INTERNATIONAL JOURNAL OF MULTIDISCIPLINARY RESEARCH AND STUDIES

VOLUME04ISSUE01

''ESTABLISHMENT OF A PHEASANT (PHASIANUS COLCHICUS) SPERMATOGONIAL VEGETATIVE CELL LINE FOR THE ASSEMBLY OF INTERSPECIES GERMLINE CHIMERAS''

PROF. HWISHART; PROF. MARTHAN

ABSTRACT

Background

Spermatogonial stem cells (SSCs) are important for the assembly of interspecies germ line chimeras. The interspecies cell transfer technique has been suggested as some way to conserve endangered birds. Our objective was to develop a way for restoring endangered birds by developing interspecies germ line chimeras between pheasant (Phasianus colchicus) and chicken (Gallus gallus) with SSCs.

Results

SSCs were isolated from the surgically removed testis of a pheasant. Growth conditions for pheasant SSCs were established by co-culturing STO (SIM mouse embryo-derived thioguanine and ouabain resistant) cells and pheasant SSCs. The colony-forming cells divided and proliferated stably to yield a longtime SSC line. Pheasant SSCs showed strong reactivity for the GDNF family receptor alpha1 (GFRα1) marker. Finally, the assembly of germ line chimeras was attempted by transferring pheasant SSCs into recipient embryos. Although final embryo survival was 5.6% (20/354), the initial survival rate was 88% (312/354). to live the percent transfer of donor SSC to gonads, the pheasant SSCs were labeled with PKH 26 dye. We observed 30% donor cells and 9.48% c-kit/CD117-positive cells within the gonads of recipient chickens. Donor SSCs were thus stably engrafted within the recipient gonads.

Conclusions

This study showed that SSCs will be used as a tool for the conservation of endangered birds and also the production of germ line chimeras. Our findings yield insights into how we may use the pheasant spermatogonial vegetative cell line for efficient production of

interspecies germ line chimeras and ultimately, to the restoration of endangered birds.

Keywords

Endangered birds vitro cultureRecipient chicken embryosSSC transplantation

1. Introduction

Germ cells are unique, with a very important role within the transmission of genetic information from one generation to the following through meiosis and mitosis. Primordial sex cells (PGCs) are the origin of the germ cell lineage [1] and a source of pluripotent germ cells after sexual maturation. within the male germ cells, gonocytes migrate through the tubule to the basement membrane and differentiate into spermatogonial stem cells (SSCs) [2]. SSCs are important germline stem cells for spermatogenesis within the testis. These male germline stem cells share characteristics with other adult stem cells and both are capable of self-renewal and differentiation into spermatozoa [3]. SSC transplantation was first reported in mice; since then, this characteristic of SSCs has been suggested as a possible tool for producing interspecies germline chimeras [4], which are of particular importance in efforts to revive species.

Although sperm cryopreservation and AI is wont to support species preservation, their utility is proscribed in non-domesticated species [5]. cell techniques are used for recent restoration efforts [6]. Studies of restoration in mammals are underway, but progress is slowed by long generation intervals. Avian species, unlike mammals, have the benefits of a comparatively brief generation interval and high productivity in order that they can grow more quickly than mammals [7]. Avian species are good models for experimental studies in basic research and biotechnology [8], but SSCs haven't been fully characterized in birds. most up-to-date studies of avian germ cells have focused on the restoration of endangered bird species. Approximately 12.5% of the 9920 bird species within the world are endangered, and conservation efforts through natural breeding still encounter difficulties [5].

Kang et al. [9,10] produced interspecies germline chimeras of pheasant and chicken by

using PGCs. The pheasant is physiologically and developmentally just like the chicken. Interspecies transplantation of PGCs and SSCs can allow the generation of donor-derived offspring [11]. In fact, restoration of endangered avian species using SSCs enables the applying of chicken germline transmission technologies to the pheasant, making it possible to supply interspecies germline chimeras in a very chicken-pheasant system. Transfer of foreign genes into chicken embryos, establishment of in vitro culture of germ cells, and production of chimeric chickens and interspecies germline chimeras [12,13,14,15,16] is used for the restoration of endangered birds. We attempted to develop interspecies cell transfer techniques and to supply interspecies germline chimeras for the restoration of endangered birds by using SSCs. during this study, we established a pheasant SSC line for transplantation to come up with interspecies germline chimeras of pheasant and chicken.

2. Materials and methods

2.1. Experimental animals

Five male person (45–50 weeks old) Korean ring-necked pheasants (Phasianus colchicus) in non-breeding season and five female chickens (Gallus gallus) were used because the donors and recipients, respectively, of SSCs. The birds were maintained at the university poultry farm of Jeju National University, Asian nation. Birds were kept in cages and therefore the poultry shed was maintained at an ambient temperature of 25 ± 1°C. Procedures for bird management, reproduction, and embryo manipulation followed the quality operating protocol of our laboratory. We followed appropriate quality standards for all experimental protocols and animal handling. Birds were offered feed and water ad-libitum.

