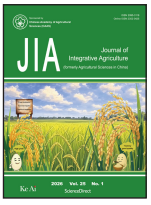
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RESEARCH ARTICLE

Establishing an induced infertile chicken line for efficient germline transmission of exogenous PGCs

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Highlights

- Generation of a novel sterile chicken host by targeting HSV-TK to the DAZL locus *via* CRISPR/Cas9.
- The sterile host eliminates endogenous PGC competition, enhancing donor PGCs transmission efficiency.

Abstract

Primordial germ cells (PGCs) are the stem-cell population of adult animal gametes, which develop into sperm or eggs. It can be propagated *in vitro* and injected into the host chicken for genome editing to obtain germline chimeric chicken. However, it has the limitation that the host embryo contains endogenous PGCs, which raises complications, resultantly donor PGCs fail to compete, and transmission efficiency reduced. Therefore, to increase the transmission efficiency, we generated a novel sterile chicken with the inducible elimination of endogenous PGCs in the host. This is the first study that applied the herpes simplex virus thymidine kinase (*HSV-TK*) cell ablation system in avian. CRISPR/Cas9-mediated homology-directed repair was performed to localize the *HSV-TK* suicide gene to the last exon of the deleted in azoospermia-like (*DAZL*) gene, and ganciclovir (GCV) was added to induce the apoptosis in the germ cells of the host embryo. The sterilized host embryo introduced genome-edited PGCs to produce chimeric chicken carrying exogenous germ cells only. It was observed that the germline transmission efficiency was 100% achieved, and the obtained chicks were purely from donor breeds. The technologies established in the current study have important applications in germplasm conservation and gene editing in chicken.

Keywords: chicken embryos, germline transmission efficiency, herpes simplex virus thymidine kinase, primordial germ cells

1. Introduction

Primordial germ cells (PGCs) are the progenitor cell type of functional gametes, which turn into either sperm or oocytes and are responsible for the transmission of genetic information between generations. The avian PGC at stage X is centrally located in the transparent zone on the ventral surface of the ectoderm (Kagami *et al*. 1997; Pilato *et al.* 2013). As the embryonic primitive strips are formed, the PGCs migrate to the germinal crescent zone, and start to enter the embryonic circulation associated with the vasculature formation. Subsequently, PGCs migrate along the dorsal mesentery to the genital ridge after actively leaving the vessels (Kuwana and Rogulska 1999; Kang *et al.* 2015). When, avian PGCs are compared with mammalian PGCs, their unique migratory mode helps to develop PGC-mediated germline transmission and transgenic systems.

Avian embryo PGCs are usually isolated and cultured from embryos at three developmental stages, including germinal crescent formation at Hamburger and Hamilton (HH) 4–8 stages, vasculature at HH14–16 stages, and gonadal ridge at HH26–28 stages. The cultured PGCs can be injected into the host chicken embryo to produce chimeric chickens (Han and Lee 2017; Altgilbers *et al.* 2021). PGCs are the germline-competent cells, and “germline chimera” usually

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refers to the mixture of gametes from different breeds or gene-edited species present in the same organism. *In vitro* culture conditions of PGCs have been continuously optimized to improve the efficiency of germline transmission and to produce germline chimeras (Song *et al.* 2014; Whyte *et al.* 2015; Lee *et al.* 2016b; Miyahara *et al.* 2016). However, long-term *in vitro* culture of PGCs faces complications and usually leads to the gradual loss of germline competence after transplantation to the host embryo, it might be the inferior of transplanted PGCs that fail to compete with the endogenous PGCs in the niche of spermatogenesis.

A feasible strategy to increase the transmission efficiency is to eliminate the endogenous germ cells by knocking out genes required for germ-cell development. In mammals, (mice, pigs, goats and cattle) a CRISPR/Cas9 gene editing model generated to knockout Nanos C2HC-type zinc finger 2 (*NANOS2*) alleles, which is reported to be devoid of germ cells while exhibiting normal testicular structures. This model of male infertility can be used for the transplantation of donor spermatogonial stem cells (Park *et al.* 2017; Ciccarelli *et al.* 2020). However, in chicken, targeting the *DDX4* (Deleted in azoospermia-like) locus in PGCs using TALE nuclease technology resulted in large deletions of the entire *DDX4* locus. In *DDX4*-knockout chickens, only female PGCs were largely lost during meiosis, leading to sterility (Taylor *et al.* 2017). Cytotoxicity induced by the suicide gene is another efficient strategy to eliminate the endogenous germline. Cell ablation strategies by suicide genes include the use of cytosine deaminase, herpes simplex virus type 1 thymidine kinase (*HSV-TK*), and induction of caspase-9 (iCaspase9). Studies have shown that iCaspase9 expressed from the *DAZL* locus selectively ablates the germ cell lineage in birds (Ballantyne *et al.* 2021). The *HSV-TK* gene combined with ganciclovir (GCV) is one of the most widely used enzyme/prodrug suicide system (Gentry 1992; Fillat *et al.* 2003). After the *HSV-TK* suicide system phosphorylates the nontoxic ganciclovir (GCV), cell kinase again phosphorylates GCV to produce GCV triphosphate, which inhibits DNA elongation and cell division and induces apoptosis (Iwasawa *et al.* 2019).

