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Possible Reproduction of Silky via. Transferred Primordial Germ Cells (PGCs)

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Abstract: This experiment aimed to develop a procedure for transferring Primordial Germ Cells (PGCs) from silkie chicken embryos into white leghorn embryos. We also examined the migratory abilities of these transferred silkie chicken PGCs. Fertilized eggs of silky were incubated until Stages 11-17 of embryonic development. Blood was collected from the blood vessels of silkie chicken embryos. PGCs were then isolated from blood cells, labeled with PKH26 and injected into the blood vessels of white leghorn embryos at Stages 11-15. Silkie chicken PGCs were found to migrate to the germinal ridges of white leghorn embryos at Stages 36. The number of PGCs that settled on the left side germinal ridges was higher as compared with that settled on the right side.

Key words: Chicken, silkie, germ line chimera, primordial germ cell, right side, settled

INTRODUCTION

Chicken Primordial Germ Cells (PGCs) are an important tool for rescuing genetic resources and production of transgenic chickens via. germline chimeras. The PGCs move to the embryonic germinal crescent regions at Stage 9 (Hamburger and Hamilton, 1951) circulate in the bloodstream of developing embryos at Stages 12-15 and finally migrate to the germinal ridge where they differentiate into spermatogonia or oogania (Kuwana, 1993). Transfer of PGCs into embryonic blood vessels is a common technique for establishing of germline chimeric chickens (Naito *et al*., 1994; Furuta and Fujihara, 1999; Furuta *et al*., 2001, 2007, 2008, 2009). The rate at which germline chimeric chickens were produced by transfer of slikie chicken PGCs was investigated chimeric rates by progeny test (Naito *et al*.,1994; Furuta *et al*., 2001). Chicken PGCs have also been observed in quail gonads using immunohistochemistry; the migratory ability of PGCs was evaluated by transferring chicken PGCs into quail embryos via. interspecies transfer of PGCs (Ishiguro *et al*., 2009). PGCs are promising tools for conserving of genetic resources in rare domestic chickens and restoring the population of other endangered birds species (Furuta *et al*., 2001). The number of circulating PGCs has been estimated in Stage 13-17 of silkie chicken embryos (Qian *et al*., 2010). However, few studies are available on PGCs migration in germline chimeric chickens.

In this study, it was investigated whether PGC migration was possible by transferring silkie chicken PGCs into developing white leghorn embryos.

MATERIALS AND METHODS

Donor PGCs: Fertilized silkie chicken eggs were supplied by the Hiroshima University Japanese Avian Bioresource Project Research Center. The eggs were incubated until the Stages 11-17 of development. Silkie chicken PGCs were collected from the blood of the embryos. Blood samples were suspended in 100 μL of 3.8% sodium citrate and 900 μL of ACK lysis buffer that containing 150 mM NH_4Cl, 1 mM $KHCO_3$ and 0.001 mM EDTA. The mixture was incubated on ice for 30 min. The samples were centrifuged at 2000 rpm 10 min at 4°C; each pellet was resuspended in 1000 μL ACK lysis buffer, incubated on ice for 15 min and washed twice in PBS (Yamamoto *et al*., 2007). The isolated PGCs were labeled using PKH26 red fluorescent cell linker (Sigma Aldrich, Tokyo, Japan) and suspended in 10 μL Kav-1 medium (Kuwana *et al*., 1996).

Recipient embryos: Fertilized eggs obtained from white leghorn maria line hens (GHEN Corporation, Gifu, Japan) were used as employed as recipient embryos. The eggs were incubated until the Stages 11-15.

Transfer of PGCs: The number of suspended PGCs in 1 μL Kav-1 medium was evaluated. A window of approximately 10 mm in diameter was opened at the sharp end of the each egg. About 4.5 μL of PGCs suspended in Kav-1 medium was injected into bloods vessel of recipient embryos. The windows on the recipient eggs were closed with tape; these eggs were incubated until Stage 36.

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Detection of donor PGCs in recipient gonad: Right and left germinal ridges were removed from the embryos. Labeled donor PGCs were observed in the germinal ridges of recipient embryos by using fluorescent microscope.

RESULTS AND DISCUSSION

On an average, 30.8 purified PGCs were observed for 1 μL ACK buffer (Table 1). The embryo survival rate after 10 days of incubation was 60.0% (Table 1). In total, 9 embryos survived the transfer of silkie PGCs (5 female and 4 males). Introduced labeled silkie chicken PGCs were found in the germinal ridges of white leghorn embryos. On an average (±SE) the numbers of exogenous PGCs in the germinal ridges of female recipient embryos were 5.0±1.64 (right germinal ridge) and 17.8±6.89 (left germinal ridge). In male, the numbers of exogenous PGCs were 5.0±4.17 and 15.5±1.08 for the right and left germinal ridges, respectively (Table 2). In females a statistically significant difference ($p<0.05$) was observed in the number of exogenous PGCs between the right and left germinal ridges of female.

Germline chimeric chickens have been previously produced by the transfer (Naito *et al*., 1994; Furuta and Fujihara, 1999; Furuta *et al*., 1999, 2007, 2008, 2009). In this procedure, blood containing PGCs were collected from the blood vessels of donor embryos. Donor PGCs were isolated and injected into the same vessels of recipient embryos. In general, mortality is found to be high in manipulated embryos population however an embryo survival rate of 60.0% was observed in our study. The number of abnormal embryos in manipulated embryos was higher than that in normal embryos (Fisher and Schoenwolf, 1983).

The PGCs transfer is a key methodology for producing donor-derived avian species it is also a useful tool for conservation of genetic resources (Furuta *et al*., 2001). In this study, the transferred silkie chicken PGCs was found to settle in the germinal ridges.

It was observed abundant settlement of transferred PGCs in the germinal ridges which is in agreement with earlier experiments (Furuta and Fujihara, 1999). The advanced functional development of the left gonad compared with the right gonad may account for this asymmetry. Silkie chicken PGCs was migrated into gonads of developing chicken embryos, there by demonstrating that germline chimeric chickens are useful tools for conserving of genetic resources of rare domestic chicken breeds.

Table 1: Number of transferred PGCs and survived recipient embryos

Number of purified (PGCs/μL)	Number of surviving embryos (%)
30.8	60.0

Table 2: Number of imigrated silky PGCs which is positive in PKH26 on recipient gonad

Sex of recipient embryos	Number of exogenous PGCs on recipient gonads	
	Right	Left
Female	5.0±1.67	17.8±6.89
Male	5.0±4.17	15.5±1.08

CONCLUSION

The present results suggest the possible proliferation of silkie chicken PGCs into the gonads of white leghorn leading to the production of germ line chimeric chicken which could help restore genetic resources.

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