Rocz. Nauk. Zoot., Vol. 49, no. 2 (2022) 91–102

THE IMPORTANCE OF CRYOBANKS OF CELL LINES FOR THE PROGRAMMES AIMED TO MAINTAIN THE BIOLOGICAL DIVERSITY OF FARM MAMMALIAN AND AVIAN SPECIES

Marcin Samiec*, **Monika Trzcińska***

National Research Institute of Animal Production
Department of Reproductive Biotechnology and Cryopreservation,
32-083 Balice near Krakow
E-mail: marcin.samiec@iz.edu.pl; E-mail: monika.trzcinska@iz.edu.pl

* Marcin Samiec and Monika Trzcińska contributed equally to this article.
Marcin Samiec ORCID: 0000-0002-4060-1893
Monika Trzcińska ORCID: 0000-0001-9758-1304

This study received funding from the research task No. 04-19-13-11 as part of the Own Research Fund of the National Research Institute of Animal Production (NRIAP).

Abstract

The current article seeks to present not only the state of the art, but also agricultural, biological and biotechnological determinants, and the importance of the National Research Institute of Animal Production (NRIAP) for creating the biorepositories of cryopreserved somatic cell lines and blastoderm-derived embryonic stem cell lines of the selected farm mammalian and avian species. The aforementioned biological repositories serve as an innovative research tool used for cryogenically-assisted and species-specific ex situ conservation. The latter ensures, in the long term, not only restitution, but also maintenance of sustainable biodiversity that underlies genotypic and phenotypic, intra- and inter-population variability within pure-breeding herds (nucleus holdings) of the national conservative livestock breeds.

Key words: biorepositories, cell lines, somatic cells, embryonic stem cells, farmed mammals and birds

Introduction

A rapid or progressive decline in the number of individuals in national subpopulations of conservation breeds of selected livestock species (sheep, goats, cattle, pigs, ducks) weakens the phenomenon of genetic drift between subpopulations of rare native breeds, as well as genetic erosion leading to lower levels of intra-population and inter-individual genotypic variation (Woelders et al., 2012; Bolton et al., 2022; Sun et al., 2022). In contrast, a relatively or extremely low degree of genetic variation in maintained subpopulations of conservation breeds of selected livestock species leads to a drastic weakening of intra- and inter-population biodiversity, which qualifies these conservation breeds to the group of rare or endangered breeds (Rege and Gibson, 2003; Blackburn, 2006). For the reasons mentioned above, an important direction of activities undertaken by the National Research Institute of Animal Production (NRIAP) in Balice is to restore and stabilize genetic biodiversity in endangered

subpopulations of indigenous breeds of selected species of livestock through the restoration of nucleus herds and increasing the number of individuals in specific subpopulations of these breeds through strategic and innovative solutions of reproductive biotechnology of farm animals and embryology applied to agricultural practice (Trzcińska and Samiec, 2021).
The tangible benefit of such activities is the sustainable preservation of *ex situ* biotechnological conservation and increased levels of genotypic biodiversity of rare, native conservation breeds of selected livestock species through comprehensive implementation of solutions in the field of assisted animal reproductive technology and embryonic genetic engineering (Samiec and Trzcinska, 2022).

Therefore, the overarching goal of this research paper is to present a wide spectrum of application possibilities of research undertaken by the NRIAP in the field of creating biorepositories in the form of cryopreserved somatic cell lines and embryonic stem cells of blastodermal origin of selected species of farmed mammals and birds.

