Animal Reproduction Science 208 (2019) 106125

Contents lists available at ScienceDirect

Animal Reproduction Science

journal homepage: www.elsevier.com/locate/anireprosci

Production of cloned cats using additional complimentary cytoplasm

Seok-Hwan Song[a,1], Kyeong-Lim Lee[a,c,1], Lianguang Xu[a,1], Myeong-Don Joo[a], Ji-Yoon Hwang[a], Seon-Hwa Oh[a], Il-Keun Kong[a,b,c,*]

[a] Division of Applied Life Science (BK21 Plus), Gyeongsang National University, Jinju, 52828, Gyeongnam Province, Republic of Korea
[b] Institute of Agriculture and Life Science, Gyeongsang National University, Jinju, 52828, Gyeongnam Province, Republic of Korea
[c] The King Kong Corp. Ltd., Gyeongsang National University, Jinju, 52828, Gyeongnam Province, Republic of Korea

ARTICLE INFO

Keywords:
Cytoplasm injection cloning technology
Somatic cell nuclear transfer
Cloning efficiency
Feline
Cloned offspring

ABSTRACT

Somatic cell nuclear transfer (SCNT) is an important technique for producing cloned animals. It, however, is inefficient when there is use of SCNT for cloned animal production. Cytoplasm injection cloning technology (CICT) was developed to overcome the inefficiencies of SCNT use of this purpose. The use of CICT involves additional cytoplasm fusing with enucleated oocytes to restore the cytoplasmic volume, thus improving the *in vitro* developmental competence and quality of cloned embryos. In this study, there was application of CICT in cats to improve the *in vitro* developmental competence of cloned embryos, as well as the production of the offspring. The results of this study were that fusion rate of the cloned embryos with use of the CICT method was greater than that with SCNT (80.0 ± 4.8% compared with 67.8 ± 11.3%, respectively), and more blastocysts developed with use of CICT than SCNT (20.0 ± 2.0% compared with 13.5 ± 5.0%, respectively). The 62 cloned embryos that were produced with use of CICT were transferred into five estrous synchronized recipients, and 151 cloned embryos produced using SCNT were transferred to 13 estrous-synchronized recipients. After the embryo transfer, there was birth from surrogate mothers of one live-born kitten that resulted using SCNT compared with three live-born kittens using CICT. The number of CICT-cloned embryos born was greater than that of SCNT-cloned embryos (4.8 ± 2.3% compared with 0.7 ± 1.3%, $P < 0.05$). These results indicate that the CICT technique can be used to produce cloned kittens, including endangered feline species.

1. Introduction

The use of somatic cell nuclear transfer (SCNT) has resulted in producing offspring in many species such as sheep (Deng et al., 2013; Zhang et al., 2013), goats (Zhou et al., 2013; Feng et al., 2015), cattle (Hoshino et al., 2009; Luo et al., 2015), buffalo (Yang et al., 2010; Lu et al., 2018), horses (Hinrichs et al., 2007; Olivera et al., 2018), pigs (Ma et al., 2016; Kwon et al., 2017), rabbits (Skrzyszowska et al., 2006; Meng et al., 2009), dogs (Jang et al., 2008; Hong et al., 2009) and cats (Choi et al., 2007; Yin et al., 2008b). Cloning with use of SCNT results in many research opportunities not only in generating and multiplying transgenic animals

* Corresponding author at: Division of Applied Life Science (BK21 Plus), Gyeongsang National University, Jinju, 52828, Gyeongnam Province, Republic of Korea.
E-mail address: ikong7900@gmail.com (I.-K. Kong).
[1] These authors contributed equally to this research.

https://doi.org/10.1016/j.anireprosci.2019.106125
Received 19 April 2019; Received in revised form 28 June 2019; Accepted 12 July 2019
Available online 15 July 2019
0378-4320/

(Yin et al., 2008a,b; Samiec and Skrzyszowska, 2011a, b; Guo et al., 2017; Wang et al., 2017), but also in genetic rescuing and re-introducing critically endangered or extinct species (Samiec, 2005; Kim et al., 2007; Gomez et al., 2008; Folch et al., 2009). With the use of SCNT, however, the efficiency of offspring production remains very low (Whitworth et al., 2009; Zhao et al., 2009; Samiec and Skrzyszowska, 2018a). The poor efficiency in offspring production using SCNT results because of epigenetic reprogramming of the somatic cell-inherited nuclear genome in the cytoplasm of recipient oocytes and descendant blastomeres of cloned embryos (Loi et al., 2016; Niemann, 2016; Sepulveda-Rincon et al., 2016; Gao et al., 2018). Removal of relatively large volumes of cytoplasm from enucleated nuclear recipient oocytes, however, contributes to the large incidence of failures in epigenetic reprogramming of somatic cell nuclei, which leads to aberrant gene expression during preimplantation development of SCNT-derived embryos (Whitworth and Prather, 2010; Kwon et al., 2015; Samiec and Skrzyszowska, 2018b). Epigenetic reprogramming of somatic cell nuclei is thought to improve with use of strategies of artificial epigenomic modulation of *in vitro* cultured nuclear donor cells, nuclear recipient oocytes and cloned embryos (Samiec and Skrzyszowska, 2012a; Samiec et al., 2015; Opiela et al., 2017; Jin et al., 2018; Saini and Selokar, 2018) or by using novel approaches to artificially activate nuclear-transferred oocytes (Samiec and Skrzyszowska, 2012b; Fernandes et al., 2014; Samiec and Skrzyszowska, 2014; de Macedo et al., 2019).

In metaphase II (MII), chromosomes are removed from the oocytes, which is an integral aspect with use of the SCNT methodology (Hua et al., 2007). In animals such as mice and rats, MII chromosomes are easily removed because the nucleus is easily visualized (Zhou et al., 2003; Kishigami et al., 2006). In other animals such as cattle and pigs, however, MII chromosomes are difficult to remove because the cytoplasm in the oocytes has a large lipid content (Abe et al., 2002; Sturmey et al., 2006). Thus, enucleation of MII-stage oocytes can be accomplished by microsurgical elimination of maternal chromosomes that have been dyed using Hoechst 33342 fluorochrome and subsequently are exposed to ultraviolet (UV) irradiation. Oocyte nuclear staining and UV irradiation has a negative effect by decreasing the oocyte development and nuclei reprogramming efficiency (Gil et al., 2012; Kim et al., 2012). To avoid damage from UV irradiation, an MII plate was randomly removed with approximately 30% of the cytoplasm using typical light procedures; however, use of this approach negatively affected embryo development. Maintenance of sufficient cytoplasmic volume in the enucleated oocytes is an important aspect of perpetuating SCNT-derived embryos with a relatively greater developmental potential (Peura et al., 1998; Sayaka et al., 2008).