2.2. Retrieval of donor SSCs

Experimental procedures for SSC retrieval were performed at the affiliated laboratories of the Jeju National University, Jeju-si, Asian country. The testes from male person (45–50 weeks old) pheasants were surgically removed and manually decapsulated by removing the tunica albuginea. The exposed parenchyma was withdraw pieces and

washed with PBS (Sigma, St Louis, MO, USA). Testicular single-cell suspensions were selected by two-step enzymatic digestion then used for individual cell separation. Testes were transferred to a digestion medium containing Dulbecco PBS (DPBS; Gibco BRL, Bethesda, MD) consisting of 1 mg/mL collagenase type IV (Sigma) and 1 mg/mL hyaluronidase (Sigma). Digestion was performed for 10 min by shaking (150 rpm) at 37°C to dissociate tubules. Testicular tissues were dissociated by gentle pipetting in 0.25% trypsin-EDTA (Gibco BRL) at 37°C for five min. Testicular cells containing SSCs were retrieved by passing through nylon mesh and so through 40 µm filters (BD Falcon, Franklin Lakes, NJ, USA). After centrifugation, the supernatant was removed and dissociated cells were washed twice in Dulbecco's Modified Eagle's Medium (DMEM; Gibco Invitrogen, Carlsbad, CA, USA).

2.3. In vitro culture of testicular cells

Dissociated cells were cultured within the 6-well plates with modified DMEM containing 10% (v/v) fetal bovine serum (FBS; Hyclone, Logan, UT, USA), nonessential amino acids (NEAA; Gibco Invitrogen), 10 ng/mL human basic fibroblast protein (bFGF; Sigma), and 100 ng/mL human insulin-like growth factor-1 (IGF-1; Sigma).

2.4. Co-culturing and growth factors of SSCs

Testicular germ cells were cultured in 24- and 6-well plates. Pheasant somatic cells were co-cultured with mitomycin C-treated STO cells and incubated for 1 d during a 5% CO2 incubator. After incubation, the cells were washed twice with PBS. Pheasant SSCs were added in DMEM with 20% FBS, 100 U/mL penicillin (Gibco Invitrogen), and 100 µg/mL streptomycin (Gibco Invitrogen) in a very humidified 5% CO2 incubator; the medium was exchanged twice per week. We studied the effect of those various factors on SSC growth and optimized the medium (Table 1). Four different media were accustomed evaluate growth factors and cell growth.

Components	Medium-1 (M1)	Medium-2 (M2)	Medium-3 (M3)	Medium-4 (M4)
Type of media	DMEM	DMEM	DMEM/F12	DMEM/F12
FBS (%)	10	10	10	5
Penicillin (U/mL)	100	100	100	100
Streptomycin (μg/mL)	100	100	100	100
NEAA (%)	–	1	1	1
LIF (U/mL)	–	10^3	–	–
bFGF (ng/mL)	–	–	10	10
IGF-1 (ng/mL)	–	–	100	100

2.5. Immunofluorescence assay

Cell surface antigen expression of cultured cells was analyzed by immunofluorescence techniques. Several antibodies, including stage-specific embryonic antigen-1 (SSEA-1), SSEA-3, SSEA-4 (all Millipore, Billerica, MA, USA), Oct4 (Santa Cruz Biotechnology, Santa Cruz, CA, USA), and GFRα1 (R&D Systems, Minneapolis, MN, USA) were accustomed trace the proliferative properties of pheasant SSCs. the subsequent primary monoclonal antibodies were wont to detect surface-antigen expression: anti-SSEA-1; anti-SSEA-3; anti-SSEA-4; anti-oct4; and anti-GFRα1. fluorescein isocyanate (FITC)-labeled secondary antibodies, appropriate to the species and isotype of the first antibody, were accustomed detect primary antibody binding. the classy cells were fixed for 15–20 min at temperature. After washing twice with 1 × rinsing buffer, cells were incubated in blocking solution for 30 min at temperature. Primary antibodies were diluted to working concentrations of 1:10–1:50 in blocking solution and incubated at temperature for 1 h, followed by incubation with secondary antibodies (final concentration 1:200) for 30 min at temperature. The stained cells were observed under an inverted microscope (IX70; Olympus, Tokyo, Japan).

2.6. Paul Karl Horan (PKH) 26 labeling

To access donor and recipient embryos, gonadal cells containing pheasant SSCs were labeled with PKH 26 fluoresceine (Sigma) for five min before transfer. The cells were suspended in 1 mL Diluent C from the PKH 26 mini kit. The egg window of the recipient embryos was sealed twice with parafilm (Bemis, Neenah, WI, USA) and positioned with the pointed end down until the following treatment. The labeled SSCs were monitored under the IX70 fluorescence microscope.

2.7. Transfer of SCCs into recipient embryos

Donor SSCs were transferred to the recipient embryos through atiny low window made at the pointed end of the eggs. Approximately 2 µL cell suspension from donor pheasant SSCs was transferred into the dorsal aorta of two.5 d old chicken embryos with a 50-µL microcapillary tube (Sigma). The egg window was sealed with parafilm and so laid down with the pointed end at the underside.

