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Opinion

Cellular Technologies (*In Vitro* Production of Embryos, Germplasm Preservation and Somatic Reprogramming) for Wisent (European Bison) Gene Pool Conservation

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Abstract

Cellular technologies are a novel approach used for the protection of endangered animals, based on advances in cell biology and biotechnology. In vitro production of embryos, germplasm cryopreservation and somatic reprogramming give new perspectives for the protection of the European bison - Wisent (Bison bonasus) considered an endangered species. These biotechniques enable the preservation of germplasm, i.e. genetic material in the form of mature and immature oocytes, spermatozoa, embryos, and also somatic cells, which can be reprogrammed. They can contribute to the program of wisent restitution, particularly in the field of preservation of genetic diversity, providing the reservoir of the gene pool for the future.

Keywords: Wisent - European bison (*Bison bonasus*), IVP, cryopreservation, gametes, embryos, iPSCs

Opinion

European bison, also called wisent (*Bison bonasus*) has a special place in the history of Poland and is considered to be the “king of the forest”, hence it is perceived as a “national treasure”. Wisent, similarly to some other species in the genus such as wood bison, to which wisent is closely related, is listed as “vulnerable” on the IUCN Red List of Threatened Species (www.iucnredlist.org). Basic threats to wisent are:

a) The small population size of this species. This situation threatens the resilience of the population, because of the limited gene pool additionally dominated by the genes of only one pair of founders.

b) The population is fragmented and isolated in herds, which limits the possibilities of preservation of genetic variability within the species, and consequently leads to inbreeding.

c) Inbreeding depression leads to both the reduction of survival and fertility of offspring of related individuals.

d) Inbreeding and specific homogeneity of genetic material result in the risk of health dangers due to various diseases which can eliminate single individuals, and even entire herds. Inbred population is extremely sensitive to the potential of particular pathogen infection.

For this reason, it is important to develop strategies for the protection and conservation of this species. There are numerous efforts described [1], based on modern biotechnology applications that may help gene pool preservation and species reintroduction. In Poland, programs for the protection of wisent, both *in situ* (in populations at liberty) and *ex situ (*in enclosures, reserves and zoological gardens) have been implemented.

There are several ways to protect wisent from the extinction thread and reproductive biotechnology is one of the major ones [2,3]. It concentrates on *in vitro* production of embryos, embryo transfer and cryopreservation of germplasm. Our team's achievement in obtaining a wisent blastocyst in 2017 was as a result of *in vitro* culture of embryos obtained after fertilization of matured oocytes by epididymal sperm [4]. Since then, we routinely produce *in vitro* wisent embryos. Furthermore, achievements in the field of conservation of endangered species [5,6] including wood bison [7-12] allow us to preserve the wisent germplasm, i.e. oocytes, spermatozoa, embryos and somatic cells. Although this strategy has good potential for the conservation of the wisent gene pool, it is restricted by the extremely limited access to germplasm, which is mainly due to the small population size. In addition, germplasm can only be taken outside the wisent reproductive season (October-March), and only from individuals eliminated from the herd. Oocytes and sperm, as well as somatic cells, are isolated *post mortem*, which can negatively affect germplasm quality. In addition, oocytes are collected from hormonally unstimulated females, which may have a negative impact on their competence for maturation and thus for fertilization and embryo development *in vitro*. Oocytes are a key element in the protection of wisent, because of possibility of their verification. Likewise, spermatozoa, which are collected from the epididymis, can be banked, although their quality and competence for fertilization are reduced. Wisent embryos obtained as a result of *in vitro* fertilization, can be easily deposited in the tissue bank, but their developmental competence after thawing is also low.

In addition to the bank of oocytes, spermatozoa and embryos, somatic cells, also primary cultures of granulosa cells, are banked in order to serve as a somatic cells for reprogramming and derivation of induced pluripotent stem cells (iPSCs) [13,14]. The iPSC technology holds great promise for purposes of the reintroduction of genetic material into breeding populations of endangered species. Wisent iPSCs would allow to conserve gene pool of living but non-reproductive or even deceased animals. Thanks to the possibility of iPSC differentiation into any somatic tissue, iPSC are going to become the valuable tool for studying diseases presenting a specific risk to the wisent population, as appropriate research models can be created after cellular reprograming of iPSCs to the tissues being afflicted by the disease. Additionally, iPSCs can be used for artificial gametogenesis as protocols for *in vitro* differentiation of iPSCs into gametes have been described [15].

