Dear Editor,

The merits of using quail as an avian experimental model include high egg production, low maintenance cost, small body size, and short generation period (approximately 6–8 weeks). Indeed, these characteristics make quail an ideal species for many biological areas including transgenic research.1 Methods of primordial germ cell (PGC)-mediated germline chimera production are considered very reliable and have been used in avian transgenesis. The production of germline chimeras for transgenic research mediated by PGCs is the most prominent system used to develop avian models, especially in the chicken.2 Several studies have also reported successful production of germline chimeric quails by transferring gonad-derived PGCs, and the method allows the production of diverse transgenic quails.3,4 However, compared with those for chickens, the methods for long-term culture and in vitro manipulation of quail PGCs are still limited for their full use.

The transplantation of male germ cells including spermatogonia and spermatogonial stem cells (SSCs) is an efficient method to study spermatogenesis and the control of male fertility. Recently, we reported successful cultivation of quail SSCs for certain periods by optimizing the culture conditions.5 Successful cultivation of quail SSCs for certain periods by optimizing the culture conditions.6

To produce germline chimeric quails using spermatogonial cells, 3×10⁶ non-cultured WP quail TCs and 14-day cultured SSCs labeled with PKH26 red fluorescence dye (Sigma-Aldrich, St. Louis, MO, USA) were transplanted into four D strain quail testes (two quails each for TC transplantation and SSC transplantation) 2 weeks after busulfan treatment (Table 1). To confirm the localization of spermatogonial cells in the transplanted testes, 20-μm-thick cryosections from one testis were stained with DAPI (Sigma-Aldrich, St. Louis, MO, USA) and examined using a fluorescence microscope (SMZ1000, Nikon Corporation, Tokyo, Japan). As a result, the transplanted TCs were identified in the inner spaces of the seminiferous tubules of the recipient testes, confirming the localization of the implanted cells (Figure 1g). According to a previous study in the quail, the fertility is increased to about 60% after about 45 days of 40 mg kg⁻¹ busulfan treatment.7 Therefore, subsequent testcross analyses were performed after 1 month from TC/SSC transplantation. The results showed that germline transmission had occurred in two of three recipients. Regarding the phenotypic characteristics, the hybrids (D/d') had dark brown feathers, whereas the donor (d'/d')-derived progenies

1Department of Agricultural Biotechnology, Research Institute of Agriculture and Life Sciences, College of Agriculture and Life Sciences, Seoul National University, Seoul 08826, Korea; 2Division of Animal Science, Faculty of Agriculture, Shinshu University, Minamininowa, Nagano 399-4598, Japan; 3Institute for Biomedical Sciences, Shinshu University, Minamininowa, Nagano 399-4598, Japan.
Correspondence: Dr. JY Han (jaehan@snu.ac.kr)
Received: 08 September 2017; Accepted: 14 December 2017

Production of germline chimeric quails following spermatogonial cell transplantation in busulfan-treated testis

Young Min Kim1, Jin Se Park1, Jong Won Yoon1, Hee Jung Choi1, Kyung Je Park1, Tamao Ono2,3, Jae Yong Han1,3

Asian Journal of Andrology (2018) 20, 414–416; doi: 10.4103/aja.aja_79_17; published online: 6 February 2018

Production of germline chimeric quails following spermatogonial cell transplantation in busulfan-treated testes

Open Access
LETTER TO THE EDITOR
had yellow and black stripes (Figure 1h). As shown in Table 1, 61 progenies were hatched from one recipient transplanted with TCs, and seven of them were identified as donor-derived progenies (11.5% germline transmission efficiency). In the two recipients of SSCs, 64 progenies were produced from recipient quail SSC #1, but no donor-derived progenies were identified. On the other hand, in recipient quail SSC #2, 42 progenies were produced, and seven of them were identified as donor-derived progenies (16.7% germline transmission efficiency) (Table 1). These germline transmission efficiencies are slightly lower than the chicken germline chimera achieved by testicular cell transplantation into the gamma-ray-irradiated testis but higher than the chicken germline chimera achieved by spermatogonial cell transplantation into the chicken testis without sterilization. We presumed that residual busulfan may inhibit donor cell’s (SSCs #1) localization or spermatogenesis in the recipient testis.