2.8. Fluorescence-activated cell sorting (FACS)

CD117 is understood because the c-kit receptor, which is expressed in germ cells. CD117 was used as a particular marker of donor-derived SSCs within the gonads of recipient chickens. Propidium iodide (PI, 1 mg/mL; Gibco Invitrogen) was wont to discriminate live cells. The FACS Calibur (Becton Dickinson, San Jose, CA, USA) was wont to identify c-kit/CD117-positive cells.

2.9. Statistical analysis

Statistical analysis was performed by analysis of variance (ANOVA) using SPSS v 12.0 software (SPSS, Chicago, IL, USA). Significant differences between the means of various groups at P values < 0.05 were analyzed by Tukey's b-test. Values are expressed as means ± standard deviations (SD).

3. Results

3.1. Proliferation efficiency of pheasant SSCs through co-culture and effect of growth factors

In this study, our aim was to co-culture pheasant SSCs, which is a longtime technique in chickens, but the foremost constraint we faced was a poor rate of growth. Therefore, we decided to use basal cells, and growth conditions were established through co-culture with the STO cells and somatic cells of pheasant. Pheasant SSCs were proliferated through co-culture. When SSCs were cultured with STO cells, proliferation efficiency increased rapidly ($P < 0.05$). However, proliferation within the absence of basal cells was very slow; after 2 weeks of culture, the cells began to die. The chicken somatic cells exhibited substantial proliferation rates. Pheasant somatic cells showed a reduced STO cell effect, but relatively high levels of cell proliferation (Fig. 1).

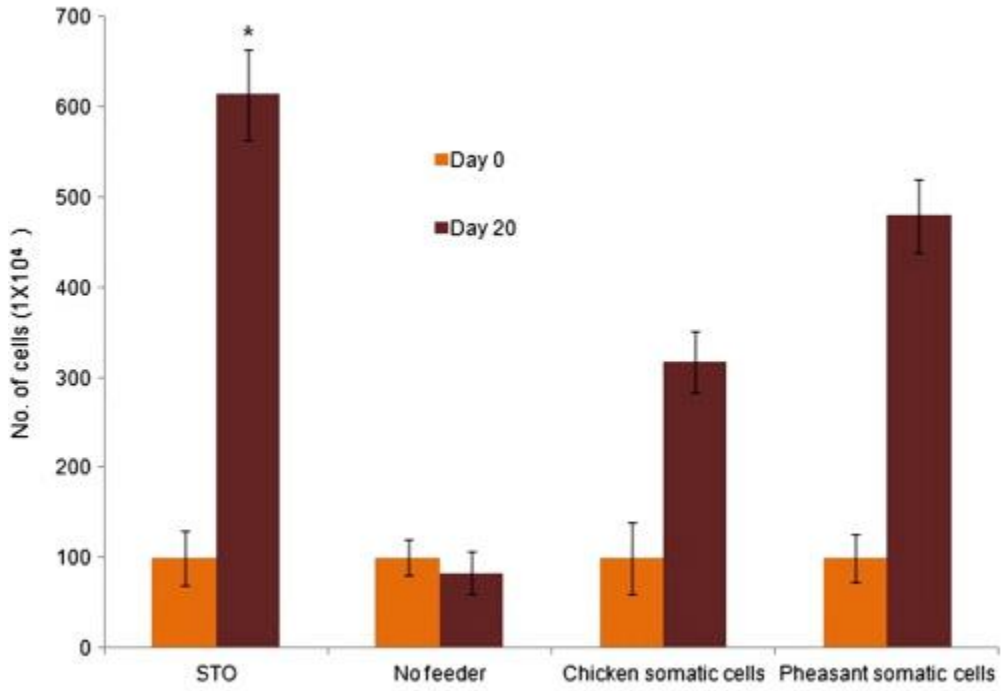

Fig. 1. STO cells and proliferation of pheasant SSCs in a co-culture system. Data are reported as mean ± SD. *Significance at $P < 0.05$.

We used this study to reveal the effect of growth factors like leukemia inhibitory factor (LIF), bFGF, IGF-1, and FBS on cell growth to optimize the medium for in vitro culture of

pheasant SSCs. The pheasant SSCs proliferated efficiently in various culture conditions with strong proliferation in M3 and M4; M1 and M2 produced lower rates of SSC growth (Fig. 2). M4 supplemented with 5% FBS, bFGF, and IGF-1 yielded significantly higher growth ($P < 0.05$) than M3 supplemented with 10% FBS. This improvement in proliferation at low-level FBS (5%) suggested these cells require lower concentrations of FBS within the growth medium.