The use of Handmade cloning (HMC) approaches allowed for overcoming of the cytoplasmic volume problem. The use of HMC approach enabled for the production of one embryo by fusing two enucleated oocytes to produce embryos with a typical cytoplasmic volume (Vajta et al., 2004; Kragh et al., 2005; Du et al., 2007). A negative feature of HMC, however, was that zona-free reconstructed embryos had greater damage due to environmental factors and potential disease transmission (Vajta et al., 2004; Verma et al., 2015).

To improve cloned embryo development and overcome the shortfalls of previous cloning methods, a new cloning method (Xu et al., 2019) described as cytoplasm injection cloning technology (CICT) was developed that allowed for restoration of the cytoplasmic volume of an enucleated oocyte. Embryos produced using CICT were of greater quality than those produced with use of SCNT, as assessed by total cell numbers and gene expression. For example, analysis of expression profiles for genes related to epigenetic reprogramming (DNA methyltransferase 1, 3a, 3b) indicated the use of CICT improved epigenetic reprogramming with use of this technique for cloning of cattle (Xu et al., 2019).

In the present study, CICT was applied in cats to produce cloned kittens. The cloning of cats was previously attempted using SCNT, but developmental capacity of cat embryos produced using SCNT was low and resulted in few live births (Yin et al., 2005, 2008a; Yu et al., 2010; Lee et al., 2010). Restoring the cytoplasm of embryos of cats using the CICT techniques will contribute to cloning both companion and endangered species of cats.

2. Materials and methods

2.1. Chemicals

Unless otherwise noted, all chemicals and reagents were obtained from Sigma-Aldrich (St. Louis, MO, USA).

2.2. Animals

Domestic female cats used as recipients and donors were individually housed in stainless steel cages measuring 1.86 m × 0.73 m × 0.65 m (W × D × H) and were fed dry food and water *ad libitum.* The animal housing room was temperature-controlled in the range of 21–25 °C and light-controlled on a 14D:10 L light cycle. The Gyeongsang National University Institute of Animal and Care and Use Committee approved all surgical procedures (GNU-140922-T0049). All cats were feline leukemia virus (FeLV)-free, feline immunodeficiency virus (FIV)-free, and feline infectious peritonitis (FIP)-free. Feline vaccines against feline herpes virus, calicivirus, panleukopenia virus and *Chlamydia psittaci* were administered (Felocell 4; Pfizer, New York, NY, USA).

2.3. Donor cell preparation

Donor somatic cells were derived from the skin tissue of domestic cats. In brief, the skin tissue was washed three times with Dulbecco's phosphate-buffered saline (D-PBS; Invitrogen, Carlsbad, CA, USA), finely cut into 1 mm^2 pieces, and digested in 0.25% (v/v) Trypsin-EDTA solution (Gibco BRL, Life Technologies, Grand Island, NY, USA) at 37 °C for 1 h. Cells were subsequently washed three times with donor cell culture medium (Dulbecco's modified Eagle's medium [DMEM, Gibco] supplemented with 15% [v/v] fetal bovine serum [FBS, Gibco], 1% [v/v] L-glutamine, 1% [v/v] nonessential amino acids, and 1% [v/v] penicillin-streptomycin [P/S]),

centrifuged at 1000 × rpm for 2 min, and seeded into a 100-mm plastic dish (Becton Dickinson, Franklin Lakes, NJ, USA). Seeded cells were subsequently cultured in donor cell culture medium at 37 °C in a humidified air atmosphere containing 5% CO_2 for 10 to 14 days. Cells were passaged three times, then frozen in DMEM supplemented with 10% (v/v) FBS and 10% (v/v) dimethyl sulfoxide and stored in liquid nitrogen. Cells were then thawed, cultured and passaged four to eight times until becoming confluent, then used for cloning.

2.4. Oocyte collection and in vitro maturation (IVM)

Two- to four-year-old female domestic cats weighing ˜5 kg were used as embryo donors. Ovarian follicular development was ensured in the donor cats by injecting 400 IU equine chorionic gonadotropin (eCG; Daesung, Seoul, Republic of Korea) and 100 IU human chorionic gonadotropin (hCG; Daesung, Seoul, Republic of Korea) 4 days apart, as previously described (Jin et al., 2012). Laparotomy was performed to access the ovaries at Gyeongsang National University Animal Medical Center. With use of general anesthesia for desensitization of the animals, immature oocytes were aspirated with an 18 G needle attached to a 1 mL syringe. Collected oocytes were cultured for 4 h in *in vitro* maturation (IVM) medium (M199, M7528) supplemented with 10% (vol/vol) FBS, 1 μg/mL estradiol-17β, 10 μg/mL follicle-stimulating hormone (FSH), 0.6 mM cysteine, 0.2 mM sodium pyruvate and 1% penicillin G/streptomycin (P4333) at 38.5 °C in a humidified atmosphere of 95% air/5% CO_2.

2.5. Nuclear transfer

Nuclear transfer was conducted as previously described (Yin et al., 2005) and all of glass pipettes (for enucleation and transfer) was autoclaved and exposed to UV irradiation to prevent the contamination. At 4 to 5 h after the onset of oocyte maturation, cumulus cells were completely removed from the cumulus oocyte complex (COCs) by gently pipetting in 0.1% bovine testicular hyaluronidase. Denuded oocytes with an extruding first polar body were selected in TCM199 supplemented with 0.3% BSA. Using a micromanipulator (Narishige, Tokyo, Japan), meiotically matured oocytes were enucleated by removal of the first polar bodies and adjacent ooplasm containing MII chromosomes. Enucleation was performed in TCM199 medium supplemented with 7.5 μg/mL cytochalasin B. Following enucleation, nuclear donor cells were trypsinized with 0.05% trypsin-EDTA solution and placed in TCM199 enriched with 0.3% BSA. Prepared donor cells were immersed in Sendai Virus (SV; Cosmo Bio, Tokyo, Japan) solution for 1 min (Azuma et al., 2018). Briefly, approximately 30% of the cytoplasm derived from donor oocytes was co-delivered to the perivitelline space of enucleated recipient oocytes using sub-zonal microinjection of single autologous (homoplasmic) cytoplasts together with nuclear donor somatic cells to induce SV-mediated fusion of triple homoplasmic cytoplast-enucleated oocyte-somatic cell couplets and to restore such a cytoplasmic volume that occurred before oocyte enucleation (Xu et al., 2019). The reconstructed oocytes were fused *via* SV-mediated fusion, then incubated in modified synthetic oviduct fluid (SOF) medium for 2 h (Mesalam et al., 2018). After fusion, the reconstructed oocytes were activated in 5 mM ionomycin for 5 min, then incubated in 2 mM 6-dimethylaminopurine under humidified conditions with 5% CO_2, at 38.5 ℃ for 4 h.

