Generation of large pig and bovine blastocysts by culturing in human induced pluripotent stem cell medium

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Summary

We investigated the effect of human induced pluripotent stem cell (hiPS) medium on porcine somatic cell nuclear transfer and bovine *in vitro* fertilized early blastocysts, in comparison with North Carolina State University (NCSU)-37 medium and *in vitro* culture (IVC)-II medium. After 2 days of culture, the diameter of the portion of the blastocyst that was extruded from the zona pellucid dramatically differed between porcine blastocysts cultured in hiPS medium and those cultured in NCSU-37 medium (221.47 ± 38.94 μm versus 481.87 ± 40.61 μm, $P < 0.01$). Moreover, the diameter of the portion of the blastocyst significantly differed between bovine blastocysts cultured in hiPS medium and those cultured in IVC-II medium (150.30 ± 29.49 μm versus 195.58 ± 41.59 μm, $P < 0.01$). Furthermore, the total number of cells per porcine and bovine blastocyst was more than two-fold higher in blastocysts cultured in hiPS medium than in those cultured in NCSU-37 medium (44.33 ± 5.28 and 143.33 ± 16.05, $P < 0.01$) or IVC-II medium (172.12 ± 45.08 and 604.83 ± 242.64, $P < 0.01$), respectively. These results indicate that hiPS medium markedly improves the quality of porcine and bovine blastocysts.

Keywords: Blastocyst cell number, Blastocyst morphological quality, Bovine blastocysts, hiPS medium, Porcine blastocysts

Introduction

The morphology and quality of mammalian blastocysts is related to the *in vitro* culture (IVC) conditions. Therefore, the number of cells in blastocysts produced by *in vitro* fertilization (IVF) or somatic cell nuclear transfer (SCNT) may be directly affected by the IVC medium in which they are cultured. Various types of IVC media have been developed in which to culture porcine embryos, such as modified Whitten's medium (Beckmann & Day, 1993), North Carolina State University 23 and 37 (NCSU-23 and 37) media (Petters & Wells, 1993), Iowa State University medium (Youngs *et al.*, 1993), Beltsville embryo culture medium 3 (Im *et al.*, 2004), and porcine zygote medium 3 (Mao *et al.*, 2012). In these media, the mean number of cells per porcine blastocyst ranges from 14 to 92 (Beckmann & Day, 1993; Petters & Wells, 1993; Youngs *et al.*, 1993; Im *et al.*, 2004; Mao *et al.*, 2012; Yamanaka *et al.*, 2009;). Moreover, various media have been reported in which to culture bovine embryos, including Charles Rosenkrans (CR) medium supplemented with amino acids (CR1-aa) (Fakruzzaman *et al.*, 2013; Ha *et al.*, 2014a,b), CR2 (Wang *et al.*, 1999), synthetic oviduct fluid (SOF) (Sagirkaya *et al.*, 2007), modified SOF (Lim *et al.*, 2003), tissue culture medium 199 (Balasubramanian & Rho, 2007), potassium simplex optimized medium (KSOM) (Arias *et al.*, 2013), G1.2/G2.2 sequential media (Lane *et al.*, 2003), bovine embryo culture medium (Lim *et al.*, 1994) and Menezo's B2 medium (Farin *et al.*, 1997). In these media, the mean number of cells per bovine blastocyst ranges from 82 to 207 (Lim *et al.*, 1994,

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2003; Farin *et al.*, 1997; Wang *et al.*, 1999; Lane *et al.*, 2003; Balasubramanian & Rho, 2007; Sagirkaya *et al.*, 2007; Arias *et al.*, 2013; Fakruzzaman *et al.*, 2013; Ha *et al.*, 2014a,b). In most studies on porcine and bovine embryos, culture in IVC media increased the percentage of embryos that developed to the blastocyst stage, but did not significantly increase the number of cells per blastocyst. To produce high-quality blastocysts that contain a higher number of cells, IVC media need to be improved.

