REPRODUCTION
RESEARCH

The reversible developmental unipotency of germ cells in chicken

Jin Gyoung Jung[1,2], Young Mok Lee[1], Jin Nam Kim[2], Tae Min Kim[1], Ji Hye Shin[2], Tae Hyun Kim[2], Jeong Mook Lim[1] and Jae Yong Han[1]

[1]*WCU Biomodulation Major, Department of Agricultural Biotechnology and Research Institute for Agriculture and Life Sciences, Seoul National University, Seoul 151-921, Republic of Korea and* [2]*Avicore Biotechnology Institute, Optifarm Solution Inc., Gunpo 435-824, Gyeonggi-Do, Republic of Korea*

Correspondence should be addressed to J Y Han; Email: jaehan@snu.ac.kr

J G Jung and Y M Lee contributed equally to this work

Abstract

We recently developed bimodal germline chimera production approaches by transfer of primordial germ cells (PGCs) or embryonic germ cells (EGCs) into embryos and by transplantation of spermatogonial stem cells (SSCs) or germline stem cells (GSCs) into adult testes. This study was undertaken to investigate the reversible developmental unipotency of chicken germ cells using our established germline chimera production systems. First, we transferred freshly isolated SSCs from adult testis or *in vitro* cultured GSCs into stage X and stage 14–16 embryos, and we found that these transferred SSCs/GSCs could migrate to the recipient embryonic gonads. Of the 527 embryos that received SSCs or GSCs, 135 yielded hatchlings. Of 17 sexually mature males (35.3%), six were confirmed as germline chimeras through testcross analysis resulting in an average germline transmission efficiency of 1.3%. Second, PGCs/EGCs, germ cells isolated from embryonic gonads were transplanted into adult testes. The EGC transplantation induced germline transmission, whereas the PGC transplantation did not. The germline transmission efficiency was 12.5 fold higher (16.3 vs 1.3%) in EGC transplantation into testis (EGCs to adult testis) than that in SSC/GSC transfer into embryos (testicular germ cells to embryo stage). In conclusion, chicken germ cells from different developmental stages can (de)differentiate into gametes even after the germ cell developmental clock is set back or ahead. Use of germ cell reversible unipotency might improve the efficiency of germ cell-mediated germline transmission.
Reproduction (2010) **139** 113–119

Introduction

An inimitable advantage of avian species as a model animal is an easy accessibility to developing embryos. Taken together with the practical embryo manipulation, avian germ cells can also be isolated and purified from developing embryonic gonads as well as adult chicken. Additionally, we can transfer these avian germ cells into embryo stages or adult reproductive tract, the seminiferous tubules of testis. Such a germ cell manipulation and re-transplantation method into developing embryos would be impossible in the mouse.

In our previous reports, we established germline chimera production systems by transferring primordial germ cells (PGCs) and embryonic germ cells (EGCs) into developing embryos (Chang *et al*. 1987, Park & Han 2000, Han *et al*. 2002, Park *et al*. 2003*a*, 2003*b*). As an alternative approach, a testis-mediated germline transmission system has been also developed (Lee *et al*. 2006). These germ cell-mediated bimodal germline chimera productions have a great advantage in the establishment and maintenance of transgenic lines, but each method has difficulties in terms of cell retrieval, *in vitro* manipulation, and transplantation. Thus, technical innovations utilizing the nature of germ cells are essential to expand the applicability of these methods to the field of agricultural biotechnology.

Deviating from the general concept of germ cell transfer protocols, we attempted either the transfer of adult-derived germ cells into embryos or the transplantation of embryo-derived germ cells into adult reproductive tissue. Such a combination might provide clues to using all of the merits of germ cell-mediated germline transmission systems and might result in a better understanding of the mechanisms of germ cell differentiation and unipotency in various environmental niches. Furthermore, the development of an effective germline transmission system would greatly accelerate transgenic fowl production in order to generate disease-resistant and model animals, and to produce bioactive materials and pharmaceutical drugs.

In this study, we examined the developmental unipotency of chicken germ cells isolated from different

© 2010 Society for Reproduction and Fertility
ISSN 1470–1626 (paper) 1741–7899 (online)
DOI: 10.1530/REP-09-0265
Online version via www.reproduction-online.org
Downloaded from Bioscientifica.com at 03/26/2025 06:52:32PM
via free access

developmental stages using our established germline chimera production systems. As the inducer of germline transmission, PGCs from embryonic gonads, EGCs derived from *in vitro* culture of PGCs, adult testicular cells containing spermatogonial stem cells (SSCs), and *in vitro* cultured GSCs were used. The blastoderm of stage X embryos, the dorsal aorta of stage 14–16 embryos, or adult testes were used as the sites of germ cell transfer.