Application of novel assisted reproductive technologies and genomic embryo engineering in the conservation of genetic resources of national conservation breeds of selected farm animal species

The priority direction of research conducted at the NRIAP is the creation of genetic reserves in the form of cryogenically protected lines of somatic cells and embryonic stem cells (derived from the blastoderm of germinal discs/embryo discs of fertilized avian eggs) for the restoration and genotypic/phenotypic stabilization of biodiversity among the few subpopulations of rare, native conservation breeds. The latter include selected species of farmed mammals and birds, such as the Heath sheep, Romanov sheep, old-type Merino, Carpathian goat, Polish Red cattle, Puławska pig, Złotnicka Spotted pig, K-2 mini duck, domestic Pekin P-33, English Pekin – synthetic line Ls-A, Danish Pekin P-8, French Pekin P-9, hybrid Kh0-01. This strategic model of research work is being implemented in the agricultural and agrobiotechnology sector through solutions in the field of innovative assisted reproduction technologies and embryonic genetic engineering of farm animals, which have been comprehensively developed at the NRIAP. The aforementioned activities in reproductive biotechnology and applied embryology include *in vitro* embryo production (IVP) based on research procedures such as somatic cloning, as well as *in vitro* fertilization (IVF) by gamete co-incubation and intracytoplasmic sperm injection (ICSI), or microinjection of exogenous DNA or RNA into fertilized oocytes (zygotes) (Mara et al., 2013; Kikuchi et al., 2016; Ryder and Onuma, 2018). The aforementioned modern instruments of livestock reproductive biotechnology can be implemented: 1) in programmes for the restoration of endangered nucleus herds, indigenous breeds of these animals, as well as 2) in programmes to restore the and stabilize genetic biodiversity in agricultural ecosystems, whose niches are the reservoir and habitat of restored subpopulations of endemic breeds of selected livestock species (Caroli and Pizzi, 2012; Smits et al., 2012; Hu et al., 2022).

Development of efficient methods for obtaining and cryogenically preserving biological materials in the form of somatic cell lines is a *sine qua non* condition not only for the establishment of genetic reserves of selected species and breeds of livestock, but also the subsequent use of these biological materials in innovative assisted animal reproduction technologies (ARTs). The latter involve cloning by somatic cell nuclear transfer (SCNT), *in vitro* fertilization by IVF and ICSI techniques, as well as embryonic genome engineering procedures such as intranuclear or intracytoplasmic microinjection of gene constructs into zygotes (Men et al., 2012; Enya et al., 2016; Gavin-Plagne et al., 2020). Among the potential benefits underlying the restoration and stabilization of biodiversity and the increased frequency of genetic drift between national subpopulations of rare and vanishing conservation breeds – through methods of reproductive biotechnology and applied embryology – will be to ensure the

sustainable conservation of valuable genetic resources in the form of endemic breeds of livestock (Liu et al., 2008; Chen et al., 2018; Dua et al., 2021; Soglia et al., 2021). Such biotechnological genotype-phenotype conservation *ex situ* of endangered breeds of farmed mammals and birds also falls within the scope of sustainable conservation of the habitat of farm animals in agricultural ecosystems, as well as the long-term preservation and stable maintenance of the dynamic ecological balance between the natural environment and anthropogenic ecosystems of farms (Bai et al., 2011; León-Quinto et al., 2014; Silyukova et al., 2020; Elyasi Gorji et al., 2021; Son et al., 2021; Bolton et al., 2022).

Generating biological repositories in the form of cryogenically protected cell lines for agricultural and reproductive biotechnology of selected species of farmed mammals and birds

The NRIAP has successfully initiated scientific research activities in the operation of biological material repositories in the form of cryogenically preserved stocks of *in vitro* cultured somatic cells and embryonic stem cells from endangered domestic breeds of sheep, goats, cattle and pigs, and from domestic nucleus herds of various breeds of domestic ducks, respectively. The effective operation of NRIAP's biorepositories, with *ex situ* deposited, cryopreserved primary cultures and somatic cell lines of vanishing, endemic breeds of livestock, including representatives of small and large ruminants, i.e. sheep, goats and cattle, as well as pigs and domestic ducks, respectively, will expand the application potential of *ex situ* conservation activities of secured genetic reserve collections (Li et al., 2009a; Liu et al., 2014; Ryder and Onuma, 2018; Elyasi Gorji et al., 2021). These activities can be targeted in the future to develop programmes to restore endangered herds of farm animals. These programmes can be effectively implemented through the use of accumulated somatic cell biorepositories for assisted mammalian reproductive technologies such as interspecies somatic cloning in intra- and interbreed variants, as well as interspecies somatic cloning – as a reproductive biotechnology tool, offering a last chance to restore a breed to the agricultural ecosystem (Li et al., 2009b; Zhang et al., 2012; Hu et al., 2013; Yang et al., 2016).