Blastocysts of an improved quality and containing more cells are important for several reasons. First, blastocysts that contain a high number of cells can assist single blastocyst analyses. Several blastocysts are required to obtain sufficient DNA for analyses, whereas only single blastocysts would be required if they contained an increased number of cells. Second, blastocyst transfer is a commonly used method in bovine transplantation trials. Hence, blastocysts with an improved morphology and a higher number of cells may improve the pregnancy rate following such transplantation. Embryonic stem (ES) cells are derived from the inner cell mass (ICM) of blastocysts and can be established from mice and humans (Evans & Kaufman, 1981; Martin 1981; Thomson *et al.*, 1995; Thomson *et al.*, 1998). Attempts to establish ES cell lines from domestic animals have been unsuccessful, due in part to the inability to develop suitable culture conditions. Therefore, development of new IVC systems to produce blastocysts with a larger ICM may increase the rate with which ES cell lines are successfully established.

In this study, we cultured porcine and bovine early blastocysts in human induced pluripotent stem cell (hiPS) medium for 2 days and then investigated the morphological quality of the blastocysts, as well as the total number of cells per blastocyst. Culture in hiPS medium increased the total number of cells per blastocyst by two-fold.

Materials and methods

All chemicals and reagents used in this study were purchased from Sigma Chemical Company (St. Louis, MO, USA). The various components of the hiPS medium were purchased from Invitrogen Inc. Cryopreserved semen was procured from Animal Husbandry Development, Inc., Yanbian City, Jilin Province, China.

Preparation and procurement of media

NCSU-37 and CR1-aa media were used to culture porcine and bovine embryos, respectively. hiPS medium was used to culture both porcine and bovine

blastocysts. Detailed information about these media is provided in Table 1.

Production of porcine SCNT embryos

Ovaries were collected at a local abattoir and transported to the laboratory at 30−35°C. Cumulus–oocyte complexes (COCs) were aspirated from antral follicles (3−6 mm) using an 18-gauge needle and a regulated vacuum pump (KMAR-5100; Cook, Eight Mile Plains, Australia) and collected into a 50 ml centrifuge tube. COCs were pooled and washed three times with HEPES-buffered Tyrode medium (TALP-HEPES). Only COCs with a uniform ooplasm were transferred to 500 μl of maturation medium (NCSU-37 medium). The detailed information was previously published (Kang *et al.*, 2013).

To prepare donor cells, a porcine fetus was obtained from a hybrid pig on day 30 of pregnancy. The tissue was cut into small pieces then washed three times and cultured at 38°C in an atmosphere of 5% (v/v) CO_2 in air in Dulbecco's modified Eagle medium (DMEM). Detailed procedures have been published previously (Jin *et al.*, 2014).

The nuclear transfer protocol was adapted from a previous study (Yin *et al.*, 2002). Mature eggs with the first polar body were enucleated. A single donor cell was inserted into the perivitelline space of each egg and electrically fused in 0.28 mol/l mannitol supplemented with 0.1 mmol/l $MgSO_4$ and 0.01% polyvinyl alcohol (v:v). Fused eggs were cultured for 1 h in medium containing 0.4 μg/ml demecolcine before electroactivation and then cultured for 4 h in medium supplemented with 2 mmol/l 6-dimethylaminopurine. The reconstructed oocytes were activated by two direct pulses of 100 V/mm for 20 μs in 0.28 mol/l manitol supplemented with 0.1 mmol/l $MgSO_4$ and 0.05 mmol/l $CaCl_2$. Activated eggs were cultured in NCSU-37 medium for 5 days at 38.5°C under a humidified atmosphere of 5% CO_2 in air. Day 5 porcine SCNT blastocysts were cultured in 500 μl of hiPS medium or NCSU-37 medium for an additional 2 days. Moreover, the number of porcine blastocysts cultured in NCSU-37 medium or hiPS medium was 16 or 16, respectively.

In vitro production of bovine embryos and embryo transfer(ET)

Ovaries were collected from a local abattoir. Embryos were produced from oocytes *in vitro* as described previously (Jeong *et al.*, 2009). In brief, COCs were recovered from follicles 2–8 mm in diameter using an 18-guage needle connected to a 10 ml syringe, as previously described (Jo *et al.*, 2014), washed with *in vitro* maturation (IVM) medium (Ha *et al.*, 2014b), and

