Somatic cell nuclear transfer in swamp buffalo

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Outline

- Somatic cell nuclear transfer in swamp buffalo
  - Effect of donor cell types
- Interspecies somatic cell nuclear transfer in swamp buffalo using bovine oocytes as recipient cytoplasm
  - Developmental potential
  - mtDNA analysis

- Swamp buffalo (*Bubalus bubalis*) is an important livestock species providing milk, meat and draught power contribute significantly to the economy in South East Asia.

- In Thailand, the number of buffalo has been dramatically decreasing during the past decade and need to conserve endangered buffalo breeds

Several assisted reproductive techniques such as

- artificial insemination
- embryo transfer
- *in vitro* fertilization (IVF)
- genome resource banking (semen, oocyte, embryo, somatic cell banks)
- somatic cell nuclear tranfer

have been implemented in buffalo production.

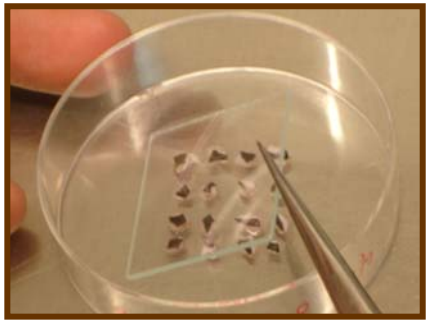
Somatic cell nuclear transfer (SCNT) in swamp buffalo

Donor cell preparation

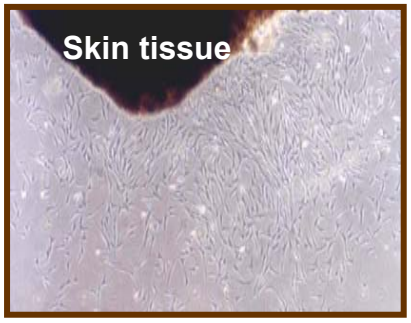
Buffalo donor

Buffalo ear skin

Skin tissue was cut into small piece



Skin tissue was cultured in αMEM + 10% FBS at 37°C under 5% CO_2 in air

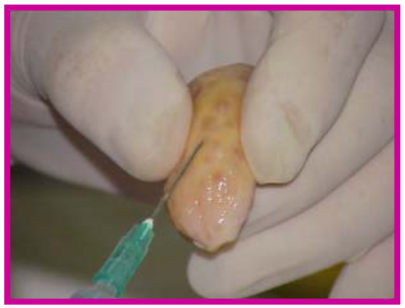


4-5 days after culture

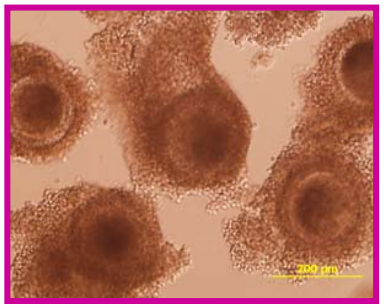
Kept in LN_2

Oocyte preparation

Buffalo ovaries from slaughterhouse

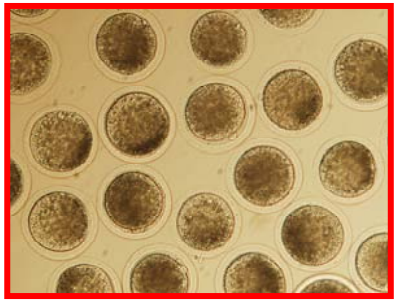


Oocytes were aspirated from follicle

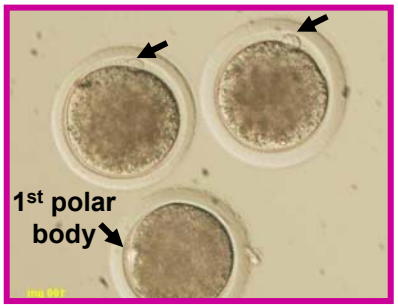


Cumulus-oocyte complexes

In vitro maturation (IVM) in IVM medium at 38.5°C under 5% CO_2 in air for 21 h



cumulus cells were mechanically removed by repeated pipetting in 0.2% hyarulonidase

