

Development of the Gonads Derived from Hetero-Sexually Transferred Primordial Germ Cells (PGCs) between Embryos in the Chicken^a

H. Furuta, H. Yamaguchi¹ and N. Fujihara*

Animal Resource Science Section, Division of Bioresource and Bioenvironmental Science
Graduate School of Kyushu University, Fukuoka 812-8581, Japan

ABSTRACT : Primordial germ cells (PGCs) of White Leghorn chicken embryos as a donor were transferred to Rhode Island Red chicken embryos as a recipient. At 48-50 h (stage 13-15) of incubation of fertilized eggs, donor PGCs, which were taken out from blood vessels of donor embryos, were injected into blood vessels of recipient embryos. Sex of the treated embryos was determined after the transfer of PGCs using remaining blood samples. In the present experiments, survival rate of the treated embryos was 33.3% for homo-sexual and 35.4% for hetero-sexual transfers of PGCs, respectively, when determined at 17 days of incubation. In this study, most of the treated embryos could not survive more than 18 days of incubation, though the reason for that was not clarified in the present work. The gonads removed from embryos that died after 18 days of incubation and the organs from newly hatched chicks were examined for morphological and histological features. The gonads removed from the embryos with homo-sexual transfer of PGCs showed normal development in appearance. On the contrary, some (35.3%) of the embryos with hetero-sexual transfer of PGCs possessed abnormal gonads similar to ovotestis by histological observation. In cases where the gonads developed to be normal organs (64.7%) the sex of embryos was the same as recipient ones. The present results suggest that hetero-sexual transfer of the PGCs may bring about the possibility of development of the embryos bearing sexually different gametes, spermatogonia or oogonia. (*Asian-Aus. J. Anim. Sci. 1999. Vol. 12, No. 8 : 1188-1191*)

Key Words : Chicken, Embryos, Gonads, Primordial Germ Cells, Transfer

INTRODUCTION

Avian primordial germ cells (PGCs) have been reported to originate from the epiblast (Eyal-Giladi et al., 1981), and subsequently appear in hypoblast of germinal crescent region. The PGCs circulate in the vascular system of developing embryos and finally migrate into the germinal ridge of the embryonic system (Fujimoto et al., 1976; Kuwana, 1993), where the cells differentiate into spermatogonia or oogonia.

Recently, it has been reported that exogenous genes could be introduced into the PGCs (Han et al., 1994a; Naito et al., 1994a; Inada et al., 1997; Eguma et al., 1999) and chimeric chickens produced by the transfer of chicken and quail PGCs or chicken blastodermal cells (Petitte et al., 1990; Han et al., 1994b; Naito et al., 1994b; Kagami et al., 1995; Ono et al., 1996; 1998; Yamaguchi et al., 1998).

Female PGCs transferred to male embryos have been demonstrated to differentiate to spermatogonia in

male gonads (Tagami et al., 1997). This result suggests the possibility of developing gonadal tissues containing PGCs from donor embryos of different sex to those of recipient embryos. If this is possible, the PGCs bearing W or Z chromosome may differentiate to spermatogonia or ova in the gonads having hetero-sexually transferred PGCs.

In this experiment, we examined the gonadal development of embryos with hetero-sexually transferred PGCs, focusing special attention on the morphological and histological features of gonads.

MATERIALS AND METHODS

Donor PGCs

Fertilized eggs obtained from Rhode Island Red chickens were used as donor PGCs. The eggs were cracked to obtain the PGCs from developing embryos after 48-50 h of incubation. At this time, the PGCs circulating in blood vessels of developing embryos could be stage 13 to 15 (Hamberger and Hamilton, 1951).

Recipient embryos

Fertilized eggs obtained from White Leghorn hens were used to produce recipient embryos. The eggs were incubated until the same stage as the donor eggs, 48-50 h (stage 13-15) after the start of incubation.