In vitro-propagated PGCs can be used for genome editing and reconstruction of rare chicken breeds. PGCs can readily be microinjected into alternative host chicken embryos, after which the embryos are hatched, raised, and matured to produce offspring from the donor cells (Lee *et al.* 2019; Altgilbers *et al.* 2021; Gessara *et al.* 2021; Lázár *et al.* 2021; Lee *et al.* 2022). The *deleted in azoospermia-like* (*DAZL*) is a germ cell determinant that maintains germ cell pluripotency (Chen *et al.* 2014; Lee *et al.* 2016a). To improve the transmission of donor genetic, we first evaluated the effect of the *HSV-TK*/GCV suicide system on the specific ablation of PGCs. Then, we knocked in the *HSV-TK* gene into the *DAZL* locus by using the CRISPR/Cas9 gene editing method and demonstrated that PGCs specifically expressing *HSV-TK* could be eliminated after the addition of GCV to the embryos. The use of *HSV-TK* sterile host chicken embryos directly improved the germline transmission efficiency after transplantation of the exogenous PGCs.

2. Materials and methods

2.1. Animals

PGCs were isolated from Donglan chicken-fertilized eggs (Guangxi Autonomous Region, China). Gene-edited *DAZL-HSV-TK-EGFP* PGCs were injected into white leghorn chicken fertilized eggs (Boehringer-Ingelheim, Beijing, China) to obtain male *HSV-TK* chimeric chickens. The derived chimeras were crossed with Roman Pink chickens to produce heterozygous *HSV-TK* in G_1 offspring. The heterozygous G_1 generation *HSV-TK* was bred to obtain the G_2 homozygous *HSV-TK* chicken and the embryos were subsequent use as host for injection.

2.2. Plasmid construction

The *HSV-TK* gene sequence was synthesized and inserted into the plasmid *DAZL* left arm-P2A\2A-*EGFP**DAZL* right arm-targeted 18T vector, which was verified by sequencing. Using the CHOPCHOP gDNA design web tool (http://chopchop.cbu.uib.no/), the single guide RNA (sgRNA) was designed for the insertion site of exon 11 on the *DAZL* gene (sgRNA sequence 5′-agggcgcatcacttcagaaaagg-3′). The annealed sgRNA oligonucleotides were constructed into the pX458 2.0 spcas9-*EGFP* vector plasmid.

2.3. Culture and transfection of PGCs

PGCs were isolated from HH 27–31 stages embryonic gonads. Gonadal tissues were treated with 0.05% trypsin-ethylenediaminetetraacetic acid (EDTA, Gibco, USA) for 10 min at 37°C, and the digestion was terminated using The Dulbecco's modified eagle medium/nutrient mixture F-12 (DMEM/F12) containing 10% fetal bovine serum. After 4–5 h of incubation, the supernatants were aspirated and centrifuged to collect the precipitate containing the cells. The cells were resuspended in knockout DMEM and inoculated in inset-containing 24-well plates with mouse embryonic fibroblasts at the bottom (Xie *et al.* 2019). To prepare *HSV-TK* PGCs, a *DAZL*-guided *HSV-TK* targeting vector and a CRISPR/Cas9 vector were transfected into the PGCs using transfection reagent *Xfect*™ transfection reagent (TaKaRa, Japan) as per the manufacturer's instructions. After culturing for 4 wk, cells positive for *HSV-TK-EGFP* were sorted using a flow cytometer (BD FACSAria II, USA). The sorted PGCs were cultured for 3 wk and cryopreserved as donor cells for transplantation.

2.4. Injection of PGCs to host embryos

The PGCs were injected into the HH 15–16 stages host chicken embryos. A total of 1×10^4 PGCs were injected into the dorsal aorta of each embryo and the windows were sealed with paraffin film. For protocol of using *HSV-TK* embryo as host, 100 μL PBS (Phosphate-Buffered Saline) containing 2% penicillin/streptomycin and 0.165 mmo L^{-1} GCV was applied to the embryos prior to PGC transplantation. After hatching, the chimeric chicks were raised to sexually mature and the males and females were mated to produce G_1 offspring. The *HSV-TK* transgene was detected in offspring by using PCR.

2.5. Cell immunofluorescence assay

PGCs were fixed in 4% paraformaldehyde at room

temperature for 10 min and permeabilized with 0.1% TritonX-100 for 10 min. The cells were washed twice with PBS, followed by the addition of 100–200 μL blocking solution containing PBS, 5% goat serum, and 1% bovine serum albumin to each cycle and incubation for 45 min at room temperature. Further, the cells were incubated with rabbit anti-*cDAZL* antibody (in-house preparation), mouse anti-*SSEA1* (Stage-Specific Embryonic Antigen-1, Hybridoma Bank, Mouse, Mc-480), or rabbit anti-*EGFP* (Bioss) for 1 h. After three washes with PBS, the PGCs were incubated with corresponding secondary antibody (ThermoFisher, A31573/A2104/A-11034) for 1 h. Finally, the nuclei were counterstained with *DAPI* ProLong Gold antifade reagent (ThermoFisher, P36941, USA) and visualized under a fluorescence microscope.

2.6. Cell proliferation assay

PGCs were seeded into 24-well plates (ThermoFisher, USA) at a density of 80,000 cells per well. Subsequently, 10 μmol L^{-1} GCV was added and the cells were incubated for 48 h at 37°C in a 5% CO_2 incubator. Cell viability was assessed by measuring the absorbance at 450 nm using the Cell Counting Kit-8 (CCK-8) as per the manufacturer's instructions (Biosharp, China). The graphs for cell proliferation were plotted using Prism 6.0 (GraphPad, USA).

2.7. Tissue histochemistry assay

Tissues were fixed with 4% paraformaldehyde for 24 h, placed in a dehydrator (Donatello, DIAPATH) under the dehydrating protocol using the gradients of ethanol. The tissue was embedded in wax using the embedding machine (HistoCore Arcadia, Germany). The trimmed wax block was cooled to –20°C, and were cut into 4-μm-thick slices using a paraffin slicer (RM2255, Leica, Wetzlar, Germany). Further, the tissue was placed on glass slides and heated in an oven at 60°C. After the dried wax was melted, the slides were taken out from the oven and stored at room temperature. Further, they were stained using hematoxylin and eosin (Servicebio, China) and observed under microscope.

