Indian Journal of Animal Sciences **88** (9): 1015–1019, September 2018/Article

Successful cleavage of cloned goat embryos using ear fibroblast cell and fetal fibroblast cell as donor karyoplast in interspecies SCNT

SITI HASLINDA MOHD SHARIF[1], ASDIANA AMRI[2], WAN KHADIJAH WAN EMBONG[3] and RAMLI BIN ABDULLAH[4]

University of Malaya, 50603 Kuala Lumpur, Malaysia

Received: 14 November 2017; Accepted: 17 May 2018

ABSTRACT

The efficiencies in producing cloned ruminant embryos and subsequent production of offspring are still low. The study was conducted to produce cloned goat and cattle embryos using inter- and intraspecies SCNT techniques. This study involved 3 different types of donor karyoplast using goat and cattle oocyte as a recipient cytoplast to produce cloned goat and cattle embryos. The results showed that fetal fibroblast cell (FFC) in interspecies SCNT gave significantly higher 2-cell (64.40 vs. 38.43%), 4-cell (54.24 vs. 24.60%), 8-cell (36.82 vs. 14.54%) and morula (22.10 vs. 7.90%) cloned goat embryos than ear fibroblast cell (EFC). As for intraspecies SCNT using cumulus cell (CC) as a donor karyoplast to produce cloned cattle and goat embryos, the values for cleavage rates were not significantly different which were; 53.57 vs. 57.17%, 33.17 vs. 46.40%, 22.15 vs. 27.30% and 11.90 vs. 15.59%, respectively for all embryo stages. Our results showed that cloned goat and cattle embryos could be produced using different types of donor karyoplast in intra- and interspecies SCNT. However, for goat-cattle interspecies SCNT, FFC was more efficient to produce cloned goat embryos compared to EFC.

Key words: Cattle, Donor karyoplast, Goat, Intraspecies SCNT, Interspecies SCNT, Recipient cytoplast

The birth of Dolly in 1997 marked an important turning point in animal reproductive cloning prospects by proving that animals could be cloned by using somatic cell nuclear transfer (SCNT) technique and opened up various new opportunities for further research and application. One of the challenges in developing countries is to produce meat, milk and the products from ruminant species to meet requirement of the countries for food security and safety. In order to overcome these issues, cloning techniques could be an integral component in the livestock management system of these countries. The situation is worsened by the difficulty to obtain the ovaries such as goat ovaries from the local abattoir to produce oocytes which are important in SCNT technique. One of the possible alternatives to intraspecies SCNT is by substituting with interspecies SCNT technique to produce cloned goat embryos using goat karyoplast and cattle cytoplast when the source of goat cytoplast is not available or in scarcity. Most of the previous studies involved in livestock (particularly cattle) cloning using intraspecies SCNT have been quite established. Cattle oocyte has been suggested to serve as a universal recipient cytoplast in interspecies SCNT due to its ability to dedifferentiate the somatic cell nuclei of other species and can carry development after the embryo had been reconstructed through SCNT (Chen *et al*. 2003). It has been reported that cattle oocytes can be reprogrammed to produce cloned embryos and subsequent fetal development as well as birth of offspring in closely related animal species (Lagutina *et al.* 2013). The birth of cloned cattle intraspecies SCNT offspring using day 60 fetus as donor karyoplast was first reported by Cibelli *et al*. (1998). Subsequently, Dominko *et al*. (1999) reported the first mammalian interspecies SCNT in sheep, pigs, monkeys and rats with ear fibroblast cells as donor karyoplast. Meanwhile, Baguisi *et al*. (1999) were the first to report the production of goat embryos by intraspecies SCNT using fetal somatic cells as donor karyoplast. Abdullah *et al*. (2011) reported that the efficiency of interspecies SCNT was similar to that of intraspecies SCNT as both to produce cloned goat embryos. Nurin (2016) successfully produced goat-cattle interspecies SCNT embryos using goat EFC as donor karyoplast and cattle cytoplast. Since then, there is not much information in goat cloning particularly in interspecies SCNT technique. Specific studies to evaluate the compatibility and efficacy of different types of karyoplast and cytoplast for goat-cattle SCNT should be carried out. Extending from the established basic intraspecies SCNT technique in cattle, potential

