F-3.2.4 **STUDIES ON IN VITRO CULTIVATION, FREEZING METHOD AND TRANSFER OF AVIAN PRIMORDIAL GERM CELLS**

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Preservation of avian genetic stocks have become important problems in biotechnology. So far, no efficient techniques have been developed for these purposes. In contrast to fertilized mammalian eggs, those of birds are big and contain much yolk, making them very difficult to handle. Primordial germ cells (PGCs) have therefore been considered as a substitute for fertilized eggs for preservation of genetic stocks. **(1) Proliferation of PGCs cultured on stroma cells from the germinal ridge;** Germinal ridges from 5-day-old chick and quail embryos were primary cultured. The used culture medium were Medium 199 supplemented with 10% FBS, human IGF-1, bovine FGF-b, and murine LIF. In 7 experiments, the number of PGCs in chick increased an average of 4.8-fold in 4 days. Intrinsic PGCs in the 5-day embryonic germinal ridge were observed loosely attached to the stroma cells, and they also increased 3.8-fold during culture for 4 days. These results indicate the possibility of application of this culture method for increase of the number of PGCs and producing transgenic bird. **(2) Freezing method of PGCs;** The 24 well culture plate with stroma cells from the germinal ridge in freezing medium were stored in deep freezer (-80°C), and then thawed rapidly. The survival rate of the PGCs of in 5 experiments were tested with trypan blue solution. The survival rate of the PGCs were 60%. **(3) Germ line chimera produced by transfer of cultured chick primordial germ cells;** Intrinsic PGCs in stage 27 (5-day-old) chick and quail embryonic germinal ridges were cultured *in vitro* for further 5 days, and shown to proliferate on stroma cells derived from the germinal ridge. To determine whether these cultured PGCs could form germinal chimera, the PGCs isolated by gentle pipetting, and labeled with PKH26 fluorescent dye, and then transferred to the blood stream of stage 17 (2.5-day-old) chick embryos. After incubation of recipient embryos until they reached stage 28, the thin sections of these embryos observed by fluorescent confocal laser microscopy. The result showed that the labeled donor PGCs had migrated into the germinal ridges in the recipients and divided at least 3-7 times. These results suggest that PGCs that had passed far beyond the migration stage *in vivo* and then been cultured *in vitro* were still able to migrate and proliferate in recipient embryonic gonads.

Key words: Primordial germ cells (PGCs), Chick, Quail, Avian, Genetic stock.