3. Result

3.1. Genome editing to produce *DAZL-HSV-TK-EGFP* chicken PGCs

To visualize the PGCs after genetic modification, the donor plasmid cassette of *HSV-TK* gene was followed by an *EGFP* reporter fused to a T2A self-cleavage peptide (Fig. 1-A). To enable the specific elimination of endogenous PGCs, *HSV-TK* was targeted to the 3′-end of the last exon of the *DAZL* gene *via* CRISPR/Cas9-mediated homologous repair (HDR) in PGCs (Appendices A–C). The plasmid of CRISPR/Cas9 and donor cassette were co-transfected into PGCs, followed by enrichment for the positive cells by flow cytometry (Fig. 1-B). Immunocytochemical analysis indicated that the transfected PGCs stably expressed the germ cell-related marker *DAZL* and *SSEA1* (Fig. 1-C). The proliferation assay revealed that the growth of PGCs containing the *HSV-TK* transgene was inhibited at micromolar concentrations of GCV compared with the WT control (Fig. 1-D), suggesting the efficiency of cell ablation by *HSV-TK* suicide system in chicken PGCs.

3.2. Production of *DAZL-HSV-TK-EGFP* transgenic chicken *via* germline transmission

DAZL-HSV-TK-EGFP PGCs were injected into the vasculature of 2.5-d-old white leghorn chicken embryos, which were subsequently sealed and incubated (Appendix D). After the chimeric males were matured, semen was collected to confirm the source of the sperm with PCR (Appendix E). G_1 offspring were obtained by crossing the male chimeras (G_0) with WT hens, and 17 donor-derived *HSV-TK* offspring (G_1) were obtained from a germline chimeric chicken (Appendix F). *EGFP* fluorescence was observed only in the gonads of *HSV-TK* embryos, and the presence of the transgene was confirmed using PCR (Appendices G–I). The *HSV-TK* G_1 chicks were raised to sexual maturity, and G_1 males were mated with females to obtain homozygous G_2. *HSV-TK*-positive offspring were verified *via* PCR expression in embryos from G_2 (Fig. 2-A; Appendix J). The results revealed that *EGFP* was specifically expressed in the gonads of *HSV-TK*-positive embryos. *DAZL-HSV-TK-EGFP* hens laid eggs at rates comparable to age-matched control hens, suggesting that gene-editing did not affect oogenesis (Appendix K).

After applying GCV into HH 16 stage embryos, significant loss of *EGFP* positive PGCs were observed at 6.5 d, suggesting the efficacy of *HSV-TK* in targeting ablation of germ cells *in vivo* (Fig. 2-B). To examine the internal structure of the gonads after sterilization by GCV treatment, tissue sections of the testes and ovaries were stained using hematoxylin and eosin (H&E) staining (Fig. 2-C). Age-matched non-treated (WT and *HSV-TK*) and GCV-treated WT groups had clear testicular tissue structure, containing spermatocytes at different stages as well as late-stage spermatids. In contrast, no spermatogenic cells were observed in GCV-treated *HSV-TK* testes, while spermatogenic tubules remained intact. The gonads of control groups females contained follicles of various sizes, whereas no follicle was observed in GCV-treated *HSV-TK* females. Collectively, these results indicated that gonadal germ cells of male or female chicken can be specifically eliminated using *HSV-TK* suicide system *in vivo*.

3.3. *HSV-TK* chicken embryos as PGCs transplant hosts achieve efficient germ-line transmission

Cryopreservation of PGCs is a method to preserve the genetic of poultry breeds. We isolated and propagated the PGCs from black chickens native in Guangxi. After cryopreservation and thaw, the PGCs were transplanted into GCV treated host *HSV-TK* embryos, and host embryos were then incubated, hatched, and cultured for chimeras to sexual maturity. After crossing the chimeric male with female, the obtained offspring were 100% derived the black chicken breeds (Appendices L and M). Moreover, the donor PGCs carrying heterozygous mCherry transgene were injected into the *HSV-TK* chicken embryos treated with GCV (Fig. 3-A). The PGCs used in the injection were labeled with mCherry, which were prepared by transfection of piggyBac transposase and XP61-CAG-mCherry into