Results

The morphology and cell surface characteristics of germ cells of different origin were evaluated (Fig. 1). PGCs isolated from 5.5-day-old embryos were clearly discernible among stromal cells by their large size (Fig. 1A), and they were subsequently transformed into colony-forming cells, named EGCs, by *in vitro* culture. The colony-formed EGCs consisted of multiple cell layers and had tight, well-delineated cell boundaries (Fig. 1B). SSCs were derived from 4-week-old chicken testes. SSCs were discernible from other stromal cells by size (Fig. 1C). Germline stem cells (GSCs) were derived from *in vitro* culture of SSCs to form colonies and these cells consisted of multiple cell layers (Fig. 1D). Each germ cell had typical reactivity with germ cell-specific markers. FITC-conjugated lectin-*Solanum tuberosum* agglutinin (STA) was detected in PGCs, EGCs, SSCs, GSCs (Fig. 1E–H), and anti-stage-specific embryonic antigen-1 (SSEA1) antibody was also detected on the cell surface of PGCs, EGCs, SSCs, and GSCs (Fig. 1I–L).

SSC/GSC transfer into developing embryos

To examine the gonadal migration ability of SSCs and GSCs in the developing embryos, we labeled these cells with Paul Karl Horan (PKH) 26 fluorescent dye and transferred into stage X or stage 14–16 embryos. PKH26 fluorescent signals were detected in the 6-day-old gonads of both the recipient embryos, stage X (Fig. 2A) and stage 14–16 (Fig. 2B) indicating that the transferred GSCs and SSCs could localize into the developing embryonic gonads. We also transferred PGCs and EGCs as a positive control and observed the gonadal migration in both EGCs as expected (Fig. 2A and B).

To confirm germline transmission to next generation, SSCs or GSCs were transferred into developing embryos of different stages and a total of 527 injections (402 and 125 for stage X and stage 14–16 embryos respectively) were conducted (Table 1). Of the embryos that received SSCs or GSCs, 135 (25.6%; 67/402 = 16.7% and 68/125 = 54.4% for blastoderm and blood vessel transfers respectively) hatched; 35 of the hatchlings (25.9%), comprising 17 males and 18 females, reached sexual maturity. Only male founders were used for testcross reproduction. Of 17 males (35.3%), six were germline chimeras; three from the SSC transfer to stage X embryos; two from the GSC transfer to stage X embryos; and one from the GSC transfer to stage 14–16 embryos. A total of 913 progenies were produced by testcross of the chimeras (Table 2). The individual efficiency of germline transmission was ranged from 0.5 to 3.8% based on the percentage of donor cell-derived progenies out of the total number of progeny. The transmission

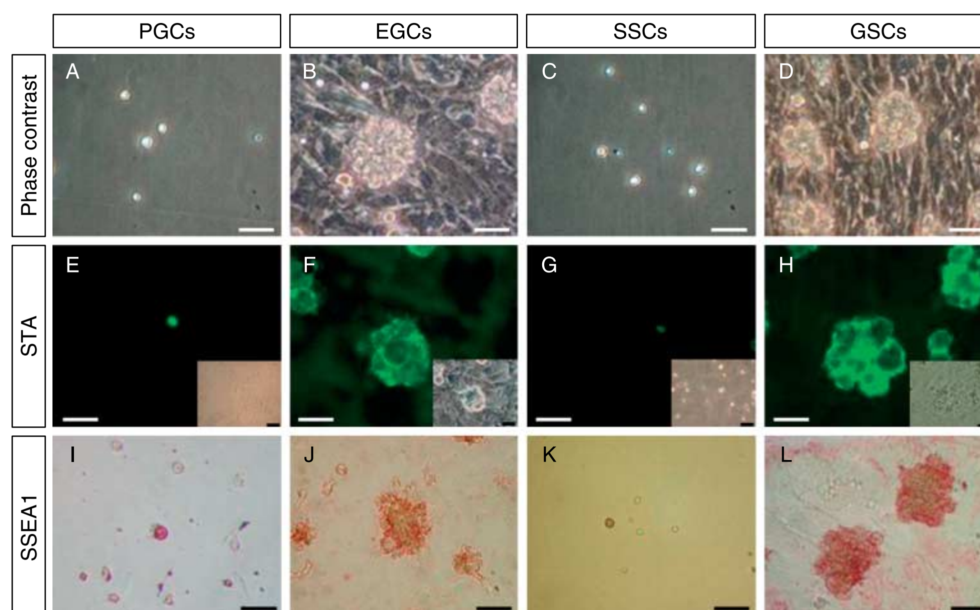

Figure 1 The morphology and cell surface characteristics of chicken embryo- and testis-derived germ cells. Germ cells collected from either testes or embryos were subcultured at least more than five times for 40 days. (A) Freshly isolated primordial germ cells (PGCs), (B) colonized EGCs, (C) freshly isolated spermatogonial stem cells (SSCs), (D) cultured germline stem cells (GSCs). (E)–(H) Immunofluorescent staining with STA on chicken germ cells, and immunohistochemical staining with anti-stage-specific embryonic antigen-1 (SSEA1) antibody on chicken germ cells. Scale bar, 50 µm.