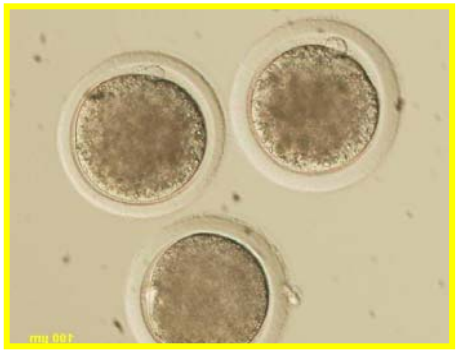


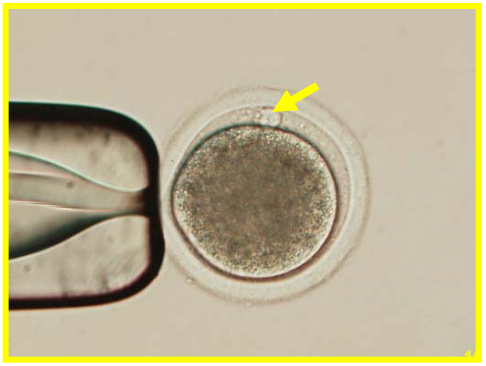
Metaphase II oocyte

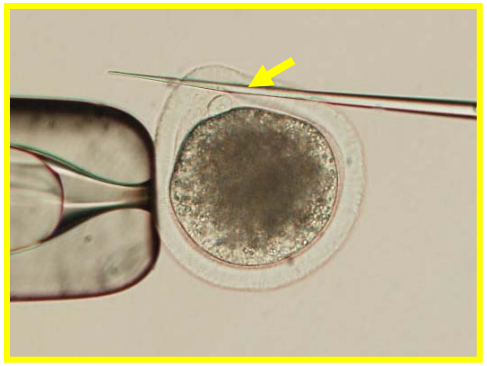
IVM medium

TCM199 supplemented with 10% fetal bovine serum (FBS), 50 IU/mL hCG, 0.02 AU/mL FSH and 1 µg/mL estradiol 17b