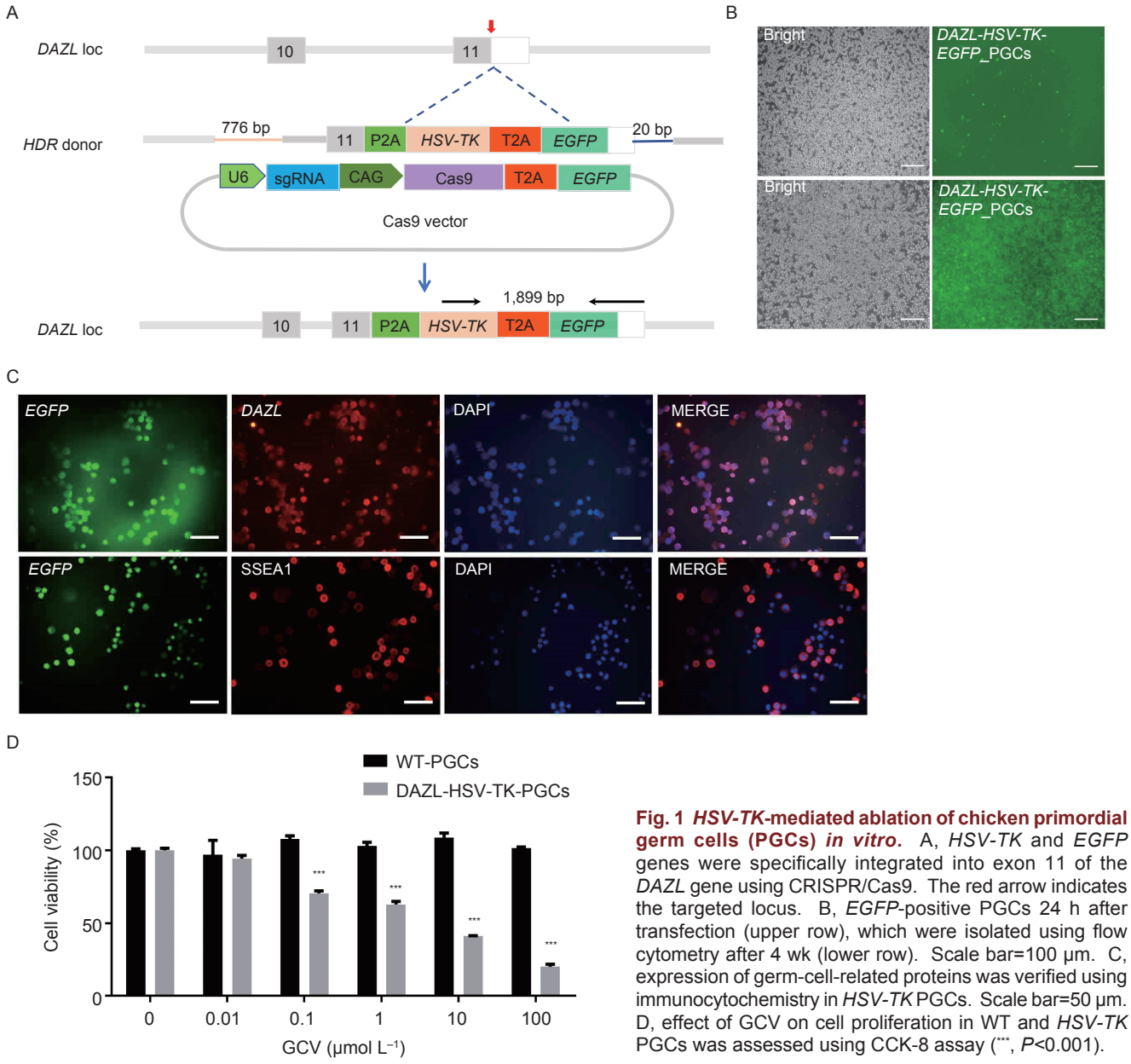


Fig. 1 *HSV-TK*-mediated ablation of chicken primordial germ cells (PGCs) *in vitro*. A, *HSV-TK* and *EGFP* genes were specifically integrated into exon 11 of the *DAZL* gene using CRISPR/Cas9. The red arrow indicates the targeted locus. B, *EGFP*-positive PGCs 24 h after transfection (upper row), which were isolated using flow cytometry after 4 wk (lower row). Scale bar=100 μm. C, expression of germ-cell-related proteins was verified using immunocytochemistry in *HSV-TK* PGCs. Scale bar=50 μm. D, effect of GCV on cell proliferation in WT and *HSV-TK* PGCs was assessed using CCK-8 assay (***, P<0.001).

the cells. The germline chimeric embryos were hatched and raised until sexual maturity. The gonads developed into normal testis and ovaries with spermatids and oocytes observed in male and female chimeras (Fig. 3-B). Direct mating of chimeric male with WT hens produced heterozygous genome-edited offspring (G_1). The *HSV-TK* transgene was undetectable in all offspring, suggesting all G_1 chicks were derived from the donor PGCs (Appendix N). Of the all chicks (63.5%) from donor mCherry-PGCs exhibited red skin. These results demonstrated that the endogenous germ cells of *HSV-TK* hosts were ablated and the use of *HSV-TK* embryos as host for PGC transplantation achieved efficient germline transmission (Fig. 3-C and D; Table 1). Thus, it is possible to regenerate a breed of chicken in gene-edited PGCs using *HSV-TK* sterile replacement hosts (Fig. 4).

4. Discussion

The rapid loss of genetic diversity in poultry breeds is significantly threatening to their unique production traits. Conventionally, living *in situ* genetic conservation is used to protect genetic traits in poultry, but it is expensive, requires special facilities, and most importantly carries risks of disease outbreaks, genetic problems, loss of reproduction, or natural disasters (Jones *et al*. 2014). PGCs are an alternative method that allows poultry genetic resources to retain intact genetics. The development of CRISPR technology and its usage in gene-editing to transfer PGCs from donor to host animal has great significance in avian genetic breeding research (Panda and Mcgrew 2022). Despite the relative tremendous progress and value of this technology, it still presents some complexities in avian genome editing that need to be addressed for better transmission efficiency of genetic traits from donor to host. The successful transmission of PGCs to the host is critical and a major challenge, which have been hinder by low transmission efficiency due to the competitive environment between endogenous and exogenous PGCs (Woodcock *et al.* 2019).