Table 1 Compositions of media used to culture porcine and bovine embryos

Component	NCSU-37	CR1-aa		hiPS
		IVC-I	IVC-II	
NaCl (mM)	108.73	114.70	114.70	–
NaHCO$_3$ (mM)	25.07	26.20	26.20	–
KCl (mM)	4.78	3.10	3.10	–
KH$_2$PO$_4$ (mM)	1.19	–	–	–
MgSO$_4$·7H$_2$O (mM)	1.19	–	–	–
CaCl$_2$·2H$_2$O (mM)	1.70	–	–	–
Glucose (mM)	5.55	–	–	–
L-Glutamine (mM)	1.00	–	–	–
Sorbitol (mM)	12.00	–	–	–
Cysteine (mM)	0.6	–	–	–
Insulin (from bovine pancreas, mg/l)	5.00	–	–	–
P/S	1%	1%	1%	–
Hemicalcium lactate (mM)	–	5	5	–
MEM NEAA (mM)	–	10	10	–
BME EAA (mM)	–	5	5	–
Glutathion (mg/ml)	–	0.31	0.31	–
BSA (mg/ml)	4	3	–	–
FBS	–	–	10%	–
KnockOut^{TM} DMEM/F-12[a]	–	–	–	1×
KnockOut^{TM} SR[a]	–	–	–	20%
MEM NEAA[a] (μM)	–	–	–	100
GlutaMAX^{TM}–I supplement	–	–	–	1×
β-Mercaptoethanol[a] (μM)	–	–	–	100
P/S[a]	–	–	–	1×
bFGF[a,b] (recombinant human, ng/ml)	–	–	–	4

bFGF, basic fibroblast growth factor; BME EAA, Basal Medium Eagle containing essential amino acids; BSA, bovine serum albumin; FBS, fetal bovine serum; MEM NEAA, Minimum Essential Medium containing non-essential amino acids; P/S, penicillin–streptomycin; SR, serum replacement.
[a]All the components of human induced pluripotent stem cell (hiPS) medium were obtained from Invitrogen Inc.
[b]hiPS medium was prepared without bFGF and then supplemented with fresh bFGF immediately before use.

transferred to a well of a 4-well dish containing 700 μl of IVM medium for 23–24 h.

COCs were fertilized with sperm obtained from frozen semen, which was thawed by warming at 38°C for 1 min and washed in Dulbecco's phosphate-buffered saline by centrifugation at 750 *g* for 5 min at room temperature. The sperm pellet was dissolved with 500 μl heparin (20 μg/ml) in fertilization medium (Ha *et al.*, 2014a) and incubated at 38.5°C in a humidified atmosphere of 5% CO$_2$ in air for 15 min. Sperm, which were presumed to be capacitated, were diluted in IVF medium (concentration, 1×10^6 sperm/ml). Matured oocytes were transferred to IVF medium containing sperm for 18–20 h.

After IVF (day 0), COCs were removed by repeated pipetting and denuded zygotes were placed into a well of a 4-well plate containing 700 μl of CR1-aa medium (Rosenkranz *et al.*, 1993), supplemented with 44 μg/ml sodium pyruvate, 14.6 μg/ml glutamine,

10 μl/ml penicillin–streptomycin, 3 mg/ml bovine serum albumin (BSA), and 310 μg/ml glutathione for 3 days (IVC-I). The presumed zygotes were cultured until day 7 of embryonic development (day 1 was the day on which IVC was performed) in the same medium, except that BSA was replaced with 10% (v:v) FBS from day 3 (IVC-II) at 38.5°C under a humidified atmosphere of 5% CO$_2$ in air. Day 7 bovine blastocysts were transferred to 500 μl of hiPS medium or IVC-II medium for another 2 days. Moreover, the number of bovine blastocysts cultured in IVC-II medium or hiPS medium was 16 or 19, respectively.

