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Quantitative analysis of mitochondrial DNA in porcine-mouse cloned embryos

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Abstract

The aim of the research is to identify that porcine oocytes can function as recipients for interspecies cloning and have the ability to develop to blastocysts. Furthermore each mitochondrial DNA (mtDNA) in interspecises cloned embryos was analyzed. For the study, mouse-porcine and porcine-porcine cloned embryos were produced with mouse fetal fibroblasts (MFF) and porcine fetal fibroblasts (PFF), respectively, introduced as donor cells into enucleated porcine oocytes. The developmental rate and cell numbers of blastocysts between intraspecies porcine-porcine and interspecies mouse-porcine cloned embryos were compared and real-time polymerase chain reaction (PCR) was performed for the estimate of mouse and porcine mtDNA copy number in mouse-porcine cloned embryos at different stages.There was no significant difference in the developmental rate or total blastocyst number between mouse-porcine cloned embryos and porcine-porcine cloned embryos (11.1 ± 0.9%, 25 ± 3.5 vs. 10.1 ± 1.2%, 24 ± 6.3). In mouse-porcine reconstructed embryos, the copy numbers of mouse somatic cell-derived mtDNA decreased between the 1-cell and blastocyst stages, whereas the copy number of porcine oocyte-derived mtDNA significantly increased during this period, as assessed by real-time PCR analysis. In our real-time PCR analysis, we improved the standard curve construction-based method to analyze the level of mtDNA between mouse donor cells and porcine oocytes using the copy number of mouse beta-actin DNA as a standard. Our findings suggest that mouse-porcine cloned embryos have the ability to develop to blastocysts in vitro and exhibit mitochondrial heteroplasmy from the 1-cell to blastocyst stages and the mouse-derived mitochondria can be gradually replaced with those of the porcine oocyte in the early developmental stages of mouse-porcine cloned embryos.

Keywords: Interspecies somatic cell nuclear transfer, Mitochondria, Porcine-mouse cloned embryo

INTRODUCTION

Interspecies-somatic cell nuclear transfer (iSCNT) is a technique wherein a donor somatic cell from one species is transferred into an enucleated oocyte of another species. iSCNT is possibly the most effective method introduced to date for preserving endangered species and maintaining biodiversity [1,2]. However, several studies on iSCNT have reported incomplete or abnormal remodeling of the donor cell nucleus within the recipient oocyte, and linked this to imprinting failure and abnormal

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Conceptualization: Jin D.
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Writing - review & editing: Shin H, Kim S, Kim M, Lee J, Jin D.

Ethics approval and consent to participate
All animal experiments were approved by the Institutional Animal Care and Use Committee (IACUC) of Chungnam National University, Korea (202003A-CNU-002).

gene expression [3]. Epigenetic reprogramming has been addressed in numerous studies [4,5]. More recently, mitochondrial heteroplasmy has been considered as an important cause of the low efficiency of iSCNT-based cloning. Mitochondria are cytoplasmic organelles that are responsible for generating energy to cells and have their own small DNA genome. Mitochondrial DNA (mtDNA) has proven useful for showing maternal relationships in forensic investigations. In normally fertilized embryos, the majority of mitochondria belonging to the sperm remain in the tail after fertilization and a minority of mitochondria in the head enter the ooplasm, where they are degraded by oocyte-derived ubiquitination allowing homogeneous with maternal mitochondria. In iSCNT, the proliferation pattern of mtDNA leading to heteroplasmy is still controversial. Several studies have analyzed the heteroplasmy of mtDNA in interspecies-somatic cell cloned embryos, such as ibex-hircus cloned embryos, ibex-rabbit cloned embryos, human-bovine cloned embryos, sheep-cattle cloned embryos, goat-sheep cloned embryos, and ovine-bovine cloned embryos [6,7]. The results of these studies supported the notion that the mitochondria of a recipient oocyte gradually substitute for those of the donor cell at the morula or blastocyst stages during the development of interspecies cloned embryos. Another study showed that the mitochondria from panda donor cells and rabbit oocytes coexisted at an early stage of embryonic development [8]. The mtDNA of rabbit oocytes represented the major population detected in blastocysts, whereas the mtDNA from the panda donor cells existed in early post-implantation fetuses. Understanding the relationship between donor- and recipient-cell mitochondria could provide new prospects for improving the rate of nuclear reprogramming in iSCNT.

