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Development of Intergeneric Canine Embryo Using Bovine and Porcine Oocyte as Cytoplasm Recipient

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Abstract

This study was conducted to increase the efficiency of canine embryo production by intergeneric somatic cell nuclear transfer (SCNT) technology. The effect of oocyte recipient for development of intergeneric canine somatic cell cloning embryos were examined. Bovine and porcine cumulus oocyte complexes (COCs) were collected from slaughterhouse ovaries and matured in TCM-199 medium depend on species. Adult dog fibroblasts collected from 3.5 years old Afghanhound dog, and cell between passage 1 and passage 10 used for intergeneric somatic cell cloning using bovine and porcine oocytes as oocyte cytoplasm donor. The result showed that oocytes from bovine and porcine can de-differentiated canine nucleus and no different between bovine and porcine oocyte in fusion and embryo development in vitro. Canine intergeneric cloned embryos developed to morula stages in vitro.

Keyword: canine, intergeneric cloning, SCNT, mSOF

Introduction

Interspecies somatic cell nuclear transfer method was firstly applied for conservation of endangered animals. The highly publicized that an adult sheep had been cloned from the nucleus of a frozen somatic cell (Wilmut *et al*., 1997) speculated that cloning technologies might be applied to increase population sizes of endangered species, or even restore them following extinction (Cohen, 1997; Wen *et al*., 2005). Interspecies nuclear transfer also provides a possible approach to clone animal species whose oocytes were difficult to obtain (Jumnian *et al*., 2002; Wen *et al*., 2003).

Several studies have shown that oocyte cytoplasm from bovine, rabbits and sheep can support early development of embryos produced by nuclear transfer of somatic cells nuclei from various mammalian species (Dominko *et al*., 1999; White *et al*., 1999; Cohen *et al*., 1997; Lanza *et al*., 1999; Chen *et al*., 2002; Wen *et al*., 2003). Recently, the successes of cloning gaur (Lanza *et al*., 2000) and mouflon (Loi *et al*., 2001) have demonstrated that the technique of interspecies cloning can be practically applied to save highly endangered species, such as the giant panda, *Ovis orientalis musimon*, buffalo, *Bos gaurus* (White *et al*., 1999; Lanza *et al*., 2000; Vogel *et al*., 2001; Chen *et al*., 2002; Jumnian *et al*., 2002; Sansisena *et al*., 2005).

The oocyte used for interspecies nuclear transfer should be easy to obtain, able to de-differentiate other species nuclei and support development the hybrid embryos (Wen *et al*., 2003; 2005). There are basically two approaches to the success of the application of nuclear transfer (NT) technology in any given species: 1) to adjust the multiple parameters that affect the overall efficiency of the NT procedure and 2) to explore the use of a more readily available, universal recipient ooplasm for NT in species were the availability of oocytes is restricted.

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The bovine ooplasm has been shown to be able to reprogram somatic cell nuclei from other species. After transfer into enucleated, metaphase II (MII) bovine oocytes, nuclei from sheep, pig, monkey and rat skin cells underwent swelling. It was reported that cell division and formation of an embryonic blastocoele cavity occurred at the time characteristic of embryos from the species of the donor nuclei (Dominko *et al*., 1999).

The first experiments of intergeneric NT in mammals, in which he transferred the rat spindle into nucleated MII mouse oocytes, but the rat-mouse hybrid embryos could not develop beyond the eigh-cell stage. Successful interspecies and intergeneric NT, either giving birth to live offspring or producing nearly termed fetuses, has been achieved by several groups concerned with the preservation of endangered species. When dermal fibroblast of andangered bovid, the gaur (*Bos gaurus*) (Lanza *et al*., 2000), granulose cells from an endangered sheep, the mouflon (*Ovis orientalis musimon*) (Loi *et al*., 2001), fetal fibroblasts from water buffalo (*Bubalus bubalis*) (Lu *et al*., 2005) and ear fibroblast of takin (*Budorcas taxicolor*) were introduced into enucleated oocyte of cow, sheep and cow, 12%, 30%, 33%, 13% and 5% of the reconstructed embryos developed to the blastocyst stage, respectively. Another experiment reported by Wen *et al.* (2005) used rabbit ooplast as recipient for panda and cat somatic nuclei and found 8%, 7% of the reconstructed embryos developed to the blastocyst stage, respectively.