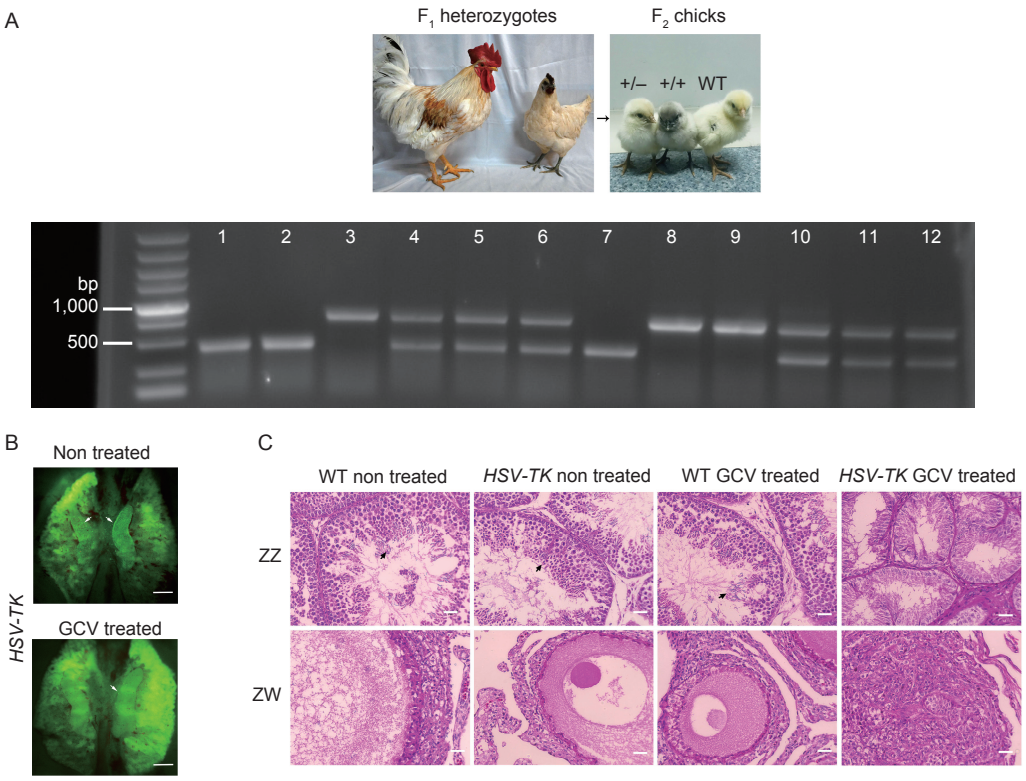


Fig. 2 *HSV-TK*-mediated ablation of chicken primordial germ cells (PGCs) *in vivo*. A, male and female heterozygous G_1 offspring were bred to obtain G_2 offspring, confirmed by PCR using *HSV-TK*-specific primers. Heterozygous: 4, 5, 6, 10, 11, and 12. Homozygous: 3, 8, and 9. WT: 1, 2, and 7. B, GCV-treated and non-treated *HSV-TK* G_2 embryos were imaged at day 6.5 of incubation. White arrows indicate the embryonic gonads. Scale bar=100 μm. C, non-treated and GCV-treated gonads were stained with hematoxylin and eosin at 28 wk. Scale bar=25 μm.

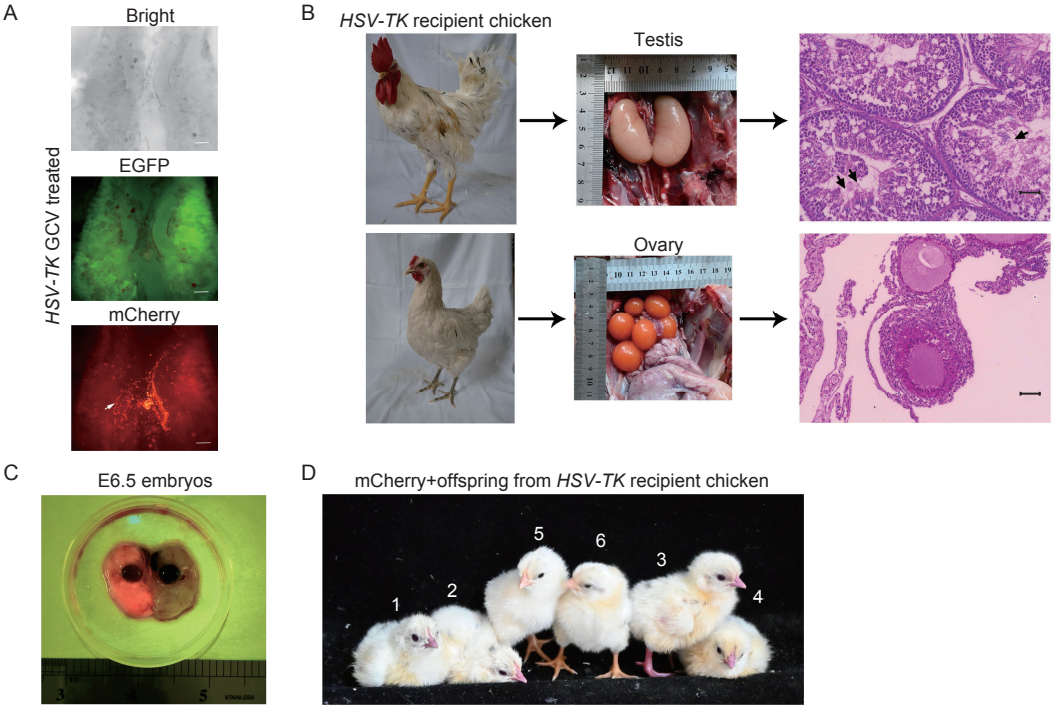


Fig. 3 Efficient germline transmission after transplantion of primordial germ cells (PGCs) to *HSV-TK* embryos. A, donor mCherry PGCs were injected into *HSV-TK* host chicken embryos along with GCV treatment and imaged at day 6.5 of incubation. Scale bar=100 μm. B, physical and gonadal appearance of adult *HSV-TK* host chickens after transplantation of mCherry PGC. Scale bar=50 μm. C, embryos produced by mating of chimeric chicken with WT were imaged at day 6.5 of incubation. D, offspring derived from donor heterozygous mCherry PGCs were differentiated by skin color, with those with red skin considered as the positive offspring (shown here as numbers 1, 2, 3, and 4).