Several methods have been applied to analyze the distribution of mtDNA in interspecies cloned embryos, including PCR-restriction fragment length, PCR-mediated single strand conformation polymorphism, and allele-specific PCR analyses. To study mitochondrial heteroplasmy, researchers have examined the copy numbers of mitochondrial RNA using real-time PCR analysis in cloned embryos [9]. However, the quantitative analysis of mtDNA in interspecies cloned embryos produced by transferring a species-far foreign somatic cell nucleus into a porcine recipient oocyte has not been reported in detail. Porcine oocytes have been used as recipients to clone rabbits and tigers, but these cloned embryos struggled to develop to the blastocyst stage [10, 11]. In the present study, to assess whether porcine oocytes can function as recipient for interspecies cloning and develop to the blastocyst stage, we produced mouse-porcine and porcine-porcine cloned embryos by inserting mouse fetal fibroblasts (MFF) and porcine fetal fibroblasts (PFF) as donor cells into enucleated porcine oocytes and real-time PCR was used to quantitatively analyze the relationship between donor cell- and recipient oocyte-derived mtDNA in mouse-porcine cloned embryos. We also improved the method of real-time PCR using beta-actin plasmid DNA to generate a standard curve and applied real-time PCR to quantitatively analyze the relationship between donor cell- and recipient oocyte-derived mtDNA in mouse-porcine cloned embryos at early development stages.

MATERIALS AND METHODS

Unless indicated, all chemicals used in this experiment were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

Donor cell preparation

Cell culture and assessment were performed as previously described [12]. PFF and MFF were used as donor cells for intraspecies and interspecies nuclear transfer, respectively. PFF were isolated from a pregnant sow at day 35 after fertilization. MEF cells were isolated from 6 to 8-week-old Institute of Cancer Research (ICR) mice at day 13.5 after mating. All cells were cryopreserved upon

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reaching confluence. Thawed fibroblasts were cultured in Dulbecco's modified Eagle's medium (DMEM, Gibco-BRL, Waltham, MA, USA) supplemented with 5% fetal bovine serum (FBS) and 5% fetal calf serum (FCS) in a cell culture dish. Before nuclear transfer, all donor cells were collected as single-cell suspensions using incubation with 0.25% trypsin and 0.5 mM ethylene-diamine-tetraacetic acid (EDTA) for 2 min in a 38.5℃ incubator. The cells were then washed twice and suspended in phosphate-buffered saline (PBS).

Collection of oocytes and *in vitro* maturation

Porcine ovaries were obtained from prepubertal gilts at a local slaughterhouse. Ovaries were transported to the laboratory in PBS containing 100 IU/mL penicillin and 50 μg/mL streptomycin at 25℃–30℃. Cumulus-oocyte complexes (COCs) were collected by aspirating 2- to 6-mm diameter ovarian follicles using a 10-mL syringe fixed with an 18-gauge needle. The follicular fluid was pooled into 50-ml conical tubes and the sediment was washed twice in TL-HEPES containing 0.1% (w/v) polyvinyl alcohol (PVA). Only oocytes with completely compact cumulus and evenly granulated cytoplasm were selected for in vitro maturation (IVM). Approximately 50 COCs were transferred into 500 μL of maturation medium in a 4-well multidish (Nunc, Roskide, Denmark) and incubated at 38.5℃ in an atmosphere of 5% CO_2 in air with maximum humidity [13]. The medium used for IVM of oocytes was TCM-199 supplemented with 10% follicular fluid, 0.57 mM L-cysteine, 0.5 μg/mL luteinizing hormone (LH), 0.5 μg/mL follicle-stimulating hormones (FSH), and 10 ng/mL epidermal growth factor receptor (EGF). After 22 h of maturation culture, the oocytes were washed three times and then further cultured in maturation medium without hormone for an additional 22 h.

Nuclear transfer and culture *in vitro*

Nuclear transfer was carried out as previously described [14]. After maturation, cumulus cells were removed from oocytes by repeated pipetting in TL-HEPES supplemented with 0.1% PVA and 0.1% hyaluronidase. Cumulus-free oocytes were enucleated by aspiration of the first polar body and metaphase II (MII) plate in a small amount of surrounding cytoplasm with a fine glass pipette in TCM 199 supplemented with HEPES, 0.3% bovine serum albumin (BSA), and 7.5 μg/mL cytochalasin B. Following enucleation, a single donor cell was placed in the perivitelline space of the oocyte to contact the oocyte membrane. Reconstructed oocytes were placed between two 0.2 mm-diameter platinum electrodes (1 mm apart) of a fusion chamber slide in fusion and activation medium consisting of 0.3 mM mannitol, 1.0 mM $CaCl_2 \cdot H_2O$, 0.1 mM $MgCl_2 \cdot 6H_2O$, and 0.5 mM HEPES. For fusion and activation, a direct current pulse of 1.1 kV/cm was applied for 30 μs with a BTX Elector-Cell Manipulator 2001. After electrical fusion, the reconstructed embryos were washed and transferred to culture medium covered with mineral oil in a 65-mm dish. Mouse-porcine cloned embryos were cultured in porcine zygote medium (PZM)-3-supplemented Chatot-Ziomek-Bavister (CZB) medium and porcine-porcine cloned embryos were cultured in PZM-3 medium. The culture environment was 5% CO_2 in air at 38.5℃ with maximum humidity.