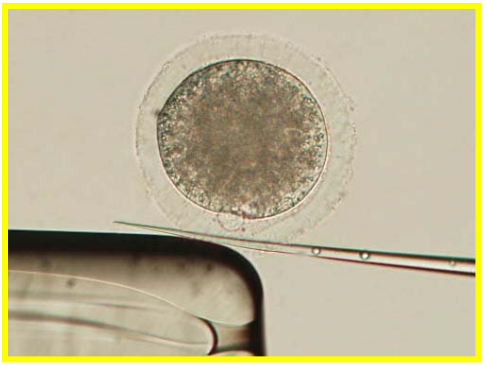
Enucleation

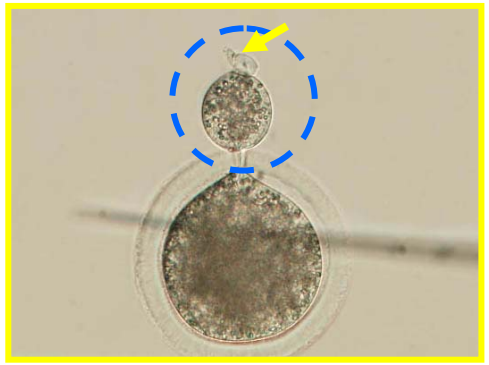


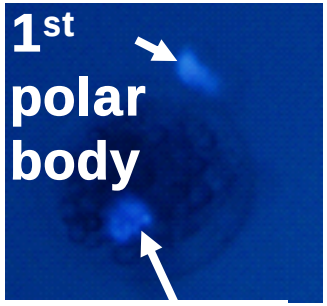




Incubated in
5 μg/ml cytochalasin B
for 5 min



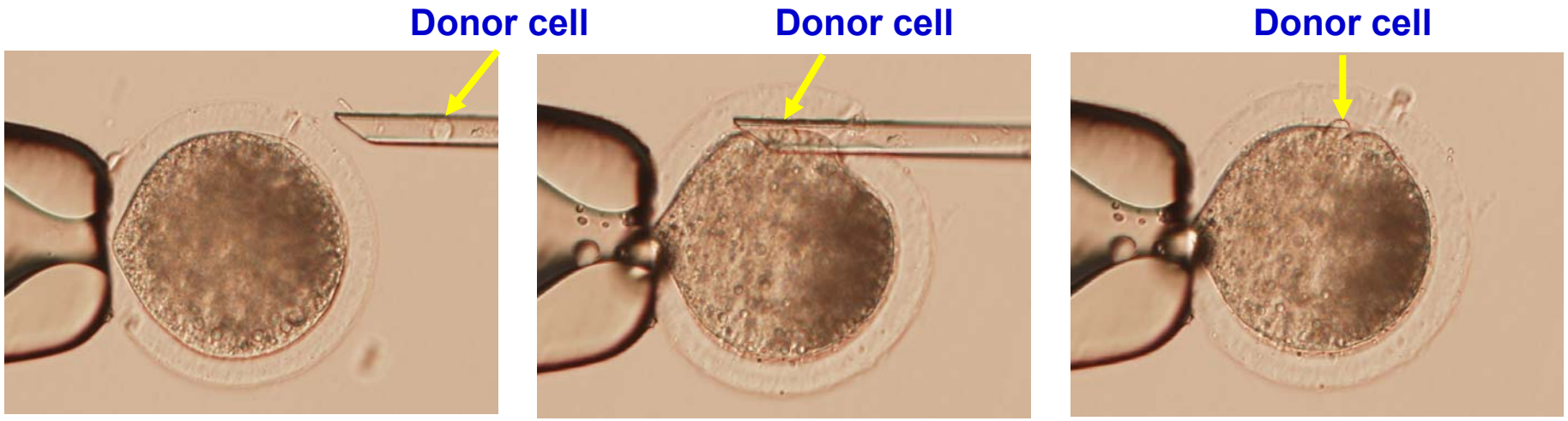




Stained with
5 μg/ml Hoechst 33342

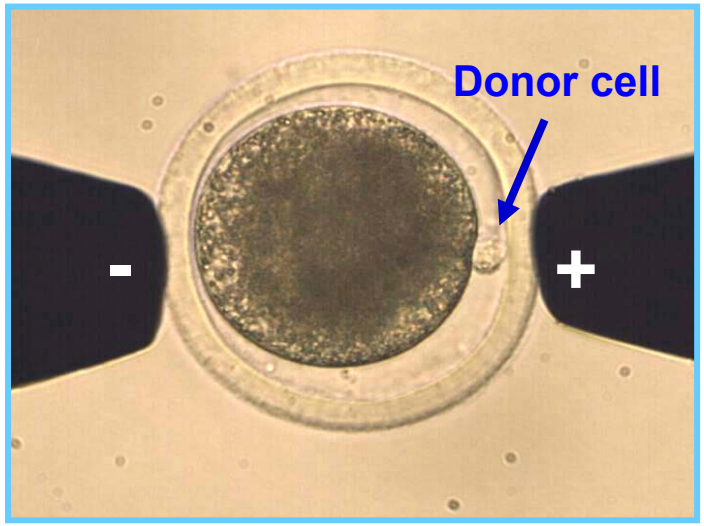
Nuclear

Injection



The donor cell was inserted into the perivitelline space of enucleated oocyte

Fusion



- Zimmermann fusion medium
- two direct current pulses (26 Volts for 17 µsec)
- a fusion machine (SUT F-1, Suranaree University of Technology)

Activation

Reconstructed embryos

Activated in 7% ethanol in TCM199-Hepes + 10% FBS

at RT for 5 min

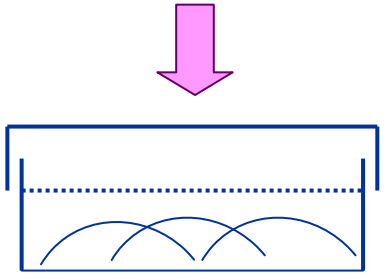
Incubated in 10 µg/mL cycloheximide

+ 1.25 µg/mL cytochalasin D

at 38.5ºC under 5% CO_2 for 5 h

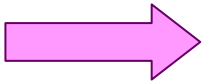
In vitro embryo culture

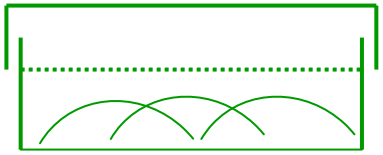
Activated embryos



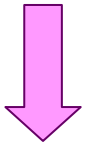
mSOFaa (Glucose free) + 3 mg/mL BSA
at 38.5˚C under 5% CO_2, 5% O_2
and 90% N_2 for 2 days

