Germline chimera production from cryopreserved primordial germ cells of a Hungarian indigenous chicken breed

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The current way of preserving most of the economically important or indigenous chicken breeds is to maintain them in *in situ* populations, which poses numerous risks, such as epidemics (e.g. avian influenza), environmental disasters or inbreeding. Therefore, preservation of the genome itself is a high priority, although it is faced with difficulties. Semen biobanking lacks the ability to conserve the W chromosome and the mitochondrial DNA, and embryo cryopreservation is not yet established. Nowadays, primordial germ cell (PGC) based biobanking is the most promising solution.

In this study, by using the unique characteristics and accessibility of PGCs (they migrate through the vascular system to reach the genital ridges), biobank for an indigenous Hungarian chicken breed – the Partridge colour Hungarian - was established and then tested.

Blood samples were collected from each embryo individually, then the isolated blood, containing the PGCs, was cultured in a PGC selective medium. After that, PGC samples were collected for DNA, RNA isolation and immunohistochemistry to characterize the quality of the cultured lines. As a next step, parallel vials were frozen from each PGC line. The cryopreserved samples have been stored in liquid nitrogen since then. To evaluate the freezing process and to prove the functional integrity and migration ability of PGCs, some of the vials were thawed and the cells were injected into recipient embryos. As a first step, the cells were labelled with an *in vivo* fluorescent dye, thus the migration of the injected cells was followed toward the developing gonads, and the ratio of the colonization was analysed. As a next step, after injection, the eggs were incubated to hatching. The hatched chicks were then kept until maturation and are going to be cross-tested with animals from the donor genotype to examine the germline transmission.

During the study, 21 PGC lines from Partridge colour Hungarian chicken were established with a derivation rate 31.1%. The PGC lines were frozen and then successfully thawed with a cell viability of 50%. The preserved cells were capable of colonizing the gonads of the recipient embryos; furthermore, we have 24 adults (13 roosters and 11 hens) which presumably contain the donor PGCs.

This is the first demonstration of a successful PGC-based cryopreservation project on a Hungarian indigenous breed and the first initiative in Hungary to establish a biobank based on PGCs.

The research was funded by Horizon 2020 (n°677353 IMAGE) and by VEKOP-2.3.2-16-2016-00012.

Keywords: primordial germ cell, cryopreservation, germplasm, biobanking, Hungarian indigenous breed, Partridge colour