Transfer of PGCs to recipient embryos

Circulating blood samples containing PGCs were

* Corresponding Author: N. Fujihara. Tel & Fax: +81-(0)92-642-2938, E-mail: nfujih@agr.kyushu-u.ac.jp.

¹ Department of Pharmacology, School of Medicine, Nagasaki University, Nagasaki 880-0023 Japan.

^a Supported by grant-in-aid for scientific research from the Ministry of Education, Science and Culture in Japan, Japanese Society for the Promotion of Science (JSPS), Heiwa Nakajima Foundation, Yuasa International Foundation and Toyota Foundation.

Received January 25, 1999; Accepted April 13, 1999

collected from the blood vessels of developing Rhode Island Red's embryos at stage 13-15, which were cracked onto petri dishes. In this case, the donor PGCs were taken into a fine glass pipette preaspirated bubble of Modified Eagle Medium (MEM, Nissui, Tokyo, Japan) supplemented with 10% fetal calf serum (FCS).

A window (ca. 10 mm in diameter) was opened at the pointed end of the recipient White Leghorn eggs. A measured amount (4 to 7 μ l) of blood was aspirated from the blood vessels of recipient embryos and subsequently the donor blood samples containing the PGCs were injected into the same vessels from which the blood samples were removed. In this study, one donor was used for one recipient, individually, instead of using pooled PGCs. The small holes on the surface of blood vessels of recipient embryos were closed with bubbles of MEM supplemented with FCS. The small windows on the recipient eggs were closed with para-film and the eggs were continued to incubate until chicks hatched.

Sex determination of donor and recipient embryos

The sex of donor and recipient embryos was determined after the transfer of PGCs by means of the PCR analysis. In brief, approximately 2 μ l of blood collected from donor and recipient embryos had DNA extracted according to the method described by Micro Extraction Kit (Stratage, Canada). The PCR analysis was carried out to multiply sequences of the W-chromosome of the chicken (Clinton, 1994).

Morphological and histological examination of gonads

The gonads of newly hatched chicks and dead embryos after 17 days of incubation were carefully examined by eye and subsequently excised. The removed gonads were fixed with Bouin's solution and processed for routine histological examination. Thin paraffin sections were stained with HE to observe under a light microscope.

Statistical analysis

All data were subjected to statistical analysis using student's T-test (Steel and Torrie, 1980).

RESULTS

The total number of embryos manipulated was 81, including 33 embryos with homo-sexual transfer and 48 of hetero-sexual transfer of the PGCs (table 1). A total of 28 embryos (34.6%) with transferred PGCs survived after 17 days of incubation. Survival rates of embryos having transferred PGCs were 33.3% for homo-sexual and 35.4% for hetero-sexual transfer of PGCs respectively with no statistical difference ($p>0.05$) between the two groups.

Table 1. Survival rate of recipient chicken embryos after 17 days of incubation, following transfer of PGCs from donor embryos

Sexes of donor or recipient embryos donor→recipient	Number of embryos manipulated	Number of surviving embryos*	Rate of survival embryos (%)
♂ → ♂	18	6	33.3
♀ → ♀	15	5	33.3
Sub-total (homo-sexual)	33	11	33.3
♂ → ♀	17	7	41.1
♀ → ♂	31	10	32.3
Sub-total (hetero-sexual)	48	17	35.4
Total	81	28	34.6

* Recorded at 17 days of incubation.

When the transfer of PGCs was carried out between embryos of the same sex, the origin of the sex could not be determined (table 2). No sign of abnormality in the gonads was observed in the embryos with homo-sexual transfer of PGCs.