To date, the following have been derived from tissue bioptates of the outer ear (explants of the dermo-integumentary tissue of the auricles), and then cryogenically preserved stable, in terms of division activity:

1) primary cultures of dermal fibroblast cells, derived from 6 juveniles of the Heath sheep breed (3 donor ewes and 3 donor rams of biological material) – in total, genetic resources in the form of 15 cellular subpopulations were collected within the biorepository dedicated to Heath sheep;
2) clonal lines of dermal fibroblast cells, derived from 6 juveniles of the Romanov sheep breed (3 donor ewes and 3 donor rams of biological material) – in total, genetic resources in the form of 32 cellular subpopulations were collected within the biorepository dedicated to Romanov sheep;
3) clonal lines of dermal fibroblast cells, derived from 6 juveniles of old-type Merino sheep (3 donor ewes and 3 donor rams of biological material) – in total, genetic resources in the form of 22 cellular subpopulations were collected within the biorepository dedicated to old-type Merino sheep;
4) primary cultures of dermal fibroblast cells, derived from 6 juveniles of the Carpathian goat breed (3 donor ewes and 3 donor goats of biological material) – in total, genetic resources in the form of 13 cellular subpopulations were collected within the biorepository dedicated to Carpathian goats;
5) clonal lines of dermal fibroblast cells, derived from reproductively mature individuals of Polish Red cattle (5 donor cows of biological material) – in total, genetic resources in the form

of 45 cellular subpopulations were collected within the biorepository dedicated to Polish Red cattle;
6) clonal lines of dermal fibroblast cells, derived from 11 reproductively mature individuals of the Puławska pig breed (10 donor sows and 1 donor boar of biological material) – in total, genetic resources in the form of 71 cellular subpopulations were collected within the biorepository dedicated to Puławska pigs.
7) clonal lines of dermal fibroblast cells, derived from reproductively mature individuals of the Złotnicka Spotted pig breed (6 donor sows of biological material) – in total, genetic resources in the form of 22 cellular subpopulations were collected within the biorepository dedicated to Złotnicka Spotted pigs.

All subpopulations of dermal fibroblasts derived from auricular explants, regardless of species, breed, individual (ontogenetic) origin, as well as the criteria of age range age and biological (genetic) sex, were characterized by high survival rate and cellular adhesion capacity to the collagen substrate of the culture dishes. In addition, a notable feature of *in vitro* cultured fibroblast cells derived from the individuals of the aforementioned breeds of sheep, goats, cattle and pigs was a high proliferative potential. This potential was reflected not only in the dynamic and stable kinetics of mitotic divisions, but also in the rapid synchronization of the mitotic cycle of cells in the G1/G0 phases due to contact inhibition of their migration and cleavage activity after reaching full confluence, i.e. a state in which the density of cultured cells limits their proliferative growth.

Table 1. Specifying the cryogenically preserved biological material in the form of somatic cell subpopulations deposited in the biorepositories collected within the framework of the National Research Institute of Animal Production in Poland

Species	**Breed**	**The type and origin of *in vitro*-proliferated somatic cells**	**Number of cryopreserved cell subpopulations**	**Number of female donors of tissue bioptates**	**Number of male donors of tissue bioptates**
Cattle	Polish Red	Mitotically stable primary cultures and/or clonal lines of fibroblast cells derived from dermo-integumentary system	45	5	-
Pigs	Puławska		71	10	1
	Złotnicka Spotted		22	6	-
Sheep	Polish Heath		15	3	3
	Romanov		32	3	3
	Old-type Merino		22	3	3
Goats	Carpathian		13	3	3