Collection of reconstructed embryos

Cloned embryos were collected from repeated nuclear transfer experiments. At the 1-cell, 2-cell, 4-cell, 8-cell, morula, and blastocyst stages, five mouse-porcine cloned embryos per stage were collected into Eppendorf tubes containing 10 μL distilled water (DW) and stored at −70℃ until PCR analysis.

Species-specific mtDNA primers for intraspecies and interspecies cloned embryos

Primers for quantifying species-specific mtDNA and mouse beta-actin were designed from the D-loop sequences of mouse mtDNA (GeneBank Accession No. NC005089), porcine mtDNA (GeneBank Accession No. AJ002189), and mouse β-actin DNA (GeneBank Accession No. NC000071.5). For conventional PCR, the mouse-specific primers were forward, 5'-TCTT TTTATTTTGGCCTAC-3' and reverse, 5'-CATCTAAGCATTTTTCAGTG-3' (length, 432 bp). The porcine-specific primers were forward, 5'-CCAAGACTCAAGGAAGGAGA-3' and reverse, 5'-GGCGCGGATACTTGCATGTG-3' (length, 1,452 bp). For real-time PCR, the mouse-specific primers were forward, 5'-GGTCTAATCAGCCCATGACC-3' and reverse, 5'-GGGTTTGGCATTAAGAGGAG-3' (length, 257 bp). The porcine-specific primers were forward, 5'-TACGAAAGCAGGCACCTACC-3' and reverse, 5'-TAGA AAGGCCAGGACCAAAC-3' (length, 229 bp).

Real-time polymerase chain reaction

Real-time PCR was performed using the SYBR Green PCR premix (Invitrogen, Waltham, MA, USA). Each 20- μL reaction consisted of 10 μL 2 × SYBR Green premix, 1 μL of forward and reverse primers (10 pmol/μL), and 2 μL standard sample or 5 μL iSCNT embryo template and 5–7 μL autoclaved water. The cycling conditions were 94℃ for 2 min followed by 45–55 cycles of denaturation at 94℃ for 30 sec, annealing at 56℃ for 30 sec, and 72℃ for 30 sec, and were applied using a Rotor Gene 2000 PCR machine (Qiagen, Redwood city, CA, USA). A melting curve was generated by slowly heating the PCR products from 56℃ to 95℃ at 0.2℃/sec. Each experiment was performed in triplicate, including amplification of the nuclear transfer embryo template, mouse beta-actin standard sample template, and water control sample. The PCR products were separated with 2% agarose gel electrophoresis.

Standard sample preparation

To produce the standard samples for real-time PCR, amplified mouse beta-actin sequences were purified from the agarose gel using a DNA extraction kit (AccuPrep®Gel Purification kit, Bioneer, Daejeon, Korea) according to the manufacturer's protocols and inserted into the pGEM®T easy vector (Promega, Madison, WI, USA). The generated plasmid was transformed into *Escherichia coli* DH5α competent cells, which were spread on Luria-Bertani (LB) agar plates with 20 μL of X-gal and 4 μL of isopropyl-beta-D-thiogalactoside (IPTG) containing ampicillin and incubated at 37℃ overnight. The inserted plasmid vectors were identified by blue-white color selection. White-colored colonies were individually transferred to 5 ml of LB medium and incubated at 37℃ overnight with shaking. The beta-actin plasmids were extracted from positive clones using a QIAprep Miniprep kit (Qiagen, Hilden, Germany). Recombinant vectors containing pGEM®T easy vectors (3.0 kb) and the inverted sequence (2.5 kb) were confirmed by 2.0% agarose gel electrophoresis. The concentration of beta-actin plasmids was quantified at wavelengths of 260 nm and 280 nm by a spectrophotometer. The plasmid DNA was isolated, diluted to 10^2 to 10^{10} copies/2 μL, and used for standard samples.

Assay of the mitochondrial DNA copy number

The copy number of mtDNA was estimated based on Avogadro's number per molar concentration. The mtDNA copy number was measured by real-time PCR [15]. The cycle threshold (CT) values within the linear exponential phase were used to measure the mtDNA copy numbers from a standard curve generated by using beta-actin DNA and mtDNA primers. All reactions were checked on the agarose gel after performing real-time PCR. Real-time PCR was carried out for

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each initial template copy number at least four times.