Present address: [1,2](lindasharif@um.edu.my, asdiana88@gmail.com), Animal Biotechnology Embryo Laboratory (ABEL), Institute of Biological Sciences, Faculty of Science. [3,4]Professor (wkhadijah@gmail.com, ramli@um.edu.my), Faculty of Bioresources and Food Industry, Sultan Zainal Abidin University, Besut Campus, 22200 Besut, Terengganu Darul Iman, Malaysia.

application of different donor karyoplasts such as EFC, FFC and CC could be evaluated using intra- and interspecies SCNT technique in goats. Since cattle and goats are closely related species, it is apparent that interspecies cloning could be relatively suitable and efficient to be adopted for research and application. Consequently, the detail of research findings on the factors affecting the efficacy of interspecies cloning such as in goats will be useful to produce cloned embryos and its application in livestock production, wildlife species conservation and human medicine. Therefore, the objective of this study was to produce cloned goat and cattle embryos using inter- and intraspecies SCNT technique using different types of donor karyoplast as well as goat karyoplast and cattle cytoplast in interspecies SCNT technique.

MATERIALS AND METHODS

Chemicals used for this experiment were obtained from Sigma-Aldrich Co. (St. Louis, Mo, USA) unless otherwise stated. All animal samples used in experiments were in accordance to the guidelines of the Institutional Animal Care and Use Committee (IACUC), University of Malaya, Malaysia.

Oocyte retrieval: Cattle and goat ovaries obtained from local abattoirs were used as recipient cytoplast in this experiment. At abattoir, the ovaries were cut out using a pair of surgical scissors and placed in the thermos flask (35°–37°C) containing normal saline supplemented with penicillin-G (60 µg/ml) and streptomycin (50 µg/ml). Upon reaching the laboratory, ovaries were washed thoroughly with warm saline (37°C) to cleanse off the remaining blood. After washing, all ovaries were placed inside a sterile beaker (100 ml) containing TL-Hepes which had already pre-warmed on a stage warmer (38.5°C). For slicing process, ovary was held with a sterile autoclaved haemostat and checkerboard slicing was made using a razor blade on the whole surface of the ovary inside the culture dish containing TL-Hepes. The sliced ovaries were then rinsed by using syringe (10 ml) containing TL-Hepes medium so that the remaining cumulus-oocyte-complexes (COC) that still stuck inside the ovary would be flushed out. A beaker containing COC was left for 5 to 10 min so that the debris settled down. Any redundant TL-Hepes was sucked out by using a sterile glass pipette. A checkerboard cut was made on a petri dish (35 mm) to assist the process of oocyte searching. Then, a petri dish (35 mm) containing TL-Hepes along with COC and debris was scanned using a stereomicroscope. The goat oocytes were obtained from laparoscopic ovum pick-up procedure as described by Abdullah *et al*. (2011).

In vitro *maturation (IVM) of oocyte:* IVM medium droplets was prepared on a small petri dish (35 mm), overlaid with silicone oil and equilibrated in the incubator (5% CO_2 in air in humidified atmosphere at 38.5°C) at least 4 h before the experiment started. After washing the COCs with TL-Hepes, 15 to 20 COCs were cultured in each microdroplet that contained 80 µl of IVM medium. The COCs were selected based on morphology of granulosa cells (more than 3 layers) for IVM. Selected COC were incubated at 38.5°C and 5% of CO_2 in a humidified air of a CO_2 incubator.

