THE USE OF BIOTECHNOLOGICAL METHODS FOR CONSERVATION OF ANIMAL GENETIC RESOURCES

SVITLANA KOVTUN, OKSANA SHCHERBAK, AZA ZYUZYUN,
NATALIIA RIEZNYKOVA, ALEXANDER DUVANOV, ALEXANDER OSYPCHUK
Institute of Animals Breeding and Genetics of the
National Academy of Agrarian Sciences of Ukraine (NAAN)
Pogrebnjaka, 1, v. Chubinsky, Boryspil District, Kyiv Region, Ukraine, 08321

For the effective development of animal husbandry in every country one needs to increase livestock number by obtaining the greatest number of offspring from genetically valuable animals. Solving of this task is possible through the use of modern methods of biotechnology, based on the rational combination of artificial insemination, cryopreservation of gametes and embryo transfer. One of the biotechnological approaches, which is gaining widespread commercial use currently is the cattle artificial insemination with sperm, previously separated for the X-and Y-chromosome (sexed sperm) (**Arruda et al.,** 2012, **Garner et al,** 2003, **Norman et al,** 2010).

The perspective of this approach is confirmed by the practical experience of many countries, including Canada, where in 2012 using sexing bull semen for cows donor insemination 2449 embryos were obtained, 1214 of which were transplanted fresh, and 1235 – after cryopreservation (www.ceta.ca).

The advantage of sexed sperm using for farmers is the possibility to get animals of desired sex that significantly increases the level of herd management (**Brogliatti et al.,** 2009, **Sá Filho et al,** 2012), though it is known that the efficiency of sexed semen use is the most economically effective at farms that have reached a high fertility level at the first insemination and have low level of stillbirth. Besides mentioned above advantage, sexed sperm using in herds of Ukraine helps to increase milk production for much shorter period by way of getting only heifers with high prediction accuracy. But as the high cost of patent and equipment for sperm sorting constrains the development of this biotechnological method in Ukraine, a number of domestic farms are buying sexed sperm that is sold in our country by foreign firms (**Chernyak et al.,** 2012). Usually such sperm is used for insemination of heifers, as they demonstrate higher rate of fertility than cows. Use of sexed semen for artificial insemination of heifers provides a higher level of income from investments into the dairy business (**Chernyak et al.,** 2012). More efficient use of such semen provides a combination of the method with embryo transfer under conditions of obtaining embryos from high performance donor cows of native breeds (**Bodmer et al.,** 2005, **Peippo et al,** 2011). Use of embryo transfer and PCR-analysis of gender will reduce cost of sexed sperm using and will increase the number of livestock, which have prior known gender. It should be mentioned, that in herds percentage of fertilization of heifers when fertilized with non sexed sperm is usually 65 %, while when inseminated with sexed one – only 35 – 45% (**Chernyak et al.,** 2012). Applying the method of embryo transfer using 2 sperm dose of such sperm per donor one can get in average from 3 to 9 embryos of desired gender for one processing. They can be cryopreserved for long-term storage or transplant to recipients for calves getting (**Bodmer et al.,** 2005, **Soares et al,** 2011).

Effective use of biotechnological methods in animal industry depends on size of their practical implementation in complex system of sustainable use of valuable genetic potential of animals. Use of native biotechnological methods, which based on complex of embryo transfer works and molecular genetic studies are currently effectively used in selection and breeding work and is part of the overall work to ensure successful implementation of the tasks of the National Project in Ukraine "Recovered cattle breeding".

Nowadays in Ukraine at the Institute of Animal Breeding and Genetics NAAS there is the Bank of Animal Genetic Resources, which according to the order of the Cabinet of Ministers of Ukraine on August 19, 2002 № 472-r is a national treasure. The basis for the realization of tasks of conservation and sustainable use of the gene pool and endangered local breeds of cattle is receiving, cryopreservation and transplantation of embryos. Among the numerous results of the realization of tasks of the program "Preserving the gene pool" is Ukraine's membership since 2009 in the European Regional Focal Point for Animal Genetic Resources (ERFP) at FAO. Efficiency of its work depends on the distribution of genetic material to the gene pool virtual cryoherds, which consist of genetic material of known origin and in an amount which is sufficient for recovering of animals of certain species or breeds.