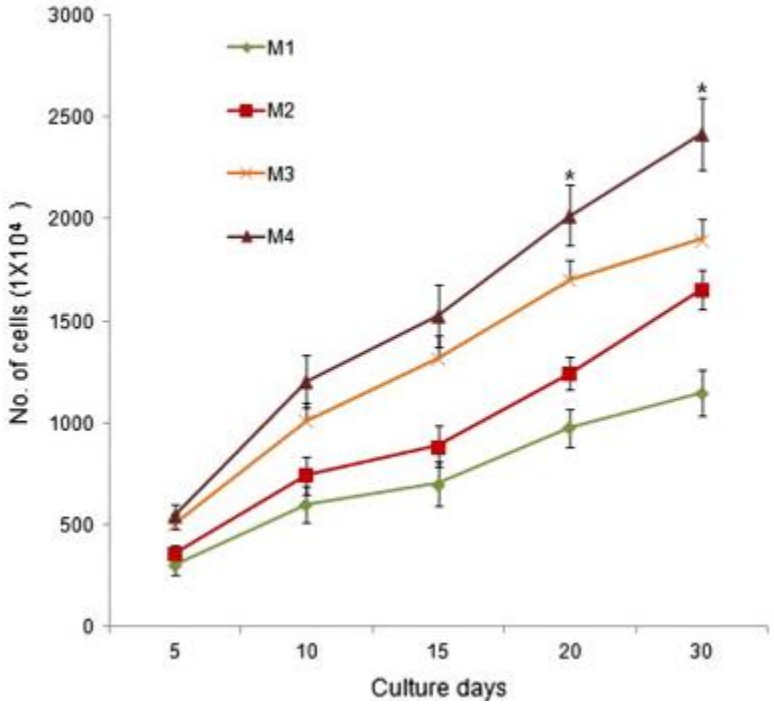

Fig. 2. Growth curves of pheasant SSCs in different media over 30 d. Medium-1 (M1) contained DMEM supplemented with 10% FBS; Medium-2 (M2) contained DMEM supplemented with 10% FBS and LIF; Medium-3 (M3) contained DMEM/F12 supplemented with 10% FBS, bFGF, and IGF-1; and Medium-4 (M4) contained DMEM/F12 supplemented with 5% FBS, bFGF, and IGF-1. M1 and M2 produced lower growth rates than did M3 and M4. M4 yielded the most rapid growth of pheasant SSCs. Data are reported as mean ± SD. *Significance at $P < 0.05$.

3.2. Establishment of pheasant SSC line and reactivity of SSCs to antibodies

The pheasant SSC line was established and it's continuing to undergo stabilization.

Cells weren't passaged over and over, but the SSC line was established. SSCs are adherent cells that proliferate by adhering to the surface of the culture vessel. A high rate of proliferation was observed within the colony-forming cells (Fig. 3).