Donor karyoplast preparation for somatic cell nuclear transfer (SCNT): Cumulus cell (CC), ear fibroblast cell (EFC) and fetal fibroblast cell (FFC) were used as donor karyoplast in this study. Goat fetuses aged 48 and 70 days were produced by natural mating induced by insertion of CIDR for 12 days at our local farm located in Batang Kali, Selangor, Malaysia. Fetuses were obtained surgically through Caesarean section and put in a culture dish (90 mm) containing PBS (-) medium. Briefly, head, internal organs, remaining tissues and bones of the fetuses were mechanically dissociated. The fibroblast tissues were then minced and washed thoroughly in PBS (-) and DMEM X 3PS medium (Gibco). The explants were cultured in a culture dish (60 mm) containing tissue culture medium (DMEM X 3PS + 10% FBS) and incubated in a humidified atmosphere of CO_2 (5%) in air at 38.5°C for 7 days. After the cells of the explants reached 80% confluency, they were detached from the surface of culture dish using trypsin-EDTA (0.25%) and then re-cultured back up to passage 2. The FFC were then cryopreserved with dimethyl sulfoxide (10% DMSO) already mixed with tissue culture medium and subsequently stored in liquid nitrogen. Prior to SCNT experiment, the frozen FFC were thawed and cultured up to 80% to be used as donor karyoplast. For EFC preparation, goat ear fibroblast tissues were biopsied and washed in PBS (-). The cartilage was removed from each ear tissue and minced into small pieces (1 mm^2) before cultured in a culture dish (60 mm). The explants were then cultured for 7–9 days in tissue culture medium incubated in a humidified atmosphere of CO_2 (5%) in air at 38.5°C (around 7 days). After reaching 80% of cell confluency, cells were trypsinized using 0.25% trypsin-EDTA and then subcultured until passage 3. For cumulus cell preparation, after 24 h IVM of COC, cumulus cells surrounding oocytes were denuded with hyaluronidase (0.1%) in TL-Hepes medium. Subsequently, the cells without the supernatant were transferred into a microcentrifuge tube (1000 µl) containing TCM and stored inside the incubator CO_2 (5%) in air at 38.5°C before the process of SCNT.

Somatic cell nuclear transfer (SCNT): After 24 h of *in vitro* maturation (IVM), CC were denuded by repeatedly pipetting in hyaluronidase (0.1%) and subsequently washed 5 times in TL-Hepes medium. Matured oocytes with visible first polar body were selected for SCNT experiment. Oocytes subjected to SCNT experiment were then enucleated with a micromanipulator by using squeezing technique (Narishige, Japan) in KSOM supplemented with FBS (10%) and cytochalasin B (5 µg/ml). Zona pellucida was punctured using laser shooting (XYClone™, Hamilton-Thorne) to create a slit adjacent to the first polar body. The first polar body along with cytoplasm (10%) was squeezed out using biopsy needle. Then, a single donor karyoplast was injected directly into cytoplasm of an enucleated cattle oocyte. For cattle and goat intraspecies SCNT, CC was used as a donor karyoplast while for goat interspecies SCNT,

the donor karyoplasts used were FFC and EFC.

Activation: Reconstructed oocytes were then induced by chemical activation with calcium ionophore (CaI; 5 µM) for 5 min and followed by with of 6-dimethylaminopurine (DMAP; 1.9 µM) for 4 h.

In vitro *culture (IVC):* After chemical activation, a group of 8–10 reconstructed oocytes were then cultured in potassium simplex optimization medium (KSOM) overlaid with silicone oil and incubated under a humidified atmosphere of CO_2 (5%) in air at 38.5°C. Embryo cleavage was monitored daily after culture for consecutive 6 days. Each embryo culture droplet was replenished every 3 days starting from day 3 onwards.

Effect of different types of donor karyoplast on both goat and cattle intraspecies SCNT (experiment 1): After IVM of oocytes, the CC was injected directly into the recipient cytoplast after which, the reconstructed oocytes (cattle oocytes) were activated using chemical treatments (CaI and 6-DMAP). After activation, the reconstructed oocytes were cultured *in vitro* CO_2 (5%) incubator at 38.5°C for embryonic development. The cleaved cloned embryos were observed under inverted microscope and recorded daily.

Effect of different types of donor karyoplast on goat-cattle interspecies SCNT (experiment 2): After IVM of oocytes, the EFC and FFC were injected directly into the recipient cytoplast (cattle oocytes) after which, the reconstructed oocytes were activated using chemical treatments (CaI and 6-DMAP). After activation, the reconstructed oocytes were cultured *in vitro* CO_2 (5%) incubator at 38.5°C for embryonic development. The cleaved cloned embryos were observed under inverted microscope and recorded daily.

Statistical analyses: Each group was replicated at least 6 times. Data were analyzed using SPSS version 20. The differences among mean percentages for all different stages of cloned embryo development rate were analyzed using one-way analysis of variance (ANOVA) followed by Duncan Multiple Range Test (DMRT). Differences were considered statistically significant if P values <0.05.