The first interspecies NT offspring was reported for the gaur by Lanza *et al*. (2000) but unfortunately died within the first 48 hours. Using domestic sheep (*Ovis aries*) as recipient cytoplasts two pregnancies were established after interspecies NT using an exotic argali (*Ovis ammon*) for donor karyoplasts, however both of these pregnancies were reported to have been lost by 59 days of gestation (White *et al*., 1999). More recently, domestic sheep (*Ovis aries*) enucleated oocytes used as cytoplasts for adult mouflon cells (*Ovis orientalis musimon*) resulted on one live offspring (Loi *et al*., 2001).

Interspecies NT studies using the bovine cytoplasts as a universal recipient included the injection of somatic cells from pigs (Lee., 2003), saolas (Bui *et al*., 2002), elands (Damiani *et al*., 2003), horses (Sansisena *et al*., 2002) and humans (Cibelli *et al*., 1998), and also Wen *et al*. (2005) using rabbit MII oocyte for panda or cat somatic nuclei, however no confirmed pregnancies have been reported. In addition, equine enucleated oocytes have been used as recipients for somatic cells from a mule fetus producing pregnancies (*Woods et al*., 2003).

The use of alternative NT techniques, such as interspecies NT, is an exciting possibility for species with limited availability of oocytes and recipients as well as for endangered or exotic species. Although some studies have shown promising results initially, the mechanisms of nuclear reprogramming by the oocyte are still unknown and the extent of the universality of the bovine cytoplast remains under investigation

The aim of these studies was to know the oocyte recipient to the best support for canine nuclei in nuclear transfer.

Materials and Methods

Collection of oocytes and in vitro maturation (IVM) of bovine oocyte

Bovine ovaries from Holstein cows were collected from a local slaughterhouse and transported to the laboratory within 2 h in 0.9% (w/v) NaCl solution at 35°C. Cumulus-oocyte complexes (COCs) were retrieved from antral follicles 2–8 mm in diameter by aspiration with an 18-gauge hypodermic needle attached to a 10 ml disposable syringe. The COCs with evenly granulated

cytoplasm and enclosed by more than three layers of compact cumulus cells were selected, washed three times in HEPES-buffered tissue culture medium (TCM)-199 (Life Technologies, Rockville, MD) supplemented with 0.5% (w/v) BSA (fatty acid free, fraction V, Sigma-Aldrich Corp., St. Louis, MO), 2 mM sodium bicarbonate and 10 mM HEPES (Sigma-Aldrich) and 1% (v/v) solution of penicillin and streptomycin (Sigma-Aldrich) (Tabel 1). For maturation, COCs were cultured in four-well dishes (30–40 COCs per well, Nunclon, Roskilde, The Netherlands) for 20–22 h in 500 µl bicarbonate-buffered TCM-199 supplemented with 10% (v/v) fetal bovine serum (FBS) (Life Technologies), 0.005 U/ml bovine FSH (Antrin®, Denka Kanagawa, Japan) and 1 µg/ml estradiol (Sigma-Aldrich) at 39°C in a humidified atmosphere of 5% CO_2 in air.