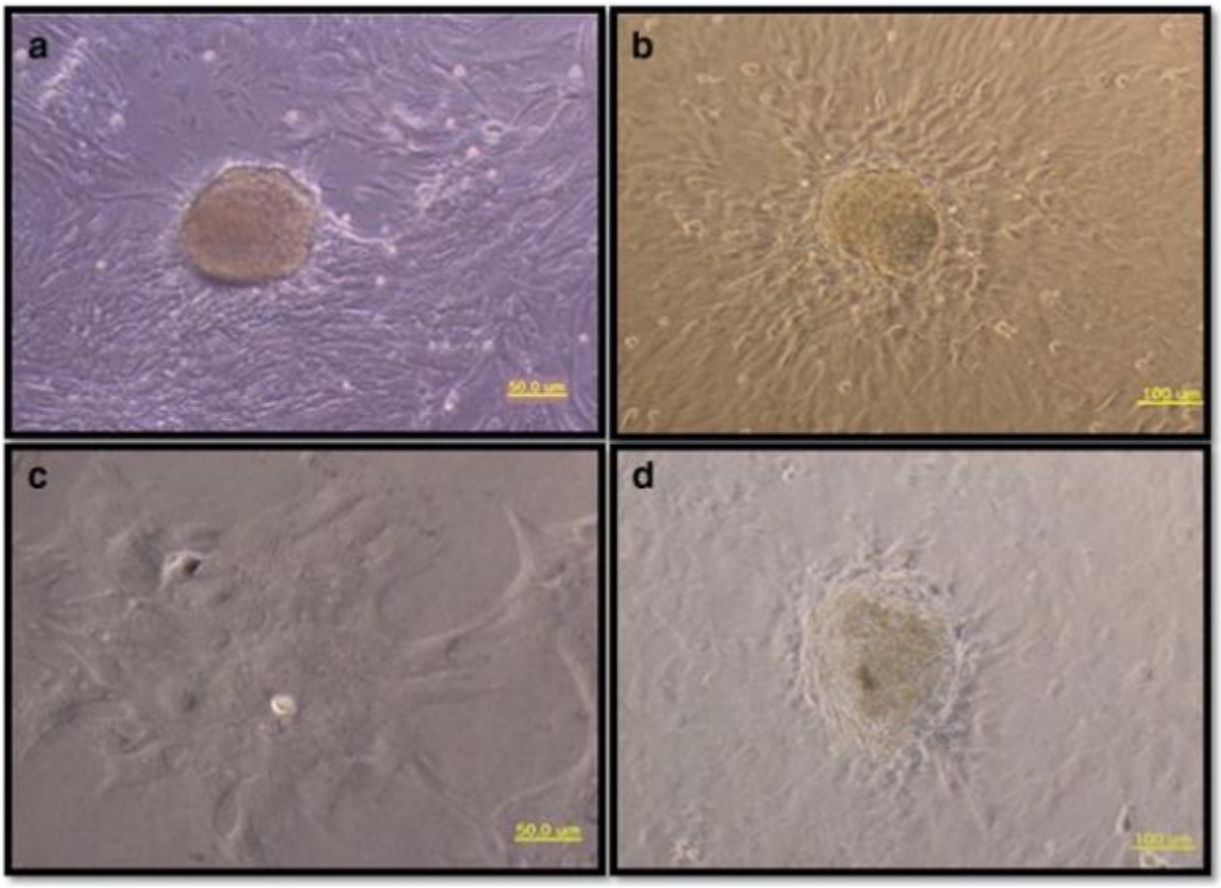

Fig. 3. Morphological characteristics of the pheasant spermatogonial stem cell line after subculture. (a) Chicken and (b) pheasant SSCs exhibited similar morphologies at 8 d; (c) pheasant SSCs maintained typical cell aggregation characteristics at 13 d (passage 3); and (d) maintained the characteristics and potential for aggregation and sphere formation at 25 d of culture (passage 6). Scale bar = 50 μm in (a) and (c); 100 μm in (b) and (d).

To analyze the proliferative properties of pheasant SSCs, the cells were examined with 5 antibodies SSEA-1, SSEA-3, SSEA-4, Oct4, and GFRα1. Reactivity was too weak to obviously detect SSCs in a very mixed vegetative cell population. Pheasant SSCs exhibited strong reactivity to GFRα1, an SSC-specific marker (Fig. 4), but didn't react with

antibodies to SSEA-1, SSEA-3, SSEA-4, and Oct4. Thus, we continued the study with GFRα1.

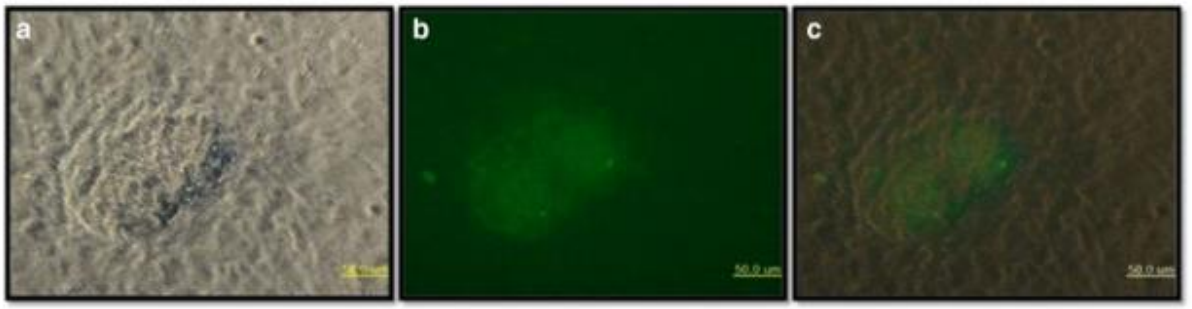

Fig. 4. Immunofluorescence detection of *GFRα1* marker on pheasant SSCs. (a) Phase-contrast microphotograph of an SSC colony; (b) green fluorescence of *GFRα1* antibody; and (c) merged figures (a) and (b). Pheasant SSCs were incubated with *GFRα1*. Scale bar; (a), (b), and (c) = 50 μm.

3.3. Production of germline chimeras by transferring donor SSCs into the recipient.

Germline chimeras were produced by transplanting pheasant SSCs into recipient chicken embryos. The pheasant SSCs were transferred into 354 recipients; after 19 d, only 24 embryos were viable. The low rate of embryo development was preceded by an initially high survival rate (312/354); only 20 chicks hatched (5.6%). Donor cells were labeled with PKH 26 absorption indicator to look at the share of donor SSCs within the recipient embryos. We transferred 10,000 and 20,000 labeled donor-derived SSCs to recipient embryos and located more cells within the gonads of chicken embryos with higher injected doses of SSCs (Fig. 5). Of 20 hatched chicks, only six had PKH 26-positive donor SSCs. As a result, 30% donor-derived SSCs were observed within the gonads of recipient chickens. Thus, we've got established stable transfer techniques, but the proportion of PKH 26-positive cells was very low (5.3%). The distribution of stem cells within the transplanted SSCs was confirmed by FACS analysis. The c-kit/CD117-positive cells were found at a rate of about 9.48%, showing stable engraftment within the recipient gonads (Fig. 6).