Morphological and histological deformities (35.3%) were observed in the embryos with hetero-sexual transfer of PGCs, for example, 2 of 7 (male to female) and 4 of 10 (female to male) (table 2). The histological observation of these morphologically abnormal embryos revealed that the organs had

Table 2. Investigation of sex and morphological and histological abnormality of recipient gonads after transfer treatment of PGCs from donor recipient

Sexes of donor or recipient embryos donor→recipient	Number of gonads investigated	Investigation of sex of gonads			Morphological and histological abnormality of gonads
		♂	♀	unclear	
♂ → ♂	6	6	0	0	0
♀ → ♀	5	0	5	0	0
♂ → ♀	7	1	5	1	2 (28.6%)
♀ → ♂	10	6	0	4	4 (40.0%)

ovotestis-like gonadal tissue.

Of 7 male to female transfers, only one embryo possessed testes very close to those of normal male embryos. However, sex of the recipient's gonads was not completely altered by transferred PGCs. The gonads with hetero-sexually transferred PGCs contained many sexual cords in the right gonads showing testis-like organ (figure 1). Five embryos possessed the organs of female birds. One of the treated embryos possessed an abnormal left gonad showing rugby ball-like organs fused each other (figure 2). In this embryo, no degeneration of sexual cords was observed, though formation of the ovotestis-like gonad was found in both left and right side organs.

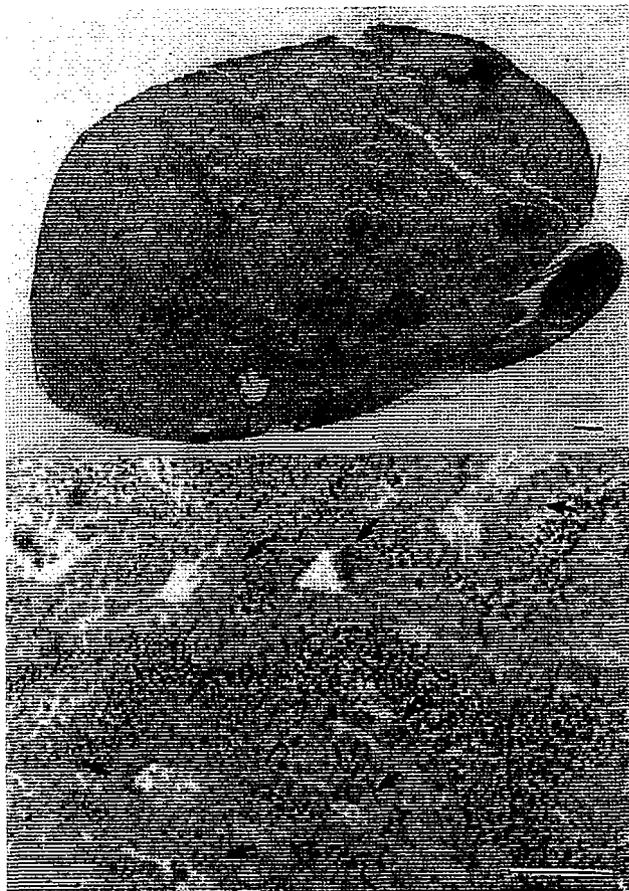


Figure 1. Gonad (right side) like testis from the chick with hetero-sexual (male to female) transfer of PGCs. Bar indicates 50 μ m. Note: sexual cords in the tissue (arrows)

When PGCs from female donor embryos were transferred to male recipient embryos, 6 of 10 embryos (60.0%) possessed male gonads very similar to the testes. The remaining 4 embryos (40.0%) had abnormal gonads, normal for the right side and abnormal for the left side organs.