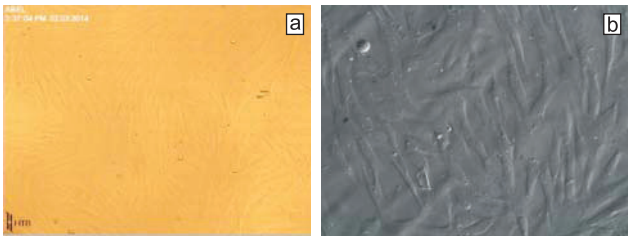

Fig. 1. Typical 80% confluency of EFC from goat (**a**) and typical 80% confluency of FFC from goat (**b**). Note: original magnification of photomicrographs (a) ×100 (b) ×200.

Table 1. Types of donor karyoplast, total number of oocyte and maturation rate in goat and cattle oocyte

Type of donor karyoplast	Total number of oocyte (n)	Maturation rate (n)
CC (cattle)	241	64.32[a] (155)
CC (goat)	99	66.67[a] (66)
EFC (goat-cattle)	352	63.63[a] (224)
FFC (goat-cattle)	476	61.76[a] (294)

[a]Means with same superscript in a column showed no significant difference (P>0.05).

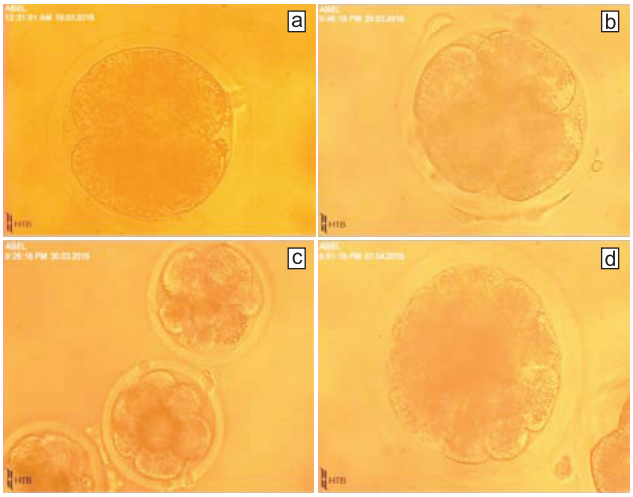

Fig. 2. Typical cattle intraspecies SCNT embryos at 2-cell stage (**a**), 4-cell stage (**b**), 8-cell stage (**c**) and morula (**d**). Note: original magnification of photomicrographs (a, b, d) × 100, (c) × 40.

RESULTS AND DISCUSSION

Typical 80% confluency of EFC and FFC as donor karyoplasts is shown in Fig. 1. The desired confluency was obtained after 3 and 2 passages, respectively, for a culture of 4 to 7 days. The maturation rate for goat and cattle oocytes ranged from 64 to 68% for both intra- and interspecies

Table 2. Percentage (mean±SEM) of *in vitro* developmental rate for intraspecies and interspecies SCNT cattle and goat embryos using different types of donor karyoplast

Type of SCNT	Type of donor karyoplast	Percentage of cleaved cloned SCNT embryos based on cell stage (n)			
		2-cell	4-cell	8-cell	Morula
Intraspecies (cattle)	Cattle cumulus cell (CC)	53.57±3.6^{abz} (33)	33.17±4.2^{ay} (21)	22.15±2.4^{ax} (14)	11.90±3.21^{abw} (8)
Intraspecies (goat)	Caprine cumulus cell (CC)	57.17±5.6^{abz} (38)	46.38±7.1^{abyz} (31)	27.25±8.7^{abx} (18)	15.59±7.0^{abw} (14)
Interspecies (goat-cattle)	Ear fibroblast cell (EFC)	38.43±5.3^{az} (59)	24.60±4.1^{ay} (29)	14.54±4.1^{ax} (24)	7.90±3.70^{aw} (14)
Interspecies (goat-cattle)	Foetal fibroblast cell (FFC)	64.41±6.8^{bz} (136)	54.24±6.4^{byz} (112)	36.82±5.3^{bx} (75)	22.10±4.55^{bw} (45)

[a,b]Means with different superscripts in a column showed a significant difference (P<0.05). [w,x,y,z]Means with different superscripts in a row showed a significant difference (P<0.05).

