MOLECULAR REPRODUCTION AND DEVELOPMENT 69:296–302 (2004)

Blastocysts Produced by Nuclear Transfer Between Chicken Blastodermal Cells and Rabbit Oocytes

SHU-ZHEN LIU,[1] ZHEN-MING ZHOU,[2] TAO CHEN,[1] YAN-LING ZHANG,[1] DUAN-CHENG WEN,[3] ZHAO-HUI KOU,[1] ZAN-DONG LI,[2] QING-YUAN SUN,[1] AND DA-YUAN CHEN[1]*

[1]*State Key Laboratory of Reproductive Biology, Institute of Zoology, Chinese Academy of Sciences, Beijing, China*
[2]*College of Biology, China Agriculture University, Beijing, China*
[3]*Guangzhou Medical College, Guangzhou, China*

ABSTRACT Interspecies nuclear transfer (INT) has been used as an invaluable tool for studying nucleus–cytoplasm interactions; and it may also be a method for rescuing endangered species whose oocytes are difficult to obtain. In the present study, we investigated interaction of the chicken genome with the rabbit oocyte cytoplasm. When chicken blastodermal cells were transferred into the perivitelline space of rabbit oocytes, 79.3% of the couplets were fused and 9.7% of the fused embryos developed to the blastocyst stage. Both M199 and SOF medium were used for culturing chicken–rabbit cloned embryos; embryo development was arrested at the 8-cell stage obtained in SOF medium, while the rates of morulae and blastocysts were 12.1 and 9.7%, respectively, in M199 medium. Polymerase chain reaction (PCR) amplification of nuclear DNA and karyotype analyses confirmed that genetic material of morulae and blastocysts was derived from the chicken donor cells. Analysis mitochondrial constitution of the chicken–rabbit cloned embryos found that mitochondria, from both donor cells and enucleated oocytes, co-existed. Our results suggest that: (1) chicken genome can coordinate with rabbit oocyte cytoplasm in early embryo development; (2) there may be an 8- to 16-cell stage block for the chicken–rabbit cloned embryos when cultured in vitro; (3) mitochondrial DNA from the chicken donor cells was not eliminated until the blastocyst stage in the chicken–rabbit cloned embryos; (4) factors existing in ooplasm for somatic nucleus reprogramming may be highly conservative. *Mol. Reprod. Dev. 69: 296–302, 2004.* © 2004 Wiley-Liss, Inc.

Key Words: early development; embryo; mitochondrial fate; reprogramming

INTRODUCTION

Studies for nucleus–cytoplasm interactions have been extensively performed since the report of Briggs and King (1952) on *Rana pipiens*. Early in 1963, Tung began to study this issue in fish using the nuclear transplantation technique, and later the hybrid larval fish was obtained from the combination of nucleus and cytoplasm from different sub-families of teleosts (Yan et al., 1985). Blastocysts were also obtained by nuclear transplantation in teleost of different families and orders (Yan et al., 1990). In mammals, several studies have shown that oocyte cytoplasm from bovine (Dominko et al., 1999), sheep (Wilmut et al., 1997; White et al., 1999), and rabbit (Chen et al., 1999) are able to dedifferentiate somatic cell nuclei from sheep, pig, monkey, rat, and giant panda, and support early development of these interspecies cloned embryos to blastocysts. Although there is no report about interactions of the mammalian oocyte cytoplasm and avian genome, some somatic cell hybrids between chicken and mammalian cells had been investigated. These previous studies demonstrated that avian gene can be stably incorporated and correctly expressed in a mammalian cell (Eun et al., 1981).

On the basis of these results, we determined to investigate whether the mammalian oocyte has the ability to reprogram chicken blastodermal cell. Previous studies in our laboratory have shown that panda–rabbit (Chen et al., 1999, 2002), cat–rabbit (Wen et al., 2003), and monkey–rabbit (Yang et al., 2003) somatic cell nuclear transfer (NT) embryos can develop to blastocysts in vitro. The results suggest that factors existing in rabbit oocyte cytoplasm for somatic nucleus reprogramming and dedifferentiation may not be species-specific in mammals (Wen et al., 2003). Establishing a model of interclass reconstructured embryo would potentially provide greater insights into nucleus–cytoplasm interaction and as well as a method in the conservation of endangered birds whose eggs are very difficult to obtain.

Shu-Zhen Liu and Zhen-Ming Zhou contributed equally to this work.

Grant sponsor: Ministry of Science and Technology, China (Climbing Project, Climbing-Special-08); Grant sponsor: Chinese Academy of Sciences (Knowledge Innovation Project); Grant numbers: KSCX1-05-01, KSCX2-SW-303.

*Correspondence to: Da-Yuan Chen, State Key Laboratory of Reproductive Biology, Institute of Zoology, Chinese Academy of Sciences, 25 Zhongguancun Road, Haidian, Beijing 100080, China. E-mail: chendy@panda.ioz.ac.cn

Received 16 January 2004; Accepted 31 March 2004
Published online in Wiley InterScience (www.interscience.wiley.com).
DOI 10.1002/mrd.20091

MATERIALS AND METHODS

Animals

Animal care and handling were in accordance with the policy on the Care and Use of Animals of the Ethical Committee, State Key Laboratory of Reproductive Biology, Institute of Zoology, Chinese Academy of Sciences. Female Japan Big Eared white rabbits were housed in stainless steel cages, and were fed with regular rabbit fodder and water ad libitum.

