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Successful biobanking of an indigenous chicken breed with cryopreserved primordial germ cell lines.
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The economically important or indigenous chicken breeds are held in in situ populations, thus they are exposed to various risks, such as epidemics, environmental disasters or management failure. Therefore, it is increasingly important to protect the genetic information these breeds represent. From the available methods, cryopreservation of primordial germ cells (PGCs) is the most promising one. The goal of the present study was to establish a biobank based on PGCs for the indigenous Hungarian chicken breeds and to test the cryopreserved cells by creating germline chimaeras with one of them; the Partridge color Hungarian. The germline chimaeras were then back-crossed to recover the donor breed. We collected blood from each embryo individually, then the isolated blood, containing the PGCs, were cultured in a medium which was selective for the PGCs. Later, samples from the cell lines were collected for DNA, RNA isolation and immunohistochemistry to characterize the quality of the cells and to perform microsatellite analysis to evaluate the representation of the genetic variability of the original population. As a next step, parallel vials were frozen from each PGC line. To evaluate the freezing process and to prove the functional integrity and migrating ability of PGCs, some of the vials were thawed and the cells were injected into recipient embryos. First, the cells were labeled 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 analyzed. As a second experiment, after injection with the frozen/thawed PG cells, the eggs were left to incubate until 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 color Hungarian chicken were established with a derivation rate of 31.1%. Based on the general diversification indexes which were used for the microsatellite analysis the cell lines represent the genetic variability of the original population. 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 initiative in Hungary to establish a biobank based on PGCs.

Key Words: biobank, cryopreservation, primordial germ cell, germline chimaera