2.6. In vitro culture of nuclear transfer embryos

After activation, the reconstructed oocytes were placed in SOF with bovine serum albumin (BSA), insulin-transferrin-sodium (ITS) and epidermal growth factor (EGF) medium droplets (15–20 embryos/droplet) and covered with mineral oil at 38.5 °C in a humidified atmosphere of 95% air/5% CO_2. The culture medium was replaced every 48 h and cloned embryos were cleaved from fusion after 2 days. The development of blastocyst competence was confirmed using a stereomicroscope (Olympus, Tokyo, Japan) 8 days after fusion.

2.7. Synchronization of stage of estrous cycle of recipient females and embryo transfer

Cloned embryos were transferred into the oviducts of recipient cats. Embryo transfer was conducted as previously described (Yin et al., 2007). The stage of the estrous cycle of the recipient cats was synchronized by injecting 200 IU eCG and 100 IU hCG, 4 days apart. Reconstructed embryos at the one-cell stage after fusion were transferred into the oviducts of the estrous-synchronized females. Recipient cats were pre-medicated with medetomidine (10 μg/kg; subcutaneous injection; Domitor, Orion Pharma, Espoo, Finland), atropine (0.04 mg/kg; subcutaneous injection; Jeil Pharmaceutical Co., Daegu, Republic of Korea), acepromazine (0.1 mg/kg; subcutaneous injection; Samu Med., Seoul, Republic of Korea), and cefazolin (25 mg/kg, intravenous [IV] injection; Chong Kun Dang Pharm Co., Seodaemun-Gu, Republic of Korea) before surgery. The cats were anesthetized using an IV administration of 2 mg/kg etomidate (B. Braun Melsungen AG, Melsungen, Germany). Anesthesia was maintained by administering 2.5% isoflurane, along with oxygen, through an endotracheal tube. Saline solution was administered IV at 10 mL/kg/h throughout the surgery. Cloned embryos were loaded into a catheter to make a column with approximately 0.5 mL of medium, and 4–15 embryos per cat were surgically implanted into four estrous-synchronized recipient cats (Fig. 1). Pregnancy was confirmed using ultrasonography (Xario® SSA-660A, TOSHIBA, Hamamatsucho, Japan) around day 45, and pregnant cats were cared for in a separate room until natural birth of offspring.

2.8. Statistical analysis

Data are expressed as the means ± standard error of the mean (SEM) and were analyzed using one-way analysis of variance

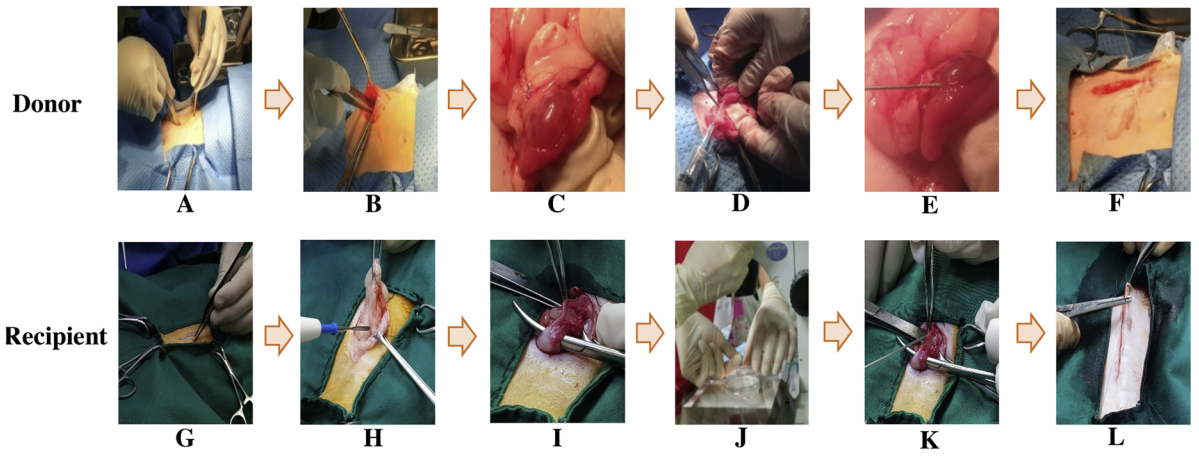

Fig. 1. Oocyte recovery and embryo transfer; Laparotomy and ovarian access of the donor and recipient (A–C, G–I); Oocyte aspiration in the donor (D–E); Abdominal sutures (F); Cloned embryo loading and transfer to the oviduct of the recipient (J–K) and abdominal sutures (L).

(ANOVA) in SPSS 18.0 (SPSS Inc., Chicago, IL, USA). Duncan's multiple range test was used to compare the groups. The probability of $P < 0.05$ was considered significant.

3. Results

3.1. Comparison of preimplantation development with use of SCNT and CICT

There was assessment of the efficiencies of the reconstructed embryos and confirmation of the cleavage of cloned embryos at 48 h and blastocyst developmental competence on day 8 of the culture period. With the used of CICT, fusion rate was greater than with use of SCNT (80.0 ± 4.8% compared with 67.8 ± 11.3%; Table 1). The 8 to 16-cell embryos had similar developmental rates with use of CICT and SCNT techniques (40.0 ± 4.0% compared with 44.0 ± 13.3%, respectively; Table 1). The percentage of cloned embryos that developed to the blastocyst stage was not different between the CICT and SCNT group (20.0 ± 2.0% compared with 13.5 ± 5.0%; Table 1).

3.2. Production and confirmation of cloned kittens

Cloned embryos ($n = 62$) derived with use of CICT techniques were transferred into five estrous-synchronized recipients, and 151 cloned embryos produced using the SCNT techniques were transferred to thirteen estrous-synchronized recipients. Three surrogate mothers were confirmed pregnant after transfer of SCNT-derived embryos, and two recipients were confirmed pregnant after transfer of CICT-derived embryos (2.6 ± 3.1% compared with 4.8 ± 2.3%, respectively) at 45 days from the time of initiation of embryo culturing (Table 2). There was birth of one live-born SCNT-derived kitten and three live-born CICT-derived kittens from surrogate queen cats (0.7 ± 1.3% compared with 4.8 ± 2.3%, respectively; Table 2). Of the three CICT-derived kittens, two were healthy and had normal behavior patterns, while one was weak and died within a few hours of birth (Fig. 2a). One initially healthy kitten later died due to insufficient management of nourishment by the surrogate queen (Fig. 2b). One CICT-derived kitten has remained healthy for about 1 ½ years and continues to be physically active with no indications of any abnormalities (Fig. 2c).

4. Discussion

Efforts have been focused on cloning of cats; however, the efficiency of production of SCNT-derived offspring has continued to be

Table 1
In vitro development of cloned embryos using different methods.

