F-3.5 Production of male and female individuals derived from a single fertilized egg

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Abstract In order to develop a novel technique for proliferating endangered animal species, a study was carried out to produce male and female individuals derived from primordial germ cells obtained from a single fertilized egg via germline chimeras. Sexing of donor primordial germ cells and recipient embryos were performed by polymerase chain reaction analysis for detecting the W-chromosome specific DNA sequence in chicken. Primordial germ cells obtained from a single embryo were divided into two parts, and a half of them was transferred into male recipient embryo and the other half was transferred into female recipient embryo. The manipulated embryos were cultured in surrogate eggshells until hatching. So far, 314 embryos were manipulated and obtained 83 (26.4%) chicks. Five pairs of chicks obtained by transferring primordial germ cells derived from the single embryos. Mating experiment for examining the germline chimerism of the putative chimeric chickens is going to be performed when they reach sexual maturation.

Key Words Primordial germ cell, Germline chimera, sexing, blastodermal cell

1. Introduction

Number of endangered animal species is now increasing and urgent research is needed to cope with this situation. The most urgent matter for rescuing endangered animal species from crisis of extinction is to increase the number of animals in a population. Effective means to do this has not yet been developed so far, it is strongly required to develop a novel technique for proliferating endangered animal species. Since it is difficult to get enough number of fertilized eggs from endangered animal species, the development of a technique to reproduce the population derived from a single fertilized egg is thought to be important. Primordial germ cells (PGCs) are progenitor cells of ova and spermatozoa[1)], so manipulation of PGCs is one of the best methods to proliferate endangered animal species artificially. In our research group, we have succeeded to produce germline chimeric chickens, with high transmission rate of donor-derived gametes, by transferring PGCs obtained from embryonic blood[2,3)]. We also succeeded to produce viable offspring derived from frozen-stored PGCs obtained from embryonic blood or gonads in liquid nitrogen (-196℃) via germline chimeric chickens[4,5)]. These techniques are very useful to develop a novel method to proliferate endangered animal species. On the other hand, it is possible to clarify the developmental ability of PGCs to differentiate into both male and female gametes (ova and spermatozoa) by transferring PGCs from male donor to female recipient or *vice versa*. In the case of developing a novel technique to proliferate endangered animal species by transferring PGCs into recipient embryos, the combination of sex between donor PGCs and recipient embryos is very important. It is also very important and an urgent matter to clarify the developmental

fate of donor PGCs in different sex recipient embryos for devising a novel technique to proliferate endangered animal species artificially.

2. Research Objective

PGCs, which appear at the early embryonic stage, are genetically male (ZZ) or female (ZW). They would have an ability to differentiate into both male and female gametes at an very early stage of embryonic development, and the differentiation to male or female gametes is decided by somatic cells surrounding PGCs. In other words, PGCs entered into male recipient embryo, the gonads differentiate to testes and the donor PGCs differentiate into spermatogonia. And PGCs entered into female recipient embryo, the gonads differentiate to ovary and the donor PGCs differentiate into oogonia[6]. It is, therefore, expected to be able to induce the differentiation of PGCs obtained from a single fertilized egg into both male and female gametes irrespective of their genetic sex by transferring them into both male and female recipient embryos. Both male and female offspring would be produced by mating male and female germline chimeric chickens produced by transfer of PGCs obtained from a single female (ZW) fertilized egg. The present study was carried out to clarify the developmental mechanisms of sexual differentiation of PGCs in the different sex gonads and to develop a novel technique to produce both male and female individuals derived from a single fertilized egg via germline chimeric chickens.

3. Research Method

In order to produce individuals derived from a single fertilized egg, PGCs should be collected from one embryo as much as possible and male and female offspring is expected to be produced via germline chimeric chickens produced by transfer of PGCs.

(1) Chicken breeds used for the experiment

The experiment was conducted using two chicken breeds; White Leghorn and Barred Plymouth Rock. White Leghorns are homozygous dominant for autosomal pigment inhibitor gene (*I/I*), while Barred Plymouth Rocks are homozygous recessive (*i/i*). PGC transfer was carried out from Barred Plymouth Rock to White Leghorn. It is, therefore, possible to distinguish offspring from germline chimeric chickens that they were derived from donor PGCs or recipient PGCs by their feather color.

(2) Sexing of donor PGCs and recipient embryos

In avian species, W-chromosome is present only in female. It has been known that DNA repetitive sequence is present on the W-chromosome, which repeating units are 0.7kb digested with restriction enzyme *Xho*I and 1.2kb digested with *Eco*RI[7]. Sexing of donor cells and recipient embryos can, therefore, be possible by detecting the presence of these repeating units. For sexing donor PGCs, DNA was extracted from blood cells and embryonic tissue of donor embryos. For sexing recipient embryos, a cluster of cells was collected from the central disc of blastoderm at stage X[8] (unincubated stage) by thin needle and DNA was extracted from these blastodermal cells. Sexing was carried out by polymerase chain reaction (PCR) method by amplifying the W-chromosome specific repetitive sequence using specific primers[9].

(3) Collection and transfer of PGCs

PGCs were collected from blood of 2 day incubated Barred Plymouth Rock embryos (stages 13-15)[10] by Ficoll density gradient centrifugation method[11]. The half of the collected PGCs was transferred to White Leghorn male recipient embryo from which blood was drawn as much as possible and the other half was transferred to the partially sterilized female

recipient embryo. The number of PGCs transferred was 30-431 per embryo. The manipulated embryos were cultured in surrogate eggshells[12, 13] until hatching.

4. Result

It was possible to determine the sex of donor PGCs and recipient embryos by analyzing DNA from donor PGCs (blood and embryonic tissues) and recipient embryos (blastodermal cells). Especially, DNA was successfully extracted from the small number of blastodermal cells obtained from recipient embryo, and sexing could be performed by PCR analysis taking a short time. So far, PGCs were transferred 314 sexed embryos, 83 (26.4%) chicks hatched, and 74 chickens (40 males and 34 females) survive now. Five pairs of male and female chicks were obtained whose donor PGCs were derived from the single fertilized egg. Sexing recipient embryos by PCR was correct because sex by PCR analysis and sex of the hatched chicks were the same in all cases. Mating experiment is going to be performed after their sexual maturation and examine the germline chimerism of the chickens.

5. Discussion

The experimental system for transferring PGCs obtained from a single fertilized egg to both male and female recipient embryos was successfully developed. For the next step, combining the technique of *in vitro* culture and proliferation of PGCs would develop more efficient system giving rise to viable offspring from PGCs via germline chimeric chickens. These new techniques will help to proliferate endangered animal species artificially. It is also required to clarify the developmental fate of PGCs in the different sex recipient embryos.

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