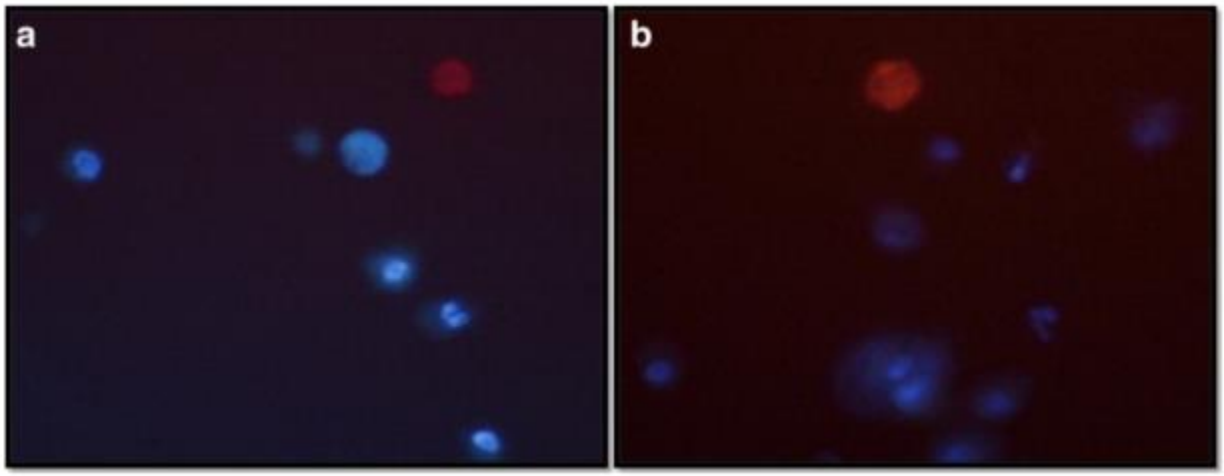

Fig. 5. PKH 26-labeled SSCs in the gonads of recipient embryos. Injection of 10,000 (a) and 20,000 (b) donor-derived SSCs. Red cells are labeled with PKH 26 cells and blue cells are PI-stained.

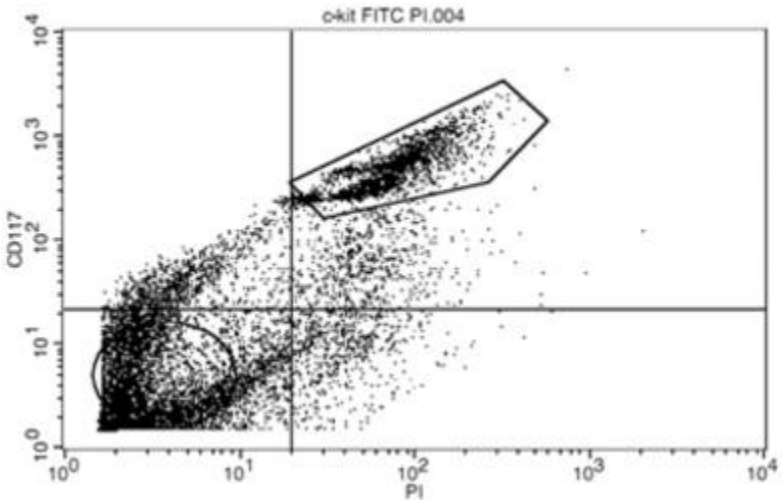

Fig. 6. The proportion of stem cells distributed in the donor PKH-26 cells.

4. Discussion

Each animal species possesses unique genetic and physiological characteristics. These features may be accustomed differentiate germ and somatic cells, analyze the expression of sex-specific genes, and conserve genetic resources by using interspecies germline chimeras. Furuta et al. [17] reported that germ cells will be used for the con-

servation of native Ehime chickens (an endangered Japanese chicken). during this study, we established an SSC line to develop an interspecies germline chimeric system which will facilitate the restoration of species.

Recent advances in chicken embryo manipulation methods have allowed researchers to supply germ line chimeras derived from the transfer of PGCs and methods for gene targeting in avian pluripotent cells are considered. Kang et al. [9] produced interspecies germline chimeras of pheasant and chicken. during this study, we used SSCs to supply interspecies germline chimeras and transfer these interspecies SSCs into recipient embryos, a technique that may be wont to restore endangered birds. Moreover, we demonstrated that SSCs will be accustomed produce chimeric birds, thus advancing the study of germ cells. Pheasant SSCs transferred into chicken embryos migrated to the gonads and entered heterologous spermatogenesis in recipient embryos. Interspecies chimeras have thus become a robust tool to preserve endangered birds [9].

It is essential to purify SSCs to extend the productivity of germline chimeras and their transmission. For in vitro culture of SSCs, it's important to retrieve highly pure SSCs from the testes. to confirm purity, several isolation methods are developed, like using surface markers for specific proteins [18,19,20] and extracellular matrix [21,22]. during this study, we treated the pheasant testes with collagenase, hyaluronidase, and trypsin to retrieve purified SSCs. Methods for in vitro culture and isolation of SSCs have recently been developed [23,24,25,26,27,28,29,30,31]. These cells were transferred into recipient testes and transgenic animals are produced continuously [32,33,34].

In general, a longtime cell line proliferates and is stably differentiated through 100 passages without apoptosis or necrosis. As shown in Fig. 3, we sub-cultured the cells continuously, thus demonstrating the establishment of the SSC line for the restoration of endangered birds. Colony-forming cells divided and proliferated stably within the positive direction. However, it's generally accepted that in vitro isolation and proliferation methods haven't been clearly established for SSCs, perhaps due to a scarcity of

data regarding appropriate in vitro culture conditions and specific markers of chicken germ line stem cells. Specifically, the concentration of SSCs within the testis is extremely low; as few as 1 in 3333 cells are reported in adult mouse testis [35], and 1 in 500 cells in adult rat testis [21]. Therefore, it's necessary to determine a highly pure SSC line from numerous styles of cells and in vitro culture systems.