Activities aimed at launching an organizational structure and developing a biotechnology workshop using techniques of derivation and cryogenic preservation of stocks of *in vitro* cultured somatic cells and embryonic stem cells, derived from endangered domestic breeds of farmed mammals and birds have served or will serve to create effectively functioning NRIAP biorepositories that are unique on a national and pan-European scale. Within these biological repositories, the following genetic reserves have been collected in the form of *ex situ* deposited, cryopreserved primary cultures and/or dermal fibroblast lines, derived from sheep of such conservation breeds as Heath, Romanov and old-type Merino, from Carpathian goats and

Polish Red cattle, as well as dermal fibroblast lines derived from endangered pigs of the Puławska and Złotnicka Spotted breeds (Table 1). The organization and maintenance of the infrastructural and technological base of the NRIAP biorepositories, with cryogenically secured somatic cell resources, is an excellent tool for *ex situ* biotechnological conservation and genetic rescue of vanishing, endemic breeds of breeding animals for the preservation of sustainable and stable biodiversity in anthropogenic agricultural ecosystems.

In addition, at this stage, research is underway to create biorepositories dedicated to breeding birds in the form of cryogenically preserved embryonic stem cell (ESC) lines, derived from blastodermal embryonic discs isolated from eggs laid approximately 24 hours after fertilization. To this end, activities are being implemented with a view to selecting female donors of fertilized eggs from among the female individuals forming nucleus flocks of the following breeds of domestic duck, covered by genetic resources conservation: domestic Pekin P-33 and/or English Pekin – synthetic line Ls-A and/or Danish Pekin P-8 and/or French Pekin P-9 and/or hybrid Kh0-01 and/or K2 mini duck. The fertilized eggs of breeding birds provide a source of embryonic disc cells (blastoderm), which in the poly- and telolecithal eggs of birds lead – as a result of incomplete (meroblastic) cleavage – to the formation, within the egg animal pole, of embryos in the blastula/discoblastula or gastrula stages. From the isolated blastoderm cells, stable lines of embryonic stem cells (primary embryonic cells) are derived. Research aimed at creating biological repositories dedicated to breeding birds will form the basis for further work in the fields of agricultural and reproductive biotechnology, *ex situ* conservation of endangered and rare conservation breeds of selected species of poultry and interdisciplinary research at the interface of biomedicine, transgenics and molecular biology and biotechnology (Wu et al., 2008; Guan et al., 2010; Na et al., 2010; Bai et al., 2013; Nandi et al., 2016; Svoradová et al., 2018; Xiong et al., 2020).

Final conclusions and future research directions

The measurable effect of the implementation of scientific research activities of the NRIAP undertaken to generate cryogenic repositories of biological material in the form of subpopulations of somatic cells is a strategic tool of modern agricultural and reproductive biotechnology and cell culture engineering, which is currently applied and/or may find application in the near future in procedures for:

1) conservation of genetic resources and creation of genetic reserves of endangered, native breeds of selected species of farmed mammals and birds (Men et al., 2012; Woelders et al., 2012; Ryder and Onuma, 2018; Gavin-Plagne et al., 2020);

2) restoration and multiplication of subpopulations of extinct, vanishing and rare breeds of livestock in order to preserve biodiversity and to increase the degree of intrapopulation and inter-individual genetic variability (Rege and Gibson, 2003; Soglia et al., 2021; Bolton et al., 2022);

3) improving indicators of breeding and performance value of livestock, including increasing their milk, meat and reproductive performance (Hoshino et al., 2009; Smits et al., 2012; Woodcock et al., 2019; Dua et al., 2021);

4) translation of development results into implementations in various sub-disciplines of the agrobiotechnology sector, which aim to create animal bioproducts for the agri-food and biopharmaceutical industries, as well as veterinary, transplantation and regenerative medicine of farm animal tissues, as well as for preclinical and clinical testing in therapies for genetically determined or acquired diseases of farm animals (Caroli and Pizzi, 2012; Hu et al., 2012; Woodcock et al., 2019; Rieblinger et al., 2021).