Preparation of recipient oocytes for somatic cell nuclear transfer (SCNT)

At 20–22 h of maturation culture, expanded cumulus cells of COCs were removed by repeated pipetting in 0.1% hyaluronidase in hCR2aa (HEPES-Charles Rosenkrans 2 aminoacid) (Table 2), and oocytes with first polar bodies were selected. Oocytes were then enucleated in hCR2aa supplemented with 10% FBS and 7 µg/ml cytochalasin B (Sigma-Aldrich) under an inverted microscope equipped with a micromanipulation system (Narishige, Tokyo, Japan). Each oocyte was held with a holding micropipette (110 µm outer diameter and 24 µm inner diameter) and the zona pellucida was partially dissected with a fine glass needle to create a slit near the first polar body. The first polar body and adjacent cytoplasm presumably containing the MII chromosomes were extruded by squeezing with the needle. Oocytes were then stained with 5 µg/ml bisbenzimide (Hoechst 33342, Sigma-Aldrich) for 15 min and observed under an inverted microscope equipped with epifluorescence at 200X magnification. Oocytes still containing DNA material were excluded. The enucleated oocytes were placed in TCM-199 supplemented with 10% FBS and used for SCNT.

Table 1. Composition of tissue culture medium 199 (TCM199) medium for IVM

Substrates	Bovine	Porcine	
	Washing	Culture	Culture
Tissue culture medium	Gibco 31100~027 (in 1 liter distilled water)	Gibco 11150~059	Gibco 11150~059
HEPES[1]	10 mM	~	
Na~pyruvate	~	1 mM	0.91 mM
Glucose	~	~	3.05 mM
$NaHCO_3$	2 mM	26.2 mM	26.2 mM
L-cysteine	~	~	0.57 mM
Calsium (lactate)			2.92 mM
PFF	~	~	10% (v/v)
Fetal Bovine Serum	~	10% (v/v)	~
Bovine Serum Albumin	0.5% (w/v)	~	~
Pen-strept	1 % (v/v)	1% (v/v)	1% (V/V)

Injection, fusion and activation oocytes

Trypsinized, a single cells with a smooth surface were selected under an inverted microscope equipped with a fluorescent isothiocyanate (FITC) filter (wavelength: excitation 489 nm and emission 508 nm) and were transferred into the perivitelline space of enucleated oocytes through the same slit that was made during enucleation. The couplets were subsequently placed in a fusion medium comprising 0.26 M mannitol, 0.1 mM $MgSO_4$, 0.5 mM HEPES and 0.05% (w/v) BSA, and transferred into a cell fusion chamber with a stainless steel electrode (3.2 mm gap; BTX Inc., San Diego, CA) after equilibration for 3 min. Fusion was induced

by two DC pulses of 1.75 kV/cm for 15 µs using an Electro-cell Manipulator 2001 (BTX Inc). The fusion of the donor cell and the ooplast was determined 1 h after electric stimulation under a stereomicroscope (Nikon Corp., Tokyo, Japan). Only fused embryos were selected and subjected to chemical activation. For transgenic embryos, fusion was confirmed by observing GFP expression in recipient cytoplasm under DIC microscopy equipped with FITC filter (Nikon Corp.). Chemical activation was induced by incubating embryos in HEPES-buffered TCM-199 (hTCM) containing 5 µM ionomycin (Sigma-Aldrich) for 4 min at 39 °C. Reconstructed embryos were then washed thoroughly in ionomycin-free hTCM and further incubated for 4 h in modified synthetic oviduct fluid (mSOF) (Table 3) medium supplemented with 1.9 mM 6-dimethylaminopurine (Sigma-Aldrich).

Table 2. Composition of Hepes-buffered Charles Rosenkrans 2 with amino acids medium for embryo handling

Substrate	Concentrations
NaCl	114 mM
KCl	3.1 mM
NaHCO3	2 mM
NaH2PO4	0.35 mM
Sod. Lactate	15 mM
CaCl2.2H2O	2 mM
MgCl2.6H2O	0.5 mM
NEAA1	1% (v/v)
ITS2	1% (v/v)
Glycine	0.37 mM
Citrate	0.33 mM
HEPES3	10.5 mM
Phenol~red	10 µg/L
FBS4	10% (v/v)
P/S5	1% (v/v)

[1]Non~essential amino acids.
[2]Insulin (10 µg/ml), transferrin (5.5 µg/ml), selenium (5 ng/ml).
[3]N~[2~Hydroxyethyl]piperazine~N~[2~ethanesulfonic acid],
[4]Fetal bovine serum,
[5] Penicillin 10,000IU, Streptomycine 10 mg/L.

