



# 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](http://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 reprogramming 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. De-

scribed 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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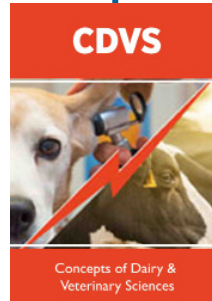
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