Groups	No. of cloned oocytes	No. (%) of fused embryos*	No. (%) ≥ 2–4 cell embryos**	No. (%) ≥ 8–16 cell embryos**	No. (%) of blastocysts***
SCNT	87	59 (67.8 ± 11.3)[a]	43 (72.8 ± 7.9) [a]	26 (44.0 ± 13.3) [a]	8 (13.5 ± 5.0) [a]
CICT	25	20 (80.0 ± 4.8) [a]	11 (55.0 ± 0.5) [a]	8 (40.0 ± 4.0) [a]	4 (20.0 ± 2.0) [a]

[a,b]Values with different superscripts in the same column differ ($P < 0.05$).
Data are expressed as the mean ± standard error of the mean (SEM).
* Fusion rates were calculated based on the number of cloned oocytes.
** Cleavage rates were calculated based on the number of fused embryos for SCNT and CICT.
*** Blastocyst development rates were calculated based on the number of fused embryos for SCNT and CICT.

Table 2
Birth of cloned embryos using different methods.

Groups	No. of cloned oocytes	No. (%) of fused embryos*	No. of transferred cloned embryos / recipients	No. (%) of implanted fetus** / recipients	No. (%) of cloned offspring from transferred embryos***
SCNT	254	173 (68.1 ± 6.0)[a]	151 / 13	4 (2.6 ± 3.1) [a] / 3	1 (0.7 ± 1.3)[a]
CICT	104	74 (71.2 ± 9.9)[a]	62 / 5	3 (4.8 ± 2.3) [a] / 2	3 (4.8 ± 2.3)[b]

[a,b]Values with different superscripts in the same column differ ($P < 0.05$).
Data are expressed as the mean ± standard error of the mean (SEM).
* Fusion rates were calculated based on the number of cloned oocytes.
** Implantation rates were calculated based on the number of transferred cloned embryos for SCNT and CICT.
*** Cloned offspring rates were calculated based on the number of transferred cloned embryos for SCNT and CICT.

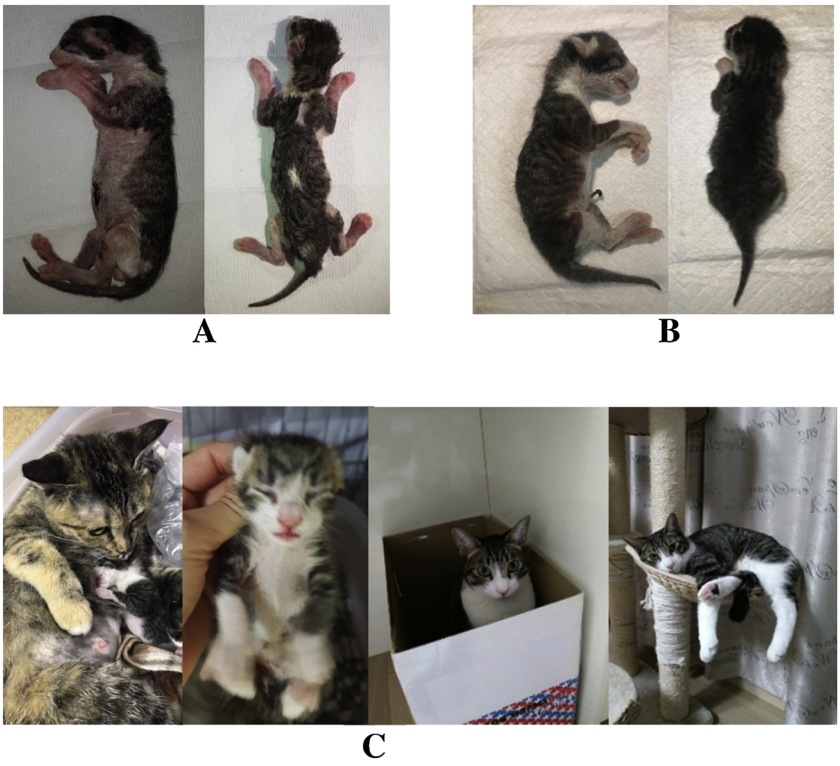

Fig. 2. Birth of cloned offspring after use of CICT for embryo production; (A)Weak kitten that died within a few hours of delivery, (B) Healthy kitten that died due to insufficient management by the surrogate mother, (C) Healthy kitten is still alive with no physical problems.

less than desired since the initial report of the birth of Dolly in 1996 (Campbell et al., 1996). To overcome this cloning inefficiency, several techniques, such as nuclear staining and handmade cloning techniques have been developed. With handmade cloning, however, zona-free embryos can easily be damaged during handling (Verma et al., 2015).

The CICT technologies were developed to overcome the shortfalls of traditional cloning methods (Xu et al., 2019). With use of the CICT technologies, there can be restoration of the cytoplasmic volume of enucleated oocytes without removing the zona pellucida and by retaining the zona pellucida there is protection from toxic substances in culture media compared with the use of handmade cloning methods. In addition, with the use of the CICT method the cytoplasm of one enucleated donor oocyte is transferred to three enucleated recipient oocytes. The CICT procedure is more efficient and effective for animals with which it is difficult to obtain oocytes. The CICT cloning technology was used for cats in the present study to enhance embryo development and production of viable offspring (Table 3).

Major factors in the efficiency of producing cloned embryos are oocyte quality, cell conditions, gene expression and methylation. Among these, reprogramming factors (gene expression and methylation) are very important when cloning is being attempted. Reprogramming factors are highly related to cytoplasmic volume (Beyhan et al., 2007; Hua et al., 2011; Liu et al., 2018). In a previous study where there was reconstruction of cytoplasmic volume, there were indications that fusion, cleavage and blastocyst formation rates were greater after using CICT than after using traditional cloning methods (Xu et al., 2019). Additionally, the expression of methylation-related genes, including DNMT 1, DNMT 3a and DNMT 3b, in CICT-derived embryos was greater than that

Table 3
In vivo development of embryos cloned using CICT in cats.

Recipients (R)	No. of CICT cloned embryos transferred	Pregnancy	Delivery (%) / at Days	Current status
R1	18	Yes	2 (11.1) / 66, 2 Live born*	2 Dead**
R2	9	No		
R3	9	No		
R4	14	Yes	1 (7.14) / 65, 1 Live born	1 Live
R5	12	No		
Total	62	3	3 (4.8)	

* After birth, one kitten was weak, while one kitten was healthy.
** The weak kitten died within a few hours of delivery, and the healthy kitten died due to insufficient management of nourishment by the surrogate mother.

when embryos were derived using traditional cloning technologies, indicating that with restoration of the cytoplasmic volume there is an increase in the demethylation of genes and reprogramming efficiency.

Feline cloning continues to be inefficient. To increase feline cloning efficiency, CICT was used in the present study to produce cloned embryos. The results indicated that with CICT-derived cat embryos, the fusion and blastocyst formation rates were greater than with use of traditional methods. These results indicate that adding cytoplasm contents into the enucleated cytoplasm led to an increased fusion rate, and in the donor cytoplast there was an enhanced adhesion between the enucleated oocytes and donor cells, and that the restored cytoplasm affected feline embryo development. As most embryos were used for embryo transfer to determine developmental competence, the numbers used in the *in vitro* study were insufficient for determining statistical differences. In previous studies, however, it has been reported that the cytoplasmic volume affected embryo development (Ribeiro et al., 2009; Panda et al., 2011). Furthermore, embryo development was related to amount of methylation (Kang et al., 2003; Reik et al., 2001), and the cytoplasmic volume affected epigenetic embryo reprogramming (Hua et al., 2011; Liu et al., 2018; Xu et al., 2019).