One of the breeds, which is classified due to "Programme of conservation of gene pool of main species of farm animals

in Ukraine for the period till 2015" as native gene pool object which is going to extinct is Grey Ukrainian cattle breed (**Guzev et al.,** 2009). Grey Ukrainian cattle breed is one of the oldest traditional Ukrainian native breed. It is characterized by a number of valuable traits – longevity, unpretentiousness to the management and feeding, resistance to epizootics, small fetus, and strong constitution, adapted to the climatic conditions of Ukraine. The population of this breed on 01.01.2012 in Ukraine is 1188 heads, kept in three farms. This population is not enough to maintain normal level of reproduction (**Guzev et al.,** 2009). So, the purpose of our study was to summarize approaches on use of advanced biotechnology techniques in system of conservation and sustainable use of animal genetic resources and ensure the enriching of the Bank of Animal Genetic Resources with embryos of Gray Ukrainian cattle breed.

MATERIAL AND METHODS

Research on the effectiveness of sexed sperm in obtaining embryos from donor cows of Holstein breed were spent on the farm "Agro-Soyuz". Studies on getting and cryopreservation of embryos of Grey Ukrainian cattle were made on the farm "Polyvanivka". As embryo donors were got clinically healthy cows without disorders of sexual cycle. Hormonal stimulation started at 8 – 12 days of heat indicators displaying. Processing of cows by hormones took 5 days and at 6-day heat was detected. Animals were inseminated 2 times with a double dose of sperm with an interval of 12 hours. In 7 – 8 days nonsurgical embryo obtaining and their cryopreservation was done due to existing methods (**Antonyk et al.,** 2004**, Sergeev et al.,** 2008). Obtained *in vivo* cattle embryos were washed in Dulbecco medium (Sigma, D-5652) with adding of 0.075 mg/ml kanamycin sulfate with 20 % fetal bovine serum (Sigma, F-7524) and were morphologically evaluated. Freezing was done in 0.25ml straw (**Kovtun et al.,** 2005).

RESULTS AND DISCUSSION

It was found that the level of formation of embryos (Figure 1) after using of sexed sperm for insemination of three donor cows was 66.7 % that did not differ significantly from the level, that was detected, using non-sexed sperm for insemination of other three cows (69.6%). But significantly lower ($P<0.05$, criterion χ^2) was the level of formation of suitable for embryo transfer embryos, when cows were inseminated with non-sexed sperm, compared with those inseminated with sexed sperm (Table 1). Owing to the complex application of modern biotechnological approaches it have been obtained and transplanted 211 Holstein sexed embryos for the last three years. Embryos that for morphological evaluation were not suitable for transplantation or cryopreservation were used for the genetic confirmation of the effectiveness of sexed sperm use (Figure 2).