References

Bai C., Wang D., Li C., Jin D., Li C., Guan W., Ma Y. (2011). Establishment and biological characteristics of a Jingning chicken embryonic fibroblast bank. Eur. J. Histochem., 55 (1): e4.

Bai C., Li X., Hou L., Zhang M., Guan W., Ma Y. (2013). Biological characterization of chicken mesenchymal stem/progenitor cells from umbilical cord Wharton's jelly. Mole. Cell Biochem., 376 (1-2): 95–102.

Blackburn H.D. (2006). The National Animal Germplasm Program: challenges and opportunities for poultry genetic resources. Poult. Sci., 85 (2): 210–215.

Bolton R.L., Mooney A., Pettit M.T., Bolton A.E., Morgan L., Drake G.J., Appeltant R., Walker S.L., Gillis J.D., Hvilsom C. (2022). Resurrecting biodiversity: advanced assisted reproductive technologies and biobanking. Reprod. Fertil., 3 (3): R121–R146.

Caroli A.M., Pizzi F. (2012). Livestock biodiversity: from genes to animal products through safeguard actions. Theor. Biol. Forum., 105 (2): 71–82.

Chen F., Zhao C., Zhao Y., Li L., Liu S., Zhu Z., Guan W. (2018). The biological characteristics of sheep umbilical cord mesenchymal stem cells. Can. J. Vet. Res., 82 (3): 216–224.

Dua S., Sharma P., Saini M., Rawat N., Rajendran R., Bansal S., Wakil A.M., Beniwal M., Parashar A., Bajwa K.K., Selokar N.L., Kumar R., Kumar D., Yadav P.S. (2021). Cryobanking of primary somatic cells of elite farm animals – A pilot study in domesticated water buffalo (*Bubalus bubalis*). Cryobiology, 98: 139–145.

Elyasi Gorji Z., Farzaneh P., Nasimian A., Ganjibakhsh M., Izadpanah M., Farghadan M., Vakhshiteh F., Rahmati H., Shahzadeh Fazeli S.A., Khaledi H., Daneshvar Amoli A. (2021). Cryopreservation of Iranian Markhoz goat fibroblast cells as an endangered national genetic resource. Mole. Biol. Rep., 48 (9): 6241–6248.

Enya S., Kawarasaki T., Otake M., Kangawa A., Uenishi H., Mikawa S., Nishimura T., Kuwahawa Y., Shibata M. (2016). Preservation and reproduction of microminipigs by cloning technology. In Vivo, 30 (5): 617–622.

Gavin-Plagne L., Perold F., Osteil P., Voisin S., Moreira S.C., Combourieu Q., Saïdou V., Mure M., Louis G., Baudot A., Buff S., Joly T., Afanassieff M. (2020). Insights into species preservation: Cryobanking of rabbit somatic and pluripotent stem cells. Int. J. Mol. Sci. 21 (19): 7285.

Guan W., He X., Li L., Liang H., Zhao Q., Pu Y., Ma Y.H. (2010). Establishment and biological characterization of fibroblast cell line from the Langshan chicken. Cell Prolif., 43 (2): 157–163.

Hoshino Y., Hayashi N., Taniguchi S., Kobayashi N., Sakai K., Otani T., Iritani A., Saeki K. (2009). Resurrection of a bull by cloning from organs frozen without cryoprotectant in a -80 degrees c freezer for a decade. PLoS One, 4 (1): e4142.

Hu P.F., Guan W.J., Li X.C., Ma Y.H. (2012). Construction of recombinant proteins for reprogramming of endangered Luxi cattle fibroblast cells. Mo.l Biol. Rep., 39 (6): 7175–7182.

Hu P.F., Guan W.J., Li X.C., Zhang W.X., Li C.L., Ma Y.H. (2013). Study on characteristics of in vitro culture and intracellular transduction of exogenous proteins in fibroblast cell line of *Liaoning cashmere goat.* Mole. Biol. Rep., 40 (1): 327–336.