Downloaded from Bioscientifica.com at 03/26/2025 06:52:32PM
via free access

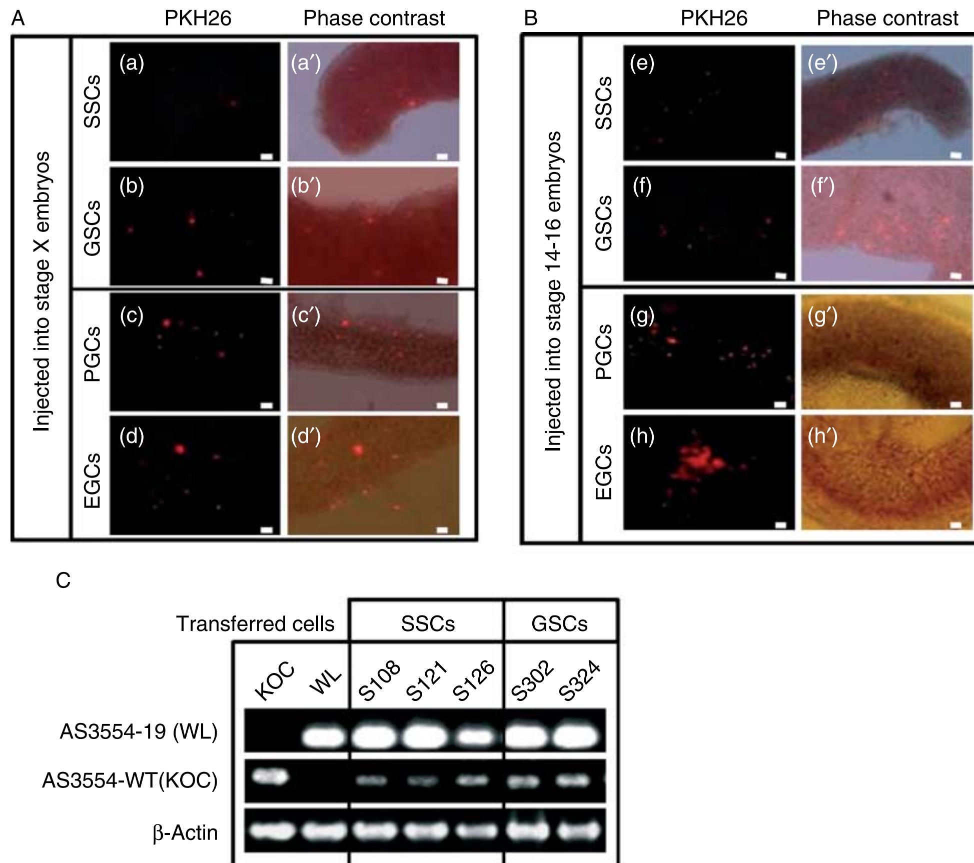

Figure 2 Detection of SSCs and GSCs derived from testicular germ cells after transfer into recipient embryos at stage X (A) or stage 14–16 (B). These SSCs and GSCs were labeled with PKH26 and transferred into the stage X blastoderm or stage 14–16 embryonic blood. To detect gonadal migration, recipient embryos were monitored at day 6. PGC- and EGC-transferred embryos were used as a positive control. (C) PCR analysis of the semen from SSC- and GSC-transferred recipients after sexual maturation was conducted to confirm differentiation of the transferred germ cells into germ cells. Scale bar, 10 μm.

efficiency was not noticeably different between SSC transfer (1.0%=4/414) and GSC transfer (0.8%=4/499). PCR analysis of the semen of the chimeras further confirmed differentiation of donor SSCs/GSCs into sperm in the recipients. We transferred SSCs/GSCs derived from Korean Oge chicken (KOC) into White Leghorn (WL) embryos and so observed KOC-specific fragment in the semen of WL recipients indicating that transferred KOC SSCs and GSCs not only migrated into the embryonic gonads but also differentiated into sperm after sexual maturation (Fig. 2C).

PGC/EGC transfer into adult testes

We also labeled PGCs and EGCs with PKH26 fluorescent dye and transplanted into adult testis to monitor localization of embryo-derived germ cells in the recipient testis. PKH26 fluorescent signals were detected in the membrane of seminiferous tubules of recipient testis 28 days after transplantation (Fig. 3). This result demonstrated that the transplanted PGCs and EGCs could settle down into the basal membrane of seminiferous tubules.