For ET, representative day 8 blastocysts were removed from IVC-I (4 days), IVC-II (3 days) and hiPS medium (1 day). Two individual embryos to be transferred were loaded into a 1/4 insemination straw, fitted onto an ET gun, and transported to the farm where ET was performed with 1 h. Single embryos were transferred non-surgically to the uterine horn
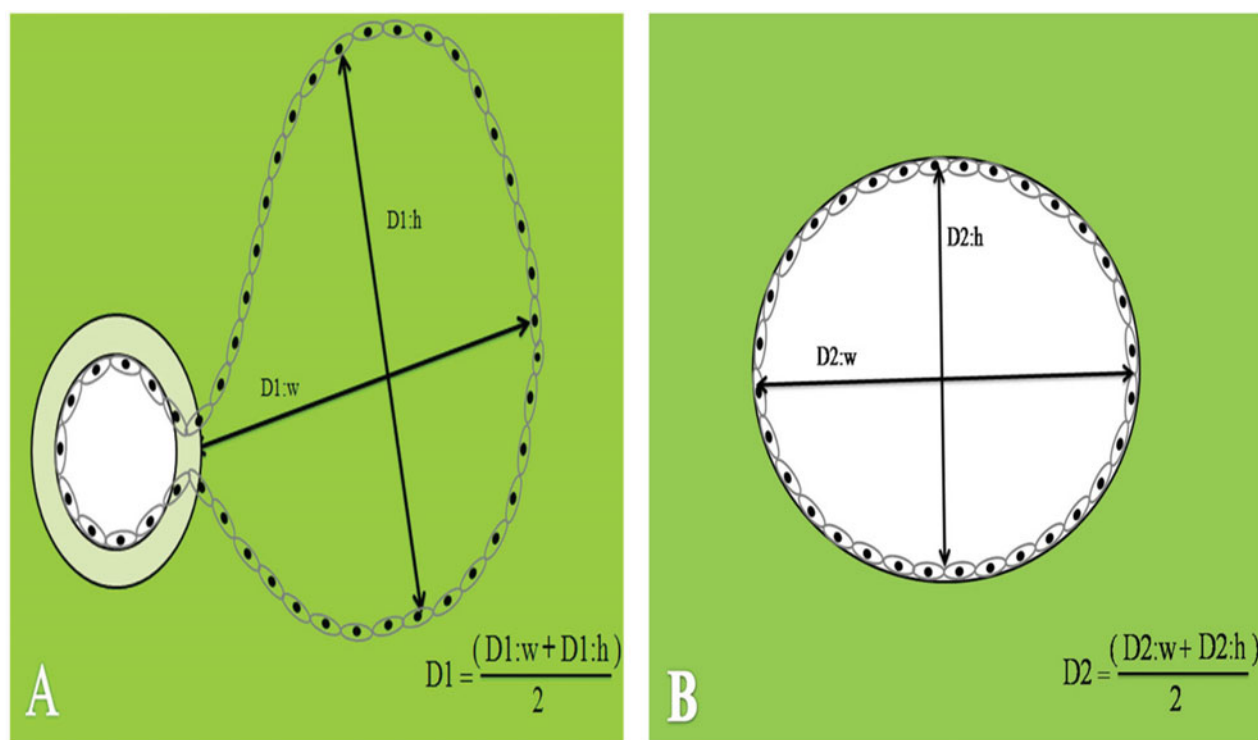
Figure 1 Methods used to evaluate the morphology of porcine somatic cell nuclear transfer (SCNT) blastocysts and bovine *in vitro* fertilization (IVF) blastocysts. (*A*) D1: diameter of the portion of the porcine blastocyst extruded from the zona pellucida. (*B*) D2: diameter of the portion of the bovine blastocyst. Method used to calculate D1 of a porcine SCNT blastocyst below. Method used to calculate D2 of a bovine IVF blastocyst below. The mean value of D1 was calculated using a previously published equation (Laowtammathron *et al.*, 2005).

ipsilateral to the corpus luteum. Day 8 blastocysts were transferred to three recipient females. The recipients were checked for pregnancy at 35–40 days after embryo transfer.

Differential staining and evaluation of morphology

The quality of day 7 porcine blastocysts was assessed by differential staining of the ICM and trophectoderm (TE) cells. The number of cells in day 9 bovine blastocyst was evaluated by Hoechst 33342 staining. The staining procedures have been previously published (Thouas *et al.*, 2001). Briefly, The TE of porcine SCNT blastocysts were stained when blastocysts were treated with a permeabilizing solution containing the ionic detergent Triton X-100 and the fluorochrome propidium iodide. Then, porcine blastocysts were incubated in a second solution containing 100% ethanol (for fixation) and the secondary fluorochrome bisbenzimide. Fixed and stained whole blastocysts were mounted and assessed for cell number using UV illumination of an epifluorescence microscope (IX71 Olympus, Tokyo, Japan).

The method used to evaluate the morphology of blastocysts is shown in Fig. 1. The mean diameter of the portion of the procine blastocyst that was extruded from the zona pellucida (D1) and the mean diameter of the portion of the bovine blastocyst (D2) were calculated as described previously (Laowtammathron *et al.*, 2005).