In conclusion, our study demonstrates that quail germline chimeras can be produced by simple transplantation of spermatogonial cells with busulfan-mediated endogenous germ cell reduction. We are the first to use this strategy to produce donor-derived progeny using adult quail germ cells. Compared with the PGC-mediated method, this strategy is simple and leads to rapid generation of quail germline chimeras. This will lead to production of transgenic models from adult germ cells and, through the production of germline chimeras, help in efforts to conserve avian species.

**AUTHOR CONTRIBUTIONS**

JYH participated in the study design and coordination. YMK participated in the design of the study, carried out the experiments and statistical analysis, and wrote the first draft of the manuscript. JSP, JWY, HJC, and KJP were involved in data interpretation. TO participated in...
writing the final version of the manuscript. All authors have read and approved the final manuscript.

**COMPETING INTERESTS**
All authors declared no competing interests.

**ACKNOWLEDGMENTS**
This work was supported by the National Research Foundation of Korea (NRF) Grant 2015R1A3A2033826 (Ministry of Science, Information and Communication Technology, and Future Planning; MSIP) and the Cooperative Research Program for Agriculture Science and Technology Development (Project PJ012866012017) from the Korean Rural Development Administration. This research was also supported by the International Research & Development Program of the National Research Foundation of Korea (NRF) funded by the Ministry of Science, ICT and Future Planning of Korea (NRF-2016K1A3A1A21005676).

**REFERENCES**
1. Huss D, Benazeraf B, Wallingford A, Filla M, Yang J, et al. A transgenic quail model that enables dynamic imaging of amniote embryogenesis. Development 2015; 142: 2850–9.
2. van de Lavoir MC, Diamond JH, Leighton PA, Mather-Love C, Heyer BS, et al. Germline transmission of genetically modified primordial germ cells. Nature 2006; 441: 766–9.
3. Kwon SC, Choi JW, Jang HJ, Shin SS, Lee SK, et al. Production of biofunctional recombinant human interleukin 1 receptor antagonist (rhIL1RN) from transgenic quail egg white. Biol Reprod 2010; 82: 1057–64.
4. Shin SS, Kim TM, Kim SY, Kim TW, Seo HW, et al. Generation of transgenic quail through germ cell-mediated germline transmission. FASEB J 2008; 22: 2435–44.
5. Pramod RK, Lee BR, Kim YM, Lee HJ, Park YH, et al. Isolation, characterization, and *in vitro* culturing of spermatogonial stem cells in Japanese Quail (*Coturnix japonica*). Stem Cells Dev 2017; 26: 60–70.
6. Tagirov M, Golovan S. The effect of busulfan treatment on endogenous spermatogonial stem cells in immature roosters. Poult Sci 2012; 91: 1680–5.
7. Jones P, Jackson H. Estimation of the duration of spermatogenesis in Japanese quail, *Coturnix Coturnix japonica*, using antispermatogonial chemicals. J Reprod Fertil 1972; 31: 319–22.
8. Bucci LR, Meistrich ML. Effects of busulfan on murine spermatogenesis: cytotoxicity, sterility, sperm abnormalities, and dominant lethal mutations. Mutat Res 1987; 176: 259–68.
9. Trefil P, Micakova A, Mucksova J, Hejnar J, Poplestein M, et al. Restoration of spermatogenesis and male fertility by transplantation of dispersed testicular cells in the chicken. Biol Reprod 2006; 75: 575–81.
10. Lee YM, Jung JG, Kim JN, Park TS, Kim TM, et al. A testis-mediated germline chimera production based on transfer of chicken testicular cells directly into heterologous testes. Biol Reprod 2006; 75: 380–6.