BUFFALO AND VARIOUS RUMINANT EMBRYOS PRODUCTION BY INTER-SPECIES, INTER-GENUS AND INTER-SUBFAMILY SOMATIC CELL NUCLEAR TRANSFER

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ABSTRACT

We have compared the effect of different nuclear -cytoplasmic relatedness on inter-species, somatic cell nuclear transfer (iSCNT) efficiency among buffalo (*Bubalus bubalis*), bovine (*Bos taurus*), goat (*Capra aegagrus hircus*) and gaur (*Bos gaurus*). on the in vitro embryo development. ISCNT is one of the techniques for species with limited availability oocytes and for endangered species. Ear fibroblast cells from buffalo, bovine and gaur were used as donor cells and recipient oocytes were obtained from buffalo, bovine and goat. The somatic cell nuclear transfer reconstructed embryos of various combinations were cultured. Our study showed that gaur-bovine (inter-species) blastocyst rate (12.10%) was higher than gaur-buffalo (inter-genus) (5.10%) and gaur-goat (inter-subfamily) (3.21%). The inter-species SCNT (gaur-bovine) blastocyst rate (12.10%) was similar to those by inter-genus SCNT (buffalo-bovine, 12.24%). The gaur-goat (inter-subfamily) showed the lowest blastocyst rate (3.21%). And buffalo media culture condition could support the development of gaur, goat and bovine iSCNT embryos. In conclusion, our study showed that blastocysts were possibly produced by inter-species, inter-genus and inter-subfamily somatic cell nuclear transfer. The inter-species SCNT and inter-genus SCNT tend to show higher development efficiency than inter-sub-family SCNT.

Keywords: *Bubalus bubalis*, buffalo, bovine, goat, gaur, iSCNT

INTRODUCTION

Interspecies somatic cell nuclear transfer provides an alternative tool for the preservation of endangered species using oocytes and recipients from related domestic species (Laguna *et al*., 2013; Yamochi *et al*., 2012.). Recently, several groups have reported the technique of interspecies somatic cell nuclear transfer (iSCNT) in which donor nuclei were transplanted into oocyte cytoplasm that had been obtained from different animal species (Yamochi *et al*., 2012). iSCNT remains an exciting tool for species with limited availability of oocytes, such as the horse, and for endangered species in which assisted reproduction is needed (Selokar *et al*., 2011; Gambini *et al*., 2016). Research in blastocyst development using interspecies nuclear transfer has succeeded, but the success rate has been low (Cordova *et al*., 2017). Many attempts have been achieved to multiply endangered species of

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ruminants by NT embryos transferred to domestic females (Folch *et al*. 2009). Gaur (*Bos gaurus*) is a protected species in Thailand and listed as a vulnerable animal by the International Union for Conservation of Nature (Srirattana *et al*., 2012). Bovine (*Bos Taurus*) oocytes have been used to support in vitro development of iSCNT embryos in various species because of the availability of ovaries from local abattoirs and an understanding of the in vivo and in vitro development (Srirattana *et al*., 2012.; Dominko *et al*. 1999; Gambini et al., 2016; Tasripoo *et al*., 2016). The report on comparison the production of interspecies SCNT between gaur fibroblast cells with swamp buffalo (*Bubalus bubalis*), bovine (*Bos taurus*) and goat (*Capra hircus*) oocytes was limited. Dominko *et al*. (1999) reported that continuation development could be a consequence of efficient reprogramming of the donor nucleus, regardless of the species, following by now embryonic gene expression. It is reasonable to assume that development in interspecies embryos was directed by introduced fibroblast nucleus. Disparity in the number of chromosome between the species used for NT does not seem to be limiting for this developmental success as expected (Dominko *et al*., 1999). The most successful progress of iSCNT was achieved using donor cells and recipient ooplast of very closely related species (Laguna *et al*., 2013). Donor cells of fibroblast origin are easier to reprogram than those of epithelial origin in interspecies SCNT (Saini *et al*., 2015; Sha *et al*., 2009) reported that a lot of published results underscored the importance of relatedness between donor cell and recipient oocytes species. The objective of this study is to compare the effect of different nuclear - cytoplasmic relatedness on inter-species, inter-genus and inter-subfamily cloning efficiency among buffalo (*Bubalus bubalis*), bovine (*Bos taurus*), goat (*Capra aegagrus hircus*) and gaur (*Bos gaurus*).