Statistical analysis

All experiments were repeated at least three times. Results are expressed as mean ± SEM or a percentage, and were analyzed with ANOVA using the SAS software program (SAS Institute). A p-value < 0.05 was considered to be statistically significant.

RESULTS

The developmental rates of intra- and interspecies cloned embryos

Porcine oocytes at MII stage were enucleated with a micromanipulator and embryos were reconstructed with individual porcine or MFF cells as donor cells. Porcine-porcine cloned embryos and mouse-porcine cloned embryos were cultured in PZM-3 and PZM-3 + CZB medium, respectively. The reconstructed embryos developed to the blastocyst stage (Fig. 1). The fusion rates were significantly different between porcine-porcine cloned embryos (82.9 ± 2.3%) and mouse-porcine cloned embryos (59.6 ± 0.7%). The cleavage rates were also significantly different between porcine-porcine cloned embryos (62.7±1.6%) and mouse-porcine cloned embryos (47.0 ± 0.9%). However, there was no significant difference in the developmental rate or total numbers of blastocysts between porcine-porcine cloned embryos (11.1 ± 0.9% and 25.0 ± 3.5, respectively) and mouse-porcine cloned embryos (10.1 ± 1.2% and 24.0 ± 6.3, respectively) (Table 1).

Detection of mitochondrialDNA by polymerase chain reaction in interspecies-somatic cell nuclear transfer embryos of different stages

To evaluate the presence of species-specific mitochondria in mouse-porcine cloned embryos, individual 2-cell, 4-cell, 8-cell, morula, and blastocyst stage embryos were analyzed by PCR

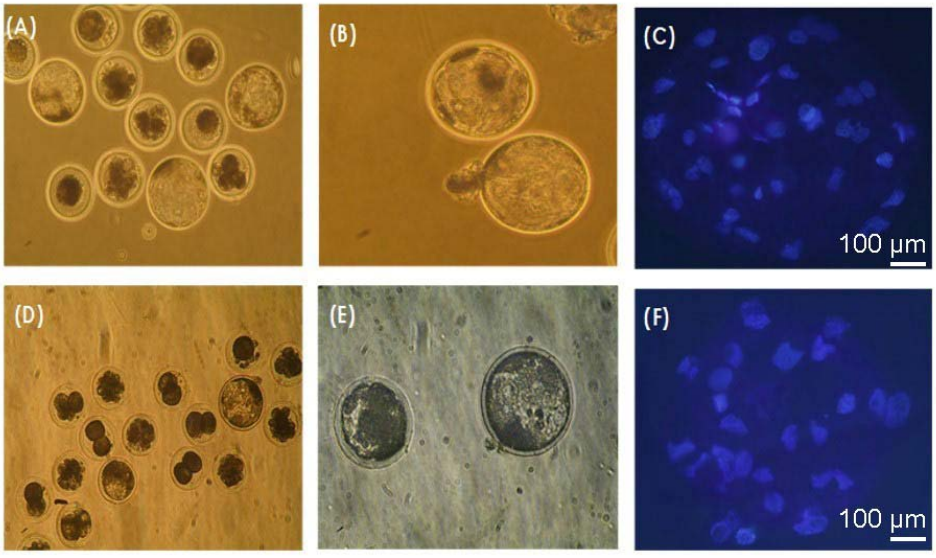

Fig. 1. Morphologies of porcine-porcine and mouse-porcine in vitro cloned embryos observed at the blastocyst stage. (A–C) Porcine-porcine cloned embryos and (C–F) mouse-porcine cloned embryos cultured in PZM-3 medium and PZM-3 + CZB medium, respectively for 7 days. (B, E) Well developed blastocysts. (C, F) Embryos observed under UV light using an epifluorescence microscope following staining with Hoechst 33342. PZM-3, porcine zygote medium-3; CZB, Chatot-Ziomek-Bavister. Scale bar = 100 μm.

Table 1. ***In vitro*** **development rates of intraspecies and interspecies cloned embryos**

Donor cell[1)]	No. of oocytes	Fusion (%)	Cleavage (%)	Morula (%)	Blastocyst (%)	No. of total cells
Porcine FF	99	82.9 ± 2.3[a]	62.7 ± 1.6[a]	12.2 ± 1.8[a]	11.1 ± 0.9[a]	25.0 ± 3.5[a]
Mouse FF	100	59.6 ± 0.7[b]	47.0 ± 0.9[b]	10.0 ± 0.7[a]	10.1 ± 1.2[a]	24.0 ± 6.3[a]

[1)]Porcine or mouse fetal fibroblast cells were used for donor cells.