Experimental design

Two experiments were conducted, and each experiment was performed more than four times. In the first experiment, porcine blastocysts were cultured in NCSU-37 medium for 5 days, and then in NCSU-37 medium or hiPS medium for an additional 2 days. Thereafter, the morphological quality of the blastocysts (day 7) was examined, as described previously (Laowtammathron *et al.*, 2005) (Fig. 1*A*). In addition, the number of cells per blastocyst was assessed by differential staining.

In the second experiment, bovine blastocysts were cultured in IVC-I medium for 3 days, then in IVC-II medium for 4 days, and then in IVC-II medium or hiPS medium for an additional 2 days. Thereafter, the diameter of the portion of the blastocyst (day 9) was calculated as shown in Fig. 1*B*. The number of cells was evaluated by Hoechst 33342 staining.
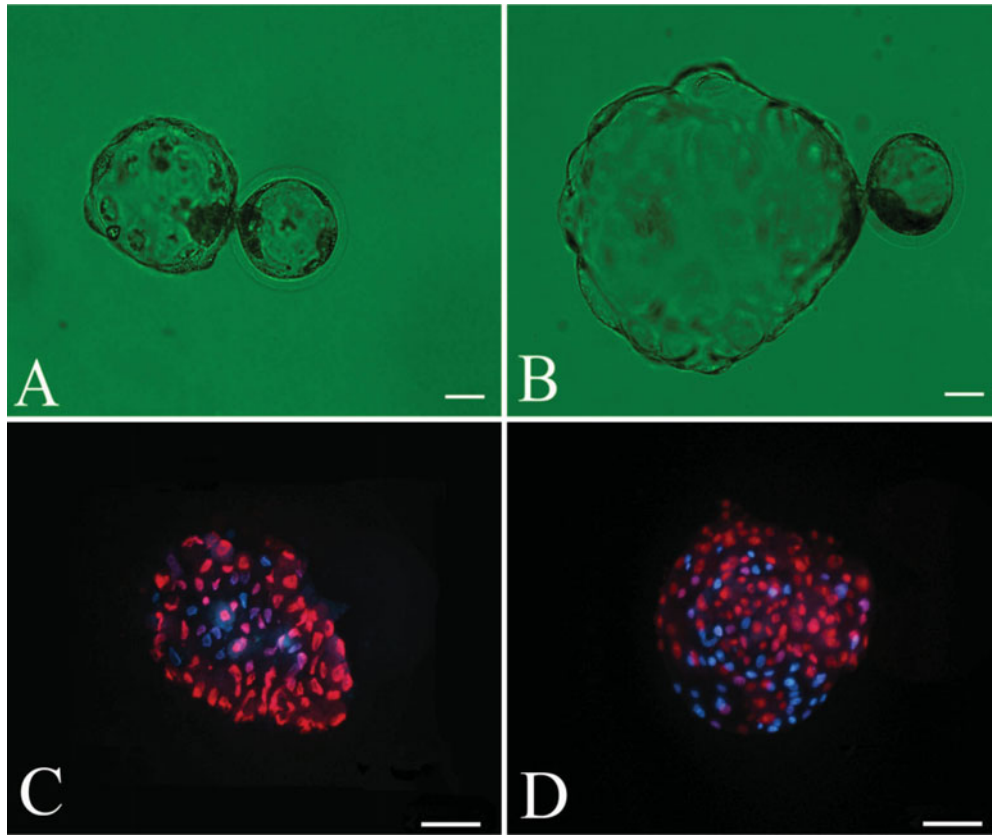
Figure 2 Differential staining of day 7 porcine blastocysts. Bright field images (*A*, *B*) and differential staining (*C*, *D*) of porcine somatic cell nuclear transfer (SCNT) blastocysts cultured in North Carolina State University (NCSU)-37 medium (*A*, *C*) or human induced pluripotent stem cell (hiPS) medium (*B*, *D*). Inner cell mass (ICM) cells and trophectoderm (TE) cells appear blue and red, respectively. (*A*, *B*) Original magnification, ×200. Scale bar = 80 µm. (*C*, *D*) Original magnification, ×200. Scale bar = 100 µm.

Day 8 bovine blastocysts cultured in hiPS medium for 1 day were transferred to synchronized recipient heifers.