In vitro culture (IVC)

A group of 5–7 reconstructed embryos were cultured in a 25 µl microdrops of SOF media supplemented with 0.8% BSA (Choi *et al.*, 2002) under embryo-tested mineral oil at 39°C with 5% CO_2, 5% O_2 and 90% N_2 in a humidified atmosphere. The embryos were evaluated at 48 and 192 h after activation for embryo development. The GFP expression in embryo was determined under a FITC filter.

Collection of oocytes and in vitro maturation (IVM) of porcine oocyte

Ovaries were obtained from a local abattoir and transported to the laboratory in physiological saline at 30 to 35°C. Antral follicles 3 to 6 mm in diameter were aspirated using an 18-gauge needle attached to a 5-ml disposable syringe. COCs with compact cumulus cells were collected from the aspirate and washed several times in TCM-199. The COCs were then placed in IVM medium (Earle's salts- and L-glutamine-containing TCM-199 supplemented with 26.2 mM $NaHCO_3$, 3.05 mM glucose, 0.91 mM sodium pyruvate, 0.57 mM L-cysteine, 75 mg/l kanamycin, 10 ng/ml epidermal growth factor (Sigma-Aldrich), equine chorionic gonadotropin (eCG, Intervet, Boxmeer, Netherland), 10 IU/ml human chorionic gonadotropin (hCG, Intervet Boxmeer Netherland), and 10% (v/v) porcine follicular fluid (pFF) (Table 1). The pFF was aspirated from superficial antral follicles 8 to 10 mm in diameter from prepubertal gilts. After centrifugation at 1,600 x g for 30 min, supernatant was collected and filtered sequentially through 1.2 µm and 0.45 µm syringe filters (Gelman

Sciences, Ann Arbor, MI, USA). Prepared pFF was then stored at -20°C until use.

A group of 50 COCs was cultured in 500 µl IVM medium at 39°C in a humidified atmosphere of 5% CO_2 and 95% air. After culturing for 22 h, COCs were transferred to eCG- and hCG-free IVM medium and cultured further for 20-22 h. At the end of the culture, oocytes were freed from cumulus cells by repeated pipetting in IVM medium containing 0.1 % hyaluronidase. Oocytes with a first polar body, intact zona pellucida, evenly granulated cytoplasm, expanded cumulus cells and distinct ooplasmic membrane were provided for SCNT of this study.

Preparation of recipient oocytes for somatic cell nuclear transfer

After 42-44 h of maturation, the oocytes were freed from cumulus cell by pipetting in HEPES-buffered NCSU-23 medium supplemented with 0.1% hyaluronidase. Oocytes were cultured in NCSU-23 containing 5 µg/ml bisbenzimide (Hoechst 33342; Sigma-aldrich Co.) and 7.5 µg/ml cytochalasin B for 30 min. Oocytes were placed in a 4 µl drop of HEPES-buffered NCSU-23 medium on working dishes. Each recipient oocyte was held with a holding micropipette (110 µm in outer and 24 µm in inner diameter) and zona pellucida was partially dissected with a fine glass needle to create a slit near the polar body. Then, the first polar body and adjacent cytoplasm containing metaphase plate were removed by squeezing. Enucleated oocyte were visually verified by ultraviolet fluorescence, keeping exposure to a minimum. The enucleated oocytes were then placed in NCSU23-D and used for SCNT.

Injection, electrofusion and activation

Injection was performed in 4 µl drop of HEPES-buffered NCSU23-W medium and covered with light mineral oil. A single cell with smooth membrane was transferred into the perivitelline space of an enucleated oocyte. Before nuclear transfer, transfected donor cell were identified emission of green fluorescence under an epifluorescent microscope using a standard FITC filter set. Round-shaped and green-colored small cell were individually injected into perivitelline space of enucleated oocytes.