[a,b]Within a column, results with different superscripts are significantly different ($p < 0.05$). The experiment was repeated four times.

FF, fetal fibroblasts.

amplification with species-specific mtDNA primers. mtDNA derived from the MFF (1,452 bp) were detected from the 2-cell to 8-cell stages, but not at the morula and blastocyst stages. Porcine mtDNA (432 bp) was detected at all stages, from the 2-cell to blastocyst stage (Fig. 2).

Polymerase chain reaction of species-specific mitochondrial DNA and beta-actin DNA

The specificity of primers was confirmed by PCR with each primer set (Fig. 3). Our results revealed that the mouse mtDNA-specific primers specifically detected mouse mtDNA (257 bp), the porcine mtDNA primers specifically detected porcine mitochondria DNA (229 bp). And the mouse beta-actin primers as a loading control detected mouse beta-actin DNA specifically in mouse-porcine cloned embryos.

Real-time polymerase chain reaction analysis of mitochondrial DNA

Using samples diluted to different concentrations of mouse β-actin DNA, we analyzed mtDNA copy numbers by real-time PCR. The results verified that species-specific mtDNA sequences in mouse-porcine cloned embryos could be amplified using species-specific mtDNA primers. mtDNA in samples representing 1-cell to blastocyst stage embryos were analyzed by real-time PCR using the mouse-specific and porcine-specific primer pairs. To check proportional

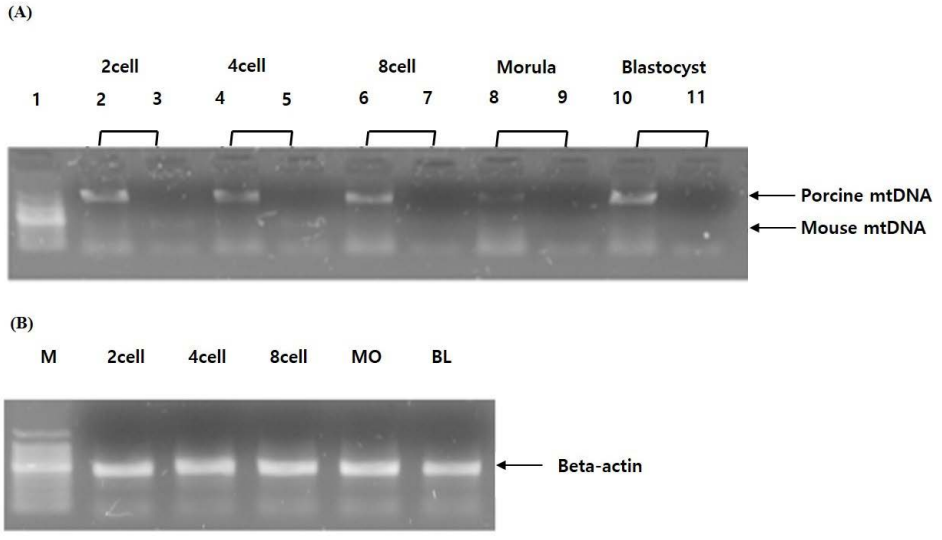

Fig. 2. mtDNA and mouse beta-actin DNA in mouse-porcine cloned embryos detected by PCR using species-specific primer pairs. (A) Lane 1: DNA marker. Lanes 2, 4, 6, 8, 10: The presence of porcine mtDNA (1,452 bp) in a mouse-porcine cloned embryo. Lane 3, 5, 7, 9, 11: The presence of mouse mtDNA (432 bp) in a mouse-porcine cloned embryo. (B) Mouse beta-actin (452 bp) in a mouse-porcine cloned embryo. M, marker; MO, morula; BL, blastocyst; mtDNA, mitochondrial DNA; PCR, polymerase chain reaction.

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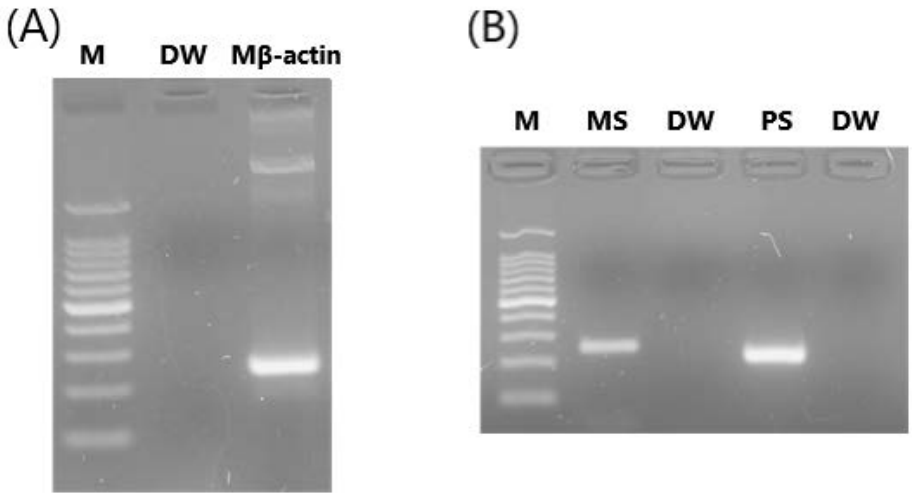