Statistical analysis

All data were obtained from more than three replicates. Diameters and nuclei numbers were analyzed by *T*-test (independent samples) using SPSS 16.0 software (SPSS Inc., Chicago, IL, USA). *P*-values <0.01 were regarded as statistically significant.

Results

Assessment of pig SCNT blastocysts morphological quality

Hatching of porcine blastocysts was clearly enhanced when they were cultured in NCSU-37 medium for 5 days and then in hiPS medium for another 2 days. The diameter of the portion of the blastocyst that was extruded from the zona pellucida was significantly lower in blastocysts that were continually cultured in NCSU-37 medium that in those that were switched to hiPS medium (221.47 ± 38.94 µm versus 481.87 ± 40.61 µm, *P* < 0.01). Therefore, the morphological quality of porcine SCNT blastocysts was significantly improved by hiPS medium. Blastocysts cultured in hiPS medium (Fig. 2*B*) were much larger than those cultured in NCSU-37 medium (Fig. 2*A*). In particular, the hatched portion of the blastocyst was much larger in the former blastocysts than in the latter blastocysts.

Cell numbers of porcine SCNT blastocysts

Table 2 shows the effects of culture in the two types of media on the quality of porcine SCNT blastocysts. The number of day 7 blastocysts evaluated was the same in the two conditions (i.e., 16). However, the total number of cells per blastocyst differed between the two conditions (44.33 ± 5.28 and 143.33 ± 16.05, *P* < 0.01). The total number of cells per blastocyst was more than two-fold higher in blastocysts cultured in hiPS medium than in those cultured in NCSU-37

Table 2 Effects of culture in the two types of medium on the quality of porcine somatic cell nuclear transfer (SCNT) blastocysts

Group	No. of day 5 blastocysts	Quality of day 7 blastocysts (mean ± SD)		
		D1 (μm)	Total number of cells per blastocyst	Highest number of cells per blastocyst
NCSU-37	16	221.47 ± 38.94^a	44.33 ± 5.28^c	60
hiPS	16	481.87 ± 40.61^b	143.33 ± 16.05^d	164

North Carolina State University (NCSU)-37: porcine SCNT embryos were cultured in NCSU-37 medium for 7 days, with a change of medium on day 5.

Human induced pluripotent stem cell (hiPS): porcine SCNT embryos were cultured in NCSU-37 medium for 5 days and then in hiPS medium for an additional 2 days (7 days in total).

D1, diameter of the portion of the blastocyst extruded from the zona pellucida.

[a,b,c,d]Different superscripts in a column indicate significant differences ($P < 0.01$).

medium (Fig. 2*C*, *D*). The highest number of cells in a blastocyst was 164 (Fig. 2*D*).

Evaluation of bovine IVF blastocysts morphological quality

Culture of bovine blastocysts in IVC-I medium for 3 days, then in IVC-II medium for 4 days, and then in hiPS medium for another 2 days improved blastocyst hatching. The number of day 9 blastocysts assessed was 11 for blastocysts that were cultured in IVC-II medium and 19 for blastocysts that were cultured in hiPS medium. The diameter of the portion of the blastocyst that was markedly lower in blastocysts cultured in IVC-II medium than in those cultured in hiPS medium(150.30 ± 29.49 μm versus 195.58 ± 41.59 μm, $P < 0.01$). Therefore, culture in hiPS medium significantly improved the morphological quality of bovine blastocysts. Blastocysts cultured in hiPS medium (Fig. 3*B*) were much larger than those cultured in IVC-II medium (Fig. 3*A*). The hatched portion of the blastocyst was much larger in the former blastocysts than in the latter blastocysts.

Cell numbers of bovine blastocysts

Table 3 shows the beneficial effects of hiPS medium on the quality of bovine blastocysts. The total number of cells per bovine blastocyst was significantly lower in blastocysts cultured in IVC-II medium than in those cultured in hiPS medium (172.12 ± 45.08 and 604.83 ± 242.64, $P < 0.01$). The total number of cells per blastocyst was more than two-fold higher in blastocysts cultured in hiPS medium than in those cultured in IVC-II medium (Fig. 3*C*, *D*). The highest number of cells in a blastocyst was 992 (Fig. 3*D*).

In summary, culture in hiPS medium generated porcine and bovine blastocysts with an increased number of cells and an improved morphology.

