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Review

AVIAN PRIMORDIAL GERM CELLS AND THEIR APPLICATION

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ABSTRACT

Primordial germ cells (PGCs) are the precursors of the progenitors of the oocytes and spermatocytes. The unique properties and accessibility of avian PGCs during early development provide an opportunity to manipulate with the avian germplasm. In this review the recent developments of the embryo manipulation techniques and the use of PGCs for the conservation of genetic resources and as a vehicle for the efficient production of transgenic chickens are described.

Key words: poultry; avian germ cells; embryo manipulation

INTRODUCTION

Germ cells play a very important role in the species identification as they transmit genetic information to the next generation. In many animal species, including birds, germ cells emerge from a small population of cells known as primordial germ cells (PGCs). Avian PGCs (Figure 1) arise from the pluripotent epiblast and are initially localized in the central disc of the pellucida area of X stage embryos. Once blood vessels form, PGCs enter the blood vessels and migrate into the forming gonad and differentiate nto mature spermatozoa and oocytes in the adult. Chicken PGCs were collected from the germinal crescent (Naito *et al*., 2001), the embryonic blood vessel (Naito *et al.*, 1994) and from the embryonic gonads (Park *et al.*, 2003). Although no phenotypic changes between the different sources of PGCs were observed, and the expression patterns of specific markers were identical (Park and Han, 2012), however the advantage of using gonadal PGCs compared with another sources of these cells is, that a greater number of PGCs can be retrieved from one embryo.

These cells can be cultured and expanded *in vitro*, without loss of germ cell integrity. PAS (periodic acid-Schiff) reaction is a generally accepted histochemical marker for differentiating PGC from surrounding somatic cells (Chojnacka-Puchta *et al.*, 2012). In addition to PAS, chicken PGCs are stained selectively by specific cell surface antigen SSEA-1, which is often used as a marker for stem cell differentiation (Jung *et al*., 2005).

The unique properties and accessibility of avian PGCs during early development provide an opportunity to manipulate with the avian germplasm. Two uses of PGCs are often reported: these cells can be used for the conservation of genetic resources and the production of transgenic birds (see Nakamura *et al*., 2013). An immediate technological application of PGCs is for the preservation of germplasm from specialised and rare breeds of poultry (see Glover and McGrew, 2012). To generate

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a genetically diverse population it will be necessary to preserve the germplasm from a minimum of 13 individuals of each sex (FAO, 1998).

Various methods have been developed to produce transgenic chickens (Table 1). However, the injection of recombinant PGCs into recipient embryos (see Chojnacka-Puchta *et al*., 2012) is currently one of the most widely used non-viral procedure for creating transgenic birds. Generation of transgenic chickens through chimeric intermediates, produced by the transfer of PGCs, has been attempted in our laboratory. This method is particularly important for studying the development of chimeric and/or transgenic organisms.

However, despite above cited achievements, currently the efficiency of the transgenic method is low

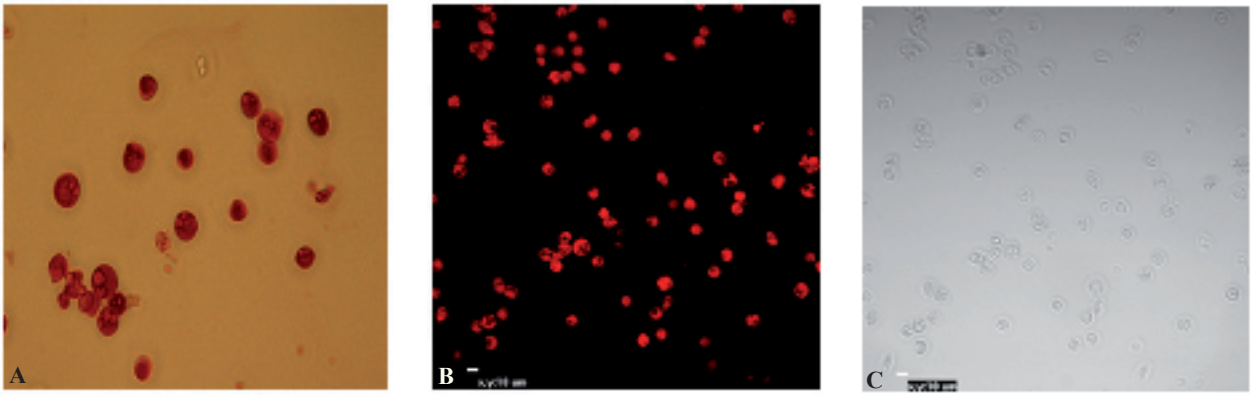

Fig. 1: PAS reaction indicates the presence of glycogen vesicles and red-stained chicken PGCs (A) (40× magnification). Immunofluorescence staining for the specific cell surface antigen SSEA-1; chicken blood PGCs were found (stages 14–16 HH) to express SSEA-1. Anti-SSEA-PerCp-positive cells are shown in red (B) and bright field (C) (adapted from Chojnacka-Puchta, 2012)

Table 1: Milestones of chicken's transgenesis

Date of publication	Achievement	Authors
1986	First successful development of transgenic chickens	Salter *et al.*
1990	First germ line chimeric chicken	Petitte *et al.*
1993	Transgenic birds produced from transformed PGCs	Vick & Simkiss
1996	Pluripotent ESC derived from blastoderm	Pain *et al.*
2003	Expression of a human interferon in egg white	Rapp *et al.*
2004	Lentiviral vectors, able to infect non-dividing cells	McGrew *et al.*
2005	Somatic chimeras produce the significant amounts of a therapeutic antibody in the egg white	Zhu *et al.*
2006	Germline transmission of grown *in vitro*, and genetically modified PGCs	van de Lavoir *et al.*
2007	Commercially significant amounts of a therapeutic protein in the egg white	Lillico *et al.*
2011	Suppression of avian influenza transmission in genetically modified chickens	Lyall *et al.*
2012	Use of transposons to modify the chicken genome	Macdonald *et al.*, Park and Han, Yang and Kim
2012	Hen's oviduct epithelial cells culture	Kasperczyk *et al*.

in many cases. On the basis of publications indexed in the Web of Knowledge some major problems concerning the current poultry transgenesis can be defined as following:

- a major problem in creation of transgenic chickens (using any methodology) by manipulating embryos is achieving a sufficient hatchability,
- only a small number of bPGCs and little larger number of gPGCs can be isolated from blood and/or gonads,
- difficult to control differentiation of cells in long-term culture systems,
- very low efficiency of integration of the transgene into the host genome by conventional transfection methods,
- the retroviral construct integrates randomly into the host genome, which may result in transgene expression at various developmental stages and in various tissues.

Some of these problems will be discussed in the light of our research.

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