Fig. 3. Template-cross PCR reactions of species-specific primer. (A) Mouse beta-actin DNA product (250 bp) of standard sample. (B) Mouse (257 bp) and porcine (229 bp) mtDNA product of mouse-porcine cloned embryos. PCR, polymerase chain reaction.

amplification between loading DNA equivalent to copy numbers and CT value during real-time PCR, the curves were generated from the samples at the threshold of exponential phase (Fig. 4). The concentration of mtDNA in each sample was obtained from exponential equations of standard samples. The copy numbers of mtDNA were calculated at the 1-cell, 2-cell, 4-cell, 8-cell, morula, and blastocyst stages with reference to the mitochondrial copy numbers deduced from the log linear phase of the sample curves (Fig. 5). The results showed that mtDNA derived from the MFF and porcine oocyte coexisted in early-stage mouse-porcine cloned embryos, but that the MFF-derived mtDNA exhibited dynamic copy number changes in late reconstructed embryos. The copy numbers of MFF-derived mtDNA were significantly lower than those of porcine oocyte-derived mtDNA from the 1-cell to blastocyst stage (Fig. 6). The numbers of porcine oocyte-derived mtDNA remained roughly consistent from the 1-cell ($1.1 \times 10^6 \pm 0.9 \times 10^6$) to blastocyst stages ($1.3 \times 10^6 \pm 0.2 \times 10^6$). However, the copy number of MFF-derived mtDNA decreased remarkably from the 1-cell ($5.7 \times 10^4 \pm 0.3 \times 10^5$) to blastocyst stage ($1.3 \times 10^2 \pm 0.3 \times 10^2$), with relatively few copies

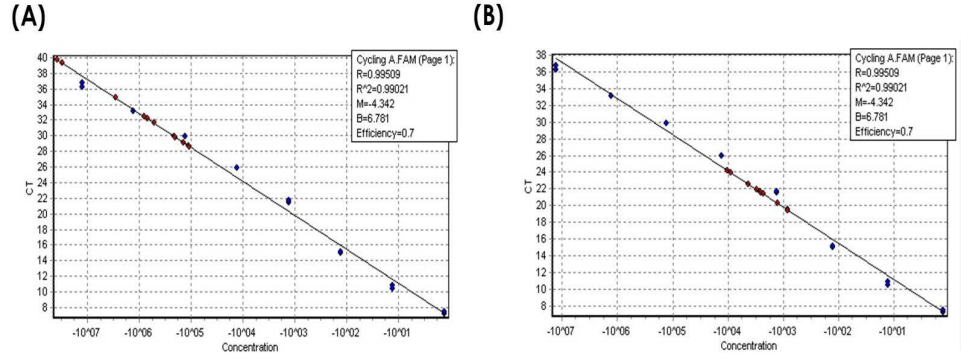

Fig. 4. Species-specific curves generated by real-time PCR to confirm proportional amplification between loading DNA of copy numbers and CT value. (A) Mouse-specific curve generated for the standard curve. (B) Porcine-specific curve generated for the standard curve. PCR, polymerase chain reaction; CT, cycle threshold.