Selected 8-C

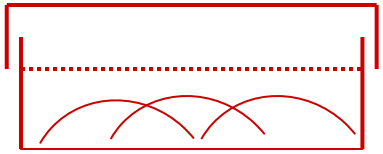




mSOFaa (0.25 mg/ml Glucose) + 3 mg/mL BSA
Co-cultured with bovine oviductal epithelial cells
at 38.5ºC under 5% CO_2 in air for 2 days



mSOFaa (0.50 mg/ml Glucose) + 3 mg/mL BSA
Co-cultured with bovine oviductal epithelial cells
at 38.5ºC under 5% CO_2 in air for 3 days



- **Although the first cloned buffalo was born in 2007 (Shi *et al*., 2007), the success rate of offspring production is still low**

- **One of key factors is the donor cell type which has a significant effect on the efficiency of SCNT**

- **Embryonic and fetal cells are reported to be more successful candidates for SCNT. On the other hand, previous studies found that adult fibroblast as cumulus cells had high potential to support embryo development.**

- **There have a few reports about the effect of donor cell types on the developmental potential of cloned buffalo embryos**

Experiment. 1 Effect of donor cell types

- Fetal fibroblasts, ear fibroblasts, granulosa cells and cumulus cells were used as donor cells in both buffalo and bovine.

- The effect of donor cell type on developmental potential of bovine and buffalo cloned embryos was investigated.

Table 1. Effect of donor cell type on developmental potential of cloned bovine and buffalo embryos*

Species	Donor cell type	No. of couplets fused (%)	No. of embryos cultured	No. of embryos cleaved (%)	No. (%) embryos developed to		
					8-Cell	Morula	Blastocyst
Bovine	FF	113/132 (85.6)^{b}	110	108 (98.2)^{a}	88 (80.0)^{a}	60 (54.5)^{a}	45 (40.9)^{a}
	EF	111/121 (91.7)^{a}	111	100 (90.1)^{ab}	78 (70.3)^{abc}	60 (54.1)^{a}	43 (38.7)^{a}
	GC	111/124 (89.5)^{ab}	111	101 (91.0)^{ab}	76 (68.5)^{ab}	51 (45.9)^{ab}	46 (41.4)^{a}
	CC	118/145 (81.4)^{c}	108	100 (92.6)^{ab}	65 (60.2)^{abc}	46 (42.6)^{ab}	40 (37.0)^{ab}
	PA	-	105	86 (81.9)^{bc}	48 (45.7)^{c}	43 (41.0)^{ab}	27 (25.7)^{cd}
Buffalo	FF	120/136 (88.2)^{ab}	119	100 (84.0)^{bc}	76 (63.9)^{abc}	38 (31.9)^{b}	26 (21.8)^{cd}
	EF	112/130 (86.2)^{b}	112	96 (85.7)^{bc}	71 (63.4)^{abc}	37 (33.0)^{b}	30 (26.8)^{cd}
	GC	108/122 (88.5)^{ab}	102	88 (86.3)^{bc}	69 (67.6)^{ab}	35 (34.3)^{b}	25 (24.5)^{cd}
	CC	117/143 (81.8)^{c}	104	86 (82.7)^{bc}	63 (60.6)^{abc}	35 (33.7)^{b}	29 (27.9)^{bc}
	PA	-	104	82 (78.8)^{c}	58 (55.8)^{bc}	36 (34.6)^{b}	20 (19.2)^{d}

*Five replicates were performed. Different superscripts within a column indicate significant differences (P<0.05).
FF= fetal fibroblasts, EF= ear fibroblasts, GC= granulosa cells, CC= cumulus cells, PA= parthenogenetic activation.