Preparation of Donor Cells

Freshly laid fertilized chicken eggs of the White leghorn breed were used to supply blastodermal cells the day they were obtained from a local hatchery. The content of egg was emptied into a petri dish. The blastodisc was dissected and immediately placed in another dish containing PBS solution without Ca^{2+} and Mg^{2+}. Scraps of the vitelline membrane and yolk fragments, if they adhered to the disc, were shaken off. The denuded blastodisc was washed several times and was transferred to a centrifuge tube containing 1 ml Dulbecco's modified Eagle medium (DMEM, Gibco-BRL, Grand Island, NY) culture medium. Six to eight blastodiscs were put into 1 ml PBS solution containing 0.25% trypsin–EDTA and incubated for 10 min at 37°C. After the inactivation of trypsin (Gibco-BRL) with 100 μl FBS, the dissociated blastomeres were harvested by centrifugation at 1,200*g* and stored at 4°C before NT.

Recipient Cytoplasm Preparation

Procedure of recipient oocyte preparation has been described previously (Yang et al., 2003). Briefly, mature female Japanese Big Eared white rabbits were superovulated by administering PMSG and hCG (Institute of Zoology, Chinese Academy of Sciences). Each rabbit was injected with 150 IU PMSG and 100 IU hCG 4 days after the PMSG injection. Rabbits were killed 14 hr after the hCG injection. Cumulus masses were collected by flushing the separated oviducts with M2 medium (Sigma Chemical Company, St. Louis, MO) and were treated with 300 IU/ml of hyaluronidase (Sigma) in M2 medium, cumulus cells were stripped from the oocyte by repeated gentle pipetting. After three washings in M2 medium, the cumulus-free eggs were transferred to M2 medium containing 7.5 μg/ml cytochalasin B (Sigma Chemical Co.), 7.5 μg/ml Hoechst 33342 (Sigma Chemical Co.), and 10% FBS for 10 min and used for micromanipulation. For enucleation, a small amount of cytoplasm from the area beneath the first polar body containing the meiotic spindle was aspirated using a 25–30 μm glass pipette, and then the aspirated karyoplast was exposed to ultraviolet light to confirm the presence of nucleus. Only the oocytes devoiding the chromosomes were used for NT (Chen et al., 1999; Li et al., 2001).

NT Procedure

The NT was conducted as previously described (Chen et al., 1999, 2002). A single donor cell was placed in the perivitelline space. The couplets were transferred to a fusion chamber consisting of two wires, 1 mm apart and overlaid with the 100 μl fusion of medium (0.25 M sorbitol, 0.5 mM magnesium acetate, 0.1 mM calcium acetate, 0.5 mM HEPES, and 100 mg/100 ml BSA) (Mitalipov et al., 1999). Fusion was induced by double DC pulses of 1.4 kV/cm for 80 μsec with an ECM2001 Electrocell Manipulator (BTX, Inc., San Diego, CA). Couplets were then washed in M199 (Gibco-BRL) supplemented with 10% FBS for three times, and incubated in the same medium for 30 min at 38°C in a humidified air containing 5% CO_2.

Activation and Embryo Culture

Couplets were checked for fusion under an inverted microscope, and fused couplets were activated either by double DC pulses of 1.4 kV/cm for 40 μsec. Some of the activated embryos were cultured in M199 + 10% FBS; Some cloned embryos were cultured in synthetic oviduct fluid (SOF) medium supplemented with 1% minimal essential medium (MEM) nonessential amino acids and 2% essential amino acids (both Gibco-BRL), and 10% FBS. All cloned embryos were cultured in M199 + 10% FBS at 38°C in a humidified air containing 5% CO_2. The developmental stages of embryos were checked twice a day.

Nuclear DNA and Mitochondrial DNA Analysis

Nuclear DNA amplification was performed in two steps using nested primers. All samples were analyzed by polymerase chain reaction (PCR) using specific primers of chicken designed according to avian feather keratin gene D, which had been sequenced (Presland et al., 1989). Primer pairs were as fellows: P1, 5′-GGAGAAGGTCCAGGGCTGACTTTA-3′; P2, 5′-ACTTCTCTTGGCAAACATG CAACC-3′ and P3, 5′-GCGTCCACCTCATCCTTAGCAG-3′. The first round amplification was performed at 94°C for 5 min, 94°C for 30 sec; 54°C for 40 sec; 72°C for 60 sec for 30 cycles, and finally 72°C for 5 min using primer P1 and P3. Aliquots of 1 μl PCR production from first round amplification were subjected to second round amplification performed at 94°C for 5 min, 94°C for 30 sec; 60°C for 40 sec; 72°C for 60 sec for 30 cycles, and finally 72°C for 5 min using primer P2 and P3. The final amplification products of 400 base pairs were separated via agar gel electrophoresis and sequenced by an auto DNA sequencer (ABI 377, Perkin-Elmer).

