F-3 Development of a new technology to restore and reproduce the threatened animals based on developmental biotechnology.

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This study aims to restore and reproduce the threatened birds by the biotechnological techniques using re-injection of primordial germ cells into the embryos of other host species similar to the technique used to obtain avian germ-line chimeras as in chick.

Firstly, the culture medium for avian embryonic cells (KAv-1 medium; Kuwana's avian 1 medium) was established according to the pH of the embryonic blood. The PGCs proliferated at over hundred times on the chick embryonic feeder cells using KAv-1 medium with cell growth factors for 7days.

Secondary, a method for the purification of PGCs from 2.5-day old quail embryos was developed. And, quail PGCs were shown to be specifically positive for the staining with monoclonal antibodies, SSEA-1 and EMA-1. Furthermore, the condition for efficient cryopreservation of quail PGCs was established.

Thirdly, to save the endemic rare or endangered species of wild animals, it is essential to exploit an inbred strain of experimental animal. In the Japanese quail, the selective breeding data over 50 generations has been analyzed by personal computer.

As a result, the reproductive ability of inbred quails changed as follows: 1) There is no trend in the egg fertility and piping death rate throughout 50 generations. 2) The hatchability declined with the progress of generation. 3) However, the hatchability of L2 quail was improved after the crisis of bottleneck effect happened at 43rd generation. After crossbreeding of H2 and L2 lines, the heterosis effect appeared in the hatchability. As this heterosis recognized only few pairs, the nicking between sire and dam might be important in the inbred lines.

Fourthly, the recovery rate of the cPGCs using a newly developed filtration method was evaluated. Result showed that the average recovery rate of cPGCs from blood collected from an individual embryo and pooled blood was 39.4±6.5 % and 54.4±6.3%, respectively. It was shown that filtration method is the practical means of recovering cPGCs from the blood collected from early chick embryos.

Lastly, In order to develop a novel technology for proliferating wild animal species, a study was carried out to produce male and female individuals derived from primordial germ cells obtained from a single fertilized egg via germline chimeras. Five pairs of chicks obtained by transferring primordial germ cells derived from the single embryos. Mating experiment for examining the germline chimerism of the putative chimeric chickens is going to be performed when they reach sexual maturation.