Srirattana et al., 2009
J Reprod Dev. 2009 Oct 8. doi:10.1262/jrd.09-135A

Table 2. Number of TE and ICM cells in bovine and buffalo blastocysts

Species	Donor cell types	No. blastocysts examined	Mean (± S.E.M.) number of cells in blastocyst*		
			TE	ICM	ICM ratio (%)
Bovine	FF	5	104.4 ± 0.83	33.6 ± 0.42	24.5 ± 0.21
	EF	5	91.6 ± 0.77	29.8 ± 0.43	24.6 ± 0.14
	GC	5	100.2 ± 1.01	33.0 ± 0.57	24.9 ± 0.22
	CC	5	105.6 ± 1.01	34.8 ± 0.57	24.8 ± 0.15
	PA	5	102.2 ± 0.79	33.4 ± 0.46	24.6 ± 0.17
Buffalo	FF	5	94.4 ± 1.16	31.4 ± 0.65	25.2 ± 0.23
	EF	5	98.0 ± 0.88	31.8 ± 0.49	24.6 ± 0.21
	GC	5	103.8 ± 0.59	34.6 ± 0.25	25.1 ± 0.21
	CC	5	115.4 ± 0.65	38.6 ± 0.35	25.1 ± 0.13
	PA	5	91.2 ± 0.87	30.4 ± 0.47	25.1 ± 0.16

*No statistical difference was obtained (P>0.05).
TE and ICM refer to trophectoderm and inner cell mass, respectively.

Srirattana et al., 2009
J Reprod Dev. 2009 Oct 8. doi:10.1262/jrd.09-135A

Experiment 1 conclusion

- The donor cell types, FFs, EFs, GCs and CCs, had the same ability to support cloned embryos to develop to the blastocyst stage within the same species

- The quality of cloned embryos derived from all four donor cells was similar in both bovine and buffalo

Interspecies somatic cell nuclear transfer (iSCNT) in swamp buffalo

- **Due to the limitation of the number of buffalo ovaries obtained from slaughterhouse and low oocyte recovery number from ovaries, SCNT is inefficient for production of buffalo**

- **iSCNT would be a valuable tool for cloning of animals whose oocytes are difficult to obtain and also for examining nucleo-cytoplasmic interactions**

Type of SCNT

1. **Intraspecies SCNT: donor cell and recipient oocyte come from same species**

Donor cell

Bos taurus

Bovine

Oocyte

Bos taurus

Bovine

2. **iSCNT: transferring a donor cell from one species into a recipient oocyte of another species**