Reconstructed oocytes were fused and activated simultaneously. The reconstructed oocytes were equilibrated for 10 sec in fusion medium (0.26 M mannitol, 0.1 mM $MgCl_2$, 0.1 mM $CaCl_2$, 0.5 mM HEPES and 0.05% BSA) and transfer to a fusion chamber with two electrodes (3.2 mm gap) overlaid with the mannitol medium. Reconstructed oocytes (5-7 oocytes) were aligned with a fine mouth-controlled Pasteur pipette in parallel with a fusion chamber.

Table 3. Composition of modified synthetic oviduct fluid (mSOF)

Ingredient	Concentrations
NaCl	100 mM
KCl	7.2 mM
NaHCO3	25.1 mM
KH2PO4	1.2 mM
Sod. Lactate	3.3 mM
CaCl2 . 12H2O	2 mM
MgCl2 .16H2O	0.5mM
EAA1	2% (v/v)
NEAA2	1% (v/v)
Sod. Pyruvate	0.3 mM
Glucose	1.5 mM
BSA3	8 mg/ml
L-Glutamine	1 mM
ITS4	0.5 % (v/v)
P/S5	1 % (v/v)

[1]Essential amino acids
[2]Non-essential amino acid
[3]Bovine serum albumin (fatty acid free, fraction V)
[4]Insulin (10 µg/ml), transferrin (5.5 µg/ml) and selenium (5 ng/ml)
[5]Penicillin 10,000 IU, Streptomycin 10 mg/L

Fusion was induced with a single DC pulse of 1.86 kV/cm for 30 µs, by on a Electro-cell Manipulator 2001. All treated oocytes were washed three times with NCSU23-W supplemented with 4 mg/ml BSA, placed in 25 µl microdrops (10-15 oocytes per drop) of NCSU23-D under mineral oil and cultured at 39°C, 5% CO_2, 5% O_2 and 90% N_2. Fused oocytes were determined one hour after the electrical pulse under microscope.

Statistical analysis

Data from all experiments were analyzed using the statistical Analysis System (SAS) program. Data were subjected to analysis of variance (ANOVA) and protected least significant different (LSD) test to determine differences among experimental groups. When a significant model effect was found in each experimental parameter, data were compared by the least squares method. Statistical significance was determined where P value was less than 0.05.

Subsequent culture

The reconstructed embryos were cultured in 25 µl drops of NCSU23-D overlaid with mineral oil at 39°C in humidified 5% CO_2, 5% O_2 and 90% N_2 atmosphere. Ten to fifteen embryos were cultured together. Cleavage rate was recorded after 48 h post fusion. On 7 days post fusion, the development of reconstructed embryos was recorded, and GFP expression rate in embryo was examined under FITC filter.

Preparation of donor canine cells

Canine fibroblast cells were isolated from ear skin. The external surface of canine ear skin was shaved and cleaned aseptically. A piece of ear skin tissue about 100 mm^3 wide and 2 mm thick was biobsied and immediately immersed in D-PBS (Life technologies). After washing, the tissues were minced by a surgical blade on a 100 mm culture dish, followed by dissociation by 0.25% (w/v) trypsin containing 1 mM EDTA for 1 to 2 h at 38°C. Trypsinized cells were washed once by centrifugation (300Xg, 2 min) and subsequently seeded into 100 mm culture dishes and cultured for 6-8 day in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% (v/v) FBS, 1 mM sodium pyruvate, 1 % (v/v) non-essential amino acid and 10 µl/ml penicillin/streptomycin solution in a humidified atmosphere of 95% air, 5% CO_2 at 38°C before removal of unattached clumps of cells or explants. The attached cells were passage by trypsinization when confluent.