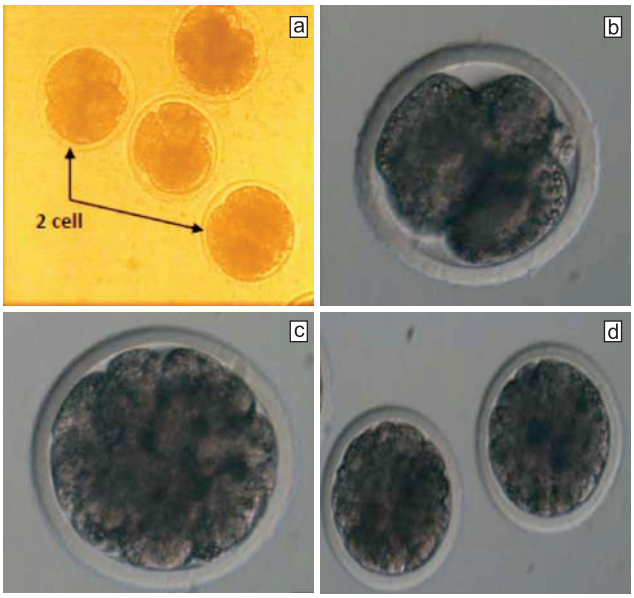

Fig. 3. Typical goat intraspecies SCNT embryos at 2-cell stage (**a**), 4-cell stage (**b**), 8-cell stage (**c**) and morula (**d**). Note: original magnification of photomicrographs (a) × 20, (b,c) × 100, (d) × 40.

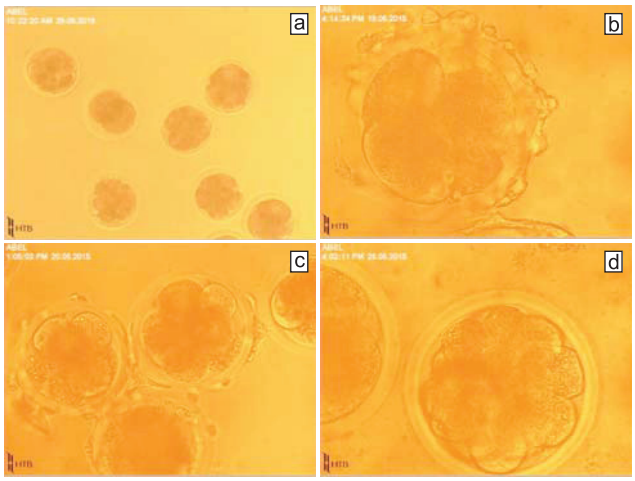

Fig. 4. Typical goat interspecies SCNT at 2–8 cell stage (**a**), 4-cell stage (**b**), 8-cell stage (**c**) and morula (**d**) (×40). Note: original magnification of photomicrographs (a) ×20, (b,d) ×100, (c) × 40.