Embryo-derived germ cells were transplanted into adult testes to confirm the germline transmission (Table 1). From 12 transplantations, three sexually mature males (25%) produced donor cell-derived progenies following artificial insemination (Tables 1 and 2). Similar to testis-derived germ cell transfer into embryo stages, PCR analysis detected a mix of donor germ cell-derived spermatozoa in the semen of the sexually mature males (data not shown). Additionally, testcross analysis of EGC-transplanted roosters with wild-type females yielded donor germ cell-derived progenies, whereas the testcross of PGC-transplanted roosters did not produce any donor germ cell-derived chick. The efficiency of germline transmission was 16.3% in the EGC recipients (EG6), which was more than 12.5 times higher than that in SSC/GSC transfer into embryos (1.3%).

Discussion

In this study, germline transmission or participation in heterogeneous gametogenesis was achieved by transferring germ cells retrieved from testicular tissue into

Downloaded from Bioscientifica.com at 03/26/2025 06:52:32PM
via free access

Table 1 Generation of germline chimeras by transfer of chicken germ cells into developing embryos of different ages or adult testes.

Germline chimera production system			Number of					Number of hatchlings	
Origin and type of germ cells	Number of cells transferred	Site of transfer (stage or age)	Recipient eggs or male	Hatchlings from recipient eggs (%)[a]	Sexually matured G_0 founders (%)[b]	Male founders used for testcross	Germline chimeras demonstrated (%)[c]	Produced from chimeras	Derived from donor cells (%)[d]
Testicular germ cells									
SSCs	3×10^3	Blastoderm (stage X)	247	31 (12.6)	7 (22.6)	3	3 (100)	370	4 (1.1)
GSCs	3×10^3	Blastoderm (stage X)	155	36 (23.2)	12 (33.3)	6	2 (33.3)	163	2 (1.2)
SSCs	3×10^3	Bloodstream (14–16)	57	34 (59.7)	4 (11.8)	2	0 (0)	0	0 (0)
GSCs	3×10^3	Bloodstream (14–16)	68	34 (50.0)	12 (35.3)	6	1 (16.7)	75	2 (2.7)
Subtotal (into embryos)			527	135 (25.6)	35 (25.9)	17	6 (35.3)	608	8 (1.3)
Embryonic germ cells									
PGCs	$0.5–1\times10^{7,e}$	Adult testes (24 weeks)	4	–	–	4	0	0	0 (0)
EGCs	$0.5–1\times10^7$	Adult testes (24 weeks)	4	–	–	4	1 (25)	49	8 (16.3)
Subtotal (into testis)			12	–	–	8	1 (12.5)	49	8 (16.3)

SSC, spermatogonial stem cell; GSC, germline stem cell; PGC, primordial germ cell; EGC, embryonic germ cell. Only sexually mature, healthy recipients were used for testcross analysis.
[a]Hatchability of recipient eggs that received donor cells. [b]Percentage of sexually mature G_0 founders among all hatchlings. [c]Percentage of germline chimeric G_0 founders among the sexually mature male recipients. [d]Percentage of hatchlings derived from germline chimeric G_0 founders.
[e]$0.5–1.0\times10^7$ gonadal cells containing $\sim4.1–8.2\times10^4$ PGCs were injected.

developing embryo stages or transplantation of EGCs into adult testes. These results indicated that chicken germ cells have the reversible developmental unipotency that germ cells can adopt themselves to developmentally discrepant environmental niche and (de)differentiate into gametes even after the germ cell developmental clock was reset.

Considering our previous productions of germline chimeras by injecting PGCs/EGCs into developing embryos (Han *et al.* 2002, Park & Han 2000, Park *et al.* 2003*b*, Kim *et al.* 2005) and the induction of germline transmission by the transplantation of testicular cells, including SSCs, into heterogeneous testes (Lee *et al.* 2006) germ cells can induce germline transmission when they are exposed to developmentally suitable gametogenic niches. In mammals, SSCs derived from postnatal males are facilitated under a defined microenvironment *ex vivo* and produced live offspring by germ cell transplantation into mice testes (Brinster & Zimmermann 1994). The involvement of transplanted germ cells in spermatogenesis in host testis has also been reported in primates (Schlatt *et al.* 2002).

In mice, SSCs can dedifferentiate into pluripotent cells after being injected into the blastocyst and can further differentiate into various somatic cell lineages (Guan *et al.* 2006). Considering that stage X chicken embryos are homologous with mammal blastocyst (Lombardi 1999), stage X embryos can provide GSCs an appropriate environment for (de)differentiating into functional gametes. In contrast, PGCs have an innate ability to migrate toward the primitive sex cords, and heterologous germ cells can develop into viable gametes (Naito *et al.* 2001, Tagami *et al.* 2007). To improve germline transmission efficiency by increasing the migrated germ cells in embryonic stages, we need to find out the critical factor(s) or surface marker(s) involved in germ cell migration in developing embryos in future studies.