MATERIALS AND METHODS

Preparation of donor cell

The samples (diameter 0.5 cm) were collected by an ear biopsy of male buffalo, male bovine and female gaur. The skin tissue was washed several times in PBS. The rest of the procedure was performed according to the method of Tasripoo *et al*. (2014; 2016).

Production of matured oocytes

Buffalo, bovine, and goat ovaries were collected from local slaughterhouse and *in vitro* maturation of buffalo, bovine, and goat oocytes were performed at 19 to 20, 20 to 21 and 20 to 21 h, respectively. The rest of the procedure was performed according to the method of Tasripoo *et al*. (2014; 2016).

Production of reconstructed embryos by iSCNT

The enucleation, nuclear transfer, fusion and activation, *in vitro* culture of reconstructed embryos was performed according to the method of Tasripoo *et al*. (2014; 2016). The same culture medium was used for all the iSCNT combination in this study.

RESULTS AND DISCUSSION

Buffalo-bovine (inter-genus) showed the highest morula rate (48.41%) in Table 1. Gaur-bovine (inter-species), gaur-buffalo (inter-genus), gaur-goat (inter-subfamily) and bovine-buffalo (inter-genus) SCNT showed similar morula rate

(25, 27.8, 16.13, 20%, respectively). But the gaur-bovine (12.10%) gave similar blastocyst rate as buffalo-bovine iSCNT (12.24%). There was no blastocyst for the bovine-buffalo iSCNT, one of the factors might be due to the fewer number of fused oocyte samples. The blastocyst rate of gaur-bovine (12.10%) and buffalo-bovine (12.24%) were not different from control (intra-species SCNT), bovine-bovine and buffalo-buffalo. It is noted that our inter-subfamily embryos (gaur-goat) could develop to blastocyst, which are different from the report by Amarnath *et al*. (2011) that there was no blastocyst in that group. It is noted that early development gaur-bovine, gaur-buffalo, gaur-goat and buffalo-bovine iSCNT embryos at 8-cell stage showed similar results (53.22, 41.67, 35.50, 65.31%, respectively), except bovine-buffalo iSCNT. Our buffalo-bovine iSCNT showed similar blastocyst rate (12.24%) to those (13.3%) reported by Lu *et al*. (2005). Our gaur-bovine blastocyst rate (12.10%) was also similar to previously reported by Lanza *et al.* (2000), in which 12% of embryos reconstructed by iSCNT of gaur fibroblast cell into bovine cytoplasts developed to blastocyst and was able to implant, and fetuses developed up to 200 days. Then in 2012, a gaur-bovine offspring was born (Srirattana *et al*., 2012). Furthermore, Mastromonaco *et al*. (2011) reported that gaur (*Bos gaurus*) is closely related to domestic cattle, having diverged fairly in evolutionary time. As a result, the gaur has been bred successfully with *Bos Taurus*, *Bos indicus*. These also agreed with Amarnath *et al*. (2011) that bovine oocytes are suitable for production of gaur embryos. Sha *et al.* (2009) has demonstrated that close nuclear-cytoplasmic relatedness can improve the post implantation development of cloned inter-species embryos. Our results agreed with Beyhan *et al*. (2007) that the ability of an iSCNT embryo to develop to the blastocyst stage decreases as the taxonomic distance between donor and recipient species increase. And as the taxonomic distance between donor and recipient species increase, the blastocyst production decrease due to lesser ability of somatic cells to be reprogrammed (Priya *et al*., 2014). Mastromonaco *et al.* (2006) demonstrates that a better understanding of the compatible of those closely related species is necessary to explain the decreased developmental capacity of these embryos. The disparity in number of chromosome between gaur and bovine (different = 4) and buffalo and bovine (different = 12) did not limit the developmental efficiency between these two groups (blastocyst rate 12.10% vs 12.24%, respectively). Our blastocyst rates of gaur-bovine (12.10%) and buffalo-bovine (12.24%) demonstrated that disparity in the number of chromosome between the species used for nuclear transfer does not seem to be limiting for developmental success as reported by Dominko *et al*. (1999). The blastocyst rate of gaur-goat (3.21%) is rather low; this may be remarkably distant (inter-subfamily) taxonomic relationships between goat recipient oocyte donors and gaur somatic cell donors. Oocytes from different species may differ in their development competence (Laguna *et al*., 2011; Cordova *et al*., 2017). It is well established that embryos from different mammalian species require species-specific embryo culture conditions (Dominko *et al*., 1999). But our study showed that the culture conditions of buffalo are possibly used for producing embryos by iSCNT among buffalo, bovine, goat and gaur. This established method is appropriate for the production of iSCNT embryos and embryo transfer will be further study. This preliminary result showed higher efficacy of bovine ooplasm for supporting nucleoli formation than buffalo and goat in producing interspecies gaur