We analyzed a region of the cytochrome b (*cytb*) gene of the mitochondrial DNA (mtDNA) by a specific PCR with two sets primers being specific to rabbit mtDNA and chicken mtDNA, respectively. Primers for amplification rabbit mitochondrial DNA were PR1, 5′-TCTACATACACGTAGGCCGCGGAA-3′ and PR2, 5′-GAGGAG AAGAATGGCTACAAGGAAA-3′. Chicken-specific primers were PC1, 5′-CCC CAGCAAACCCACTAGTA-3′ and PC2, 5′-TGGTCTAGGGTTCCGATTGT-3′. The final amplification products of 353 base pairs were separated via

agar gel electrophoresis and also sequenced by an auto DNA sequencer (ABI 377, Perkin-Elmer).

Karyotype Analysis of Reconstructed Blastocyst

Karyotype of chicken and rabbit fibroblast cells was analyzed according to a method described previously (Lanza et al., 2000). In brief, 24 hr after plating, rabbit, and chicken cells were treated with 0.3 μg/ml demecolcine for 3 hr, and then exposed to KCl (0.075 M) for 20 min at 37°C. The cells were then fixed in acetic acid:methanol (1:3 v:v) and drops of cells suspension were spread on clean microscope slides. The chromosomes were stained with 5% Giemsa for 5–10 min.

The karyotype of blastocysts was conducted as follows: first, blastocysts were exposed to 0.3 μg/ml demecolcine solution for 4–5 hr at 37°C and then treated with KCl (0.075 M) for 40 min, transferred to clean slides. Drops of the fixed solution were added to the blastocysts in order to make chromosomes spread. Air or fire-dried slides were stained with 5% Giemsa for 10 min. Total 21 reconstructed blastocysts were examined. The numbers of chromosomes were counted under a light microscope at 1,000× magnification.

Statistical Analysis

Percentages of embryos at different stages were compared between groups using χ^2 analysis. Significant difference was determined at $P < 0.05$ or $P < 0.01$.

RESULTS

Developmental Capacity of Chicken–Rabbit Cloned Embryos

After chicken blastodermal cells were fused with enucleated metaphase II rabbit oocytes, pronuclear formation was observed between 5 and 7 hr after activation. Cloned embryos began to compact on day 3, the fused embryos developed to the blastocyst stage on day 6 and escaped from the zona pellucida on day 7 (Fig. 1). When the chicken–rabbit cloned embryos were cultured in SOF medium, the reconstructed embryos arrested at the 8-cell stage and no morula was obtained. However, when cultured in M199 medium, though most of the cloned embryos arrested before the 8-cell stage, 9.7% of the cloned embryos could develop into blastocysts (Table 1).

Analysis of Nuclear DNA and Mitochodrial DNA in Cloned Embryos

PCR amplification was conducted on nuclear DNA from chicken, rabbit, and reconstructured 8-cell embryos, morulae, and blastocysts. The specific avian feather keratin gene D of 400 base pairs (bp) was detected in 8-cell embryos, morulae and blastocysts (Fig. 2). PCR products of cloned morulae and blastocysts were sequenced (repeated three times) and matched the DNA sequences from the donor cells. The three sequences were all identical to the sequence of avian feather keratin gene D (1,163 bp) from base 336–735 accessed in GenBank (GenBank code: X17509) (Fig. 3).

Chromosomes of chicken and rabbit were easily distinguishable from physiognomic form and numbers (Fig. 4). The chicken karyotype consists of 39 pairs of chromosomes which were delimited into three groups, determined on the basis of size: six pairs of large, morphologically distinguishable macrochromosomes, four pairs of intermediate, and 29 pairs of very small individually indistinguishable microchromosomes (Bloom et al., 1993). While the rabbit karyotype consists of 22 pairs of distinguishable chromosomes. Visual observations suggested that the chromosomes of chicken–rabbit cloned embryos were the same as chicken cells.

Chicken–rabbit cloned morulae and blastocysts were used for evaluating mitochondrial DNA. Both rabbit and chicken mitochondrial DNA could be detected in embryos (Fig. 5). PCR products of donor cells and cloned embryos were sequenced (repeated three times) and shown to be identical to the sequence of the *cytb* gene (1,140 bp) of White leghorn deposited in GenBank (GenBank code: AY029582) from base 771–1,123 (Fig. 6).