Experimental studies

Effect of oocyte recipient on development rate of intergeneric canine embryo

In vitro developmental rates of NT embryos produced by transferring canine cell into enucleated MII bovine and porcine oocytes was examined. Forty eight h after cultured, embryos were examined for cleavage rate and 172 h for final development. Embryos derived from nuclear transfer of canine donor cells to bovine or porcine oocyte were randomly distributed and cultured for 192 h in mSOF for bovine and 144 h in NCSU23-D for porcine, development of embryo control by the 48 h after cultured in media.

Results

Effect of cytoplasmic recipient on development of canine interspecies embryo

A total 274 bovine oocyte and 303 porcine oocyte are used in this experiment. The fusion rate of reconstructed almost similar between couplets canine cell and bovine or porcine oocyte and have no statistically different between them. Porcine oocyte have 68% fusion rate and bovine give 66% fusion with canine cell. The cleaved rate from bovine oocyte have 58% and from porcine oocyte 72% and embryo

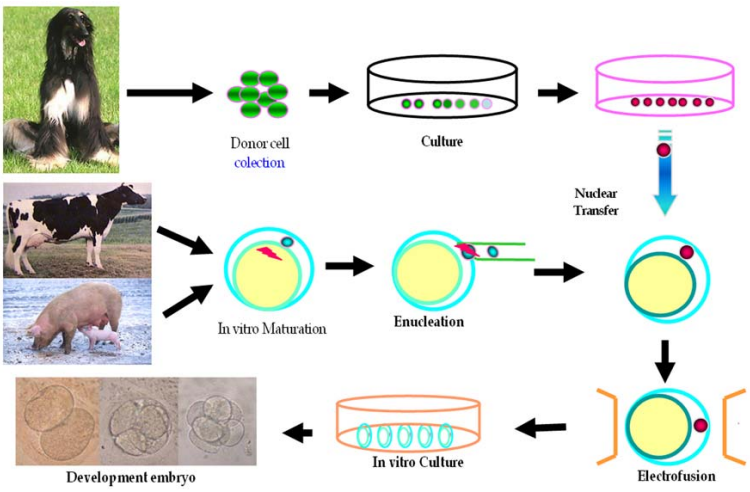

Figure 1. Scheme of somatic nuclear transfer technology

development between both of oocyte recipient almost same at 8-16 cell stage 54% for porcine and 51% for bovine and the same morula stage rate within porcine and bovine 1%.

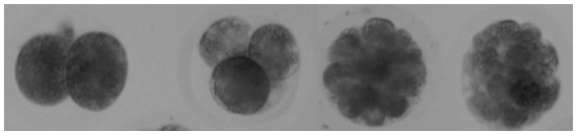

Figure 2. Development of intergeneric cloned embryo using bovine oocyte recipient

Discussion

In this study wanted to learn about intergeneric cloned dog using bovine and porcine oocyte as cytoplasm donor. We used both kind of oocyte because limitation of oocytes from canine species and ones of the reason we used interspecies nuclear transfer was limitation of oocyte source and several studies using cytoplasm. This reason also supported by delivery of mouflon (Loi *et al.*, 2001) and gaur (Lanza *et al.*, 2000) which using interspecies too and also successful of bovine oocyte to support development of embryos from another species (Dominko *et al.*, 1999; Arat *et al.*, 2001; Hwang *et al.*, 2001; Bui *et al.*, 2002).

In this present study we found that canine intergeneric nuclear transfer using pigs and cows oocytes as recipient oocytes have no different result in fusion condition and this result almost same with result used in porcine cloning around 68-75% (Hao *et al.*, 2003; Hyun *et al.*, 2003). A majority of the fused embryos in this study initiated cleavage and developed to the 16 cell stage of development. However, development rate after 16-cell is very low and no blastocyst stage achievement in this experiment. It would be expected that intra- or inter-generic reconstituted embryos would undergo cleavage because maternally inherited gene products that are stored in the oocyte regulate the earliest stages of embryogenesis (Memili and First, 2000). The earliest stages of embryogenesis in normal embryos are regulated by maternally inherited gene products stored within the oocyte cytoplasm. Progression of development becomes dependent on embryonic gene activation at a species-specific developmental stage (Telford *et al.*, 1990). This occurs at the 2- to

4-cell stage in rats (Ansai *et al*., 1994), late 4-cell stage in cattle (Jones *et al*., 2001) and pig (Prather, 1993), and 8-cell stage in sheep (Crosby *et al*., 1988) and at 4- to 8-cell stage in human embryos (Braude *et al*., 1988).