Hu T., Taylor L., Sherman A., Keambou Tiambo C., Kemp S.J., Whitelaw B., Hawken R.J., Djikeng A., McGrew M.J. (2022). A low-tech, cost-effective and efficient method for safeguarding genetic diversity by direct cryopreservation of poultry embryonic reproductive cells. eLife, 25 (11): e74036.

Kikuchi K., Kaneko H., Nakai M., Somfai T., Kashiwazaki N., Nagai T. (2016). Contribution of in vitro systems to preservation and utilization of *porcine genetic resources.* Theriogenology, 86 (1): 170–175.

León-Quinto T., Simón M.A., Cadenas R., Martínez A., Serna A. (2014). Different cryopreservation requirements in foetal versus adult skin cells from an endangered mammal, the Iberian lynx (*Lynx pardinus*). Cryobiology, 68 (2): 227–233.

Li L.F., Guan W.J., Li H., Zhou X.Z., Bai X.J., Ma Y.H. (2009a). Establishment and characterization of a fibroblast cell line derived from Texel sheep. Biochem. Cell Biol., 87 (3): 485–492.

Li L.F., Guan W.J., Hua Y., Bai X.J., Ma Y.H. (2009b). Establishment and characterization of a fibroblast cell line from the Mongolian horse. In Vitro Cell. Dev. Biol. Anim., 45 (7): 311–316.

Liu C., Guo Y., Guan W., Ma Y., Zhang H.H., Tang X. (2008). Establishment and biological characteristics of Luxi cattle fibroblast bank. Tissue Cell, 40 (6): 417–424.

Liu C., Guo Y., Lu T., Li X., Guan W., Ma Y. (2014). Establishment and genetic characteristics analysis of *in vitro* culture a fibroblast cell line derived from Wuzhishan miniature pig. Cryobiology, 68 (2): 281–287.

Mara L., Casu S., Carta A., Dattena M. (2013). Cryobanking of farm animal gametes and embryos as a means of conserving livestock genetics. Anim. Reprod. Sci., 138 (1-2): 25–38.

Men H., Walters E.M., Nagashima H., Prather R.S. (2012). Emerging applications of sperm, embryo and somatic cell cryopreservation in maintenance, relocation and rederivation of swine genetics. Theriogenology, 78 (8): 1720–1729.

Na R.S., Bai C.Y., Jin D.P., Su X.H., Feng B.G., Guan W.J., Ma Y.H. (2010). Establishment and biological characteristics of Qingyuan partridge chicken fibroblast line. Poult. Sci., 89 (6): 1207–1216.

Nandi S., Whyte J., Taylor L., Sherman A., Nair V., Kaiser P., McGrew M.J. (2016). Cryopreservation of specialized chicken lines using cultured primordial germ cells. Poult. Sci. 95 (8): 1905–1911.

Rege J.E.O., Gibson J.P. (2003). Animal genetic resources and economic development: issues in relation to economic valuation. Ecol. Econ., 45 (3): 319–330.

Rieblinger B., Sid H., Duda D., Bozoglu T., Klinger R., Schlickenrieder A., Lengyel K., Flisikowski K., Flisikowska T., Simm N., Grodziecki A., Perleberg C., Bähr A., Carrier L., Kurome M., Zakhartchenko V., Kessler B., Wolf E., Kettler L., Luksch H., Hagag I.T., Wise D., Kaufman J., Kaufer . BB., Kupatt C., Schnieke A., Schusser B. (2021). Cas9-expressing chickens and pigs as resources for genome editing in livestock. Proc. Natl. Acad. Sci. USA, 118 (10): e2022562118.

Ryder O.A., Onuma M. (2018). Viable Cell Culture Banking for Biodiversity Characterization and Conservation. Annu. Rev. Anim. Biosci., 6: 83–98.

Samiec M., Trzcińska M. (2022). Perpetuating biological diversity in threatened indigenous livestock breeds by creating biorepositories for the purpose of assisted reproductive technologies. Anim. Sci. Reuters. Rep. 40 (4), in press.