DISCUSSION

Sperm-induced oocyte activation is triggered by intracellular Ca^{2+} oscillations during fertilization (Fissore et al., 1992). This stimulus triggers the inactivation of maturation-promoting factor (MPF), composed of cyclin B and cdc2, which is present at high levels in MII oocytes (Well et al., 1997; Wu et al., 1997). With the aim to

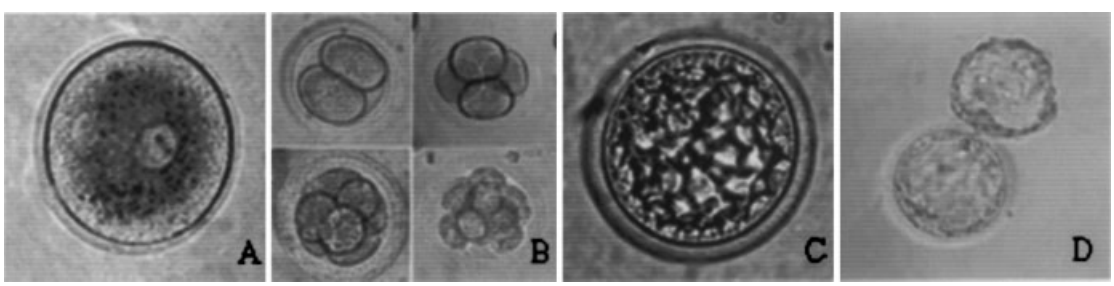

Fig. 1. In vitro development process of interclass reconstructured embryos from chicken donor cells. **A**: Blastodermal cell NT embryo at the pronuclear stage (×200), (**B**) NT embryo at the 2-cell to 16-cell stage (×100), (**C**, **D**) NT embryo at the blastocyst stage (×200; ×100).

TABLE 1. In Vitro Development of Chicken–Rabbit Interclass Cloned Embryos in Different Culture Media*

Culture medium	NT units	Fused (%)	2-cell (%)	4-cell (%)	8-cell (%)	Morula (%)	Blastocyst (%)
M199 +10% FBS	416	330 (79.3)[a]	120 (36.4)[a]	96 (29.1)[a]	74 (22.4)[a]	40 (12.1)	32 (9.7)
SOF	198	156 (78.8)[a]	55 (35.3)[a]	35 (22.4)[a]	24 (15.4)[a]	0	0

*Fused (%), fused oocytes/nuclear transfer (NT) units; development rate of embryos at different stages, number of embryos/number of fused oocytes. Values with different superscripts within each column are significantly different ($P < 0.05$).

activate the MII rabbit enucleated oocytes, electrical activation was used in this study. This method was proved to be efficient for NT embryos from the rabbit oocyte as recipient (Chen et al., 1999, 2002; Wen et al., 2003; Yang et al., 2003).

The chicken–rabbit embryos were cultured in different media, aiming to examine the effects of different culture systems on the preimplantation embryo development. SOF a chemical defined medium, has been widely used to culture embryos from various mammalian species (Walker et al., 1996; Krisher et al., 1999; Freistedt et al., 2001; Donnay et al., 2002). But our results showed that the embryos were arrested at 8-cell stage in the SOF medium. In our previous reports, M199 was able to support the panda–rabbit (Chen et al., 1999, 2002), cat–rabbit, and rabbit-rabbit (Wen et al., 2003) embryo development in vitro. When chicken–rabbit embryos were cultured in M199 the embryo development can reach the blastocyst stage. Our results show that M199 is more suitable for chicken–rabbit embryo culture in vitro.

In mammalian species, the maternal-zygotic transition (MZT) or activation of the embryonic genome (EGA) occurs at earlier embryonic stages and is often associated with embryo compaction and developmental block (Frei et al., 1989; Telford et al., 1990). During this period, protamines are replaced by histones, the methylated haploid parental genomes undergo demethylation following formation of the diploid zygote, and maternal control of development is succeeded by zygotic control. Superimposed on this activation of the embryonic genome is the formation of a chromatin-mediated transcriptionally repressive state requiring enhancers for efficient gene expression (Kanka, 2003). For somatic NT embryos, there is a similar transition associated with the nucleus reprogramming (Campbell, 1999). However, we do not know whether the time of this transition is recipient-specific or nucleus-specific in interclass cloned embryos. The time of MZT for rabbit occurs at the 8- to 16-cell stage (Manes, 1973; Henrion et al., 1997), while there is no investigation on early cleavage of chick embryo in vitro. Repeated cell division occurs after fertilization and by the time the egg is laid (about 24 hr) the chick embryo possesses 32,000–42,000 cells (Stepinska and Olszanska, 1983). In the present study, most chicken–rabbit embryos were blocked at the 8- to 16-cell stage and only small part of them can compact and continue to develop. Our results imply that the MZT for chicken–rabbit cloned embryos might occur at 8- to 16-cell stage and after that chicken nuclei take full control of the hybrid embryos. The stage of MZT for the interclass cloned embryos is likely oocyte-specific as that of interspecies cat–rabbit cloned embryos (Wen et al., 2003).

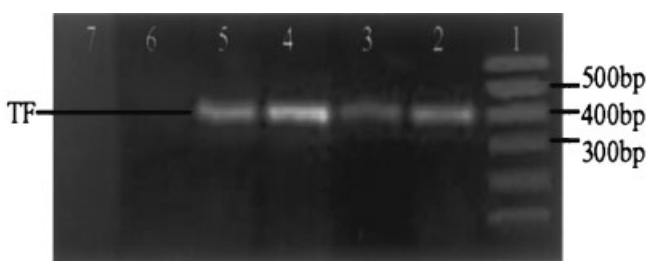

Fig. 2. Electrophoretic analysis of polymerase chain reaction (PCR) product using specific avian feather keratin gene D primer. **Lane 1**: Markers (100 bp ladder), (**lane 2**) chicken donor cells, (**lane 3**) 8-cell cloned embryo, (**lane 4**) blastocyst stage, (**lane 5**) morula stage, (**lane 6**) water (negative control), (**lane 7**) rabbit somatic cells; TF, target fragment.