SCNT groups (Table 1). As for intraspecies SCNT using CC as donor karyoplast to produce cloned cattle and goat embryos (Table 2), the values for cleavage rates were 53.57 vs. 57.17%, 33.17 vs. 46.4%, 22.15 vs. 27.3% and 11.90 vs. 15.59%, respectively, for 2-cell, 4-cell, 8-cell and morula, embryo stages were not significantly different. The reconstructed goat cloned embryos with the respective values of 64.40 vs. 38.43%; 54.24 vs. 24.60%; 36.82 vs. 14.54%; 22.10 vs. 7.90%, at all stages mentioned above for interspecies SCNT using goat FFC and goat EFC as donor karyoplasts and cattle oocyte as recipient cytoplast, respectively (Table 2). Typical embryos produced by intra- and interspecies SCNT are shown in Figs 2–4. Our findings showed that the cattle oocytes were successfully matured and the percentage of maturation (66.73%) was comparable with the results obtained by previous researchers (78.10%, Kwong (2012); 57.67%, Soh (2012); 72.04%, Asdiana (2014)). The reconstructed cloned embryos of both cattle and goats derived from intra- and interspecies SCNT approaches seemed to have the embryo developmental competence comparable to previous studies (Baguisi 1999, Baldassarre and Karatzas 2004). Types of donor karyoplast could impact the efficiency of cloning (Powell *et al*. 2004). Adult cells are the most commonly used as donor karyoplasts in SCNT since they are easy to produce and result in no injuries to animals (Yang *et al*. 2010). However, it still remains unclear which types of donor karyoplast are more efficient in animal cloning. The FFC has been extensively used for the production of cloned animals, especially in domestic animals; however, its application is still scarce in livestock animal cloned embryo production (Soh 2012). From this study, FFC gave higher cleavage rate when compared to CC and EFC. FFC has been widely used as donor karyoplast nowadays since it is superior to adult fibroblast cells and it can perform better in term of pregnancy and delivery rates (Forsberg *et al*. 2002). Kues *et al*. (2000) reported that FFC could proliferate rapidly and have less genetic damage. Donor karyoplast from fetal fibroblast of cattle (Saikhun *et al.* 2002), pig (Lee *et al.* 2003) and buffalo (Shah *et al.* 2009) also gave higher SCNT efficiency rate when compared to adult fibroblast. Wakayama *et al*. (2001) also found that the efficiency of SCNT was higher with FFC as donor karyoplasts (2.2%) compared to adult cells (1.7%). The present results showed that CC gave higher cleavage rate than EFC. This finding was in agreement with Hosseini *et al*. (2008) in which CC gave higher cleavage rate in sheep SCNT compared with EFC. Shah *et al*. (2009) and Yang *et al*. (2010) reported that CC gave better cleavage rate than EFC in buffalo species which was similar with the present study in goat SCNT. The findings from this study showed that both intra- and interspecies SCNT using CC, EFC and FFC could be used as donor karyoplasts. In some tropical countries such as Malaysia, it is difficult to obtain goat ovary samples from local slaughterhouse due to the low number of goat slaughtered and the scarcity of goat population as well as poor quality animals. Thus, cattle oocyte can be an alternative to be used as a recipient cytoplast in goat-cattle interspecies SCNT since it is relatively easier to obtain. Furthermore, cattle oocyte has been used as a recipient cytoplast in interspecies SCNT research due to similarity of diploid chromosome number (60n) present in both goat and cattle species (Soh 2012). The closer genetic distance between recipient cytoplast and donor karyoplast could improve *in vitro* cloned embryo development better than the diverse genetic background (Li *et al*. 2006). In conclusion, these results are useful to be considered in goat production of localities, whereby there are constraints to obtain goat ovaries in both quantity and quality to produce oocytes as recipient cytoplasts in intraspecies SCNT. Therefore, interspecies SCNT using readily available cattle cytoplasts and goat karyoplasts could be incorporated into

the strategic planning to produce superior goats in larger scale at a rapid rate in developing ASEAN region for food security and safety.

ACKNOWLEDGEMENTS

The authors would like to thank all ABEL members and staff of Livestock Research Centre (LSC), University of Malaya for their support during this research. This study was supported by e-Science grant (MOSTI) SF015-13

REFERENCES

Abdullah R B, Khadijah W E and Kwong P J. 2011. Comparison of intra- and inter-species nuclear transfer techniques in the production of cloned caprine embryos. *Small Ruminant Research* **98**(1–3): 196–200.

Asdiana A. 2014. 'Production of cloned intra- and inter-species caprine embryos through somatic cell nuclear transfer technique with special reference to donor karyoplast type and recipient cytoplast source.' Masters Thesis, University of Malaya.

Baguisi A, Behboodi E, Melican D T, Pollock J S, Destrempes M M, Cammuso C, Williams J L, Nims S D, Porter C A, Midura P, Palacios M J, Ayres S L, Denniston R S, Hayes M L, Ziomek C A, Meade H M, Godke R A, Gavin W G, Overstrom E W and Echelard Y. 1999. Production of goats by somatic cell nuclear transfer. *Nature Biotechnology* **17**(5): 456.

Baldassarre H and Karatzas C N. 2004. Advanced assisted reproduction technologies (ART) in goats. *Animal Reproduction Science* **82**: 255–66.

Cibelli J B, Stice S L and Golueke P J. 1998. Cloned transgenic calves produced from nonquiescent fetal fibroblasts. *Science* **280**: 1256–58.

Dominko T, Mitalipova M, Haley B, Beyhan Z, Memili E, McKusick B and First N L. 1999. Bovine oocyte cytoplasm supports development of embryos produced by nuclear transfer of somatic cell nuclei from various mammalian species. *Biology of Reproduction* **60**: 1496–502.

Forsberg E J, Strelchenko N S, Augenstein M L, Betthauser J M, Childs L A, Eilertsen K J, Enos J M, Forsythe T M, Golueke P J, Koppang R W, Lange G, Lesmeister T L, Mallon K S, Mell G D, Misica P M, Pace M M, Pfister-Genskow M, Voelker G R, Watt S R and Bishop M D. 2002. Production of cloned cattle from *in vitro* systems. *Biology of Reproduction* **67**: 327–33.

