Establishment of a system for cryopreservation of poultry genetic resources using primordial germ cells

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Cryopreservation of germplasm in poultry has been restricted to the use of semen, preventing preservation of the W chromosome and mitochondrial DNA. A further challenge is posed by the structure of their eggs, which prevents the freezing of ova and fertilized eggs, a technique widely used for mammalian species. Primordial germ cell (PGC) are the first germ cell population established during development. A unique biological property of avian PGCs which circulate temporarily in the bloodstream during early development allows the collection and transplantation of PGCs. However, the low efficiencies in each step related to manipulation of PGCs have prevented practical use of PGC-mediated cryobanking. Here, a system for cryopreservation of poultry genetic resources was established through technical development and improvement of PGC manipulation techniques.

Collection of PGCs for cryobanking involves the sacrifice of donor embryos, which have the potential to hatch out. This inconsistency makes them impractical when applied to endangered species. The unique accessibility of avian PGCs during early development provides the opportunity to combine the reproduction of living birds with cryopreservation of PGCs. This prospect was applied to the cryopreservation of Gifujidori fowl, which is a Japanese natural monument. Using valuable 88 fertilized eggs of Gifujidori fowl, both reproduction of 12 living birds with normal reproductive capabilities and cryopreservation of 4562 PGCs were achieved. Moreover, live offspring of Gifujidori fowl originating from frozen-thawed PGCs were successfully regenerated by mating female and male host chickens, with the frequency of 6.0％[1].

PGC-mediated cryobanking requires regeneration of living birds following transplantation of PGCs with high efficiency. A unique drug delivery method was developed for chicken embryos by using their biological property, which remains on the top of the yolk even when the egg is rotated. The principle of this method is as follows: a sustainable emulsion containing drug (busulfan) rises readily when it is injected into the yolk, then makes contact with the chicken embryos, which are lighter than the yolk contents. This method allows the removal of endogenous PGCs at a constant level in a dose-dependent manner. A dose of 100 μg was found to provide the best outcome in terms of preparing host embryos lacking endogenous PGCs, with acceptable hatchability. After transplantation, donor PGCs can migrate toward and repopulate the gonads of sterile host embryos. Of 11 host chickens, 7 produced only donor-derived offspring, suggesting that these produced only donor-derived spermatozoa or ova in the host's gonads [2].

On the basis of the recent technical advances in manipulation of poultry PGCs described above, collection and cryopreservation of PGCs from both industrial and indigenous breeds has started. To date, our cryobanking contains 19 chicken breeds, including nine indigenous breeds that are designated as Japanese natural monuments, and 3 lines of Japanese quail [3].

References

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@article{Nakamura2016PoultryGR, title={Poultry genetic resource conservation using primordial germ cells}, author={Yoshiaki Nakamura}, journal={The Journal of Reproduction and Development}, year={2016}, volume={62}, pages={431 - 437} }. Yoshiaki Nakamura. Published 2016. Biology, Medicine. The Journal of Reproduction and Development.Â However, in situ conservation of poultry genetic resources always carries the risk of loss owing to pathogen outbreaks, genetic problems, breeding cessation, or natural disasters. Cryobanking of germplasm in birds has been limited to the use of semen, preventing conservation of the W chromosome and mitochondrial DNA. A further challenge is posed by the structure of avian eggs, which restricts theâ€¦ Expand. View PDF. Primordial germ cells are single cells that under certain culture conditions can form colonies of cells which morphologically resemble undifferentiated embryonic stem cells (ES cells) (Resnick et al., 1992). These cells can be maintained on feeder layers for extended periods of time and can give rise to embryoid bodies and to multiple differentiated cell phenotypes in monolayer culture and in tumours in nude mice.Â Gonadal colonization is mediated by CXCL12 (previously called SDF1) and its receptor CXCR4 and influenced by CXCR7. In the first few months of gestation, PGCs undergo multiple cycles of mitotic division. In the testis, a self-renewing population of germ cells exists. Production of germline chimeras via primordial germ cells for avian transgenesis. â€œGermline chimeraâ€ usually refers to the presence of mixed gametes from different breeds or species in one individual.Â Cryopreservation of PGCs can enable the preservation of avian genetic resources and restore endangered bird species.Â Prior to the establishment of long-term in vitro PGC culture systems, the major transgenic technology used in birds was based on injecting viruses into EGK stage X embryos.Â In particular, high-risk infectious poultry diseases such as avian influenza and Marekâ€™s disease cause serious problems in various countries and adversely affect the poultry industry. The purpose of cryopreservation is to store cells indefinitely by halting the cellâ€™s metabolism with ultralow temperatures. The freeze-thaw process is stressful to all cells and tissues. Therefore, effective techniques were developed to prevent cell death and damage.Â Rapid cooling may be used for cryopreservation by the vitrification technique. Vitrification is a fast cryopreservation method that avoid ice crystals formation within the cell. Vitrification method is usually successful when cryopreservation solutions contain high concentration of cryoprotective agents and it is used routinely for the cryopreservation of gametes and embryos. Cryoprotectants improve osmotic imbalance and dehydration during slow cooling. Fast Cooling.