In Vitro Development and Postvitrification Survival of Cloned Feline Embryos Derived from Preadipocytes

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Abstract. The aim of the present study was to optimize the conditions for in vitro development and postvitrification survival of somatic cell cloned feline embryos. To determine the effects of cell cycle synchronization of the nuclear donor cells, we cultured preadipocytes under serum starvation or conventional conditions. After two days in serum starvation culture, the proportion of synchronized donor cells at the G0/G1 phase was 91.6%. This was significantly higher than the proportion of non-synchronized cells in the proliferative phase (72.6%, P<0.05). The in vitro development of somatic cell nuclear transfer (SCNT) embryos reconstructed using donor cells treated under serum starvation conditions (normal cleavage rate of 65.7%, 46/70, and blastocyst formation rate of 20.0%, 14/70) was comparable to that of the serum supplemented group (52.5%, 31/59, and 20.3%, 12/59). Use of in vitro or in vivo matured oocytes as recipient cytoplasts equally supported development of the SCNT embryos to the blastocyst stage (11.9%, 5/42, vs. 9.5%, 2/21). SCNT-derived blastocysts were vitrified using the original minimum volume cooling (MVC) or the modified (stepwise) MVC method. Although none (n=10) of the SCNT blastocysts survived following vitrification by the original MVC method, the stepwise MVC method resulted in 100% survival after rewarming (n=11). In conclusion, we demonstrated that feline somatic cell cloned embryos with a high developmental ability can be produced irrespective of cell cycle synchronization of donor cells using either in vitro or in vivo matured oocytes. Furthermore, by utilizing a stepwise vitrification method, we showed that it is possible to cryopreserve cloned feline blastocysts.

Key words: Cryopreservation, Domestic cat, Preadipocyte, Somatic cell nuclear transfer, Vitrification (J. Reprod. Dev. 57: 273–279, 2011)

In 1997, the world’s first cloned sheep was produced by somatic cell nuclear transfer (SCNT) [1]. Since then, cloned animals have been reported in other species such as the mouse [2], cow [3], goat [4], pig [5] and rabbit [6]. In 2002, cats became the first carnivorous species to be cloned [7]. Feline SCNT techniques can be applied to the conservation of endangered wild feline species [8] or the preparation of feline embryonic stem cells (ES cells) [9]. However, the current utility of SCNT in such applications is limited because the production efficiency of cloned cats is low [7, 8, 10–12]. Thus, it is essential to establish a more efficient system to promote the use of SCNT in cats.

Several factors such as donor cell type [13–15], cell cycle synchronization [16–18] and recipient oocyte origin (in vitro or in vivo matured oocytes) [19, 20] are known to affect SCNT efficiency and subsequent embryonic development in many animal species. However, there have been few studies investigating such factors in cats [10, 11, 21, 22], so further research is necessary. We had previously demonstrated that cloned piglets could be efficiently obtained using preadipocytes (derived from mature adipocytes) as donor cells in porcine SCNT [23, 24]. Therefore, we selected preadipocytes as donor cells to perform feline SCNT in this study.

To produce cloned cats, it is necessary to transfer numerous embryos into a female cat [8]. Unlike livestock species with which ovaries can be easily obtained from slaughterhouses, it is difficult to obtain cat ovaries. Therefore, it is difficult to prepare a sufficient number of SCNT embryos for production of cloned cats. In addition, cats breed seasonally, and there is no established method for artificially controlling the estrous cycle of the cat, making it difficult to obtain female cats that are suitable as surrogate recipients. These are several factors that contribute to the difficulty of cloning cats. However, if proper techniques for cryopreserving SCNT embryos can be established so that embryos can be stored until an appropriate time, the efficiency of producing cloned cats will be improved. So far, there have been no reports on the cryopreservation of feline SCNT embryos. Furthermore, production of cats from cryopreserved in vitro fertilized embryos has had limited success [25, 26].

Cryopreservation of porcine embryos, which had been difficult to perform [27], was made possible through improved vitrification methods. In fact, we showed that by applying the minimum volume cooling (MVC) method, in vitro matured-fertilized embryos and cloned embryos could be cryopreserved [28, 29]. Based on these findings in pigs, we postulated that the MVC method would also be useful for cryopreserving feline SCNT embryos.

In this study, we investigated the effects of cell cycle synchroni-
ization of feline preadipocytes on the development of SCNT embryos. We also compared the usage of two types of recipient oocytes, i.e., in vivo and in vitro matured oocytes. Lastly, we vitrified feline SCNT embryos and determined their in vitro development after rewarming.

Materials and Methods

Animal care

All of the animal experiments in this study were approved by the Institutional Animal Care and Use Committee of Meiji University (IACUC 05-001). The cats used for this study (Narc:Catus, Narc, Chiba, Japan) were housed under controlled light (12-h light/12-h dark) and temperature (22 ± 3 °C) in a facility that specializes in contract breeding of experimental animals (Narc). These cats were supplied with water ad libitum and food once a day.

Chemicals

All chemicals were obtained from Sigma Chemical (St. Louis, MO, USA) unless otherwise indicated.