The CICT technique has been adapted for both *in vitro* development and for producing kittens. Results of previous studies indicated offspring birth rates were very low, which is consistent with results in other mammalian species (Lagutina et al., 2007; Wei et al., 2013). Birth rates using CICT were greater than with use of SCNT. With use of SCNT, the rates of fetal implantation were less than those with use of CICT and most fetuses died or were aborted during the gestation period, while there was one birth in the present study. With all CICT-derived fetuses for which there was implantation, there was birth of normal fetuses at the expected dates for these births. The data indicate that the restored cytoplasm positively affected the birth rate; however, further study is needed to validate the findings in the present study.

With the use of the CICT techniques, the cytoplasm of donor oocytes is artificially injected into the perivitelline space of enucleated oocytes. Because of imposing this methodology, some cloned embryos have heteroplasmy, which is characterized by the presence of more than one mitochondrial DNA (mtDNA) type within the embryo. Results of a previous study indicated that mtDNA heteroplasmy was related to a mitochondrial disease in humans (Wallace and Chalkia, 2013). Furthermore, mtDNA heteroplasmy was associated with a lesser physical activity, food intake and cognitive impairment in mice (Lane, 2012; Sharpley et al., 2012). To avoid mtDNA heteroplasmy in the present study, the homoplasmy state was induced by simultaneous sub-zonal microinjection of single nuclear donor somatic cells (NDSCs) and autologous (homoplasmic) cytoplasts derived from the same oocyte/NDSC donor female cat into the perivitelline space of enucleated oocytes and there was subsequent SV-mediated fusion of triple complexes comprised of such homoplasmic cytoplasts, enucleated oocytes and somatic cells. The birth rate results were that two of the three kittens were healthy and physically and behaviorally normal, but one kitten was weak with a small body size. The reason for the weak kitten development requires further study. Furthermore, cloned embryo studies involving homoplasmy and heteroplasmy in cats are needed for successful cloning.

5. Conclusions

In conclusion, the results of the present study indicate the use of CICT resulted in restoration of the cytoplasmic volume of reconstructed embryos and improved the production of kittens from cloned cat embryos. The results of this study may assist in producing cloned animals, especially for transgenic and endangered cats.

Authors' contributions

All authors contributed to this manuscript. Seok-Hwan Song, Kyeong-Lim Lee and Lianguang Xu designed and performed the experiments. Myeong-Don Joo, Ji-Yoon Hwang and Seon-Hwa Oh analyzed the data and assisted with culture media preparation. Il-Keun Kong wrote the manuscript and managed this work.

Ethics approval and consent to participate

The Gyeongsang National University Institute of Animal and Care and Use Committee (GNU-140922-T0049), Gyeongsang National University, Republic of Korea approved this study, which was conducted at the Animal Research Facility and Veterinary School of Gyeongsang National University, Republic of Korea.

Funding

This research was partly supported by a grant from the IPET through Agri-Bio Industry Technology Development Program, funded by MAFRA (Grant numbers: 117029-3 and 315017-5), Production of Cloned Cat; Jaerong (2017-0814). Seok-Hwan Song, Kyeong-Lim lee, Lianguang Xu, Myeong-Don Joo, Ji-Yoon Hwang and Seon-Hwa Oh were supported by scholarships from the BK21 Plus Program.

References

Abe, H., Yamashita, S., Satoh, T., Hoshi, H., 2002. Accumulation of cytoplasmic lipid droplets in bovine embryos and cryotolerance of embryos developed in different culture systems using serum-free or serum-containing media. Mol. Reprod. Dev. 61, 57–66.

Azuma, R., Miyamoto, K., Oikawa, M., Yamada, M., Anzai, M., 2018. Combinational treatment of trichostatin A and vitamin C improves the efficiency of cloning mice by somatic cell nuclear transfer. J. Vis. Exp. 134. https://doi.org/10.3791/57036.

Beyhan, Z., Forsberg, E.J., Eilertsen, K.J., Kent-First, M., First, N.L., 2007. Gene expression in bovine nuclear transfer embryos in relation to donor cell efficiency in producing live offspring. Mol. Reprod. Dev. 74, 18–27.

Campbell, K.H., McWhir, J., Ritchie, W.A., Wilmut, I., 1996. Sheep cloned by nuclear transfer from a cultured cell line. Nature 380, 64–66.

Choi, E.G., Yin, X.J., Lee, H.S., Kim, L.H., Shin, H.D., Kim, N.H., Kong, I.K., 2007. Reproductive fertility of cloned male cats derived from adult somatic cell nuclear transfer. Cloning Stem Cells 9, 281–290.

de Macedo, M.P., Glanzner, W.G., Rissi, V.B., Gutierrez, K., Currin, L., Baldassarre, H., Bordignon, V., 2019. A fast and reliable protocol for activation of porcine oocytes. Theriogenology 123, 22–29.

Deng, S., Li, G., Zhang, J., Zhang, X., Cui, M., Guo, Y., Liu, G., Li, G., Feng, J., Lian, Z., 2013. Transgenic cloned sheep overexpressing ovine toll-like receptor 4. Theriogenology 80, 50–57.

Du, Y., Kragh, P.M., Zhang, Y., Li, J., Schmidt, M., Bogh, I.B., Zhang, X., Purup, S., Jorgensen, A.L., Pedersen, A.M., Villemoes, K., Yang, H., Bolund, L., Vajta, G., 2007. Piglets born from handmade cloning, an innovative cloning method without micromanipulation. Theriogenology 68, 1104–1110.

Feng, X., Cao, S., Wang, H., Meng, C., Li, J., Jiang, J., Qian, Y., Su, L., He, Q., Zhang, Q., 2015. Production of transgenic dairy goat expressing human alpha-lactalbumin by somatic cell nuclear transfer. Transgenic Res. 24, 73–85.

Fernandes, C.B., Devito, L.G., Martins, L.R., Blanco, I.D., de Lima Neto, J.F., Tsuribe, P.M., Goncalves, C.G., da Cruz Landim-Alvarenga, F., 2014. Artificial activation of bovine and equine oocytes with cycloheximide, roscovitine, strontium, or 6-dimethylaminopurine in low or high calcium concentrations. Zygote 22, 387–394.

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

Gao, R., Wang, C., Gao, Y., Xiu, W., Chen, J., Kou, X., Zhao, Y., Liao, Y., Bai, D., Qiao, Z., Yang, L., Wang, M., Zang, R., Liu, X., Jia, Y., Li, Y., Zhang, Y., Yin, J., Wang, H., Wan, X., Liu, W., Zhang, Y., Gao, S., 2018. Inhibition of aberrant DNA Re-methylation improves post-implantation development of somatic cell nuclear transfer embryos. Cell Stem Cell 23, 426–435 e5.