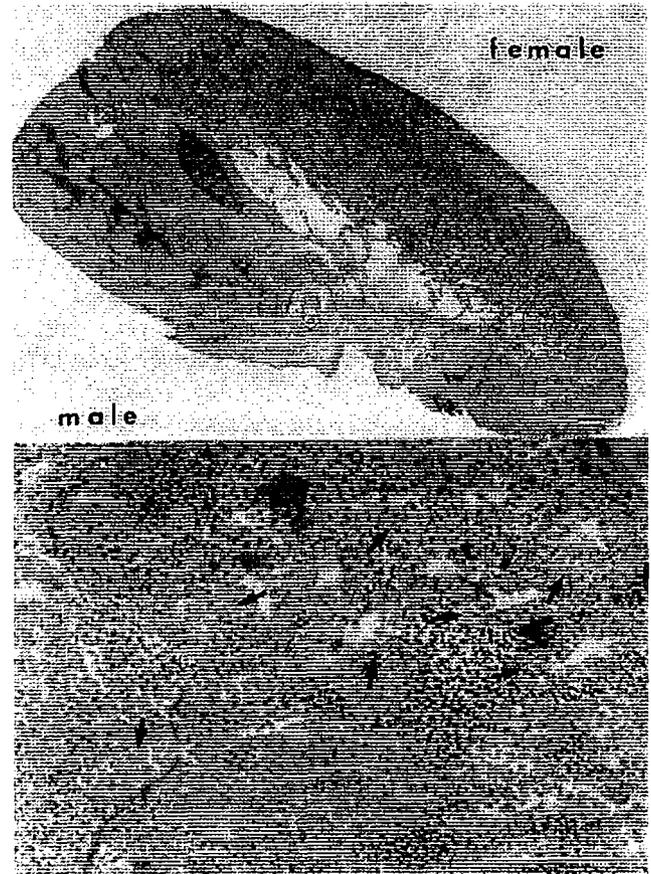


Figure 2. Gonad (left side) from the chick with hetero-sexual transfer (female to male) of PGCs. Bar indicates 50 μ m. Note: Ovotestis-like organ showing numerous sexual cords in the tissue (arrows)

DISCUSSION

Chimeric chickens have already been produced by the transfer of PGCs from embryos of White Leghorn chickens to the embryos of Barred Plymouth Rock birds (Naito et al., 1994a). Recently, chimeric birds representing inter-specific hybrids have also been produced by the transfer of PGCs from quail to chicken embryos (Ono et al., 1996; 1998). However, it has not been elucidated yet whether PGCs of donor embryos might be concerned to the sex determination of recipient embryos. In general, the PGCs have been said to have no bearing on the sex of the embryos which is dependent upon the sex of the somatic cells.

In this experiment, however, the sex of individual embryos was determined immediately after the transfer of PGCs, unlike the previous study in which pooled PGCs (mixed cells from male and female donors) were used following centrifugation of the cells (Yasuda et al., 1992). In the present works, therefore, previously sexed PGCs were transferred to the recipient embryos which were also sex-determined

prior to treatment. However, in this case, it was not possible to determine whether or not the recipient's gonads originated from donor's cells when the PGCs having same sex were transferred.

On the contrary, in the experiment of hetero-sexual transfer of PGCs, around 70% for the case from male to female, and 60% for the case from female to male showed normal development of gonadal tissues of the recipient embryos. However, only one case of hetero-sexual transfer of PGCs developed donor-derived gonads, from male to female, possessing normal testes in place of the ovary. Other embryos having hetero-sexual transfer of PGCs showed ovotestis-like gonads. In addition, around 40% of embryos with hetero-sexual transfer of PGCs possessed abnormal gonads, not clearly testis or ovary. Even in the case of hetero-sexual transfer of PGCs, approximately 60% of the treated embryos showed normal development of the gonads. Regarding this matter, it has been reported that female chicken PGCs differentiated to spermatozoa in male gonads (Tagami et al., 1997).

The results obtained from the present experiments suggest that hetero-sexual transfer of PGCs may induce the case of development of the gonads having opposite sex to somatic cells, which may also produce gametes bearing opposite sex in the future.

ACKNOWLEDGEMENT

The authors are grateful to Dr. T. Kuwana for kind guidance to collect avian PGCs from early embryos with special skilful techniques. Special financial support for this study was provided by grant-in-aid for scientific research from the Ministry of Education, Science and Culture in Japan, Japanese Society for the Promotion of Science (JSPS), Heiwa Nakajima Foundation, Yuasa International Foundation and Toyota Foundation.