Table 1 Germline transmission of *HSV-TK* hosts injected with mCherry-primordial germ cells (PGCs)

HSV-TK host mating groups	Fertility (% of eggs incubated)	Number of chicks hatched (fertile eggs, %)	Positive of mCherry	Positive for *HSV-TK* transgene (%)[1)]
*HSV-TK*_♂×WT_♀(30)	124 (57.9%)	85 (68.5%)	54 (63.5%)	0

[1)] Number of offspring PCR$^+$ for *HSV-TK* transgene.

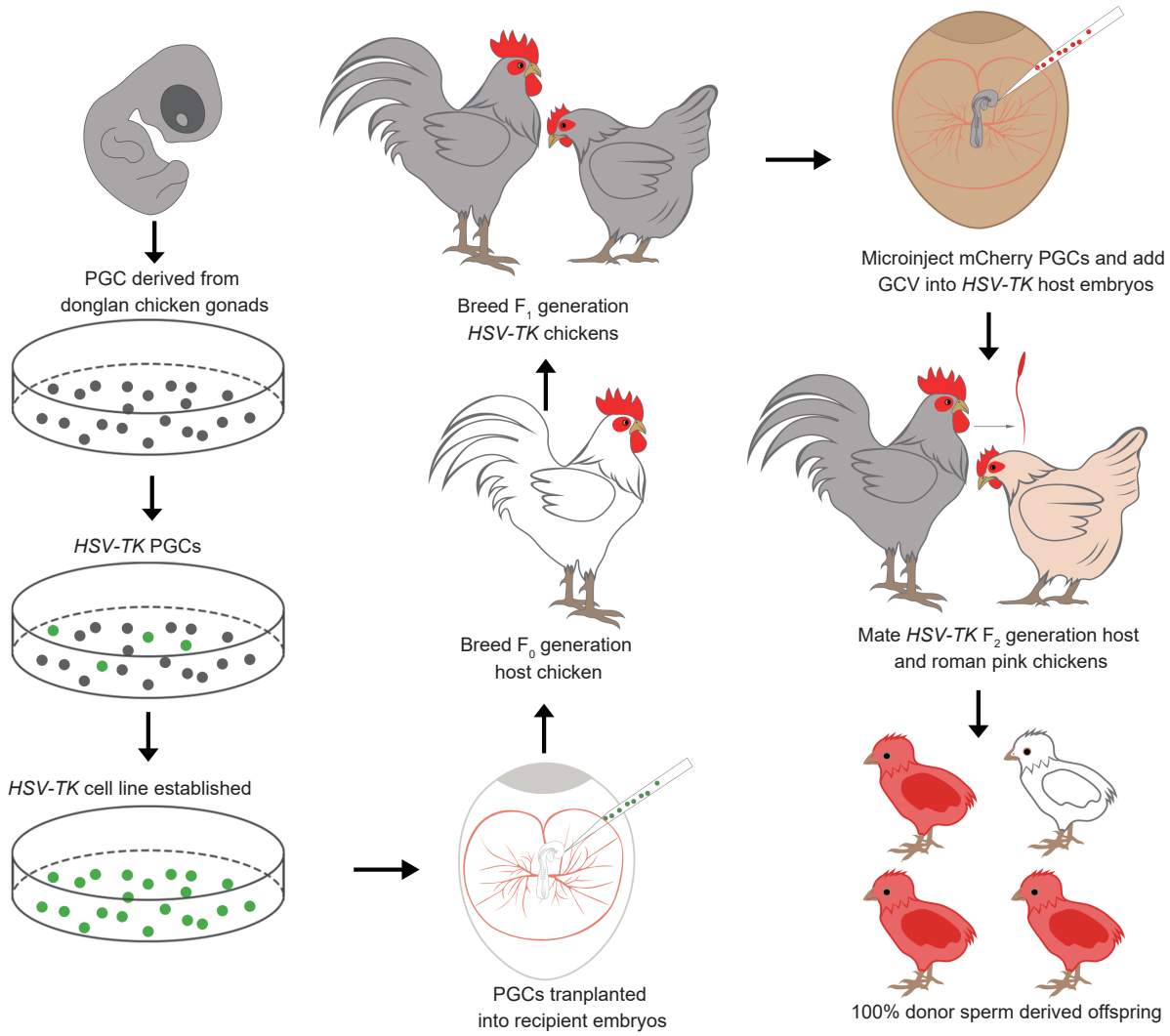


Fig. 4 Diagram of the preparation and application of *HSV-TK* sterile chicken.

In the process for producing genome-edited chicken, donor PGCs are transplanted into the host embryo and compete with the endogenous PGCs for spermatogenesis in the same niche. However, the exogenous PGCs subjected to long-term culture usually result in a gradual loss of the ability to compete with endogenous germ cells and, thus complications of low germline transmission efficiency can be observed. The genetic transmission efficiency of donor-derived PGCs is key to ensuring that genetic recovery is effective enough for the population. Therefore, we prepared *HSV-TK* sterile chicken embryos to improve the efficiency of genetic offspring after transplanting either male or female PGCs, and it can be used as a new strategy for germplasm conservation and genetic modification in chicken.

The *HSV-TK*/GCV system is widely adopted in genetic engineering to utilize the TK enzyme and GCV to kill targeted cells for specific modification in cancer therapies. Previously, it has been extensively studied in animal model trials, and its safety and efficacy have been demonstrated in stem-cell-related transplants and gene therapy for different cancers (Wang *et al.* 2004; Qasim *et al.* 2005; Li *et al.* 2006; Amano *et al.* 2009). However, this is the first study of its kind that successfully applied the *HSV-TK* cell ablation system in an avian species to produce the induced sterile chicken. In the current study, results confirmed efficacy of the suicide gene *HSV-TK* for efficient ablation of endogenous germ cells and high-grade germline transmission of donor PGCs in the chimeras.

