연제 10

High Transmission Rate of Germline Chimerism Using Cultured Primordial Germ Cells in Chickens.

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Abstracts

Although primordial germ cells (PGCs) have been used in the production of germline chimera, efficiency has not been satisfactory. The present study was conducted to improve efficiency of germline chimera production using the cultured gonadal PGCs (gPGCs).

Germline chimeric chickens were produced by transfer of cultured gonadal primordial germ cells from Korean Ogol Chicken (KOC) to White Leghorn (WL). Gonadal PGCs were isolated from KOC embryonic gonads at stage 27 (5.5-day-old) and cultured *in vitro* for 10 days. Approximately 200 gPGCs were injected into the bloodstream through the dorsal aorta of stage 12-13 (2-day-old) recipient embryos from which blood had been withdrawn via the dorsal aorta prior to the injection. Recipient embryos were incubated until hatching.

Germline chimerism of the chickens reaching maturity was examined by mating them with Korean Ogol Chicken. Donor-derived offspring were identified as germline chimeric chickens based on their feather color. The frequency of germline transmission of donor PGCs ranged 1.9~60.7%. There was no difference between both sexes. Therefore, it can be concluded that efficiency of germline chimerism can be improved via using cultured gPGCs.

Introduction

Primordial germ cells (PGCs) have all the genomic information of an individual species. Therefore, PGCs have been considered as a gene transfer vehicle in the production of transgenic chicken. Various attempts have been made to produce germline chimeras by the transfer of PGCs into the host embryos. A limited number of PGCs are present in the embryonic blood, their *in vitro* culture has been found to be difficult. However, a larger number of PGCs can be collected and successfully cultured from developing gonads. It has been shown that, germline chimeric chicken could be produced by transfer of cultured gPGCs to recipient embryo (Chang et al., 1995;1997). In the present study, we could improve efficiency of germline chimerism by transfer of gPGCs culture *in vitro*.

Materials and Methods

Fertilized eggs from KOC stock were incubated at 37.5℃ for 5.5 days. The gPGCs were cultured for 10 days by Chang's method (1995) and transfected with plasmids carrying the chicken interferon-gamma gene by electroporation method. Approximately 200 cultured gPGCs from KOCs were injected into the bloodstream through the dorsal aorta of WL embryos. The hated WL chickens injected with KOC PGCs are referred as WL(KOC). WL(KOC) that survived to sexual maturity were mated with KOC by artificial insemination. The black color indicated that the progenies were derived from donor PGCs(KOC) and the white with small black patches were from the recipient PGCs(WL) when the parent was WL(KOC).

Results and Discussion

From the hatched recipient chickens, 62 males and 84 females were brought up to maturity. These WL(KOC) were mated with KOCs, resulting in 248 germline chimeric offsprings (Table 1). A total of progenies were identified as germline chimeras by black feather color of their progenies. At 4-13 weeks of test period, the frequency of germline transmission of donor gPGCs were 1.9~60.7% (30.7% average) for 13 chimeras. Thus, the donor PGCs were normally developed and differentiated into ova and spermatozoa in chimeric embryos and chickens, despite being surrounded by genetically-different somatic cells.

Table 1. Frequencies of germline chimera progenies

Sex	*No. of individual*	*Test period (week)*	*No. of chickens hatched*	*No. of black chickens (No. death)*	*% of black chickens*
Male	027	13	89	5(2)	5.6
	032	13	84	51(5)	60.7
	001*	4	25	14(2)	56.0
	016	13	102	2	1.9
	017*	13	144	77(14)	53.5
	004	13	16	1	6.3
Female	034	13	45	1	2.2
	040	13	42	2	4.8
	043*	13	64	27(1)	42.2
	044	13	51	1	1.9
	001	13	45	18(1)	40.0
	004	13	32	18(4)	56.3
	008	13	70	31(3)	44.3
Total			809	248(32)	30.7

* N/A : recently not available

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References

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