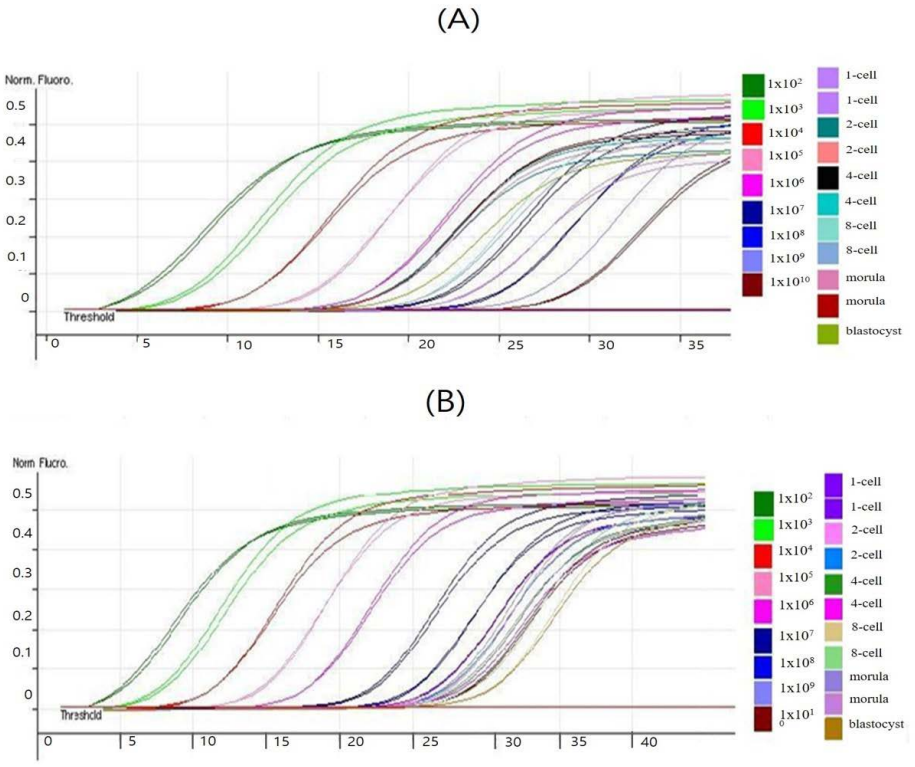

Fig. 5. Real-time PCR curves used for the estimate of mtDNA copy numbers in mouse-porcine were used cloned embryos at different developmental stages. Log linear phases of real-time PCR of porcine mtDNA (A) and mouse mtDNA (B) in mouse-porcine cloned DNA were used for the calculation of mtDNA copy number. The fluorescence levels are shown with the threshold cycles of the standards. PCR, polymerase chain reaction; mtDNA, mitochondrial DNA.

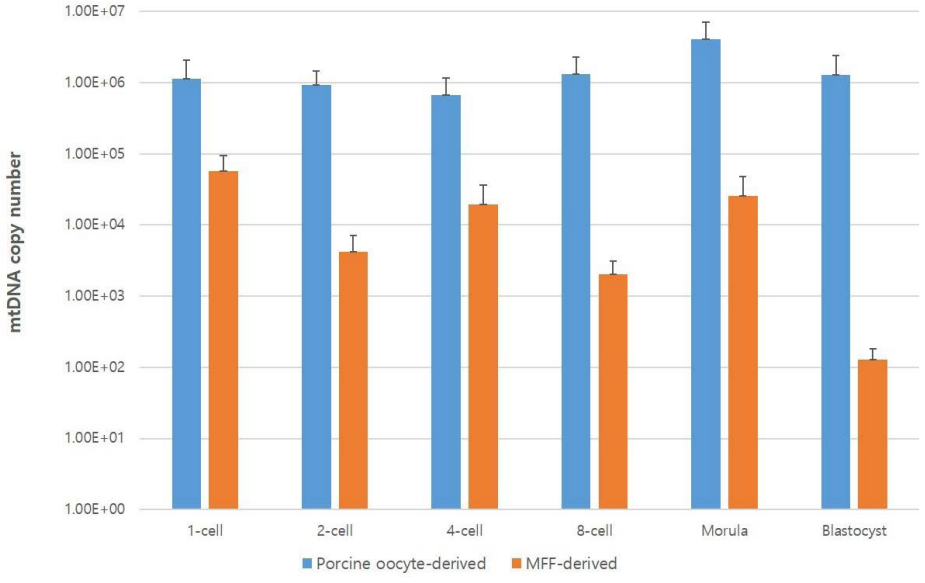

Fig. 6. Porcine and mouse mtDNA copy numbers in mouse-porcine cloned embryos at various developmental stage. mtDNA, mitochondrial DNA; MFF, mouse fetal fibroblasts.

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Table 2. Mitochondrial DNA copy numbers derived from porcine oocyte and mouse donor cells at different developmental stages of mouse-porcine cloned embryos[1)]

Stages	The mtDNA copy numbers		MFF/porcine oocyte mtDNA (%)
	Porcine oocyte mtDNA	MFF mtDNA	
1-cell	$1.1 \times 10^6 \pm 0.9 \times 10^6$	$5.7 \times 10^4 \pm 0.3 \times 10^5$	5.0 ± 0.04
2-cell	$9.3 \times 10^5 \pm 0.5 \times 10^6$	$4.2 \times 10^3 \pm 1.0 \times 10^3$	0.5 ± 0.01
4-cell	$6.6 \times 10^5 \pm 0.2 \times 10^6$	$1.9 \times 10^4 \pm 0.6 \times 10^4$	2.9 ± 0.04
8-cell	$1.3 \times 10^6 \pm 0.3 \times 10^6$	$2.0 \times 10^3 \pm 1.1 \times 10^3$	0.2 ± 0.00
Morula	$4.1 \times 10^6 \pm 0.8 \times 10^6$	$2.5 \times 10^4 \pm 1.1 \times 10^4$	0.6 ± 0.01
Blastocyst	$1.3 \times 10^6 \pm 0.2 \times 10^6$	$1.3 \times 10^2 \pm 0.3 \times 10^2$	0.009 ± 0.01

[1)]Experiments were repeated four times.

seen at the latter stage (Table 2).

