 2). For artificial insemination using sperm of three bulls breed of White Headed Ukrainian (Orel 235, Neapol' 561, Signal 721). Semen of bulls kept in the Bank of Animal Genetic Resources Institute of Animal Breeding and Genetics nd. a. M.V.Zubets National Academy of Agrarian Science of Ukraine from 12 to 45 years.

As a result of non-surgical flushed was 82 cells, the number of embryos formed was at 69.5 % (57 of 82 embryos washed cells). The morphological analysis shown that embryos obtained full and no signs of degeneration at an appropriate stage of embryos were 30, representing 36.6 % of the flushed cells (table 1). Of the estimated 30 embryos at the stage of middle and late morula embryos were 27 (90 % of high-grade embryos) and three embryos were at the stage of early blastocysts (10 %).



Fig. 1. Nonsurgical flushed embryos cow donor number 3843 (Norka)

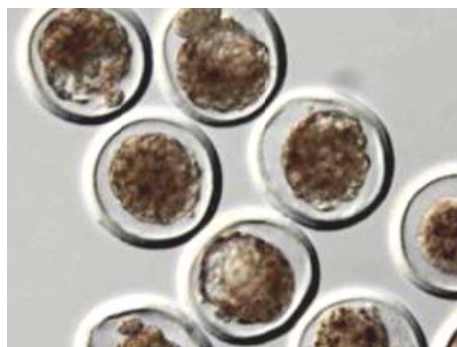


Fig. 2. Life photo of embryos obtained from donor cows bilogolova ukrainian breed

All cryopreserved embryos were transferred to the Bank of Animal Genetic Resources Institute of Animal Breeding and Genetics nd. a. M. V. Zubets National Academy of Agrarian Science of Ukraine for long-term storage. By using biotechnological approaches to the bank laying on saving cryopreserved viable embryos 30 of White Headed Ukrainian Breed of cattle. Of these, 27 morphological assessments identified as morula and blastocyst three excellent quality.

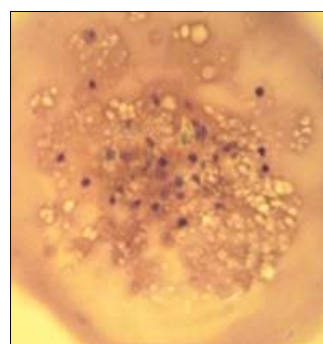
**1. Results of obtaining embryos from donor cows bilogolova ukrainian breed**

Name and individual donor number, date of birth	Total № of oocytes/embryos collected	№ of oocytes collected	№ of embryos collected	№ of embryos frozen
Naryadnaya 3676, 20.04.10	14	1	13	13
Norka 3843, 04.09.10	11	-	11	5
Baronessa 0064, 10.08.04	14	1	13	6
Vihra 9318, 04.08.09	11	5	6	3
Brava 9333, 21.08.09	6	2	4	3
Nezhna 3302, 01.05.09	2	-	2	-
Symna 3390, 10.05.09	6	3	3	-
Malvina 5839, 05.07.02	12	11	1	-
Verba 9317, 11.08.09	6	2	4	-
Total	82	25	57	30

Oocytes and embryos with signs of degeneration used for cytogenetic analysis to diagnose causes stop embryogenesis (fig. 3). According to the results of cytogenetic analysis found that almost all embryos (21 of 27) by morphological features as viable, had degenerated nucleus and their number did not correspond stage of the sexual cycle of cows. Six embryos were number of cores corresponding to the stage, but were core features of progressive degeneration (fig. 4).



**Fig. 3. Obtained in vivo morula with signs of degeneration**



**Fig. 4. Degenerative embryos at the morula stage, n = 29 nucleus**

Analysis of cytogenetic preparations oocytes confirmed that all 25 investigated ova identified correctly. They had degenerated chromatin at different stages of meiosis and was identified pronuclear, to indicate their fertilization.

**Conclusions.** The results of the studies found that the termination of the development of the embryo at a certain stage of stop embryogenesis to lack of energy resource maternal cells may polyovulation conditional or living conditions, because the stage of embryo development did not correspond to the stage of the sexual cycle of cows at the time of leaching. We combined morphological and cytogenetic analysis of bovine embryos and show the relationship between visual assessment and as chromatin nuclei. Applied detailed morphological and cytogenetic analysis

of the nucleus of embryos has provided an objective assessment of the viability of embryos and forecast vital parameters embryos intended for non-surgical transplant recipients.

With these approaches, the resulting material for analyzing and evaluating the effectiveness of potential donor cows of White Headed Ukrainian Breed livestock farms Private Corporation «Podilskyi gospodar».

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УДК 636.22./28.082.13

## РОЗВЕДЕННЯ, ВИРОЩУВАННЯ ТА ЗБЕРЕЖЕННЯ ГЕНОФОНДУ БУРОЇ КАРПАТСЬКОЇ ПОРОДИ У ГІРСЬКІЙ ЗОНІ УКРАЇНСЬКИХ КАРПАТ

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*Досліджено стан розведення, вирощування, збереження вітчизняного генофонду бурої карпатської породи гірського типу у гірській зоні Українських Карпат Закарпатської області.*

*Вивчено рівень наявності, продуктивності, плодючості, розвитку екстер'єру корів цієї породи, та динаміку росту ремонтних телиць в умовах гірської зони українських Карпат Закарпатської області.*

**Ключові слова:** буро-карпатська порода, збереження, генофонд, корова, плодючість, продуктивність

**BREEDING, KEEPING AND PRESERVING THE GENE POOL OF BROWN CARPATHIAN BREED IN THE MOUNTAIN AREA OF UKRAINIAN CARPATHIANS**

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