In this study, there was a discrepancy in the number of germ cells being transplanted in different transfer systems and it would be hard to quantitatively compare the activity of each germ cell to induce germline transmission in different systems. However, in our previous reports (Park *et al.* 2003*a*, 2003*b*, Lee *et al.* 2006, Kim *et al.* 2009), we monitored migration of exogenous germ cells after being transferred into recipients and optimized the injection cell number for either embryo or testis. According to our standardized injection protocol, 3000 SSCs/GSCs were transferred into blood vessel of the recipient embryos at stage 14–16, while $0.5–1.0\times10^7$ gonadal cells/EGCs were injected into testis. Additionally, due to a technical issue, we cannot inject more than 3000 germ cells into recipient embryonic blood vessel at stage 14–16 and 3000 SSCs/GSCs were too low number for injection into testis to confirm germline chimerism. Thus, we transferred the different injection cell numbers according to embryos or testis. We could not produce any germline chimera from PGC injection into testis. Although the reason is unknown, this negative result might result from the suboptimal conditions of transfer such as number or purity of donor germ cells. The other

Downloaded from Bioscientifica.com at 03/26/2025 06:52:32PM
via free access

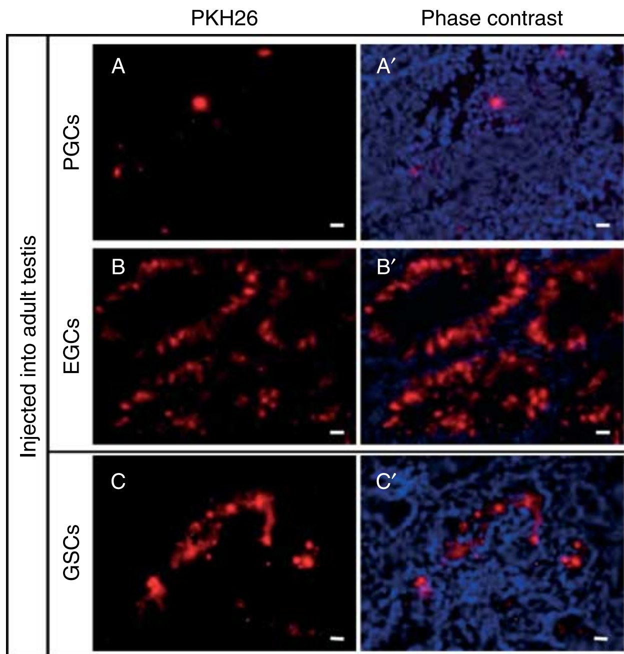

Figure 3 Detection of PGCs and EGCs after transplantation into adult testes. PGCs, EGCs were labeled with PKH-26 and subsequently transplanted into the testes of 24-week-old roosters. GSCs were used as a positive control. On 28 days of germ cell transplantation, localization of transplanted PGC (A), EGCs (B) and GSCs (C) into the seminiferous tubules was monitored under a fluorescence microscope. Scale bar, 10 μm.

possibility is that PGCs freshly isolated and transferred into adult testis could not overcome the developmental gap for (de)differentiation and maturation or actually, the transplanted PGCs were a mixed population retrieved from male and female embryos which probably affected germ cell differentiation in the recipient testis.

Although both the PGC/EGC-to-embryo and SSC/GSC-to-testis germline transmission systems can generate germline chimeras or transgenic animals (van de Lavoir *et al*. 2006, Kalina *et al*. 2007, Shin *et al*. 2008, Han 2009), each possesses different merits. The classical embryo-mediated system is more practical than the testis-mediated system and detailed information is available for the chicken. However, very few donor cells can be retrieved from embryos, and the collection technique is difficult. Furthermore, the embryo-mediated system is time consuming because of the necessity of undertaking testcross analysis to produce progeny. The testis-mediated system has an advantage in donor cell preparation, and the *de novo* creation of chimeric status in recipient testes needs short time period for testcross analysis and saves the extra time for testing.

In this study, we did not quantitatively compare the activity of each germ cell to induce germline transmission since the germline transmission systems being employed in this study were different. So, there is apparent limitation to conclude potential feasibility and efficiency of each germline transmission system using germ cells of different types, but our results simply showed the activity of each germ cell to induce germline transmission. Large scale experiments with long-term observations are necessary for the practical application of our results and increase in embryos and hatchlings for evaluating germline transmission may contribute to obtaining more conclusive data. Considering that each procedure suggested has different advantages in inducing germline transmission, alteration of the combinations of germ cell-mediated germline chimera production protocols and retrieval of a sufficient number of germ cells might be examined for optimization of the germline chimera production. Such an approach will give a better understanding of germ cell differentiation mechanisms and unipotency under various environmental niches and will improve germ cell-mediated germline chimera production for developing an efficient generation of transgenic aves.