DISCUSSION

Mitochondrial heteroplasmy is critically important yet under-studied. iSCNT is a good tool for examining basic questions related to nucleus-cytoplasm mitochondrial interactions between different species. Although porcine oocytes have been applied for interspecies nuclear transfer as recipients [16–18] and the cloned embryos were able to develop to blastocysts, the overall cloning efficiency was very low. A few studies used porcine oocytes as recipients in interspecies nuclear transfer and assessed whether the embryos could develop to blastocysts. For example, porcine oocytes were used as recipients for goat, Minke whale, and rabbit donor cells. However, the reconstructed embryos did not develop to blastocysts [19].

In the present study, we reconstructed mouse-porcine embryos, collected them at different developmental stages, and studied the mitochondrial heteroplasmy from donor and recipient cells for the first time. We reveal that mouse-porcine cloned embryos can develop to the blastocyst stage *in vitro*, as can porcine-porcine cloned embryos. To our surprise, we did not observe any significant difference in the blastocyst rate or total blastocyst number in mouse-porcine versus porcine-porcine cloned embryos. This may reflect our use of a modified *in vitro* culture medium (PZM-3 + CZB) for the interspecies nuclear transfer. Most components in two mediums are similar, but Na/K ratio, potassium and hypotaurine concentrations differ between PZM-3 and CZB [20,21]. PZM-3 was previously shown to provide a superior culture condition for the *in vitro* development of preimplantation porcine intraspecies [22] and interspecies nuclear transfer embryos.

To investigate mitochondrial heteroplasmy during the early development of mouse-porcine cloned embryos, we used real-time PCR, which is considered a precise and feasible method for quantifying the percentage of donor mtDNA in cloned embryos. Recent studies reported on the fate of mtDNA in goat-sheep cloned embryos using species-specific sequences inserted in plasmid vectors to generate standard samples for real-time PCR [23]. We modified this previous method by preparing standard samples curve to check linear amplification between loading DNA of copy numbers and CT values during real-time PCR. Our results showed that the nuclei of MFFs were gradually dedifferentiated in enucleated porcine oocytes, but some mouse mtDNA still remained in mouse-porcine cloned embryos through blastocyst stage. These results appear to be consistent with previous observations when bovine cells and rabbit oocytes were used for iSCNT and bovine mtDNA was detected in interspecies cloned embryos. Somatic cell nuclei can be reprogrammed in xenogenic oocytes, although iSCNT-derived embryos exhibit a low cloning efficiency [11]. The dynamics described herein for the fate of mitochondria from recipient porcine oocytes and donor

MFFs showed that the copy numbers of MFF-derived mtDNA were relatively consistent from the 2-cell to morula stages, and then decreased at the blastocyst stage. Meanwhile, the copy numbers of porcine oocyte-derived mtDNA were stably maintained from the 1-cell to blastocyst stage. This contrasts with previous reports that recipient oocyte-derived mtDNA increases at the blastocyst stage [24–26]. This apparent discrepancy may relate to the relatively distant relationship of the species studied which might destabilize the localization of mitochondria between donor cells and recipients. We speculated that few copy numbers of mouse-derived mtDNA could be processed to the hatched blastocyst or post-implantation stages. Our results suggest that nuclei from mouse donor cells can completely support the biogenesis of mitochondria derived from porcine oocytes, and that mitochondria derived from mouse cells may not be seriously needed in late-stage reconstructed embryos and for implantation. These findings are consistent with the programming of mitochondria described for panda-rabbit cloned embryos [24,27,28].

In conclusion, the results of this study suggest that mouse-porcine interspecies cloned embryos cultured in PZM-3 + CZB medium have the capacity to develop to blastocysts at a rate similar to that seen for porcine-porcine intraspecies cloned embryos. Our successful construction of mouse-porcine interspecies cloned embryos indicates that porcine oocytes can represent candidate recipient cells for interspecies cloned embryos. Furthermore, we show that heteroplasmy exists in mouse-porcine cloned embryos, and that mitochondria derived from porcine oocytes will gradually replace those from the donor cell.

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