Overall, all these efforts will help in the progress and development of other rescue tools for endangered species. Described above bio techniques holds great promise for purposes of the reintroduction of genetic material into breeding populations of endangered species. Even if habitat conservation is the cornerstone of all preservation efforts, the cellular technologies provide the tool to rescue and reintroduce genes that would be otherwise lost. In conclusion, these bio techniques can support a program of wisent restitution, particularly preservation of genetic diversity, and a controlled and wide reserve of the gene pool for the future.

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References

1. Saragusty J, Diecke S, Drukker M, Durrant B, Friedrich Ben-Nun I, et al. (2016) Rewinding the Process of Mammalian Extinction. Zoo Biology 35: 280-292.
2. Duszewska AM, Trzeciak P, Compa A, Rąpała Ł (2010) Selected issues concerning biotechnology of farm animals breeding - a review. Animal Science Papers and Reports 28(4): 295-306.
3. Duszewska AM, Rąpała Ł, Trzeciak P, Dąbrowski S, Piliszek A (2012) Obtaining farm animal embryos in vitro. Journal of Animal and Feed Sciences 21: 217–233.
4. Duszewska AM, Gręda P, Baraniewicz M, Bielecki W, Niżański W, et al. (2018) Obtaining wisent early blastocyst *in vitro* is a basic for protection and creation of biodiversity for this threatened species. Reproduction in Domestic Animals. 5(3): 818-821.
5. De Souza JMG, Batista RITP, Melo LM, Freitas VJF (2011) Reproductive biotechnologies applied to the conservation of endangered ruminant - past, present and future. Revista Portuguesa Ciencias Veterinaris 106: 31-38.
6. Silva AR, Lima GL, Peixoto GChX, Souza ALP (2015) Cryopreservation in mammalian conservation biology: current applications and potential utility. Research and Reports in Biodiversity Studies 4: 1-8.
7. Thundathil J, Whiteside D, Shea B, Ludbrook D, Elki B, et al. (2007) Preliminary assessment of reproductive technologies in wood bison (Bison bison athabascae): Implications for preserving genetic diversity. Theriogeneology 68: 93-99.
8. Krishnakumar S, Whiteside DP, Elkin B, Thundathil JC (2015) Efect of reproductive seasonality on gamete quality in the north american bison (Bison bison bison). Reproduction in Domestic Animals 50: 206-215.
9. Cervantes MP, Manuel Palomino J, Anzar M, Mapletoft RJ, Adams GP (2016) In vivo and *in vitro* maturation of oocytes collected from superstimulated wood bison (Bison bison athabascae) during the anovulatory and ovulatory seasons. Animal Reproduction Science 173: 87-96.
10. Cervantes MP, Palomino JM, Anzar M, Mapletoft RJ, Mastromonaco GF, et al. (2017) *In vitro* embryo production in wood bison (Bison bison athabascae) using in vivo matured cumulus-oocyte complexes. Theriogenology 89: 122-130.
11. Barfield J (2019) *In vitro* production of bison embryos. In Jason R. Herrick (Eds.), Comparative Embryo Culture: Methods and Protocols, Methods in Molecular Biology pp. 165-177.
12. Takahashi K, Tanabe K, Ohnuki M, Narita M, Ichisaka T, et al. (2007) Induction of Pluripotent Stem Cells from Adult Human Fibroblasts by Defined Factors. Cell 131: 861-872.

13. Han X, Han J, Ding F, Cao S, Lim SS, et al. (2011) Generation of induced pluripotent stem cells from bovine embryonic fibroblasts cells. Cell Research 21: 1509-1512.

14. Easley CA, Simerly CR, Schatten G (2014) Gamete derivation from embryonic stem cells, induced pluripotent stem cells or somatic cell nuclear transfer-derived embryonic stem cells: state of the art. Reproduction Fertility Development 27: 89-92.

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