Gil, M.A., Maside, C., Cuello, C., Parrilla, I., Vazquez, J.M., Roca, J., Martinez, E.A., 2012. Effects of Hoechst 33342 staining and ultraviolet irradiation on mitochondrial distribution and DNA copy number in porcine oocytes and preimplantation embryos. Mol. Reprod. Dev. 79, 651–663.

Gomez, M.C., Pope, C.E., Kutner, R.H., Ricks, D.M., Lyons, L.A., Ruhe, M., Dumas, C., Lyons, J., Lopez, M., Dresser, B.L., Reiser, J., 2008. Nuclear transfer of sand cat cells into enucleated domestic cat oocytes is affected by cryopreservation of donor cells. Cloning Stem Cells 10, 469–483.

Guo, Y., Li, H., Wang, Y., Yan, X., Sheng, X., Chang, D., Qi, X., Wang, X., Liu, Y., Li, J., Ni, H., 2017. Screening somatic cell nuclear transfer parameters for generation of transgenic cloned cattle with intragenomic integration of additional gene copies that encode bovine adipocyte-type fatty acid-binding protein (A-FABP). Mol. Biol. Rep. 44, 159–168.

Hinrichs, K., Choi, Y.H., Varner, D.D., Hartman, D.L., 2007. Production of cloned horse foals using roscovitine-treated donor cells and activation with sperm extract and/or ionomycin. Reproduction 134, 319–325.

Hong, S.G., Jang, G., Kim, M.K., Oh, H.J., Park, J.E., Kang, J.T., Koo, O.J., Kim, D.Y., Lee, B.C., 2009. Dogs cloned from fetal fibroblasts by nuclear transfer. Anim. Reprod. Sci. 115, 334–339.

Hoshino, Y., Hayashi, N., Taniguchi, S., Kobayashi, N., Sakai, K., Otani, T., Iritani, A., Saeki, K., 2009. Resurrection of a bull by cloning from organs frozen without cryoprotectant in a -80 degrees c freezer for a decade. PLoS One 4, e4142.

Hua, S., Zhang, H., Su, J.M., Zhang, T., Quan, F.S., Liu, J., Wang, Y.S., Zhang, Y., 2011. Effects of the removal of cytoplasm on the development of early cloned bovine embryos. Anim. Reprod. Sci. 126, 37–44.

Hua, S., Zhang, Z., Zhang, C., Zhang, Y., 2007. An improved enucleation method of bovine somatic cell nuclear transfer. J. Genet. Genomics 34, 491–496.

Jang, G., Hong, S.G., Oh, H.J., Kim, M.K., Park, J.E., Kim, H.J., Kim, D.Y., Lee, B.C., 2008. A cloned toy poodle produced from somatic cells derived from an aged female dog. Theriogenology 69, 556–563.

Jin, L., Guo, Q., Zhang, G.L., Xing, X.X., Xuan, M.F., Luo, Q.R., Luo, Z.B., Wang, J.X., Yin, X.J., Kang, J.D., 2018. The histone deacetylase inhibitor, CI994, improves nuclear reprogramming and in vitro developmental potential of cloned pig embryos. Cell. Reprogram. 20, 205–213.

Jin, Y.X., Cui, X.S., Yu, X.F., Lee, S.H., Wang, Q.L., Gao, W.W., Xu, Y.N., Sun, S.C., Kong, I.K., Kim, N.H., 2012. Cat fertilization by mouse sperm injection. Zygote 20, 371–378.

Kang, Y.K., Lee, K.K., Han, Y.M., 2003. Reprogramming DNA methylation in the preimplantation stage: peeping with Dolly's eyes. Curr. Opin. Cell Biol. 15, 290–295.

Kim, E.Y., Park, M.J., Park, H.Y., Noh, E.J., Noh, E.H., Park, K.S., Lee, J.B., Jeong, C.J., Riu, K.Z., Park, S.P., 2012. Improved cloning efficiency and developmental potential in bovine somatic cell nuclear transfer with the oosight imaging system. Cell. Reprogram. 14, 305–311.

Kim, M.K., Jang, G., Oh, H.J., Yuda, F., Kim, H.J., Hwang, W.S., Hossein, M.S., Kim, J.J., Shin, N.S., Kang, S.K., Lee, B.C., 2007. Endangered wolves cloned from adult somatic cells. Cloning Stem Cells 9, 130–137.

Kishigami, S., Wakayama, S., Thuan, N.V., Ohta, H., Mizutani, E., Hikichi, T., Bui, H.T., Balbach, S., Ogura, A., Boiani, M., Wakayama, T., 2006. Production of cloned mice by somatic cell nuclear transfer. Nat. Protoc. 1, 125–138.

Kragh, P.M., Du, Y., Corydon, T.J., Purup, S., Bolund, L., Vajta, G., 2005. Efficient in vitro production of porcine blastocysts by handmade cloning with a combined electrical and chemical activation. Theriogenology 64, 1536–1545.

Kwon, D.J., Kim, D.H., Hwang, I.S., Kim, D.E., Kim, H.J., Kim, J.S., Lee, K., Im, G.S., Lee, J.W., Hwang, S., 2017. Generation of alpha-1,3-galactosyltransferase knocked-out transgenic cloned pigs with knocked-in five human genes. Transgenic Res. 26, 153–163.

Kwon, S., Jeong, S., Park, J.S., Kang, Y.K., 2015. Quantifying difference in gene expression profile between bovine blastocysts derived by in vitro fertilization and somatic cell nuclear transfer. Gene Expr. Patterns 19, 14–20.

Lagutina, I., Lazzari, G., Duchi, R., Turini, P., Tessaro, I., Brunetti, D., Colleoni, S., Crotti, G., Galli, C., 2007. Comparative aspects of somatic cell nuclear transfer with conventional and zona-free method in cattle, horse, pig and sheep. Theriogenology 67, 90–98.

Lane, N., 2012. The problem with mixing mitochondria. Cell 151, 246–248.

Lee, H.S., Yu, X.F., Bang, J.I., Cho, S.J., Deb, G.K., Kim, B.W., Kong, I.K., 2010. Enhanced histone acetylation in somatic cells induced by a histone deacetylase inhibitor improved inter-generic cloned leopard cat blastocysts. Theriogenology 74, 1439–1449.

Liu, X., Luo, C., Deng, K., Wu, Z., Wei, Y., Jiang, J., Lu, F., Shi, D., 2018. Cytoplasmic volume of recipient oocytes affects the nucleus reprogramming and the developmental competence of HMC buffalo (Bubalus bubalis) embryos. J. Vet. Med. Sci. 80, 1291–1300.