Silyukova Y.L., Stanishevskaya O.I., Dementieva N.V. (2020). The current state of the problem of *in vitro* gene pool preservation in poultry. Vavilovskii Zhurnal Genet. Selektsii, 24 (2): 176–184.

Smits K., Hoogewijs M., Woelders H., Daels P., Van Soom A. (2012). Breeding or assisted reproduction? Relevance of the horse model applied to the conservation of endangered equids. Reprod. Domest. Anim., 47 (4): 239–248.

Soglia D., Sartore S., Lasagna E., Castellini C., Cendron F., Perini F., Cassandro M., Marzoni M., Iaffaldano N., Buccioni A., Dabbou S., Castillo A., Maione S., Bianchi C., Profiti M., Sacchi P., Cerolini S., Schiavone A. (2021). Genetic diversity of 17 autochthonous Italian chicken breeds and their extinction risk status. Front. Genet., 14 (12) :715656.

Son Y.B., Jeong Y.I., Jeong Y.W., Yu X., Cai L., Choi E.J., Hossein M.S., Tinson A., Singh K.K., Rajesh S., Noura A.S., Hwang WS. (2021). Vitrification of camel skin tissue for use as a

resource for somatic cell nuclear transfer in *Camelus dromedarius*. In Vitro Cell. Dev. Biol. Anim., 57 (5): 487–492.

Sun Y., Li Y., Zong Y., Mehaisen G.M.K., Chen J. (2022). Poultry genetic heritage cryopreservation and reconstruction: advancement and future challenges. J. Anim. Sci. Biotechnol., 13 (1): 115.

Svoradová A., Kuželová L., Vašíček J., Olexíková L., Chrenek P. (2018). Cryopreservation of chicken blastodermal cells and their quality assessment by flow cytometry and transmission electron microscopy. Biotechnol. Threshold 34 (3): 778–783.

Trzcińska M., Samiec M. (2021). *Ex situ* conservation and genetic rescue of endangered Polish cattle and pig breeds with the aid of modern reproductive biotechnology. Ann. Anim. Sci., 21 (4): 1193–1207.

Woelders H., Windig J., Hiemstra SJ. (2012). How developments in cryobiology, reproductive technologies and conservation genomics could shape gene banking strategies for (farm) animals. Reprod. Domest. Anim., 47 (4): 264–273.

Woodcock M.E., Gheyas A.A., Mason A.S., Nandi S., Taylor L., Sherman A., Smith J., Burt D.W., Hawken R., McGrew M.J. (2019). Reviving rare chicken breeds using genetically engineered sterility in surrogate host birds. Proc. Natl. Acad. Sci., 116 (42): 20930–20937.

Wu H., Guan W., Li H., Ma Y. (2008). Establishment and characteristics of white ear lobe chicken embryo fibroblast line and expression of six fluorescent proteins in the cells. Cell Biol. Int., 32 (12): 1478–1485.

Xiong C., Wang M., Ling W., Xie D., Chu X., Li Y., Huang Y., Li T., Otieno E., Qiu X., Xiao X. (2020). Advances in isolation and culture of chicken embryonic stem cells *in vitro*. Cell. Reprogram., 22 (2): 43–54.

Yang J., Zhao Q., Wang K., Liu H., Ma C., Huang H., Liu Y. (2016). Isolation and biological characterization of tendon-derived stem cells from fetal bovine. In Vitro Cell Dev. Biol. Anim., 52 (8): 846–856.

Zhang Y.L., Liu F.J., Zhuang Y.F., Wang X.A., Zhai X.W., Li H.X., Hong Z.Y., Chen J.J., Zhong L.C., Zhang W.C. (2012). Blastocysts cloned from the Putian Black pig ear tissues frozen without cryoprotectant at –80 and –196 degrees Celsius for 3 yrs. Theriogenology, 78 (5): 1166–1170.

Accepted for printing: 8 December 2022