Histochemistry results showed that the gonads tissue section of the *HSV-TK* roosters treated with GCV during the embryonic stage, testes contained intact seminiferous

tubules but no spermatogenic cells, and similarly no follicles seen in the female ovaries. Furthermore, PCR results revealed that chimeras derived offspring by injecting donors into *HSV-TK* hosts were found *HSV-TK* transgene free. Wherase, chicks derived from the donors were 63.5% PGCs positive for mCherry transgene. These results indicated the endogenous germ cells of the host were ablated and the use of *HSV-TK* surrogate hosts achieved efficient germline inheritance from the donor PGCs. Many suicide gene safety systems have been reported to date, including *HSV-TK* and iCaspase9, both of which are widely validated in clinical settings. In a previous study, donor PGCs treated with AP20187 compounds were added into iCaspase9/aviCaspase9 host embryos and then hatched; the offspring of the aviCaspase9 surrogate hosts detected the iCaspase9 transgene, indicating incomplete ablation of endogenous germ cells in this aviCaspase9 hosts (Ballantyne *et al.* 2021). The *HSV-TK* host is comparable to the reported iCaspase9 host, whose endogenous germ cells can be completely eliminated by the inducers. And as the endogenous PGCs were efficiently eliminated in the host and the transgene *HSV-TK* was not detected in the derived chicken, the leakage of the foreign gene is very unlikely while using the inducible infertile host for genetic conservation.

5. Conclusion

It is confirmed that effectively ablated endogenous germ cells is crucial for the success of this technique in genome editing, and the *HSV-TK*/GCV suicide gene system can be applied to endogenous germ cell ablation in avian. Efficient germline transmission of donor PGCs achieved *HSV-TK* sterile host chicken embryos to directly produce other varieties of genome-edited chicken. The ablation method eliminates this competition, allowing donor PGCs to more efficiently establish in the recipient's reproductive system. It has important application values in germ cell development research, genome-edited chicken conservation and germplasm resource protection.

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Declaration of competing interest

The authors declare that they have no conflict of interest.

Declaration of generative AI and AI-assisted technologies in the writing process

The authors declare that they did not use AI in the preparation and writing of this manuscript.

Ethical approval

The animal management and embryo manipulation in this experiment was approved by the Animal Experimental Committee of Guangxi University, China.

Appendices associated with this study are available at https://doi.org/10.1016/j.jia.2024.08.009

References

Altgilbers S, Klein S, Dierks C, Weigend S, Kues W A. 2021. Cultivation and characterization of primordial germ cells from blue layer hybrids (Araucana crossbreeds) and generation of germline chimeric chickens. *Scientific Reports*, **11**, 12923.

Amano S, Li S, Gu C, Gao Y, Koizumi S, Yamamoto S, Terakawa S, Namba H. 2009. Use of genetically engineered bone marrow-derived mesenchymal stem cells for glioma gene therapy. *International Journal of Oncology*, **35**, 1265–1270.

Ballantyne M, Woodcock M, Doddamani D, Hu T, Taylor L, Hawken R J, Mcgrew M J. 2021. Direct allele introgression into pure chicken breeds using Sire Dam Surrogate (SDS) mating. *Nature Communications*, **12**, 659.

Chen H H, Welling M, Bloch D B, Muñoz J, Mientjes E, Chen X, Tramp C, Wu J, Yabuuchi A, Chou Y F, Buecker C, Krainer A, Willemsen R, Heck A J, Geijsen N. 2014. DAZL limits pluripotency, differentiation, and apoptosis in developing primordial germ cells. *Stem Cell Reports*, **3**, 892–904.

Ciccarelli M, Giassetti M I, Miao D, Oatley M J, Robbins C, Lopez-Biladeau B, Waqas M S, Tibary A, Whitelaw B, Lillico S, Park C H, Park K E, Telugu B, Fan Z, Liu Y, Regouski M, Polejaeva I A, Oatley J M. 2020. Donor-derived spermatogenesis following stem cell transplantation in sterile NANOS2 knockout males. *Proceedings of the National Academy of Sciences of the United States of America*, **117**, 24195–24204.

Fillat C, Carrió M, Cascante A, Sangro B. 2003. Suicide gene therapy mediated by the Herpes Simplex virus thymidine kinase gene/Ganciclovir system: Fifteen years of application. *Current Gene Therapy*, **3**, 13–26.

Gentry G A. 1992. Viral thymidine kinases and their relatives. *Pharmacology & Therapeutics*, **54**, 319–355.

Gessara I, Dittrich F, Hertel M, Hildebrand S, Pfeifer A, Frankl-Vilches C, Mcgrew M, Gahr M. 2021. Highly efficient genome modification of cultured primordial germ cells with lentiviral vectors to generate transgenic songbirds. *Stem Cell Reports*, **16**, 784–796.

Han J Y, Lee B R. 2017. Isolation and characterization of chicken primordial germ cells and their application in transgenesis. *Methods in Molecular Biology*, **1650**, 229–242.

Iwasawa C, Tamura R, Sugiura Y, Suzuki S, Kuzumaki N, Narita M, Suematsu M, Nakamura M, Yoshida K, Toda M, Okano H, Miyoshi H. 2019. Increased cytotoxicity of herpes simplex virus thymidine kinase expression in human induced pluripotent stem cells. *International Journal of Molecular Sciences*, **20**, 810 .