Donor cell

Bubalus bubalis

Buffalo

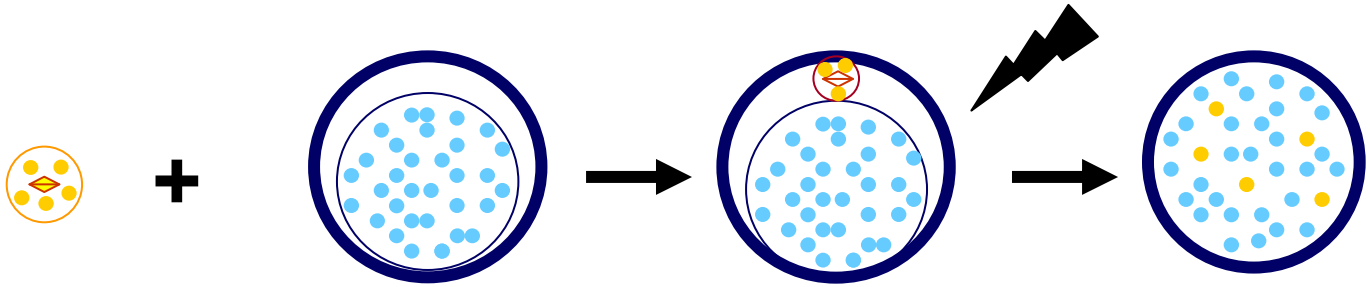
Oocyte

Bos taurus

Bovine

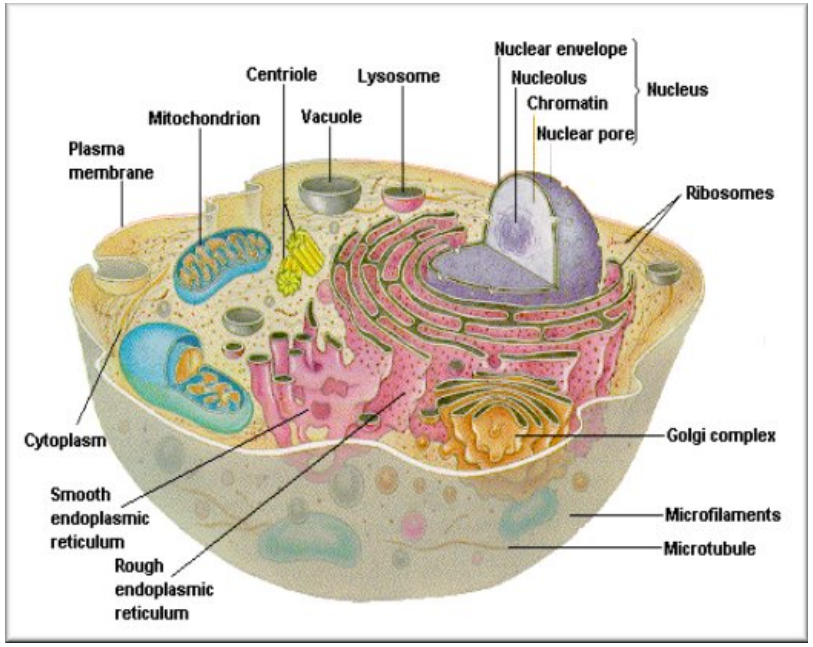
Bovine oocyte has been used to support embryo development of various species because of availability of ovaries from slaughterhouse and a great understanding of *in vivo* and *in vitro* development on bovine embryos

- **In the process of nuclear transfer, the transfer of donor cell mitochondria with nuclei into recipient oocytes has resulted in mitochondrial heteroplasmy**

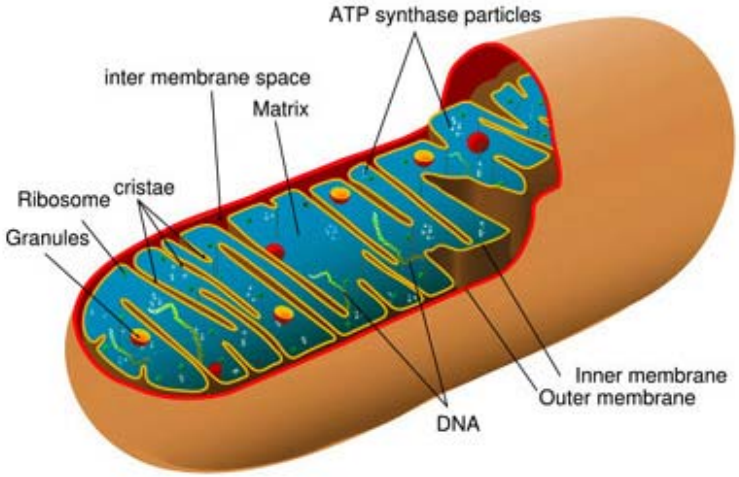


Donor cell **Enucleated oocyte** **Heteroplasmy**

Mitochondria from donor cell and recipient oocyte



Mitochondria generate most of the cell's supply of adenosine triphosphate (**ATP**), are involved in a cell signaling, cellular differentiation, cell death, as well as the control of the cell cycle and cell growth



Mitochondrial DNA (mtDNA): double-stranded circular genome encodes **13 of the proteins of the electron transfer chain** associated with the process of **oxidative phosphorylation**

- Interactions between nuclear and mtDNA are critical for the subsequent development of the embryos
- Quantitative analysis of mtDNA would be a powerful tool to study the interaction between donor nucleus and mitochondria after nuclear transfer
- A better understanding of the nuclear-cytoplasmic compatibility is necessary to improve iSCNT efficiency

Experiment 2. mtDNA in buffalo iSCNT embryos

- To investigated the developmental potential of buffalo iSCNT embryos derived from swamp buffalo fibroblast and bovine enucleated oocyte

- The mtDNA content in buffalo iSCNT embryos during pre-implantation development was examined by real-time PCR of the species-specific cytochrome b gene.