REFERENCES

- Clinton, M. 1994. A rapid protocol for sexing chick embryos. *Anim. Gen.* 25:361-362.
- Eguma, K., T. Soh, M. A. Hattori and N. Fujihara. 1999. *In vitro* transfer of foreign DNA into primordial germ cells (PGCs) of embryos. *Asian-Aus. J. Anim. Sci.* 12:520-524.
- Eyal-Giladi, H., M. Ginsburg and A. Farbarov. 1981. Avian primordial germ cells are of epiblastic origin. *J. Embryol. Exp. Morphol.* 65:139-147.
- Fujimoto, T., T. Ninomiya and A. Ukeshima. 1976. Observations of the primordial germ cells in blood samples from the chick embryos. *Dev. Biol.* 49:278-282.
- Hamberger, V. H. and L. Hamilton. 1951. A series of normal stages in the development of the chick embryos. *J. Morphol.* 8:49-92.
- Han, J. Y., R. N. Shoffner and K. S. Guise. 1994a. Gene transfer by manipulation of primordial germ cells in the chicken. *Asian-Aus. J. Anim. Sci.* 7:303-457.
- Han, J. Y., D. S. Seo and R. N. Shoffner. 1994b. Primordial germ cells in aves. *Asian-Aus. J. Anim. Sci.* 7:459-600.
- Inada, S., M. A. Hattori, N. Fujihara and K. Morohashi. 1997. *In vivo* gene transfer into the blastoderm of early developmental stage of chicken. *Reprod. Nutr. Dev.* 37:13-20.
- Kagami, H., M. E. Clark, A. M. V. Gibbins and R. J. Etches. 1995. Sexual differentiation of chimeric chickens containing ZZ and ZW cells in the germline. *Mol. Reprod. Dev.* 42:379-387.
- Kuwana, T. 1993. Migration of avian primordial germ cell toward the gonadal anlage. *Develop. Growth Differ.* 35:237-243.
- Naito, M., E. Sasaki, M. Ohtaki and M. Sakurai. 1994a. Introduction of exogenous DNA into somatic and germ cells of chicken by microinjection into the germinal disk of fertilized ova. *Mol. Reprod. Dev.* 37:167-171.
- Naito, M., A. Tajima, Y. Yasuda and T. Kuwana. 1994b. Production of germline chimeric chickens, with high transmission rate of donor-derived gametes, produced by transfer of primordial germ cells. *Mol. Reprod. Dev.* 39:153-161.
- Ono, T., R. Yokoi and H. Aoyama. 1996. Transfer of male or female primordial germ cells of quail into chick embryonic gonads. *Exp. Anim.* 45:347-352.
- Ono, T., R. Yokoi, S. Maeda, T. Nishida and H. Aoyama. 1998. Settlement of quail primordial germ cells in chicken gonads. *Anim. Sci. Technol. (Jpn.)* 69:546-555.
- Petite, J. N., M. E. Clark, G. Liu, A. M. V. Gibbins and R. J. Etches. 1990. Production of somatic and germline chimeras in the chicken by transfer of early blastodermal cells. *Development.* 108:185-189.
- Steel, R. G. D. and J. H. Torrie. 1980. *Principles and Procedures of Statistics*, 2nd Ed. McGraw-Hill, New York.
- Tagami, T., Y. Matsubara, H. Hanada and M. Naito. 1997. Differentiation of female chicken primordial germ cells into spermatozoa in male gonads. *Develop. Growth Differ.* 39:267-271.
- Yamaguchi, H. and N. Fujihara. 1998. Inter embryonic (home- and hetero- sexual) transfer of primordial germ cells (PGCs) between chicken embryos. *Cytotechnology.* 26 (In press).
- Yasuda, Y., A. Tajima, T. Fugimoto and T. Kuwana. 1992. A method to obtain avian germ-line chimaeras using isolated primordial germ cells. *J. Reprod. Fert.* 96:521-528.