Although the rate of the blastocysts was very low, a small portion of the chicken–rabbit embryos broke the MZT block, developing to blastocyst stage. We propose that continuation of development could be a consequence of reprogramming of the donor nucleus, regardless of the species and some key embryonic genes expressed in the early development stage may be homologous. Though there was no report about this, de novo transcription of chick genes and the synthesis of both globin and constitutive proteins can be detected after introducing inactive chick erythrocyte nuclei into mammalian cell cytoplasm (Zuckerman et al., 1982). When HPRT-deficient mouse and Chinese hamster ovary cells are fused with chick embryo erythrocytes containing genetically inactive nuclei, the HPRT activity is expressed in cell hybrids produced by the chick *HPRT* gene (Eun et al., 1981). Studies have shown that the acid α-glucosidase in the chick erythrocyte-human fibroblast heterokaryons is of chick origin, and is localized in the same lysosomes as the human lysosomal enzymes (Sips et al., 1986). The mouse TCR ζ-chain-deficient cells were transfected with the chicken TCR ζ-chain gene. Unexpectedly, the chicken ζ-chain was able to rescue the surface expression and function of the mouse TCR (Thomas et al., 1998). According to our studies, panda–rabbit (Chen et al., 1999, 2002), cat–rabbit (Chen et al., 2002), macaca–rabbit (Yang et al., 2003), and chicken–rabbit cloned embryos all can develop to blastocyst stage. We may draw the conclusion that some key ooplasm reprogramming factors required from early embryo development are conserved in animal kingdom.

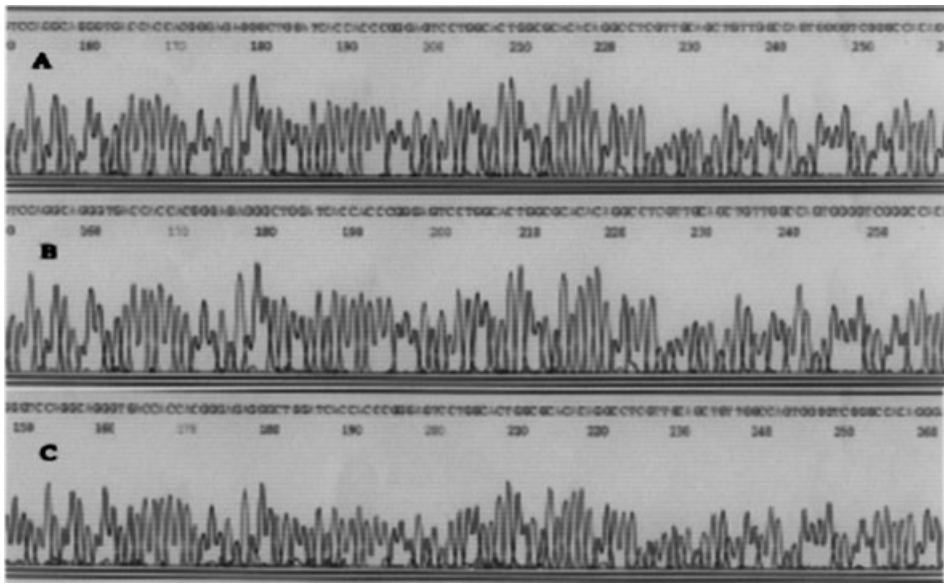

Fig. 3. Sequences of PCR products by using specific avian feather keratin gene D primer. **A**: Chicken donor cells, (**B**) morula stage, (**C**) blastocyst stage NT embryo.

In the process of NT, mitochondrial DNA of donor cells, together with the nucleus, are transferred into the recipient oocyte. There are three conclusions about mitochondrial DNA in animals cloned by NT: (1) homoplasmy of recipient oocyte mtDNA (Kaneda et al., 1995; Evans et al., 1999; Takeda et al., 1999; Lanza et al., 2000; Loi et al., 2001; Meirelles et al., 2001); (2) homoplasmy of donor cell mtDNA (Chen et al., 2002); (3) heteroplasmy of both donor and recipient mtDNA (Steinborn et al., 1998; Hiendleder et al., 1999; Nagao et al., 1997; Sutovsky et al., 1999; Yang et al., 2003). Our results are consistent with the third conclusion that chicken and rabbit mtDNAs coexist before the blastocyst stage. This is perhaps caused by compatibility and coordination between chicken nuclei and rabbit cytoplasm. The protein genes in mitochondrial genome of the White leghorn chicken are highly similar to their mammalian counterparts and are translated according to the same variant genetic code. The avian genome encodes the same set of genes (13 proteins, 2 rRNAs, and 22 tRNAs) as do other vertebrate mitochondrial DNAs and is organized in a very similar economical fashion (Desjardins and Morais, 1990). Anyway this is the first report showing avian and mammalian mitochondrial DNA coexist in interclass cloned embryos; whether this is a general phenomenon needs further investigation.