Materials and Methods

WL (genotype *I/I*) chickens and KOCs (genotype *i/i*) were used and were maintained at the University Animal Farm, Seoul National University, South Korea. All procedures for animal management, reproduction, and surgery were performed in accordance with the standard protocols of Seoul National University.

Experimental design

For transplantation of EGCs into adult testis, PGCs or EGCs were injected into the seminiferous tubules of adult testis, and SSCs or GSCs were transferred into the dorsal aorta of stage 14–16 embryos for adult testicular germ cells into embryo stages. Each germ cell was characterized before transfer and gonadal migration of the transferred cells or their localization in the basal membrane of the seminiferous tubules was subsequently monitored using PKH26-labeled germ cells (see below). The germline chimerism was demonstrated by testcross and PCR analysis.

Isolation of PGCs and in vitro *culture of EGCs*

Stage 28 (5.5-day-old) KOC embryos were collected and rinsed with PBS to remove residual yolk and blood. The gonads of retrieved embryos were collected by medial dissection of the abdomen with sharp tweezers under a stereomicroscope. The gonadal cells, containing PGCs, were dissociated from the gonads by gentle pipetting in 0.05% (v/v) trypsin solution supplemented with 0.53 mM EDTA. The dissociated cells were subsequently centrifuged at 200 ***g*** for 5 min and used for either the testis injection or EGC culture. *In vitro* culture was conducted according to standard protocols (Park & Han 2000, Han *et al*. 2002). Briefly, the seeded cells were then cultured in an incubator with 5% CO_2 in air at 37 °C and 60–70% relative humidity. To confirm PGCs or EGCs, these cells were immunostained with FITC-conjugated lectin-STA (Sigma–Aldrich) and SSEA-1 antibody (Developmental Studies Hybridoma Bank, Iowa City, IA, USA) for immunocytochemical analyses (Jung *et al*. 2005).

Downloaded from Bioscientifica.com at 03/26/2025 06:52:32PM
via free access

Table 2 Individual germline transmission efficiency of germline chimeras generated by transferring germ cells from different developmental origins.

Germline chimera production system				
Origin and type of germ cells	Site of transfer (stage or age)	**ID of male founder**	**Number of hatched**	**Number of donor cell-derived progeny** (%, IDs)[a]
Testicular germ cells				
SSCs	Blastoderm (stage X)	S108	197	1 (0.5, BLG1)
		S121	52	2 (3.8, BLG5,6)
		S126	121	1 (0.8, BLG2)
GSCs	Blastoderm (stage X)	S302	59	1 (1.7, BLG4)
		S306	8	0
		S310	87	0
		S315	12	0
		S320	103	0
		S324	104	1 (1.0, BLG3)
SSCs	Bloodstream (stage 14–16)	G202	37	0
		G404	7	0
GSCs	Bloodstream (stage 14–16)	G503	9	0
		G510	75	2 (2.7, BVG1,2)
		G511	14	0
		G519	6	0
		G523	3	0
		G527	19	0
	Subtotal (into embryos)		913	8 (0.9)
Embryonic germ cells				
PGCs	Adult testes (24 weeks)	PG1	46	0
		PG2	69	0
		PG3	95	0
		PG4	45	0
EGCs	Adult testes (24 weeks)	EG5	58	0
		EG6	49	8 (16.3, TG1–8)
		EG7	87	0
		EG8	40	0
	Subtotal (into testis)		489	8 (1.6)

[a]Percentage of donor-derived progenies among the hatched progenies.

Isolation of SSCs and in vitro *culture of GSCs*

SSCs were obtained from KOC and WL testes and GSCs were cultured according to standard protocols (Jung *et al.* 2007). Briefly, after the dissociation of testicular tissue, $\sim 1\times 10^6$ dissociated cells were cultured in a 100 mm culture dish in DMEM (Gibco Invitrogen) supplemented with 10% fetal bovine serum, 2% chicken serum, 1× antibiotic–antimycotic, 10 mM nonessential amino acids, 10 mM Hepes, 0.55 mM β-mercaptoethanol (Gibco Invitrogen), 2 ng/ml human leukemia inhibitory factor, 0.5 ng/ml human basic fibroblast growth factor, and 10 ng/ml human insulin-like growth factor 1 (Sigma–Aldrich). The seeded cells were cultured in an incubator with 5% CO_2 in air at 37 °C. SSCs were harvested by gentle agitation after short-term incubation of 3–5 days for the embryo injection, and the colonized GSCs from testis-derived germ cells were obtained at the end of the fifth subculture. Both cell types were also characterized using germ cell-specific markers.