Hosseini S M, Moulavi F, Foruzanfar M, Hajian M, Abedi P, Rezazade-Valojerdi M and Nasr-Esfahani M H. 2008. Effect of donor cell type and gender on the efficiency of *in vitro* sheep somatic cell cloning. *Small Ruminant Research* **78**(1): 162–68.

Kues W A, Anger M, Carnwath J W, Paul D, Motlik J and Niemann H. 2000. Cell cycle synchronisation of porcine fetal fibroblasts effects of serum deprivation and reversible cell cycle inhibitors. *Biology of Reproduction* **62**: 412–19.

Kwong P J. 2012. 'Development of intra- and inter-species somatic cell nuclear transfer protocols using ear fibroblast cells as donor karyoplasts for production of cloned caprine embryos.' Doctor of Philosophy Thesis, University of Malaya.

Lagutina I, Fulka H, Lazzari G and Galli C. 2013. Interspecies somatic cell nuclear transfer: advancements and problems. *Cellular Reproramming* **15**(5): 374–84.

Lee J W, Wu S C, Tian X C, Barber M, Hoagland T, Riesen J, Lee K H, Tu C F, Cheng W T and Yang X. 2003. Production of cloned pigs by whole-cell intracytoplasmic microinjection. *Biology of Reproduction* **69**(3): 995–1001.

Li Y, Li Y, Dai Y, Du W, Zhao C, Wang H, Wang L, Li R, Liu Y, Wan R and Li N. 2006. Cloned endangered species takin (*Budorcas taxicolor*) by inter-species nuclear transfer and comparison of the blastocyst development with yalk (*Bos grunniens*) and bovine. *Molecular Reproduction and Development* **73**: 189–95.

Nurin F N. 2016. 'Production of interspecies cloned caprine embryos and mouse embryonic fibroblast feeder cell layer to culture embryonic stem cell.' Masters Thesis, University of Malaya.

Powell A M, Talbot N C, Wells K D, Kerr D E, Pursel V G and Wall R J. 2004. Cell donor influences success of producing cattle by somatic cell nuclear transfer. *Biology of Reproduction* **71**: 210–16.

Saikhun J, Pavasuthipaisit K, Jaruansuwan M and Kitiyanant Y. Xenonuclear transplantation of buffalo (*Bubalus bubalis*) fetal and adult somatic cell nuclei into bovine (*Bos indicus*) oocyte cytoplasm and their subsequent development. *Theriogenology* **57**(7): 1829–37.

Selokar N L, George A, Saha A P, Sharma R, Muzaffer M, Shah R A, Palta P, Chauhan M S, Manik R S and Singla S K. 2011. Production of interspecies handmade cloned embryos by nuclear transfer of cattle, goat and rat fibroblasts to buffalo (*Bubalus bubalis*) oocytes. *Animal Reproduction Science* **123**(3–4): 279–82.

Shah R A, George A, Singh M K, Kumar D, Anand T, Chauhan M S, Manik R S, Palta P and Singla S K. 2009. Pregnancies established from handmade cloned blastocysts reconstructed using skin fibroblasts in buffalo (*Bubalus bubalis*). *Theriogenology* **71**: 1215–19.

Soh H H. 2012. 'Production of cloned-caprine embryos *in vitro* obtained from interspecies nuclear transfer using bovine cytoplast and caprine karyoplast.' Masters Thesis, University of Malaya.

Yang C Y, Li R C, Pang C Y, Yang B Z, Qin G S, Chen M T, Zhang X F, Huang F X, Zheng H Y, Huang Y J and Liang X W. 2010. Study on the inter-subspecies nuclear transfer of river buffalo somatic cell nuclei into swamp buffalo oocyte cytoplasm. *Animal Reproduction Science* **121**(1–2): 78–83.

Ying C, He Z X, Liu A, Wang K, Mao W W, Chu J X, Lu Y, Fang Z F, Shi Y T, Yang Q Z and Da Yuan C H. 2003. Embryonic stem cells generated by nuclear transfer of human somatic nuclei into rabbit oocytes. *Cell Research* **13**: 251–63.

Wakayama T and Yanagimachi R. 2001. Mouse cloning with nucleus donor cells of different age and type. *Molecular Reproduction and Development* **58**(4): 376–83.