It is important to optimize the culture conditions (culture medium, co-cultured cells, and growth factors) for successful in vitro culture. Nagano et al. [23] confirmed the influence of in vitro culture conditions on SSCs. They also demonstrated the influence of substance, co-cultured cells, and growth factors on the proliferation and differentiation of SSCs. The success of long-term proliferation in culture relies on cultural conditions [36]. For effective differentiation of SSCs, our cells were co-cultured with Sertoli cells. during this study, SSCs were co-cultured with STO cells and proliferated by co-culturing both SSCs and STO cells (Fig. 1). Furthermore, Ryu et al. [19] found that SSCs proliferated within the presence of growth factors including somatic cell line-derived neurotrophic factor (GDNF), basic fibroblast protein (bFGF), and GFRα1. GFRα1 is thought to market the proliferation of SSCs. Interestingly, we detected significant expression of GFRα1 in pheasant SSCs and showed strong reactivity for GFRα1 (Fig. 4). during this study, we cultured SSCs in growth media with and without growth factors to spot the optimal culture conditions for pheasant SSCs. We obtained the simplest results with DMEM supplemented with 5% FBS, 100 U/mL penicillin, 100 µg/mL streptomycin, nonessential amino acids, 10 ng/mL bFGF, and 100 ng/mL IGF-1 (Fig. 2). These findings clearly indicated that growth factors like LIF, bFGF, IGF-1, and FBS are important for the expansion and proliferation of SSCs.

Lee et al. [37] established a germline chimeric system for transferring testicular cells into heterologous testes. The testicular cells from juvenile and adult Korean Ogol chickens (KOC) were transplanted into White Leghorn (WL) testes. during this study, we transferred SSCs into 354 recipient embryos. Although embryo survival incidence was low (20/354), initial survival rates were sufficient. Subsequently, 30% donor-derived

SSCs were observed within the gonads of recipient chickens. We confirmed the soundness of our transfer techniques, but the proportion of PKH 26-positive cells was very low (5.3%). We believe that our findings support the event of an interspecies reproductive cell transfer system for the restoration of endangered birds.

Financial support
This study was supported by a grant from the National Research Foundation of Korea (No. 2013–0968).

Acknowledgment
The authors are grateful to the laboratory of the gene-splicing and vegetative cell Biology of the Jeju National University and Animal Genetic Resource Station of the National Institute of Animal Science, Asian country.

References

[1]E. Magnúsdóttir, M.A. Surani
How to make a primordial sex cell
Development, 141 (2014), pp. 245-252, 10.1242/dev.098269
CrossRefView Record in ScopusGoogle Scholar

[2]R.L. Brinster
Germline vegetative cell transplantation and transgenesis
Science, 296 (2002), pp. 2174-2176, 10.1126/science.1071607
View Record in ScopusGoogle Scholar

[3]J.M. Oatley, R.L. Brinster
The germline somatic cell niche unit in mammalian testes
Physiol Rev, 92 (2012), pp. 577-595, 10.1152/physrev.00025.2011

CrossRefView Record in ScopusGoogle Scholar

[4]R.L. Brinster, J.W. Zimmermann
Spermatogenesis following male germ-cell transplantation
Proc Natl Acad Sci U S A, 91 (1994), pp. 11298-11302, 10.1073/pnas.91.24.11298
CrossRefView Record in ScopusGoogle Scholar

[5]R.J. Pereira, A. Napolitano, F.L. Garcia-Pereira, C.F. Baldo, S.T. Suhr, L.E. King, et al.
Conservation of avian germplasm by xenogeneic transplantation of spermatogonia from sexually mature donors
Stem Cells Dev, 22 (2013), pp. 735-749, 10.1089/scd.2012.0497
CrossRefView Record in ScopusGoogle Scholar

[6]M. Roe, N. McDonald, B. Durrant, T. Jensen
Xenogeneic transfer of adult quail (Coturnix coturnix) spermatogonial stem cells to embryonic chicken (Gallus gallus) hosts: A model for avian conservation
Biol Reprod, 88 (2013), p. 129, 10.1095/biolreprod.112.105189
Google Scholar

[7]H. Sang
Transgenic chickens — Methods and potential applications
Trends Biotechnol, 12 (1994), pp. 415-420, 10.1016/0167-7799(94)90030-2
ArticleDownload PDFView Record in ScopusGoogle Scholar

[8]K.J. Park, T.M. Kim, H.C. Lee, H.J. Jang, G.H. Song, J.Y. Han
Effects of gamma-irradiation on the sterilization of primordial germ cells in quail
Korean J Poult Sci, 37 (2010), pp. 139-143, 10.5536/KJPS.2010.37.2.139
CrossRefView Record in ScopusGoogle Scholar

[9]S.J. Kang, J.W. Choi, S.Y. Kim, K.J. Park, T.M. Kim, Y.M. Lee, et al.
Reproduction of untamed birds via interspecies sex cell transplantation
Biol Reprod, 79 (2008), pp. 931-937, 10.1095/biolreprod.108.069989
CrossRefGoogle Scholar

[10]S.J. Kang, J.W. Choi, K.J. Park, Y.M. Lee, T.M. Kim, S.H. Sohn, et al.
Development of a pheasant interspecies primordial reproductive cell transfer to chicken embryo: Effect of donor cell sex on chimeric semen production
Theriogenology, 72 (2009), pp. 519-527, 10.1016/j.theriogenology.2009.04.007
ArticleDownload PDFView Record in ScopusGoogle Scholar

