Development of Germline Chimera Production System by Spermatogonial Stem Cell Transplantation in Chicken

Young Mok Lee[1], Jin Nam Kim[1], Tae Sub Park[1], Duk Kyung Kim[1], Yeong Ho Hong[2], Jeong Mook Lim[1], and Jae Yong Han[1]
[1]*School of Agricultural Biotechnology, Seoul National University, Korea*
[2]*Avicore Biotechnology Institute, Korea*

Abstract

최근 생쥐에서 정원세포를 이용한 생식선 카이메라의 생산이 보고되었다. 정원세포의 경우 성축으로부터 세포를 다량으로 얻기가 쉬우며, 수용체 정소 내로 이식될 경우 생식선 카이메라의 생산능력이 있어서 이전의 배아줄기 세포를 이용할 때의 문제점을 효율적으로 해결할 수 있다. 또한 유전자가 도입된 정원세포의 이식에 의한 수용체 정소 내에서의 정자형성의 보고는 정원세포를 이용한 형질전환 동물의 생산 시스템으로의 개발 가능성을 보여준다.

본 실험에서는 닭에서 기존에 이용되어 왔던 형질전환 동물 생산 시스템의 문제점을 극복하고자 주령별 정원세포의 분리 및 이식을 통하여 조류에서 정원세포의 이식방법을 확립하고 생식선 카이메라 생산 효율을 증진시키기 위하여 불임제인 부설판 등을 이용한 불임화 기술을 확립하여, 결국 조류에서의 형질전환 조류 생산 시스템으로서의 개발가능성을 제시하고자 한다.

(Key words : 닭 정원세포, 생식선 카이메라, 부설판, 불임화, 형질전환 조류생산 시스템)

Introduction

Recently, a transplantation system of mouse testicular cells from a fertile male to the seminiferous tubules of an infertile recipient male has been developed. When the donor germ cells are transplanted into the testes of infertile recipient animals, they could proliferate in recipient seminiferous tubules, generate colonies and produce donor-derived progenies. The spermatogonial transplantation method has been utilized on studying the biology of male germline stem cells, restoring fertility in infertile animals and introducing foreign gene into the germline to produce transgenic animals.

Testicular cell-transplantation has now been performed using donor testis cells from experimental animals, livestock and human. Despite economical and experimental benefits, no attempt have been made to apply the transplantation technique In birds. However, its anatomical structures, physiological characteristics and the spermatogenesis are almost the same process like mammalian species. Thus, In this study, we developed transplantation method for avian species and optimized adequate sterilization conditions for transplantation using busulfan treatment.

Materials and Methods

Testes were obtained from male Korean Ogol chickens at 4 weeks and 24 weeks. Testicular cells were collected by a two-step enzymatic digestion method. Testicular cells were placed on a feeder layer of testicular stromal cells in a six-well tissue culture plate at 2×10^6 cells/well. Cells were cultured at 37 ℃ for 0~15 days in an atmosphere of 5 % CO_2 in air. In vitro-cultured cells for 0, 5, 10, 15 days, respectively, were transplanted to recipient White Leghorn(WL) testis and testcross was conducted with Korean Ogol female. To study sterilization effects, busulfan was treated to recipient White Leghorn male(40 mg/kg).

Results

As a result, germline chimeras were produced and transplanted donor cell-derived progenies were induced. Busulfan treatment showed the disruption of spermatogenesis and so decreased sperm number in ejaculated semen. Our Results showed that the spermatogonial transplantation technique which used in mammalian species was successfully applicable in chicken and busulfan treatment would enhance germline chimerism efficiency.

Reference

- Brinster RL, Zimmermann JW. Spermatogenesis following male germ-cell transplantation. *Proc Natl Acad Sci* 1994; 91:11298-11302.
- Hemsworth BN, Jackson H. Effect of busulphan on the developing gonad of the male rat. *J Reprod Fertil* 1963; 5:187-194.
- Ogawa T, Avarbock MR, Brinster RL. Transplantation of testis germinal cells into mouse seminiferous tubules. *Int J Dev Biol* 1997; 41:111-122.