

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 Gifuji-dori fowl, which is a Japanese natural monument. Using valuable 88 fertilized eggs of Gifuji-dori fowl, both reproduction of 12 living birds with normal reproductive capabilities and cryopreservation of 4562 PGCs were achieved. Moreover, live offspring of Gifuji-dori 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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