Loi, P., Iuso, D., Czernik, M., Ogura, A., 2016. A New, Dynamic Era for Somatic Cell Nuclear Transfer? Trends Biotechnol. 34, 791–797.

Lu, F., Luo, C., Li, N., Liu, Q., Wei, Y., Deng, H., Wang, X., Li, X., Jiang, J., Deng, Y., Shi, D., 2018. Efficient generation of transgenic buffalos (Bubalus bubalis) by nuclear transfer of fetal fibroblasts expressing enhanced green fluorescent protein. Sci. Rep. 8 6967-018-25120-5.

Luo, Y., Wang, Y., Liu, J., Lan, H., Shao, M., Yu, Y., Quan, F., Zhang, Y., 2015. Production of transgenic cattle highly expressing human serum albumin in milk by phiC31 integrase-mediated gene delivery. Transgenic Res. 24, 875–883.
Ma, J., Li, Q., Li, Y., Wen, X., Li, Z., Zhang, Z., Zhang, J., Yu, Z., Li, N., 2016. Expression of recombinant human alpha-lactalbumin in milk of transgenic cloned pigs is sufficient to enhance intestinal growth and weight gain of suckling piglets. Gene 584, 7–16.
Meng, Q., Polgar, Z., Liu, J., Dinnyes, A., 2009. Live birth of somatic cell-cloned rabbits following trichostatin A treatment and cotransfer of parthenogenetic embryos. Cloning Stem Cells 11, 203–208.
Mesalam, A., Lee, K.L., Khan, I., Chowdhury, M.M.R., Zhang, S., Song, S.H., Joo, M.D., Lee, J.H., Jin, J.I., Kong, I.K., 2018. A combination of bovine serum albumin with insulin-transferrin-sodium selenite and/or epidermal growth factor as alternatives to fetal bovine serum in culture medium improves bovine embryo quality and trophoblast invasion by induction of matrix metalloproteinases. Reprod. Fertil. Dev. 31 (2), 333–346.
Niemann, H., 2016. Epigenetic reprogramming in mammalian species after SCNT-based cloning. Theriogenology 86, 80–90.
Olivera, R., Moro, L.N., Jordan, R., Pallarols, N., Guglielminetti, A., Luzzani, C., Miriuka, S.G., Vichera, G., 2018. Bone marrow mesenchymal stem cells as nuclear donors improve viability and health of cloned horses. Stem Cells Cloning 11, 13–22.
Opiela, J., Samiec, M., Romanek, J., 2017. In vitro development and cytological quality of inter-species (porcine–&bovine) cloned embryos are affected by trichostatin A-dependent epigenomic modulation of adult mesenchymal stem cells. Theriogenology 97, 27–33.
Panda, S.K., George, A., Saha, A.P., Sharma, R., Manik, R.S., Chauhan, M.S., Palta, P., Singla, S.K., 2011. Effect of cytoplasmic volume on developmental competence of buffalo (Bubalus bubalis) embryos produced through hand-made cloning. Cell. Reprogram. 13, 257–262.
Peura, T.T., Lewis, I.M., Trounson, A.O., 1998. The effect of recipient oocyte volume on nuclear transfer in cattle. Mol. Reprod. Dev. 50, 185–191.
Reik, W., Dean, W., Walter, J., 2001. Epigenetic reprogramming in mammalian development. Science 293, 1089–1093.
Ribeiro, E.S., Gerger, R.P., Ohlweiler, L.U., Ortigari Jr, I., Mezzalira, J.C., Forell, F., Bertolini, L.R., Rodrigues, J.L., Ambrosio, C.E., Miglino, M.A., Mezzalira, A., Bertolini, M., 2009. Developmental potential of bovine hand-made clone embryos reconstructed by aggregation or fusion with distinct cytoplasmic volumes. Cloning Stem Cells 11, 377–386.
Saini, M., Selokar, N.L., 2018. Approaches used to improve epigenetic reprogramming in buffalo cloned embryos. Indian J. Med. Res. 148, S115–S119.
Samiec, M., 2005. The effect of mitochondrial genome on architectural remodeling and epigenetic reprogramming of donor cell nuclei in mammalian nuclear transfer-derived embryos. J. Anim. Feed Sci. 14 (3), 393–422.
Samiec, M., Skrzyszowska, M., 2012a. High developmental capability of porcine cloned embryos following trichostatin A-dependent epigenomic transformation during *in vitro* maturation of oocytes pre-exposed to *R*-roscovitine. Anim. Sci. Pap. Rep. 30 (4), 383–393.
Samiec, M., Skrzyszowska, M., 2018a. Can reprogramming of overall epigenetic memory and specific parental genomic imprinting memory within donor cell-inherited nuclear genome be a major hindrance for the somatic cell cloning of mammals? – a review. Ann. Anim. Sci. 18 (3), 623–638.
Samiec, M., Opiela, J., Lipinski, D., Romanek, J., 2015. Trichostatin A-mediated epigenetic transformation of adult bone marrow-derived mesenchymal stem cells biases the in vitro developmental capability, quality, and pluripotency extent of porcine cloned embryos. Biomed Res. Int. 2015, 814686.
Samiec, M., Skrzyszowska, M., 2011a. The possibilities of practical application of transgenic mammalian species generated by somatic cell cloning in pharmacology, veterinary medicine and xenotransplantology. Pol. J. Vet. Sci. 14, 329–340.
Samiec, M., Skrzyszowska, M., 2011b. Transgenic mammalian species, generated by somatic cell cloning, in biomedicine, biopharmaceutical industry and human nutrition/dietetics–recent achievements. Pol. J. Vet. Sci. 14, 317–328.
Samiec, M., Skrzyszowska, M., 2012b. Roscovitine is a novel agent that can be used for the activation of porcine oocytes reconstructed with adult cutaneous or fetal fibroblast cell nuclei. Theriogenology 78, 1855–1867.
Samiec, M., Skrzyszowska, M., 2014. Biological transcomplementary activation as a novel and effective strategy applied to the generation of porcine somatic cell cloned embryos. Reprod. Biol. 14, 128–139.
Samiec, M., Skrzyszowska, M., 2018b. Intrinsic and extrinsic molecular determinants or modulators for epigenetic remodeling and reprogramming of somatic cell-derived genome in mammalian nuclear-transferred oocytes and resultant embryos. Pol. J. Vet. Sci. 21, 217–227.
Sayaka, W., Satoshi, K., Van Thuan, N., Hiroshi, O., Takafusa, H., Eiji, M., Thuy, B.H., Masashi, M., Teruhiko, W., 2008. Effect of volume of oocyte cytoplasm on embryo development after parthenogenetic activation, intracytoplasmic sperm injection, or somatic cell nuclear transfer. Zygote 16, 211–222.