At present, we do not have sufficient data supporting the conclusion that rabbit oocyte cytoplasm reprograms chicken nucleus. Molecular descriptors of successful reprogramming in different species have not yet been obtained. Now we only found that 2C9 monocloned antibody, which is special to the antigen of chicken PGCs, was positive to the reconstructed chicken–rabbit embryo (unpublished data). Next, we will prepare to transplant the chicken–rabbit reconstructed embryos to the egg of chicken to see if they can progress into organogenesis. If the experiment succeeds, the method could be used for rescuing the endangered birds.

In conclusion, enucleated rabbit ooplasm can support chicken blastodermal cells develop to blastocyst stage.

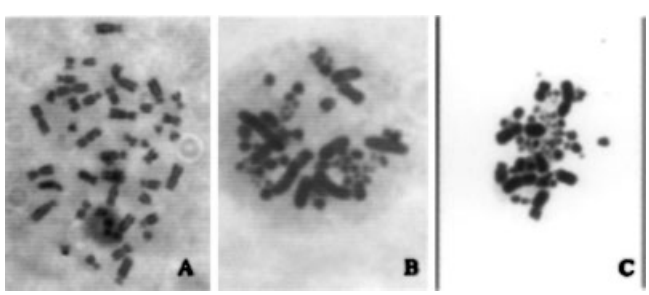

Fig. 4. Chromosomes of rabbit somatic cell, chicken–rabbit reconstructed blastocyst and chicken somatic cell. **A**: Rabbit somatic cell, (**B**) reconstructed blastocyst, (**C**) chicken somatic cell.

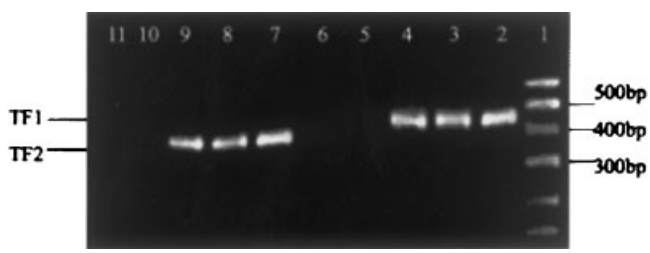

Fig. 5. Electrophoretic analysis of PCR product using specific rabbit and chicken cytochrome primers, respectively. **Lane 1**: Markers, (**lane 2**) rabbit somatic cells, (**lanes 3** and **8**) morula stage, (**lanes 4** and **9**) blastocyst stage, (**lane 5**) chicken somatic cells; (**lanes 6** and **11**) water (lanes 2–6, using rabbit cytb primers); (**lane 7**) chicken somatic cells; (**lane 10**) rabbit somatic cells (lanes 7–11 using chicken cytb primers); TF1, TF2, target fragment.

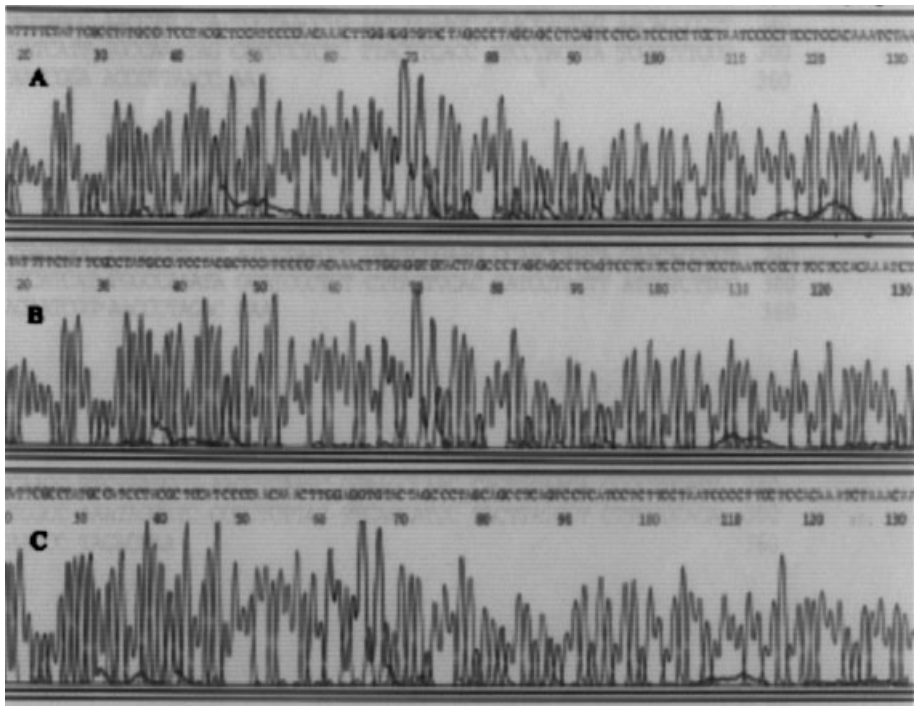

Fig. 6. Sequences of PCR products by using specific rabbit and chicken cytochrome b primers, respectively. **A**: Chicken somatic cells, (**B**) morula stage, (**C**) blastocyst stage NT embryo.