Table 3 Developmental potential of bovine SCNT and buffalo iSCNT embryos

Donor cell (No. of experiments)	Fused (%)	Cultured	Cleaved (%)	No. of embryos (%)		
				8-cell	morula	blastocyst
Bovine (6)	86/97 (88.7)[a]	71	43 (60.6)	29 (40.8)	19 (26.8)	15 (21.1)
Male Buffalo (6)	79/100 (79.0)[b]	66	33 (50.0)	22[†] (33.3)	0	0
Female Buffalo (2)	40/44 (90.9)	39	30 (76.9)	15[†] (38.5)	0	0

[†]Arrested at 8- to 16- cell stage
*Values were significantly different by ANOVA ($P < 0.05$).

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Animal Science Journal, submitted

Table 4 The copy number of bovine and buffalo mtDNAs in buffalo iSCNT embryos

Donor cell	Stage of embryos*	No. analyzed	Mean ± SD copy number of mtDNA		Ratio of buffalo to total mtDNAs (%)
			Bovine	Buffalo	
Male buffalo	Injected	6	$9.7\times10^{5} \pm 2.0\times10^{5}$	$6.3\times10^{2} \pm 1.9\times10^{2}$	0.07
	Fused	7	$8.8\times10^{5} \pm 1.7\times10^{5}$	$5.9\times10^{2} \pm 2.2\times10^{2}$	0.07
	Activated	6	$7.7\times10^{5} \pm 1.7\times10^{5}$	$5.4\times10^{2} \pm 2.4\times10^{2}$	0.07
	Arrested at 8- to 16-cell	9	$9.2\times10^{5} \pm 2.0\times10^{5}$	$6.2\times10^{2} \pm 2.2\times10^{2}$	0.07
Female buffalo	Injected	3	$8.2\times10^{5} \pm 1.5\times10^{5}$	$1.0\times10^{3} \pm 2.2\times10^{2}$	0.13
	Fused	3	$8.4\times10^{5} \pm 2.4\times10^{5}$	$6.3\times10^{2} \pm 1.6\times10^{2}$	0.08
	Activated	3	$9.0\times10^{5} \pm 1.6\times10^{5}$	$1.1\times10^{3} \pm 2.3\times10^{2}$	0.12
	Arrested at 8- to 16-cell	12	$7.6\times10^{5} \pm 2.3\times10^{5}$	$7.4\times10^{2} \pm 3.6\times10^{2}$	0.10

*The data from different stages were not statistically different by ANOVA ($P > 0.05$).