Cell labeling, transfer, and tracing

In our previous report (Lee *et al.* 2006), we optimized the cell number for germ cell transplantation into the testes (1.0×10^7 cells/testis). So, in this study, $0.5–1\times 10^7$ cells were surgically transplanted into the recipients' testes after anesthetization with 50 mg/kg ketamine (Yuhan Pharmaceutical Corp., Seoul, South Korea). After *in vitro* expansion of EGCs by subpassages (more than 40 days), EGCs were harvested by gentle agitation. In the case of PGC injection into testis, we transferred $0.5–1\times 10^7$ gonadal cells including gonadal stroma cells and PGCs. Cell transplantation was performed using a Hamilton syringe (Hamilton, Reno, NV, USA). Approximately, 17 days are required for the completion of spermatogenesis in chicken (Etches 1996). Testcross reproduction analysis was conducted 1 month after transplantation and, to monitor the localization of transferred cells, germ cells were labeled with PKH26. The localization of PKH-labeled cells was evaluated 28 days after the transfer. To further evaluate localization after PGC/EGC injection into the testes, testicular tissues were cryosectioned and subsequently stained with 4′-6-diamidino-2-phenylindole. The stained tissue was examined under an inverted fluorescence microscope.

The cell preparation method and cell number to transfer into embryo stages, blastoderm at stage X and embryonic blood vessel at stage 14–16, were determined according to our previous experiment (Kim *et al.* 2009). Likely to EGCs, GSCs were harvested by gentle agitation at the end of culture (more than 40 days) and SSCs for embryo injection were also collected by gentle agitation after short-term culture of 3–5 days. In the testis-derived germ cell transfer into the blastoderm, a small window was made on the lateral part of a recipient egg, and the shell membrane was removed for the transfer. SSCs or GSCs (3000 cells) were then injected into

Downloaded from Bioscientifica.com at 03/26/2025 06:52:32PM
via free access

the subgerminal cavity of the embryo at stage X using a microcapillary pipette (Sigma–Aldrich). The window of the recipient egg was sealed twice with parafilm, and the egg was maintained with the pointed end facing down until hatching. For the testis-derived germ cell transfer into stage 14–16 embryos, cells were injected into the dorsal aorta of recipient embryos at stage 14–16 (H&H stage). For the injection, a small window was made at the pointed end of recipient eggs. Hatched chickens were maintained for up to 6 months.

PCR analysis of breed-specific polymorphisms

In the *PMEL17* gene, a WL-specific DNA probe (AS3554-I9) primers amplified a 222-bp fragment (5′ primer, 5′-AGCAGCGGCGATGAGCGGTG-3′; 3′ primer, 5′-CTG CCTCAACGTCTCGTTGGC-3′) and a KOC-specific DNA probe (AS3554-WT) amplified a 213-bp fragment (5′ primer, 5′-AGC AGC GGC GAT GAG CAG CA-3′; 3′ primer, 5′-CTG CCT CAA CGT CTC GTT GGC-3′; Choi *et al*. 2007). The blood or feather pulp of chimeras or the semen of founders was used for DNA extraction. The PCR reaction was conducted in a 25 μl solution containing 50–100 ng genomic DNA, 0.2 mM dNTPs, 0.4 pmol forward and reverse primers, and 1 unit Taq polymerase. The thermocycling conditions were 10 min at 94 °C; 35 cycles of 30 s at 94 °C; 30 s at 65 °C, and 2 min at 72 °C; and finally, 10 min at 72 °C.

Declaration of interest

The authors declare that there is no conflict of interest that would prejudice the impartiality of this scientific work.

Funding

This work was supported by a grant from BioGreen 21 Program (20070401034010), Rural Development Administration, and was also supported by WCU (World Class University) program through the Korea Science and Engineering Foundation funded by the National Research Foundation of Korea (R31-10056).

References

Brinster RL & Zimmermann JW 1994 Spermatogenesis following male germ-cell transplantation. *PNAS* **91** 11298–11302.

Chang IK, Jeong DK, Hong YH, Park TS, Moon YK, Ono T & Han JY 1987 Production of germline chimeric chicken by transfer of cultured chick primordial germ cells. *Cell Biology International* **21** 495–499.

Choi JW, Lee EY, Shin JH, Zheng Y, Cho BW, Kim JK, Kim HB & Han JY 2007 Identification of breed-specific DNA polymorphisms for a simple and unambiguous screening system in germline chimeric chickens. *Journal of Experimental Zoology* **307** 241–248.