Bovine embryo transfer

Bovine embryos were cultured in IVC-I (4 days), IVC-II (3 days) and hiPS medium (1 day). Then day 8 bovine blastocysts were transferred to three recipient heifers (two embryos/recipient). One of three recipients was in pregnancy.

Discussion

The present study demonstrated that *in vitro*-produced porcine and bovine early blastocysts was significantly improved by culture in hiPS medium for 2 days. Similar observations were occasionally made during a trial of human–pig chimera production in our experiment. In this trial, hiPS were injected into pig early blastocysts (day 5), and then the blastocysts were cultured in hiPS medium. After 2 days, the blastocysts were rather larger than control blastocysts. Subsequently, we investigated the *in vitro* development of porcine and bovine embryos cultured in NCSU-37, CR1, and hiPS medium.

NCSU-37 and CR-1aa have been reported in which to culture porcine and bovine embryos, respectively. hiPS medium is a comprehensive medium for hiPS cells with minimal spontaneous differentiation, high levels of pluripotency markers, differentiation potential and possession of a normal karyotype. DMEM/F-12, a 1:1 mixture of DMEM and Ham's F-12 Nutrient Mixture, serves as a basal medium consisting of typical amino acids, glucose, salts and vitamins. It has broad applicability in the serum-free culture of not only normal mouse and chicken cells, but also transformed cells, whose growth characteristics are likely altered in some way. The growth-promoting factors and hormones in DMEM/F-12 may have important effects on porcine and bovine blastocysts. Addition of essential amino acids (EAAs) and non-essential amino acids (NEAAs) to

Table 3 Comparison of the quality of bovine *in vitro* fertilization (IVF) blastocysts cultured in the two types of media

Group	Blastocysts at day 7	Hatched blastocysts at day 9 (%)	Quality of day 9 blastocysts (mean $\pm$ SD)		
			D2 (μm)	Total number of cells per blastocyst	Highest number of cells per blastocyst
CR1	59	45 (76.27)	150.302 $\pm$ 9.49[a]	172.12 $\pm$ 45.08[c]	241
hiPS	40	35 (87.50)	195.58 $\pm$ 41.59[b]	604.83 $\pm$ 242.64[d]	992

CR1: bovine IVF embryos were cultured in *in vitro* culture (IVC)-I medium for 3days and then in IVC-II medium for 6 days; the IVC-II medium was changed once on day 7.

Human induced pluripotent stem cell (hiPS): bovine IVF embryos were cultured in IVC-I medium for 3 days, in IVC-II medium for 4 days, and then in hiPS medium for an additional 2 days (9 days in total).

D2, diameter of the portion of the blastocyst within the zona pellucida.

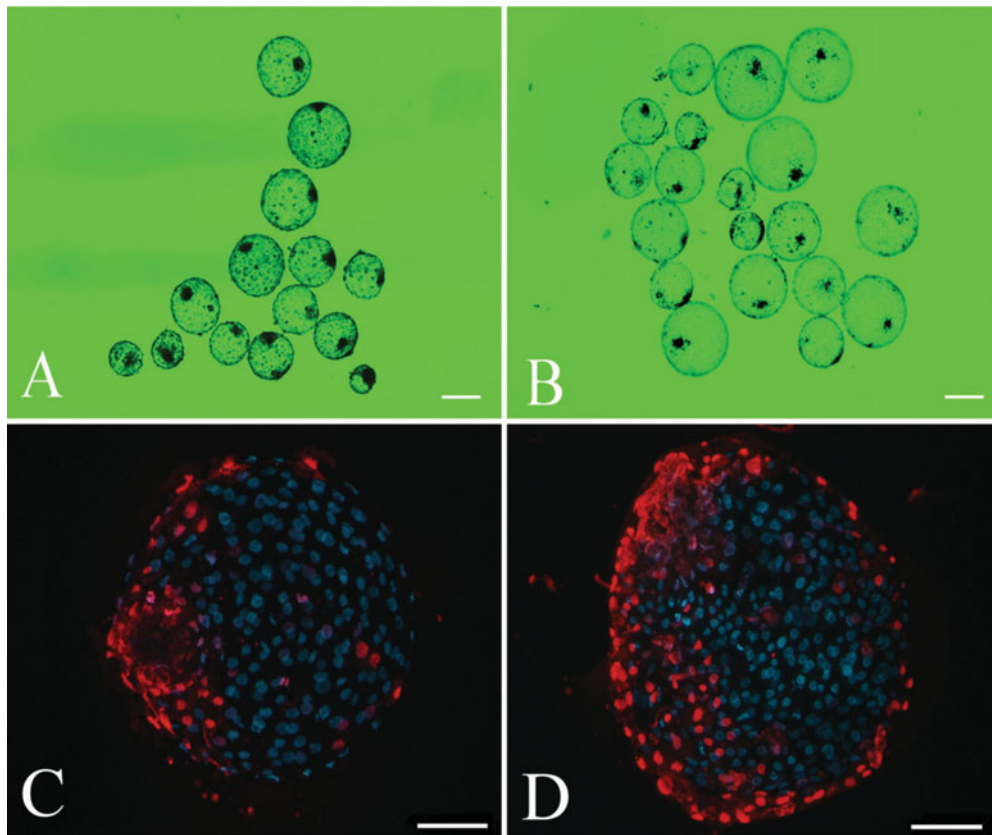[a,b,c,d]Different superscripts in a column indicate significant differences ($P < 0.01$). SD, standard deviation.