Chicken and rabbit mtDNAs coexist in the chicken–rabbit cloned embryos.

REFERENCES

Bloom SE, Delany ME, Muscarella DE. 1993. Constant and variable features of avian chromosomes. In: Etches RJ, editor. Manipulation of the avian genome. Boca Raton: CRC. pp 39–59.

Briggs R, King TJ. 1952. Transplantation of living nuclei from blastula cells into enucleated frog's eggs. Proc Natl Acad Sci USA 38:455–463.

Campbell KH. 1999. Nuclear equivalence, nuclear transfer, and the cell cycle. Cloning 1:3–14.

Chen DY, Sun QY, Liu JL, Li GP, Lian L, Wang MK, Han ZM, Song XF, Li JS, Sun Q, Chen YC, Zhang YP, Ding B. 1999. The giant panda (*Ailuropoda melanoleuca*) somatic nucleus can dedifferentiate in rabbit ooplasm and support early development of the reconstructed egg. Sci China (Series C) 29:324–330.

Chen DY, Wen DC, Zhang YP, Sun QY, Han ZM, Liu ZH, Shi P, Li JS, Xiangyu JG, Lian L, Kou ZH, Wu YQ, Chen YC, Wang PY, Zhang HM. 2002. Interspecies implantation and mitochondria fate of panda–rabbit cloned embryos. Biol Reprod 67:637–642.

Desjardins P, Morais R. 1990. Sequence and gene organization of the chicken mitochondrial genome. A novel gene order in higher vertebrates. J Mol Biol 212:599–634.

Dominko T, Mitalipova M, Haley B, Beyhan Z, Memili E, Mckusick B, First NL. 1999. Bovine oocyte cytoplasm supports development of embryos produced by nuclear transfer of somatic cell nuclei from various mammalian species. Biol Reprod 60:1496–1502.

Donnay I, Feugang JM, Bernard S, Marchandise J, Pampfer S, Moens A, Dessy F. 2002. Impact of adding 5.5 mM glucose to SOF medium on the development, metabolism, and quality of in vitro produced bovine embryos from the morula to the blastocyst stage. Zygote 10:189–199.

Eun CK, Paik SG, Goldwasser P, Shin S, Klinger HP. 1981. Immunochemical identification of the chick HPRT gene transferred from chick erythrocytes to mammalian somatic cells. Cytogenet Cell Genet 29:116–121.

Evans M, Gurer C, Loike JD, Wilmut I, Schnieke AE, Schon EA. 1999. Mitochondrial DNA genotypes in nuclear transfer-derived cloned sheep. Nat Genet 23:90–93.

Fissore RA, Dobrinsky JR, Balise JJ, Duby RT, Robl J. 1992. Patterns of intracellular Ca^{2+} concentrations in fertilized bovine eggs. Biol Reprod 47:960–969.

Frei RE, Schultz GA, Church RB. 1989. Qualitative and quantitative changes in protein synthesis occur at the 8–16-cell stage of embryogenesis in the cow. J Reprod Fertil 86:637–641.

Freistedt P, Stojkovic M, Wolf E. 2001. Efficient in vitro production of cat embryos in modified synthetic oviduct fluid medium: Effects of season and ovarian status. Biol Reprod 65:9–13.

Henrion G, Brunet A, Renard JP, Duranthon V. 1997. Identification of maternal transcripts that progressively disappear during the cleavage period of rabbit embryos. Mol Reprod Dev 47:353–362.

Hiendleder S, Schmutz SM, Erhardt G, Green RD, Plante Y. 1999. Transmitochondrial differences and varying levels of heteroplasmy in nuclear transfer cloned cattle. Mol Reprod Dev 54:24–31.

Kaneda H, Hayashi J, Takahama S, Taya C, Lindahl KF, Yonekawa H. 1995. Elimination of paternal mitochondrial DNA in intraspecific crosses during early mouse embryogenesis. Proc Natl Acad Sci USA 92:4542–4546.

Kanka J. 2003. Gene expression and chromatin structure in the pre-implantation embryo. Theriogenology 59:3–19.

Krisher RL, Lane M, Bavister BD. 1999. Developmental competence and metabolism of bovine embryos cultured in semi-defined and defined culture medium. Biol Reprod 60:1345–1352.

Lanza RP, Cibelli J, Diaz F, Moraes C, Farin PW, Farin CE, Hammer CJ, West MD, Damiani P. 2000. Cloning of an endangered species using interspecies nuclear transfer. Cloning 2:79–90.

Li GP, Chen DY, Lian L, Sun QY, Wang MK, Liu JL, Li JS, Han ZM. 2001. Viable rabbits derived from reconstructed oocytes by germinal vesicle transfer after intracytoplasmic sperm injection (ICSI). Mol Reprod Dev 58:180–185.

Loi P, Ptak G, Barboni B, Fulka J, Cappai P, Clinton M. 2001. Genetic rescue of an endangered mammal by cross-species nuclear transfer using post-mortem somatic cells. Nat Biotech 19:962–964.

Manes C. 1973. The participation of the embryonic gnome during early cleavage in the rabbit. Dev Biol 32:453–459.