Srirattana *et al*., 2009
Animal Science Journal, submitted

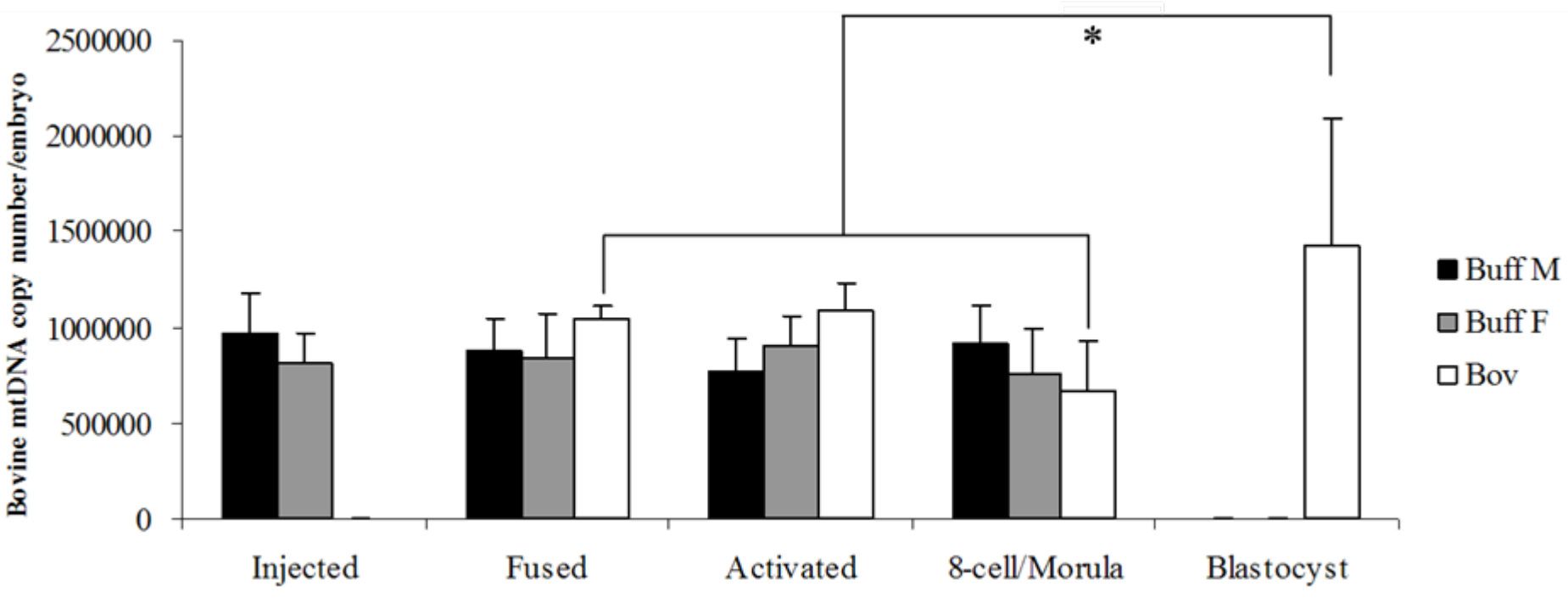

Fig. 1. Bovine mtDNA copy numbers in buffalo iSCNT and bovine SCNT embryos during early development.

***(P<0.05)**

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Experiment 2 conclusion

- Buffalo iSCNT and bovine SCNT embryos showed similar rates of cleavage and development to the 8-cell stage. However, buffalo iSCNT embryos did not develop beyond the 16-cell stage.

- In case of arrested buffalo iSCNT, both the donor cell and recipient cytoplast mtDNAs of the buffalo iSCNT embryos coexisted and were constant throughout iSCNT process until 8-16 cell stage.

Overall conclusion

- Fetal fibroblasts, ear fibroblasts, granulosa cells and cumulus cells had the same ability to support cloned embryo development to the blastocyst stage
- No change were observed in the mtDNA copy numbers of the undeveloped buffalo iSCNT embryos
- Further studies should be performed to establish the effective SCNT and iSCNT technique for buffalo production.

Acknowledgments

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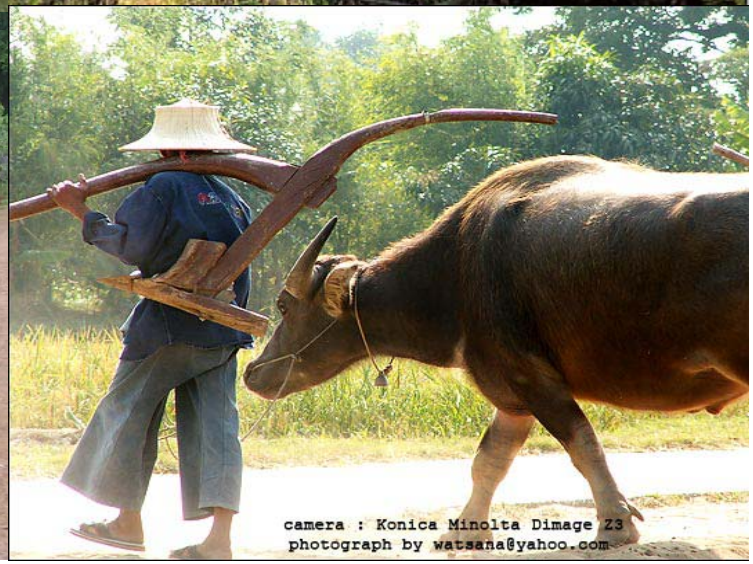
  National Institute of Livestock and Grassland Science, NARO
  Tsukuba, Japan

Thank you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