[11]C. Liu, K.A. Khazanehdari, V. Baskar, S. Saleem, J. Kinne, U. Wernery, et al.
Production of chicken progeny (Gallus gallus domesticus) from interspecies germline chimeric duck (Anas domesticus) by primordial cell transfer
Biol Reprod, 86 (2012), p. 101, 10.1095/biolreprod.111.094409
ArticleDownload PDFCrossRefView Record in ScopusGoogle Scholar

[12]M.A. Kim, T.S. Park, J.N. Kim, H.J. Park, Y.M. Lee, T. Ono, et al.
Production of quail (Coturnix japonica) germline chimeras by transfer of gonadal primordial germ cells into recipient embryos
Theriogenology, 63 (2005), pp. 774-782, 10.1016/j.theriogenology.2004.04.014
ArticleDownload PDFView Record in ScopusGoogle Scholar

[13]T.S. Park, M.A. Kim, J.M. Lim, J.Y. Han
Production of quail (Coturnix japonica) germline chimeras derived from in vitro-cultured gonadal primordial germ cells
Mol Reprod Dev, 75 (2008), pp. 274-281, 10.1002/mrd.20821
CrossRefGoogle Scholar

[14]S.J. Kang, S.H. Sohn, K.S. Kang, H.C. Lee, S.K. Lee, J.W. Choi, et al.
Molecular and biological aspects of early reproductive cell development in interspecies hybrids between chickens and pheasants
Theriogenology, 75 (2011), pp. 696-706, 10.1016/j.theriogenology.2010.10.010
ArticleDownload PDFView Record in ScopusGoogle Scholar

[15]J.N. Kim, T.S. Park, S.H. Park, K.J. Park, T.M. Kim, S.K. Lee, et al.
Migration and proliferation of intact and genetically modified primordial germ cells and therefore the generation of a transgenic chicken
Biol Reprod, 82 (2010), pp. 257-262, 10.1095/biolreprod.109.079723
View Record in ScopusGoogle Scholar

[16]L. Chojnacka-Puchta, K. Kasperczyk, G. Płucienniczak, D. Sawicka, M. Bednarczyk
Primordial germ cells (PGCs) as a tool for creating transgenic chickens
Pol J Vet Sci, 15 (2012), pp. 181-188, 10.2478/v10181-011-0132-6
CrossRefView Record in ScopusGoogle Scholar

[17]H. Furuta, K. Kinoshita, Y. Maeda, N. Fujihara
Restoration of genetic resources from Ehime native chicken via transferred primordial germ cells (PGCs)
J Poult Sci, 38 (2001), pp. 302-307, 10.2141/jpsa.38.302
CrossRefView Record in ScopusGoogle Scholar

[18]B.Y. Ryu, K.E. Orwig, H. Kubota, M.R. Avarbock, R.L. Brinster
Phenotypic and functional characteristics of spermatogonial stem cells in rats
Dev Biol, 274 (2004), pp. 158-170, 10.1016/j.ydbio.2004.07.004
ArticleDownload PDFView Record in ScopusGoogle Scholar

[19]B.Y. Ryu, H. Kubota, M.R. Avarbock, R.L. Brinster
Conservation of spermatogonial vegetative cell self-renewal signaling between mouse

and rat
Proc Natl Acad Sci U S A, 102 (2005), pp. 14302-14307, 10.1073/pnas.0506970102
CrossRefView Record in ScopusGoogle Scholar

[20]K. Guan, F. Wolf, A. Becker, W. Engel, K. Nayernia, G. Hasenfuss
Isolation and cultivation of stem cells from adult mouse testes
Nat Protoc, 4 (2009), pp. 143-154, 10.1038/nprot.2008.242
CrossRefView Record in ScopusGoogle Scholar

[21]K.E. Orwig, T. Shinohara, M.R. Avarbock, R.L. Brinster
Functional analysis of stem cells within the adult rat testis
Biol Reprod, 66 (2002), pp. 944-949, 10.1095/biolreprod66.4.944
CrossRefView Record in ScopusGoogle Scholar

[22]J. Luo, S. Megee, I. Dobrinski
Asymmetric distribution of UCH-L1 in spermatogonia is related to maintenance and differentiation of spermatogonial stem cells
J Cell Physiol, 220 (2009), pp. 460-468, 10.1002/jcp.21789
CrossRefGoogle Scholar

[23]M. Nagano, B.Y. Ryu, C.J. Brinster, M.R. Avarbock, R.J. Brinster
Maintenance of mouse male germline stem cells in vitro
Biol Reprod, 68 (2003), pp. 2207-2214, 10.1095/biolreprod.102.014050
View Record in ScopusGoogle Scholar

[24]H. Kubota, M.R. Avarbock, R.L. Brinster
Culture conditions and single growth factors affect fate determination of mouse spermatogonial stem cells
Biol Reprod, 71 (2004), pp. 722-731, 10.1095/biolreprod.104.029207
View Record in ScopusGoogle Scholar

AUTHOR AFFILIATION

PROF. HWISHART; PROF. MARTHAN