Meirelles FV, Bordignon V, Watanabe Y, Watanabe M, Dayan A, Lobo RB, Garcia JM, Smith LC. 2001. Complete replacement of the mitochondrial genotype in a Bos indicus calf reconstructed by nuclear transfer to a *Bos taurus* oocyte. Genetics 158:351–356.

Mitalipov SM, White KL, Farrar VR, Morrey J, Reed WA. 1999. Development of nuclear transfer and parthenogenetic rabbit embryos activated with inositol 1,4,5-trisphosphate. Biol Reprod 60:821–827.

Nagao Y, Totsuka Y, Atomi Y, Kaneda H, Yonekawa H, Imai H. 1997. Heterogeneous mitochondrial DNA introduced by nuclear transfer influences the developmental ability of mouse embryos in vitro. Theriogenology 47:233 (Abstract).

Presland RB, Gregg K, Molloy PL, Morris CP, Crocker LA, Rogers GE. 1989. Avian keratin gene I. molecular analysis of the structure and expression of a group of feather keratin gene. J Mol Biol 209: 549–559.

Sips HJ, Reuser AJ, van der Veer E. 1986. Synthesis and intracellular localization of chick acid α-glucosidase in chick erythrocyte-human fibroblast heterokaryons. A model system for the study of lysosomal enzyme synthesis. Exp Cell Res 162:555–561.

Steinborn R, Zakhartchenko V, Wolf E, Muller M, Brem G. 1998. Non-balanced mix of mitochondrial DNA in cloned cattle produced by cytoplast–blastomere fusion. FEBS Lett 426:357–361.

Stepinska U, Olszanska B. 1983. Cell multiplication and blastoderm development in relation to egg envelope formation during uterine defelopment of quail (*Coturnix cotunix*) embryo. J Exp Zool 228:505–510.

Sutovsky P, Moreno RD, Ramalho-Santos J, Dominko T, Simerly C, Schatten G. 1999. Ubiquitinatin tag sperm mitochondria. Nature 402:371–372.

Takeda K, Takahashi S, Onishi A, Goto Y, Miyazawa A, Imai H. 1999. Dominant distribution of mitochondrial DNA from recipient oocytes in bovine embryos and offspring after nuclear transfer. J Reprod Fertil 116:253–259.

Telford NA, Watson AJ, Schultz GA. 1990. Transition from maternal to embryonic control in early mammalian development: A comparison of several species. Mol Reprod Dev 26:90–100.

Thomas WF, Luca B. 1998. Cutting edge: The chicken TCR-chain restores the function of a mouse T cell hybridoma. J Immunol 160: 1552–1554.

Walker SK, Hill JL, Kleemann DO, Nancarrow CD. 1996. Development of ovine embryos in synthetic oviductal fluid containing amino acids at oviductal fluid concentrations. Biol Reprod 55:703–708.

Well DN, Misica PM, Mcmillan WH, Tervit HR. 1997. Production of cloned lambs from an established embryonic cell line: A comparison between in vivo and in vitro matured cytoplasts. Biol Reprod 57:385–393.

Wen DC, Yang CX, Cheng Y, Li JS, Liu ZH, Sun QY, Zhang JX, Lei L, Wu YQ, Kou ZH, Chen DY. 2003. Comparison of developmental capacity for intra- and inter-species cloned cat (*Felis catus*) embryos. Mol Reprod Dev 66:38–45.

White KL, Bunch TD, Mitalipov S, Reed WA. 1999. Establishment of pregnancy after the transfer of nuclear transfer embryos produced from the fusion of Argali (*Ovis ammon*) nuclei into domestic sheep (*Ovis aries*) enucleated oocytes. Cloning 1:47–54.

Wilmut I, Schnieke AE, McWhir J, Kind AJ, Campbell KH. 1997. Viable offspring derived from fetal and adult mammalian cells. Nature 385:810–813.

Wu B, Ignotz G, Currie B, Yang X. 1997. Dynamics of maturation-promoting factor and its constituent protins during maturation of bovine oocytes. Biol Reprod 56:253–259.

Yan SY, Lu DY, Du M, Li GS, Han Z, Yang HY, Wu ZG, Yang YQ, Xia DQ, Liu AZ, Zhang XZ. 1985. Nuclear transplantion in teleosts. Chin J Biotechnol 1:15–26.

Yan SY, Tu M, Yang HY, Mao ZG, Zhao ZY, FU LJ, Li GS, Huang GP, Li SH, Jin GQ. 1990. Developmental incompatibility between cell nucleus and cytoplasm as revealed by nuclear transplantation experiments in teleost of different families and orders. Int J Dev Biol 34:255–266.

Yang CX, Han ZM, Wen DC, Sun QY, Zhang KY, Zhang LS, Wu YQ, Kou ZH, Chen DY. 2003. In vitro development and mitochondrial fate of macaca-rabbit cloned embryos. Mol Reprod Dev 65:396–401.

Zuckerman SH, Linder S, Ringertz NR. 1982. Transcription of chick genes by mammalian RNA polymerase II in chick erythrocyte-mammalian cell heterokaryons. J Cell Physiol 113:99–104.