## Summary

# The production of somatic nuclear transferred chickens using primordial germ cells

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The somatic nuclear transfer technique is an important tool for breeding domestic animals, conserving genetic resources, producing transgenic animals, and studying developmental biology. Although somatic clones of several mammals have been produced, this technique has not been applied to avian species because of the morphological and structural properties of their ova.

Primordial germ cells (PGCs) are candidate nuclear recipients, because germline chimeric chickens can be produced by PGC injection.

This study examined the production of somatic nuclear transferred chickens using PGCs as the nuclear recipient. This involved the functional enucleation of PGCs by UV irradiation, the production of somatic nuclear-transferred PGCs (NT-PGCs) by electrofusion, and the production of germline chimeric embryos using NT-PGCs.

It is difficult to remove the nuclei from PGCs because of their large size. Therefore, we inactivated the nuclei using UV irradiation to obtain functional enucleated PGCs. UV-irradiated PGCs had significant DNA damage, normal viability, and normal migratory ability. These results suggest that PGCs are possible nuclear recipients.

Somatic nuclear transfer to PGCs was performed by electrofusion.

PGCs and embryonic blood cells (EBCs), as the somatic nuclear donor, in the fusion fluid were aligned using an AC field, and then fusion between cells in close contact was induced by DC pulses. Approximately 10 % PGCs were fused with EBCs by a 350-V/cm AC field for 60 sec, with three 4-kV/cm DC pulses, using 0.25 M fusion fluid.

The NT-PGCs produced by UV irradiation and electrofusion were injected into 2-day-old recipient embryos. NT-PGCs were detected from gonads of 7-day-old recipient embryos. This experiment showed that NT-PGCs can migrate to the gonadal ridge and produce germline chimeric embryos.

In conclusion, NT-PGCs can be produced by functional enucleation with UV irradiation, followed by nuclear transfer with electrofusion. Furthermore, the injection of recipient embryos with NT-PGCs can produce germline chimeric embryos.

Future studies should examine ways to improve the efficiency of producing NT-PGCs, conduct a physiological analysis of NT-PGCs, examining proliferation, meiosis, and differentiation, and ultimately produce somatic nuclear transferred chickens.