Jones B S, Lamb L S, Goldman F, Di Stasi A. 2014. Improving the safety of cell therapy products by suicide gene transfer. *Frontiers in Pharmacology*, **5**, 254.

Kagami H, Tagami T, Matsubara Y, Harumi T, Hanada H, Maruyama K, Sakurai M, Kuwana T, Naito M. 1997. The developmental origin of primordial germ cells and the transmission of the donor-derived gametes in mixed-sex germline chimeras to the offspring in the chicken. *Molecular Reproduction and Development*, **48**, 501–510.

Kang K S, Lee H C, Kim H J, Lee H G, Kim Y M, Lee H J, Park Y H, Yang S Y, Rengaraj D, Park T S, Han J Y. 2015. Spatial and temporal action of chicken primordial germ cells during initial migration. *Reproduction*, **149**, 179–187.

Kuwana T, Rogulska T. 1999. Migratory mechanisms of chick primordial germ cells toward gonadal anlage. *Cellular and Molecular Biology*, **45**, 725–736.

Lázár B, Molnár M, Sztán N, Végi B, Drobnyák Á, Tóth R, Tokodyné Szabadi N, Mcgrew M J, Gócza E, Patakiné Várkonyi E. 2021. Successful cryopreservation and regeneration of a partridge colored Hungarian native chicken breed using primordial germ cells. *Poultry Science*, **100**, 101207.

Lee H C, Choi H J, Lee H G, Lim J M, Ono T, Han J Y. 2016a. *DAZL* expression explains origin and central formation of primordial germ cells in chickens. *Stem Cells and Development*, **25**, 68–79.

Lee H C, Lim S, Han J Y. 2016b. Wnt/β-catenin signaling pathway

activation is required for proliferation of chicken primordial germ cells *in vitro*. *Scientific Reports*, **6**, 34510.

Lee J, Kim D H, Karolak M C, Shin S, Lee K. 2022. Generation of genome-edited chicken and duck lines by adenovirus-mediated *in vivo* genome editing. *Proceedings of the National Academy of Sciences of the United States of America*, **119**, e2214344119.

Lee J, Ma J, Lee K. 2019. Direct delivery of adenoviral CRISPR/Cas9 vector into the blastoderm for generation of targeted gene knockout in quail. *Proceedings of the National Academy of Sciences of the United States of America*, **116**, 13288–13292.

Li Z B, Zeng Z J, Chen Q, Luo S Q, Hu W X. 2006. Recombinant AAV-mediated *HSVtk* gene transfer with direct intratumoral injections and tet-on regulation for implanted human breast cancer. *BMC Cancer*, **6**, 66.

Miyahara D, Oishi I, Makino R, Kurumisawa N, Nakaya R, Ono T, Kagami H, Tagami T. 2016. Chicken stem cell factor enhances primordial germ cell proliferation cooperatively with fibroblast growth factor 2. *The Journal of Reproduction and Development*, **62**, 143–149.

Panda S K, Mcgrew M J. 2022. Genome editing of avian species: Implications for animal use and welfare. *Laboratory Animals*, **56**, 50–59.

Park K E, Kaucher A V, Powell A, Waqas M S, Sandmaier S E, Oatley M J, Park C H, Tibary A, Donovan D M, Blomberg L A, Lillico S G, Whitelaw C B, Mileham A, Telugu B P, Oatley J M. 2017. Generation of germline ablated male pigs by CRISPR/Cas9 editing of the *NANOS2* gene. *Scientific Reports*, **7**, 40176.

Pilato G, D'urso V, Viglianisi F, Sammartano F, Sabella G, Lisi O. 2013. The problem of the origin of primordial germ cells (PGCs) in vertebrates: Historical review and a possible solution. *The International Journal of Developmental Biology*, **57**, 809–819.

Qasim W, Gaspar H B, Thrasher A J. 2005. T cell suicide gene therapy to aid haematopoietic stem cell transplantation. *Current Gene Therapy*, **5**, 121–132.

Song Y, Duraisamy S, Ali J, Kizhakkayil J, Jacob V D, Mohammed M A, Eltigani M A, Amisetty S, Shukla M K, Etches R J, De Lavoir M C. 2014. Characteristics of long-term cultures of avian primordial germ cells and gonocytes. *Biology of Reproduction*, **90**, 15.

Taylor L, Carlson D F, Nandi S, Sherman A, Fahrenkrug S C, Mcgrew M J. 2017. Efficient TALEN-mediated gene targeting of chicken primordial germ cells. *Development*, **144**, 928–934.

Wang J, Lu X X, Chen D Z, Li S F, Zhang L S. 2004. Herpes simplex virus thymidine kinase and ganciclovir suicide gene therapy for human pancreatic cancer. *World Journal of Gastroenterology*, **10**, 400–403.

Whyte J, Glover J D, Woodcock M, Brzeszczynska J, Taylor L, Sherman A, Kaiser P, Mcgrew M J. 2015. FGF, insulin, and SMAD signaling cooperate for Avian primordial germ cell self-renewal. *Stem Cell Reports*, **5**, 1171–1182.

Woodcock M E, Gheyas A A, Mason A S, Nandi S, Taylor L, Sherman A, Smith J, Burt D W, Hawken R, Mcgrew M J. 2019. Reviving rare chicken breeds using genetically engineered sterility in surrogate host birds. *Proceedings of the National Academy of Sciences of the United States of America*, **116**, 20930–20937.

Xie L, Lu Z, Chen D, Yang M, Liao Y, Mao W, Mo L, Sun J, Yang W, Xu H, Lu K, Lu Y. 2019. Derivation of chicken primordial germ cells using an indirect co-culture system. *Theriogenology*, **123**, 83–89.

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