In this study we get development block after 16 sel stage with show the lower development to the morula and no blastocyst stages. There are many factors that can interfere in embryo development causing embryo cleavage to cease, and two main mechanisms are believed to cause the embryo developmental block are inability to overcome the chromatin repression and (i) active transcription of important developmental genes and/or (ii) to react to injuries caused by environment. Most of the embryo block occurs during the fourth or between the fourth and fifth cell cycle transition (Memili and First, 2000). This developmental block is observed in many species, with the peculiarity that it shows up at different stages in different species. This species-specific block moment is concurrent with maternal-embryo transition, the development stage when embryos conclude the mayor genome activation and must rely on the mRNAs transcribed from its own genome to continue development (Meirelles *et al*., 2004).

Table 4. Effect of bovine and porcine oocyte as cytoplasm donor in canine intergeneric nuclear transfer

Oocyte recipient	No. of oocytes	Fusion (%)	Cleavage (%)	No. (%) of intergeneric embryo		
				2/4 cell	8/16 cell	morula
Bovine	274	181 (66)	106 (58)	49 (45)	55 (51)	2 (1)
Porcine	303	209 (68)	152 (72)	66 (43)	83 (54)	3 (1)

In vitro developmental block has been observed using interspecies methods. It has been suggested that mitochondrial mismatch between donor cell and recipient oocyte could be a cause for the embryonic developmental arrest. Continuation of development could be a consequence of efficient reprogramming of the donor nucleus, regardless of the species, followed by now embryonic gene expression. Alternatively, it could indicate incompatibilities between the new components synthesized by the donor nucleus and the components left over from the recipient cytoplasm. In this case, the introduced fibroblast nucleus would be directing cell proliferation, and the resulting multicellular structure would have few or no embryonic characteristics. Observations of embryo-like structures containing a high number of cells that would not undergo compaction support the latter scenario (Lee *et al*., 2003). Expression of specific genes has been shown to be required for compaction and cavitation in developing embryos. Absence of compaction in some embryos in this study would suggest lack of or impairment in transcription of genes required for these differentiation events to occur (De sousa *et al*., 1999; Watson *et al*., 2000).

Several studies have reported inheritance of mitochondrial and mitochondrial DNA (mtDNA) after nuclear transfer procedure. Sheep (Evans *et al*., 1999) and calves (Takeda *et al*., 1999) produce by somatic nuclear transfer have been reported to inherit their mitochondria entirely from the oocyte and not from the donor cell. However, indication of the presence of heteroplasmy after NT and the potential for mitochondrial mismatch could possibly impair embryo development (Nagao *et al*., 1998). Steinborn *et al*. (2000) reported that mtDNA heteroplasmy in cloned cattle generated from fetal and adult donor cell.

mtDNA also reported to be present in somatic cell NT-derived embryos by allele-specific PCR, direct squencing and DNA chromatography of the D-loop region (Do *et al*., 2002). Interestingly, Nagao *et al*. (1998) reported, decreasing physical performance in congenic mice with mismatch between the nuclear and the mitochondrial genome. When foreign mitochondrial are introduced in conducting interspecies NT procedures, it is possible that mitochondrial heteroplasmy, as well as chromosome number, may be key factors that affect embryonic development and in utero survivability of interspecies NT embryos (Sansinena *et al*., 2005).

Conclusion

For intergeneric nuclear transfer, oocytes from bovine and porcine can de-differentiated canine nucleus and no different between bovine and porcine oocyte in fusion or embryo development in vitro. Canine intergeneric cloned embryos developed to morula stages in vitro.

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