Table 1. Developmental potential of interspecies, inter-genus and inter-subfamily somatic cell nuclear transfer embryos using various recipient oocytes.

Donor Cell (no. chromosome)	Oocyte (no. chromosome)	Oocytes in MII (%)	No. of oocytes fused	Cleavage Rate (%)	Development of reconstructed embryos (%)				
					8-cell	Morula	Blastocyst	Hatched blastocyst	Taxonomical relations
Gaur (56)	Bovine (60)	195 (67.24)	124	101 (81.45)	66 (53.22)	31 (25)	15 (12.10)	5 (4.03)	Inter species
Gaur (56)	Buffalo (48)	59 (53.20)	36	27 (75)	15 (41.67)	10 (27.80)	2 (5.10)	-	Inter genus
Gaur (56)	Goat (60)	37 (69.81)	31	18 (58.10)	11 (35.50)	5 (16.13)	1 (3.21)	-	Inter subfamily
Bovine (60)	Bovine (60)	77 (57.04)	60	49 (81.67)	35 (58.33)	17 (28.33)	10 (16.67)	4 (6.67)	Intra species
Buffalo (48)	Bovine (60)	74 (62.71)	49	41 (83.67)	32 (65.31)	24 (48.41)	6 (12.24)	2 (4.08)	Inter genus
Buffalo (48)	Buffalo (48)	12 (34.29)	10	7 (70)	4 (40)	2 (20)	1 (10)	-	Intra species
Bovine (60)	Buffalo (48)	5 (29.41)	5	3 (60)	1 (20)	1 (20)	-	-	Inter genus

Note: Part of this work has presented in CUVC 2016.

embryos (Tasripoo *et al*., 2016). And buffalo media culture condition could support the development of gaur, goat and bovine embryos. In conclusion, our study showed that blastocysts were possibly produced by inter-species, inter-genus and inter-subfamily somatic cell nuclear transfer.

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REFERENCES

Amarnath, D., I. Choi, A.R. Moawad, T. Wakayama and K.H.S. Campbell. 2011. Nuclear-cytoplasmic incompatibility and inefficient development of pig-mouse cytoplasmic hybrid embryos. *Reproduction*, **142**: 295-307.

Beyhan, Z., A.E. Lager and J.B. Gibelli. 2007. Interspecies nuclear transfer: Implications for embryonic stem cell biology. *Cell Stem Cell*, **1**: 502-512.

Cordova, A., W.A. King and G.F. Mastromonaco. 2017. Choosing a culture medium for SCNT and iSCNT reconstructed embryos: from domestic to wildlife species. *J. Anim. Sci. Tech*., **59**:**24**: 1-14.

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

Folch, J., M.J. Cocero, P. Chesne, J.L. Alabart, V. Mominguez, Y. Cognie, A. Roche, Fernandez-Arias, J.I. Marti, P. Sanchez, E. Echegoyen, J.F. Beckers., A. Sanchez Bonastre and X. Vignon. 2009. First birth of an animal from an extinct subspecies (Capra pyrenaica) by cloning. *Theriogenology*, **71**: 1026-1034.

Gambini, A., A. de Stefano, J. Jarazo, C. Buemo, F. Karlanian and D.F. Salamone. 2016. Embryo aggregation does not improve the development of interspecies somatic cell nuclear transfer embryos in the horse. *Theriogenology*, **86**: 1081-1091.

Laguna, I., H. Fulka, G. Lazzari and C. Galli. 2013. Interspecies somatic cell nuclear transfer: Advancements and problems. *Cell Reprog*., **15**(5): 374-384.

