The transgenic chickens obtained by microinjection of DNA in the ovarian follicles

Liudmila Korshunova, Ruben Karapetyan, Osiya Ziadinova, Vladimir Fisinin

Federal Scientific Center "All-Russian Research and Technological Institute of Poultry" of Russian Academy of Sciences; Sergiev Posad, Moscow Province, Russia

Corresponding author: lg@vnitip.ru

The technology of the transgenesis applied is based upon a surgery providing external access to the ovary and subsequent natural deposition of membranes resulting in eggs suitable for incubation. The microinjection of foreign DNA directly into the blastodiscs of large pre-ovulated follicles within the ovarian hierarchy improved the operation rate in compare to microinjections of ovulated follicles through the wall of the infundibulum. Though the access to ovary is more difficult than to infundibulum, and follicular membrane is harder and thicker than infundibular wall, this technique has certain important advantages including the lack of necessity in the determination of ovulatory cycle timing and ovulation times for every follicle to be injected. The technique gives 2-3 injected follicles per surgery per hen without possible problems related to the irregular deposition of tertiary membranes. The trials were performed on White Leghorn chicken; the injected construct was linearized plasmid pSMTHG9 containing metallothionein promoter and gene of growth hormone (GH). 45 chickens were operated; 120 follicles were injected; 56 whole injected eggs were obtained and incubated; 40 day-old chicks were hatched and studied for the transgenicity. GH was found in the blood of 10 chicks in concentrations of 0.5-1.0 ng/ml; in 2 chicks the nucleotide sequences of GH were found in the DNA of whole blood. It can result from the mosaicism of the obtained transgenic birds.

Keywords: transgenesis, microinjection of DNA, chicken

Efficiency of Sleeping Beauty transposase mediated transfection of chicken PGCs in vivo and in vitro and analysis of the derived cell chimeric roosters

Stefanie Altgilbers, Ronald Wittig, Meike Stünkel, Steffen Weigend, Wilfried Kues, Sabine Klein

Friedrich-Loeffler-Institut, Federal Research Institute for Animal Health, Institute of Farm Animal Genetics Mariensee, Höltystr. 10, D-31353 Neustadt, Germany

Corresponding author: sabine.klein@fli.de

Transfections of Primordial Germ Cells (PGCs) either in vivo or in vitro are now important tools for gene transfer experiments in avian embryos. However, there are few reports on the functionality of germ line transmission especially for in vivo transfections. This study reports on efficiency comparison for both transfection strategies using the Sleeping Beauty (SB) transposon system encoding a ubiquitously expressed Venus fluorophore reporter.

In vivo transfections of SB components as plasmid/liposome complexes were performed into chicken embryos at embryonic day (E) 3. We recorded up to 50% of embryos with Venus-positive cells in the gonads at E10. Immuno-histochemical labeling with SSEA1 confirmed small clusters of primordial stem cells migrating into the gonads. In two experiments with 80 and 76 embryos injected, a total of 24 and 28 chicks hatched, respectively. Total 19 roosters were successfully raised to maturity and provided semen samples. However only three cocks showed 0.05 to 0.20% Venus-positive sperm in their ejaculates by flow cytometry.

Cultured PGCs were electroporated with the SB and Venus plasmids and Venus positive cells were enriched by FACS. After injection of the Venus-positive PGCs into 80 host embryos at E3, total 13 roosters could be raised and trained for semen collection. All 13 cocks provided between 1 and 8% Venus-positive spermatozoa in their ejaculates. The best seven roosters are currently mated to White Leghorn hens for progeny testing. Comparing results of the two transfection strategies, transfer of in-vitro pre-transformed PGCs proved advantageous over in vivo transfections.

Keywords: primordial germ cells, *in vivo* transfection, Sleeping Beauty transposase

The effect of a diet high in methyl donors on DNA methylation patterns across multiple generations

Chelsea A. Phillips and Chris M. Ashwell

Prestage Department of Poultry Science, NC State University, Raleigh, NC 27695 USA

Corresponding author: cmashwel@ncsu.edu

The impact of the environment on epigenetic regulation and ultimately phenotypes has attracted considerable interest in animal agriculture. In many studies, environmentally induced changes in gene expression are associated with altered DNA methylation patterns or with altered histone modifications. This study focused on the systematic evaluation of DNA methylation across