Sepulveda-Rincon, L.P., Solanas Edel, L., Serrano-Revuelta, E., Ruddick, L., Maalouf, W.E., Beaujean, N., 2016. Early epigenetic reprogramming in fertilized, cloned, and parthenogenetic embryos. Theriogenology 86, 91–98.
Sharpley, M.S., Marciniak, C., Eckel-Mahan, K., McManus, M., Crimi, M., Waymire, K., Lin, C.S., Masubuchi, S., Friend, N., Koike, M., Chalkia, D., MacGregor, G., Sassone-Corsi, P., Wallace, D.C., 2012. Heteroplasmy of mouse mtDNA is genetically unstable and results in altered behavior and cognition. Cell 151, 333–343.
Skrzyszowska, M., Smorag, Z., Slomski, R., Katska-Ksiazkiewicz, L., Kalak, R., Michalak, E., Wielgus, K., Lehmann, J., Lipinski, D., Szalata, M., Plawski, A., Samiec, M., Jura, J., Gajda, B., Rynska, B., Pienkowski, M., 2006. Generation of transgenic rabbits by the novel technique of chimeric somatic cell cloning. Biol. Reprod. 74, 1114–1120.
Sturmey, R.G., O'Toole, P.J., Leese, H.J., 2006. Fluorescence resonance energy transfer analysis of mitochondrial:lipid association in the porcine oocyte. Reproduction 132, 829–837.
Vajta, G., Bartels, P., Joubert, J., de la Rey, M., Treadwell, R., Callesen, H., 2004. Production of a healthy calf by somatic cell nuclear transfer without micro-manipulators and carbon dioxide incubators using the Handmade Cloning (HMC) and the Submarine Incubation System (SIS). Theriogenology 62, 1465–1472.
Verma, G., Arora, J.S., Sethi, R.S., Mukhopadhyay, C.S., Verma, R., 2015. Handmade cloning: recent advances, potential and pitfalls. J. Anim. Sci. Biotechnol. 6 43-015-0043-y. eCollection 2015.
Wallace, D.C., Chalkia, D., 2013. Mitochondrial DNA genetics and the heteroplasmy conundrum in evolution and disease. Cold Spring Harb Perspect. Biol. 5, a021220.
Wang, Y., Ding, F., Wang, T., Liu, W., Lindquist, S., Hernell, O., Wang, J., Li, J., Li, L., Zhao, Y., Dai, Y., Li, N., 2017. Purification and characterization of recombinant human bile salt-stimulated lipase expressed in milk of transgenic cloned cows. PLoS One 12, e0176864.
Wei, H., Qing, Y., Pan, W., Zhao, H., Li, H., Cheng, W., Zhao, L., Xu, C., Li, H., Li, S., Ye, L., Wei, T., Li, X., Fu, G., Li, W., Xin, J., Zeng, Y., 2013. Comparison of the efficiency of Banna miniature inbred pig somatic cell nuclear transfer among different donor cells. PLoS One 8, e57728.
Whitworth, K.M., Li, R., Spate, L.D., Wax, D.M., Rieke, A., Whyte, J.J., Manandhar, G., Sutovsky, M., Green, J.A., Sutovsky, P., Prather, R.S., 2009. Method of oocyte activation affects cloning efficiency in pigs. Mol. Reprod. Dev. 76, 490–500.
Whitworth, K.M., Prather, R.S., 2010. Somatic cell nuclear transfer efficiency: how can it be improved through nuclear remodeling and reprogramming? Mol. Reprod. Dev. 77, 1001–1015.
Xu, L., Mesalam, A., Lee, K.L., Song, S.H., Khan, I., Chowdhury, M.M.R., Lv, W., Kong, I.K., 2019. Improves the in vitro developmental competence and reprogramming efficiency of cloned bovine embryos by additional complimentary cytoplasm. Cell. Reprogram. 21, 51–60.
Yang, B.Z., Yang, C.Y., Li, R.C., Qin, G.S., Zhang, X.F., Pang, C.Y., Chen, M.T., Huang, F.X., Li, Z., Zheng, H.Y., Huang, Y.J., Liang, X.W., 2010. An inter-subspecies cloned buffalo (Bubalus bubalis) obtained by transferring of cryopreserved embryos via somatic cell nuclear transfer. Reprod. Domest. Anim. 45, e21–5.
Yin, X.J., Lee, H.S., Kim, L.H., Shin, H.D., Kim, N.H., Kong, I.K., 2007. Effect of serum starvation on the efficiency of nuclear transfer using odd-eyed white cat fibroblasts. Theriogenology 67, 816–823.
Yin, X.J., Lee, H.S., Lee, Y.H., Seo, Y.I., Jeon, S.J., Choi, E.G., Cho, S.J., Cho, S.G., Min, W., Kang, S.K., Hwang, W.S., Kong, I.K., 2005. Cats cloned from fetal and adult somatic cells by nuclear transfer. Reproduction 129, 245–249.
Yin, X.J., Lee, H.S., Yu, X.F., Choi, E., Koo, B.C., Kwon, M.S., Lee, Y.S., Cho, S.J., Jin, G.Z., Kim, L.H., Shin, H.D., Kim, T., Kim, N.H., Kong, I.K., 2008a. Generation of cloned transgenic cats expressing red fluorescence protein. Biol. Reprod. 78, 425–431.
Yin, X.J., Lee, H.S., Yu, X.F., Kim, L.H., Shin, H.D., Cho, S.J., Choi, E.G., Kong, I.K., 2008b. Production of second-generation cloned cats by somatic cell nuclear transfer. Theriogenology 69, 1001–1006.
Yu, X.F., Cho, S.J., Bang, J.I., Lee, H.S., Lee, Y.S., Kwon, T.H., Deb, G.K., Kong, I.K., 2010. Effect of equine chorionic gonadotropin on the efficiency of superovulation induction for in vivo and in vitro embryo production in the cat. Theriogenology 73, 413–420.
Zhang, P., Liu, P., Dou, H., Chen, L., Chen, L., Lin, L., Tan, P., Vajta, G., Gao, J., Du, Y., Ma, R.Z., 2013. Handmade cloned transgenic sheep rich in omega-3 Fatty acids.

PLoS One 8, e55941.
Zhao, J., Ross, J.W., Hao, Y., Spate, L.D., Walters, E.M., Samuel, M.S., Rieke, A., Murphy, C.N., Prather, R.S., 2009. Significant improvement in cloning efficiency of an inbred miniature pig by histone deacetylase inhibitor treatment after somatic cell nuclear transfer. Biol. Reprod. 81, 525–530.
Zhou, Q., Renard, J.P., Le Friec, G., Brochard, V., Beaujean, N., Cherifi, Y., Fraichard, A., Cozzi, J., 2003. Generation of fertile cloned rats by regulating oocyte activation. Science 302, 1179.
Zhou, Z.R., Zhong, B.S., Jia, R.X., Wan, Y.J., Zhang, Y.L., Fan, Y.X., Wang, L.Z., You, J.H., Wang, Z.Y., Wang, F., 2013. Production of myostatin-targeted goat by nuclear transfer from cultured adult somatic cells. Theriogenology 79, 225–233.