Lagutina, I., V. Zakharchenko, H. Fulka, S. Colleoni, E. Wolf, J. Fulka Jr, G. Lazzari and C. Galli. 2011. Formation of nucleoli in interspecies nuclear transfer embryos derived from bovine, porcine, and rabbit oocytes and nuclear donor cells of various species. *Reproduction*, **141**: 453-465.

Lanza, R.P., J.B. Cibelli, F. Diaz, C.T. Moraes, P.W. Farin, C. E. Farin, C.J. Hammer, M.D. West and P. Damiani. 2000. Cloning of an endangered species (*Bos gaurus*) using interspecies nuclear transfer. *Cloning*, **2**(1): 79-90.

Lu, F.H., D.H. Shi, J.W. Wei, S.F. Yang and Y.M. Wei. 2005. Developement of embryos reconstructed by interspecies nuclear of adult fibroblasts between buffalo (*Bubalus bubalis*) and cattle (*Bos indicus*). *Theriogenology*, **64**: 1309-1319.

Mastromonaco, G.F., L.A. Favetta, L.C. Smith, F.

Filion and W.A. King. 2007. The influence of nuclear content on developmental competence of gaur x cattle hybrid in vitro fertilized and somatic cell nuclear transfer embryos. *Biol. Reprod*., **76**: 514-523.

Priya, D., N.L. Selokar, A.K. Raja, M. Saini, A.A. Sahare, N. Nala, P. Palta, M.S. Chauhan, R.S. Manik and S.K. Singla. 2014. Production of wild buffalo (*Bubalus arnee*) embryos by interspecies somatic cell nuclear transfer using domestic buffalo (*Bubalus bubalis*) oocytes. *Reprod. Domest. Anim*., **49**: 343-351.

Saini, M., N.L. Selokar, A.K. Raja, A.A. Sahare, S.K. Singla, M.S. Chauhan, R.S. Manik and P. Palta. 2015. Effect of donor cell type on developmental competence, quality, gene expression, and epigenetic status of interspecies cloned embryos produced using cells from wild buffalo and oocytes from domestic buffalo. *Theriogenology*, **84**: 101-108.

Selokar, N.L., A. George, A.P. Saha, R. Sharma, M. Muzaffer, R.A. Shar, P. Palta, M.S. Chauhan, R.S. Manik and S.K. Singla. 2011. Production of interspecies handmade cloned embryos by nuclear transfer of cattle, goat and rat fibroblasts to buffalo (*Bubalus bubalis*). *Anim. Reprod. Sci*., **123**: 279-282.

Sha, H., P. Wang, P. Zhang, G. Cheng and J. Chen. 2009. Close relatedness between exotic nucleus and cytoplast can improve the postimplantation development rate of cloned intersubspecies embryos. *Cloning and Stem Cells*, **11**(3): 347-353.

Srirattana, K., S. Imsoonthornruksa, C. Laowtammathorn, A. Sangmalee, W. Tunwattana, T. Thongprapai, C. Chaimongkol, M. Ketuda-Cairns and R. Parnpai. 2012. Full-term development of gaur-bovine interspecies somatic cell nuclear transfer embryos: Effect of trichostatin a treatment. *Cell Reprog.*, **14**: 248-257.

Tasripoo, K., K. Srisakwattana and S. Sophon. 2016. Production of gaur embryos by inter-species somatic cell nuclear transfer using buffalo, bovine and goat oocytes, p. 219-220. *In Proceedings of the 15th Chulalongkorn University Veterinary Conference*, Bangkok, Thailand.

Tasripoo, K., W. Suthikrai, S. Sophon, R. Jintana, W. Nualchuen, S. Usawang, A. Bintvihok, M. Techakumphu and K. Srisakwattana. 2014. First cloned swamp buffalo produced from adult ear fibroblast cell. *Animal*, **8**(7): 1139-1145.

Yamochi, T., Y. Kida, N. Oh, S. Ohta, T. Amano and M. Anzai. 2013. Development of interspecies cloned embryos reconstructed with rabbit (*Oryctolagus cuniculus*) oocytes and cynomolgus monkey (*Macaca fascicularis*) fibroblast cell nuclei. *Zygote*, **21**(4): 358-366.