It is known that the length of cattle Y-chromosome spe-

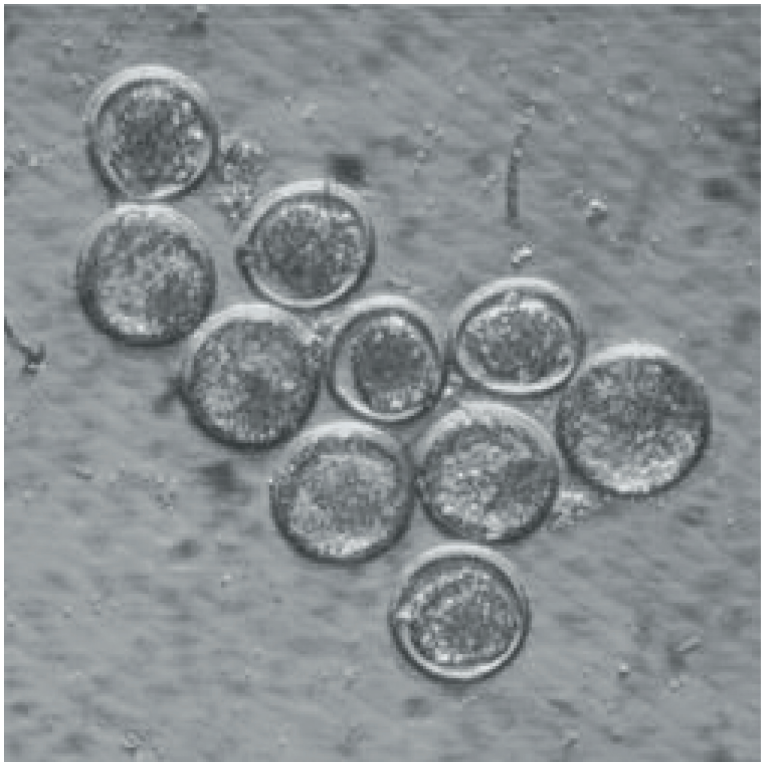

Fig. 1. **Photo of embryos obtained from donor cow N688/6914**

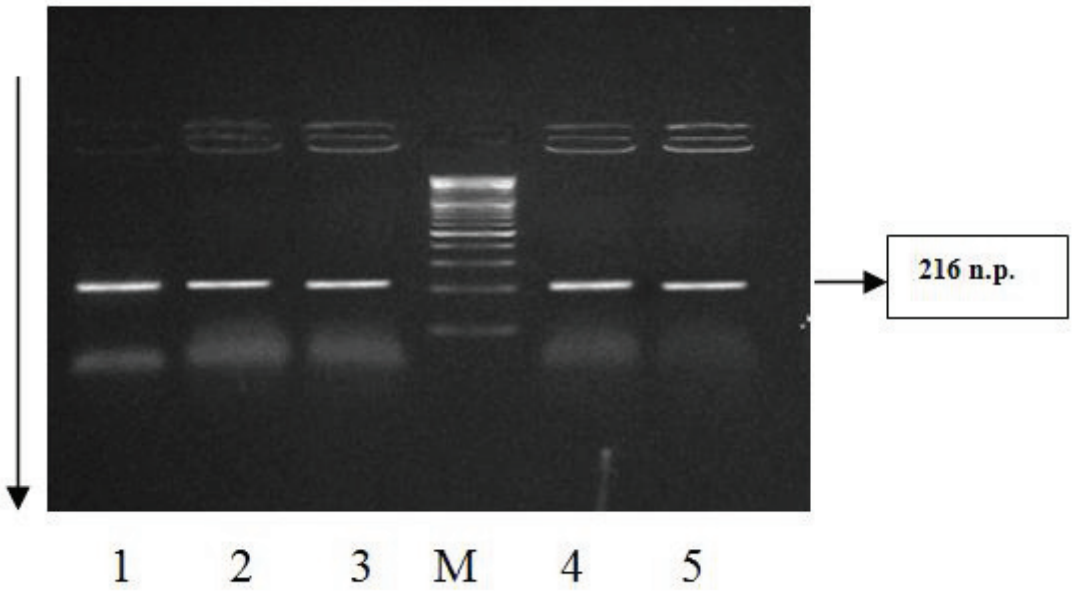

Fig. 2. **Electrophonogram of the amplification products with X-and Y-specific primers. Tracks: molecular weight marker DNA-Ladder 50bp. 1 – 5 – embryos, M-marker. All investigated cattle embryos appeared to be females**

Table 1. **Embryoproduction of cows at use of sexed sperm**

Type of sperm	Total number of embryos and oocytes, *n*	Oocytes, *n* (% ±m)	Total number of embryos *n* (% ±m)	Embryos, *n* (% ±m)	
				suitable for transplantation	degenerated
Sexed	27	9[a] (33.3±9.0)	18[b] (66.7±9.0)	10[c] (55.6±11.7)	8[d] (44.4±11.7)
Non-sexed	59	26[a] (44.0±6.5)	32[b] (54.2±6.5)	23[c] (71.9±7.9)	4[e] (12.5±5.8)

Note. d:e, $P<0.05$, χ^2 criterion

Table 2. **Embryoproduction of Grey Ukrainian cows and heifers**

Donors	Total number of obtained cells, *n*	Oocytes, *n* (% ±m)	Embryos, *n* (% ±m)	
			suitable for cryopreservation	unsuitable for cryopreservation
Cows (*n*=6)	72	34 (47.2±5.9)	25 (34.7±5.6)	16 (22.2±4.8)
Heifers (*n*=8)	18	12 (66.7±11.1)	5 (27.7±10.6)	2 (11.1±7.4)

cific amplification product is 173 nucleotide pairs (np) long and X-specific fragment – 216 np. As a result of PCR it was observed in cows one amplicon sized 216 np, while in bulls – two, sized 173 np and 216 np (**Kopylov et al.,** 2008)**.** Use of PCR analysis showed that all studied embryos were female's, which fully confirmed the effectiveness of sperm sorting.