Figure 3 Staining of day 9 bovine IVF blastocysts. Bright field images (*A*, *B*) and Hoechst 33342 staining (*C*, *D*) of bovine blastocysts cultured in *in vitro* culture (IVC)-II medium (*A*, *C*) or human induced pluripotent stem cell (hiPS) medium (*B*, *D*). (*A*, *B*) Original magnification, $\times$50. Scale bar = 150 μm. (*C*, *D*) Original magnification, $\times$200. Scale bar = 50 μm.

culture medium promotes the development of murine (Gardner & Lane, 1993), bovine (Takahashi & First, 1992; Liu & Foote, 1995), and porcine (Van Thuan *et al.*, 2002; Suzuki & Yoshioka, 2006). NEAAs could provide favorable conditions for early cleavage and the expansion of porcine parthenogenetic diploid blastocysts, whereas EAAs could increase the total number of cells and the number of ICM cells (Van Thuan *et al.*, 2002). However, EAAs and NEAAs were added even in the IVC-II medium, the cell numbers in

bovine blastocysts cultured in IVC-II medium were not improved. Hence, the abnormal cell number in bovine blastocysts from hiPS medium might be due to EAAs and NEAAs interact with other ingredients. Besides DMEM/F-12 and NEAAs, hiPS medium also comprises other components, namely, KnockOut™ Serum Replacement, GlutaMAX™-1, β-mercaptoethanol, and basic fibroblast growth factor (bFGF). KnockOut™ Serum Replacement is a solution of BSA containing lipids, vitamins, trace elements, and low concentration

of transferring and insulin. GlutaMAX™-1 contains a stabilized form of a dipeptide form of L-glutamine, namely, L-alanyl–L-glutamine which prevents degradation and ammonia build-up even during long-term cultures. β-Mercaptoethanol, a hybrid of ethylene glycol, is used to reduce disulfide bonds and can act as a biological antioxidant by scavenging hydroxyl radicals. bFGF is a bioactive protein intended for use in cell culture applications. It is involved in a number of biological processes, including embryonic development and differentiation, and the proliferation of cells of mesodermal and endodermal origins. The critical component of hiPS medium that plays a critical role in the development of porcine and bovine blastocyst is unknown; however, we are currently undertaking experiments to identify this factor. We have cultured porcine reconstructed embryos in hiPS medium for 7 days, during which time the medium was changed once, on day 5. In this condition, the blastocyst formation rate and blastocyst quality were not improved (unpublished data); thus hiPS medium may be unsuitable for the early development of porcine SCNT embryos. So far, only hiPS medium broke through the limit of number of cells per porcine and bovine blastocyst. Human iPS medium is suitable for the culture of early porcine and bovine blastocysts, but additional mechanistic studies and the expression levels of developmentally important genes need to be researched. Moreover, the effects of hiPS medium on embryos of other species also need to be investigated in the future.

Based on results of this study, we concluded that hiPS medium improves the morphological quality of porcine and bovine blastocysts, as well as the number of cells per blastocyst (more than two-fold higher than in other media).

Acknowledgements

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