Etches RJ 2006 *Reproduction in Poultry*, pp 208–214. New York: CAB International.

Guan K, Nayernia K, Maier LS, Wagner S, Dressel R, Lee JH, Nolte J, Wolf F, Li M, Engel W *et al.* 2006 Pluripotency of spermatogonial stem cells from adult mouse testis. *Nature* **440** 1199–1203.

Han JY 2009 Germ cells and transgenesis in chickens. *Comparative Immunology, Microbiology and Infectious Diseases* **32** 61–80.

Han JY, Park TS, Hon YH, Jeong DK, Kim JN, Kim KD & Lim JM 2002 Production of germline chimeras by transfer of chicken gonadal primordial germ cells maintained *in vitro* for an extended period. *Theriogenology* **58** 1531–1539.

Jung JG, Kim DK, Park TS, Lee SD, Lim JM & Han JY 2005 Development of novel markers for the characterization of chicken primordial germ cells. *Stem Cells* **23** 689–698.

Jung JG, Lee YM, Park TS, Park SH, Lim JM & Han JY 2007 Identification, culture, and characterization of germline stem cell-like cells in chicken testes. *Biology of Reproduction* **76** 173–182.

Kalina J, Senigl F, Micáková A, Mucksová J, Blazková J, Yan H, Poplstein M, Hejnar J & Trefil P 2007 Retrovirus-mediated *in vitro* gene transfer into chicken male germ line cells. *Reproduction* **134** 445–453.

Kim MA, Park TS, Kim JN, Park HJ, Lee YM, Ono T, Lim JM & Han JY 2005 Production of quail (*Coturnix japonica*) germline chimeras by transfer of gonadal primordial germ cells into recipient embryos. *Theriogenology* **63** 774–782.

Kim JN, Park TS, Park SH, Park KJ, Kim TM, Lee SK, Lim JM & Han JY 2009 Migration and proliferation of intact and genetically modified primordial germ cells and the generation of a transgenic chicken. *Biology of Reproduction* [in press]. DOI: 10.1095/biolreprod.109.079723.

van de Lavoir MC, Diamond JH, Leighton PA, Mather-Love C, Heyer BS, Bradshaw R, Kerchner A, Hooi LT, Gessaro TM, Swanberg SE *et al.* 2006 Germline transmission of genetically modified primordial germ cells. *Nature* **441** 766–769.

Lee YM, Jung JG, Kim JN, Park TS, Kim TM, Shin SS, Kang DK, Lim JM & Han JY 2006 A tested-mediated germline chimera production based on transfer of chicken testicular cells directly into heterologous testes. *Biology of Reproduction* **75** 380–386.

Lombardi J 1999 *Comparative Vertebrate Reproduction*, pp 199–223. Heidelberg: Springer.

Naito M, Sano A, Matsubara Y, Harumi T, Tagami T, Sakurai M & Kuwana T 2001 Localization of primordial germ cells or their precursors in stage X blastoderm of chickens and their ability to differentiate into functional gametes in opposite-sex recipient gonads. *Reproduction* **121** 547–552.

Park TS & Han JY 2000 Derivation and characterization of pluripotent embryonic germ cells in chicken. *Molecular Reproduction and Development* **56** 475–482.

Park TS, Jeong DK, Kim JN, Song GH, Hong YH, Lim JM & Han JY 2003*a* Improved germline transmission in chicken chimeras produced by transplantation on of gonadal primordial germ cells into recipient embryos. *Biology of Reproduction* **68** 1657–1662.

Park TS, Hong YH, Kwon SC, Lim JM & Han JY 2003*b* Birth of germline chimeras by transfer of chicken embryonic germ (EG) cells into recipient embryos. *Molecular Reproduction and Development* **65** 389–395.

Schlatt S, Foppiani L, Rolf C, Weinbauer GF & Nieschlag E 2002 Germ cell transplantation into X-irradiated monkey testes. *Human Reproduction* **17** 55–62.

Shin SS, Kim TM, Kim SY, Kim TW, Seo HW, Lee SK, Kwon SC, Lee GS, Kim H, Lim JM *et al.* 2008 Generation of transgenic quail through germ cell-mediated germline transmission. *FASEB Journal* **22** 2435–2444.

Tagami T, Kagami H, Masubara Y, Harumi T, Naito M, Takeda K, Hanada H & Nirasawa K 2007 Differentiation of female primordial germ cells in the male testes of chicken (*Gallus gallus domesticus*). *Molecular Reproduction and Development* **74** 68–75.

Received 27 June 2009
First decision 30 July 2009
Revised manuscript received 2 September 2009
Accepted 22 September 2009

Downloaded from Bioscientifica.com at 03/26/2025 06:52:32PM
via free access