Obtaining of Grey Ukrainian cattle embryos was carried out in the herd of "Polyvanivka" farm from heifers and cows that have been treated hormonally with follicle stimulating hormone (FSH). For artificial insemination of donors the sperm of Grey Ukrainian bulls, which is stored in a Bank of Genetic Resources in average 36 years was used.

It was found that obtaining of embryos from heifers of Grey Ukrainian breed is impractical due to a complex process of obtaining, physiological structure of sexual organs and low embryoproduction. This is confirmed by the number and quality of obtained embryos. From three donor heifers only five embryos were selected for cryopreservation out of 18 obtained, that confirms the effectiveness of embryoproduction at 28% level. From six donor cows 25 embryos with excellent and good characteristics were obtained, which were cryopreserved and 47 oocytes and embryos that were unsuitable for cryopreservation.

Although the level of formation of high-grade embryos

from donor cows (34.7%) and donor heifers (27.7%) does not differ significantly, the calculation of embryoproduction per head showed the difference – 4.17 at donor cows and only 1.67 at donor heifers (Table 2). So, as from donor cows we receive 2,5 times more valuable embryos than from heifers, further work on the obtaining and cryopreservation of embryos of Grey Ukrainian breed should be carried out using donor cows.

Totally after cryopreservation into the Bank of Animal Genetic Resources were laid 30 embryos, of which 10 morulas and 20 blastocysts of good and excellent quality.

The rest of obtained embryos and oocytes were used for cytogenetic analysis (42 ones) and genotyping (18 ones) by PCR-analysis (**Dobryanska**., 2012). These studies were carried out to analyze the reasons of embryonic development stop, evaluation of early embryogenesis, the finding out of embryos' sex.

It was found that the termination of embryos' development at certain stage of embryogenesis is linked to lack of energy resources in maternal cell that may be caused by poliovulation or unsatisfactory management conditions.

The sex of embryos was determined, using PCR-analysis. It was found that 67 % of embryos are females, that allow to predict, that among 30 laid to the Bank embryos about 20 ones are heifers and ten – bulls.

CONCLUSION

Complex application of genetic-and-biotechnological approaches, including embryo transfer and PCR analysis of their gender, reduces costs when use sexed sperm and provides genetic sperm quality control stated in the documentation by the manufacturer. Using biotechnology techniques, to the Bank of Animal Genetic Resources was laid 30 viable embryos of Grey Ukrainian cattle, which belongs to the national gene pool object that is on the verge of extinction. The effectiveness of the implementation of biotechnological methods into animal breeding depends on the volume of their practical realization at complex system of rational use of valuable genetic potential of animals. The methods of biotechnology, based on a set of works on embryo transfer, cytogenetic and molecular genetic studies are currently being implemented effectively into the selection and breeding work and is part of the overall work package to ensure successful realization of the tasks of the National Project "Recovered cattle-breeding" in Ukraine.

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S. Kovtun, OK. Shcherbak, A. Zyuzyun, N. Rieznykova, A. Duvanov, A. Osypchuk
Institute of Animals Breeding and Genetics of the
National Academy of Agrarian Sciences of Ukraine (NAAN)
Pogrebnjaka, 1, v. Chubinsky, Boryspil District, Kyiv Region, Ukraine, 08321

SUMMARY

In the research there were proved the efficiency of modern biotechnological methods application for the conservation of gene pool and improving of the profitability of cattle-breeding. There were combined the sexed sperm artificial insemination method with transfer of Holstein bovine embryos in "Agro-Soyuz" cattle farm. It was found that embryo forming level after using of sexed semen for insemination of donor cows did not differ significantly compared with the use of non-sexed semen. But at using of sexed semen for insemination of cows there were detected much lower level (P <0.05, the criterion $\chi 2$) of suitable for embryo transplantation embryo forming (55.6%, 10 of 18 embryos) when compared with non-sexed semen (87.5 %, 23 of 32 embryos). For the last years we got and transplanted 211 sexed embryos at the farm "Agro-Soyuz". Our DNA research of Holstein degenerated embryos, using PCR-analysis, showed that all researched embryos were female's.

Embryo transfer was used at small Grey Ukrainian cattle keeping farm for more effective expanded use of valuable adaptive genetic potential of this breed, animals of which are used longer and are healthier, than other breeds.

Key words: *biotechnological methods, genetic resources, sexed semen of bulls, bovine embryo transfer*

E-mail: kovtun_si@i.ua