Establishing Germline Chimeric Chickens Using Primordial Germ Cells

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Chicken primordial germ cells (PGCs) are used to restore and conserve genetic resources of some endangered birds or flocks of domestic chickens. When PGCs are transferred from a donor to a recipient, they can survive and gain sexual maturity in the recipient chicken. This indicates that PGCs retaining exogenous genes can proliferate and develop in recipient gonads, and proves that transferred PGCs are effective sources for establishing transgenic and germline chimeric chickens. The PGC transfer method has been used to produce germline chimeric chickens and may become popular in the field of reproductive science. Germline chimeric chickens have the potential to reconstruct and reproduce endangered birds, including both domestic and wild species.

Key words: PGCs, Germline chimeric chicken, Transgenic chicken

Production of Chimeric Chickens

Germline chimeric chickens are considered effective means for the conservation of endangered birds and for the production of transgenic chickens. Blastodermal Cells

Somatic cell chimeras have been produced by transferring blastoderm at stage X. Freshly oviposited fertilized eggs were used as donor and recipient embryos. Donor blastodermal cells were transferred to a recipient blastoderm, and subsequently, the donor-derived cells developed and differentiated in the recipient gonads (Petitte et al., 1990; Carsience et al., 1993; Kagami and Hanada 1997; Trefil et al., 2002).

Implantation and Immigration of PGCs

Germline chimeric chickens can be produced using PGCs. Fertilized eggs are incubated until embryonic developmental stages 12–15. PGCs are collected from the veins of the
developing donor embryos and injected into the blood vessels of developing recipient embryos. Donor-derived offspring were obtained from germine chimeric chickens (Naito et al., 1994; Furuta et al., 2001; Kuwana et al., 2006). When the Japanese quail was used, chicken and quail hybrid chimeras were produced (Nakamura et al., 1992; Ono et al., 1996, 1998a,b). However, when PGCs were introduced into the germinal crescent of recipient embryos at stage 9, the donor PGCs immigrated to the recipient gonads via embryonic blood vessels (Furuta and Fujihara 1999).

**Endogenous PGCs of Recipient Embryos**

PGCs obtained from a donor are used to produce germine chimeric chickens. Previously attempts have been made to decrease the PGC concentration in recipient chicken embryos. Busulfan is known to inhibit the proliferation of germ cells, and has been administered to recipient embryos to decrease the number of PGCs (Wentworth et al., 1988; Aigegil and Simikiss 1991; Halltt and Wentworth 1991; Furuta and Fujihara 1999). In a study that investigated whether the rate of donor PGCs to settle in the germinal ridges of chicken embryos increased on busulfan treatment, exogenous PGCs proliferation was found to be higher in busulfan-treated embryos than in untreated embryos (Furuta and Fujihara 1999). The PGC concentration in embryos has been analyzed after irradiation with ultraviolet, γ rays, and soft X-rays (Reynaud 1976; Carsience et al., 1993; Kagami et al., 1995). The abovementioned methods are considered to have the same results as other methods used to decrease the number of PGCs.

**Restoration and Conservation of Genetic Resources**

PGCs obtained from native Japanese quail were introduced into the germinal crescent of recipient embryos at stage 9 with and without heterosexually transferred PGCs. Gonadal development of embryos with homosexually transferred PGCs was normal, whereas gonads with heterosexually transferred PGCs showed abnormal development. This effect was observed in the female, probably due to the effect of endogenous ovarian aromatase produced by the ovary. Anti-Müllerian hormone (AMH) is responsible for the regression of the Müllerian ducts in males during embryonic development (Vigier et al., 1987). The inhibition of aromatase activity can cause the differentiation of the right and left testes or ovotestes in female chickens (Abinawanto et al., 1996; Burke and Henry, 1999; Elbrecht and Smith, 1992). The gonadal development of day 15 embryos and newly hatched chicks as well as AMH expression in day 7 embryos after heterosexual transfer of PGCs have been examined.

PGCs were taken from the blood vessels of donor White Leghorn embryos and transferred to recipient Rhode Island Red embryos between developmental stages 12 and 15. The gender of the treated embryos was determined after the transfer of PGCs using the remaining blood samples. The gonadal development of embryos with homo- and heterosexually transferred PGCs was examined. Gonads with homosexually transferred PGCs showed normal development, while some of those gonads with heterosexually transferred PGCs showed abnormal development on histological examination. No significant difference was found in the AMH gene expression level between gonads of 7 day embryos with and without heterosexually transferred PGCs in both sexes (Furuta et al., 2008).

**Introduction of Exogenous Genes into Early Chicken Embryos**

Previously, attempts have been made to introduce genes into chicken embryos to produce transgenic chickens. Exogenous genes have been injected into the blastoderm at stage X, immediately after oviposition or in the germinal crescent region at stage 9, and have been transferred into cells using a transfection reagent or electroporation. The expression of exogenous genes was observed in chicken embryos, extra embryos, and PGCs that were collected from blood at developmental stages 11–15 (Inada et al., 1997; Eguma et al., 1999; Furuta and Fujihara 2000; Furuta et al., 2000). Thus, it is possible to introduce an exogenous genes into embryonic tissues for subsequent gene functions.

**Heterosexual Transfer of PGCs**

PGCs transferred from females to male embryos have been demonstrated to differentiate into spermatogonia in male gonads (Tagami et al., 1997). Thus, it may be possible to produce W chromosome spermatogonia using this method. However, heterosexual (female to male or male to female) transfer of PGCs has been shown to cause abnormal development in the gonads of chimeric chicken embryo (Furuta et al., 1999; 2008; Yamaguchi et al., 2000). In avians, estrogen is necessary to initiate feminization in normal females and cytochrome P450 aromatase is an important enzyme for the conversion of testosterone to estrogen. During the aromatization of testosterone to estradiol in the male gonad, the absence of P450 aromatase results in the accumulation of testosterone (Woods and Erton 1978; Yoshida et al., 1996), which then induces masculinization. However, the masculinizing effect of testosterone treatment in ovo is only temporary in the female, probably due to the effect of endogenous ovarian aromatase produced by the ovary. Anti-Müllerian hormone (AMH) is responsible for the regression of the Müllerian ducts in males during embryonic development (Vigier et al., 1987). The inhibition of aromatase activity can cause the differentiation of the right and left testes or ovotestes in female chickens (Abinawanto et al., 1996; Burke and Henry, 1999; Elbrecht and Smith, 1992). The gonadal development of day 15 embryos and newly hatched chicks as well as AMH expression in day 7 embryos after heterosexual transfer of PGCs have been examined.

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Introduction of Exogenous Genes in Blastodermal Cells and PGCs

Exogenous genes have been introduced in blastodermal cells obtained from unincubated fertilized eggs (Brazolot et al., 1991; Zhu et al., 2005). These cells were transferred to recipient embryos that differentiated into various embryonic tissues. PGCs are a useful means for producing transgenic chickens because they can differentiate into gametes. PGCs collected from embryos were cultured in order to transfer exogenous genes and used for the production of transgenic chicks via germinal chimeric chickens (Vick exogenous genes and used for the production of transgenic chickens because they can differentiate into gametes. PGCs collected from embryos were cultured in order to transfer exogenous genes and for the production of transgenic chicks via germinal chimeric chickens (Vick et al., 1993b; Shin et al., 2008; Furuta et al., 2010). Presence and expression of exogenous genes were detected in the next generation (van de Lavoir et al., 2006).

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References

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