Preparation of preadipocytes

A primary culture of preadipocytes was established as previously reported [23, 30]. Subcutaneous fat tissue was obtained from a 9-month-old female cat by abdominal incision under general anesthesia with intravenous injection of propofol (8 mg/kg, Rapinovet; Intervet, Osaka, Japan) and inhalation of isoflurane (1.0–1.5% O2, Forane; Abbott Japan, Tokyo, Japan). Fat tissue was washed with Dulbecco’s phosphate-buffered saline (PBS; Nissui Pharmaceutical, Tokyo, Japan) and supplemented with 0.1% (w/v) polyvinyl alcohol (PVA), 75 μg/ml penicillin G and 50 μg/ml streptomycin. Connective tissue surrounding the fat tissue was removed using sterile scissors. Small pieces of fat tissue were then excised and incubated in Dulbecco’s Modified Eagle’s Medium (DMEM) containing 10% (v/v) fetal bovine serum (FBS; JRH Biosciences, Lenexa, KS, USA). 20% (v/v) porcine follicular fluid and 0.3% (w/v) BSA for 24–28 h in a humidified atmosphere of 5% CO2 in air at 37 °C.

Preparation of recipient oocytes

Feline ovaries were obtained from local veterinary clinics following routine ovariohysterectomy. The ovary donors included American Shorthair, Russian Blue, Siamese and crossbred cats of unknown backgrounds (>80%). The ages of these cats ranged from 5 months to 2 years and 4 months. Collected ovaries were transported to the laboratory within 2–5 h in PBS containing 0.1% (w/v) PVA, 75 μg/ml penicillin G and 50 μg/ml streptomycin at 30–38 °C. The ovaries were washed several times in PBS and minced with a scalpel blade in order to release cumulus-oocyte complexes (COCs). COCs with several layers of compact cumulus cells were selected and cultured in TC199 medium (Nissui Pharmaceutical) supplemented with 0.36 mM pyruvate, 1.12 mM cysteine, 25 ng/ml EGF, 0.5 IU/ml eCG (ASKA Pharmaceutical, Tokyo, Japan), 1 IU/ml hCG (ASKA Pharmaceutical), 1 μg/ml estradiol, 10% (v/v) porcine follicular fluid and 0.3% (w/v) BSA for 24–28 h in a humidified atmosphere of 5% CO2 in air at 38 °C.

In vivo matured oocytes were collected from sexually mature female cats (age: 3 years and 9 months to 4 years). Female cats without any indication of estrus for at least three weeks were selected, and superovulation was carried out using the following methods: follicular growth was induced by injecting 100 IU of eCG (ASKA Pharmaceutical) intramuscularly, followed by 50 IU of eCG intramuscularly 24 h later. At 120 h after the first eCG injection, 100 IU of hCG (Kawasaki Pharmaceutical, Tokyo, Japan) was injected intravenously. At 23 ± 1 h after hCG administration, the ovaries were surgically excised, and in vivo matured oocytes were collected by mincing ovarian tissue with a scalpel blade as
described above. Oocytes having an expanded cumulus layer and a first polar body were used for the experiments.

**Nuclear transfer**

Nuclear transfer of feline preadipocytes was performed according to the method used previously for porcine SCNT, with slight modification [23, 32–34]. Oocytes matured in vivo or in vitro were treated with 1 mg/ml hyaluronidase and denuded of cumulus cells by pipetting. Matured oocytes that contained a first polar body were enucleated by aspirating the polar body and adjacent cytoplasm using a beveled pipette (diameter, 27–30 μm).  Enucleation was confirmed by staining the cytoplasts with 0.1 mM MgSO4 and 0.01% (w/v) PVA and held between two electrode needles. Membrane fusion was induced with a somatic hybridizer (ET3-AC; Fujihira Industry, Tokyo, Japan) by applying two 40-μsec direct current (DC) pulses of 300 V/mm at intervals of 0.1 sec were applied to oocytes or SCNT embryos using an electrical pulsing machine (ET3-AC). This was followed by treatment with 10 μg/ml cycloheximide and 5 μg/ml CB for 5 h.

**In vitro culture of embryos**

In vitro culture of SCNT or parthenogenetic embryos was performed on a plastic Petri dish in 20-μl droplets of PZM-5 medium under paraffin oil. The dish was maintained under a humidified atmosphere of 5% CO2, 5% O2 and 90% N2 at 38 C. After 96 h, 2-μl (10%) FBS was added to each culture droplet. During the culture period for 7–8 days, embryos were monitored for cleavage on Days 1 and 2 and for blastocyst formation on Days 5 to 8.

**Vitrification of embryos**

Cryopreservation of embryos was carried out by vitrification using the MVC method [35] or the modified MVC method (stepwise MVC, Table 1). Control parthenogenetic embryos and SCNT embryos that were reconstructed with in vitro matured oocytes and cell cycle synchronized or nonsynchronized cells were vitrified at the blastocyst stage after culture for 6–7 or 5–6 days, respectively. All solutions used during vitrification and rewarming were prepared with a basal medium composed of TCM199 containing 20 mM Hepes, 4.2 mM NaHCO3, 75 μg/ml potassium penicillin G, 50 μg/ml streptomycin sulfate and 20% (v/v) calf serum (CS; catalog no. 12133–500M, lot no. 40214; JRH Biosciences).

For the MVC method, embryos were kept for 4 min in equilibration solution containing 7.5% (v/v) ethylene glycol (EG; Nacalai Tesque) and 7.5% (v/v) dimethyl sulfoxide (DMSO; Wako Pure Chemical) followed by exposure to a vitrification solution containing 15% EG, 15% DMSO and 0.5 M sucrose (Nacalai Tesque). Embryos were then loaded onto an MVC plate (Cryotop; Kitazato Supply, Shizuoka, Japan) and immediately plunged into liquid nitrogen (LN). The whole process, from exposure of the embryos to a vitrification solution to plunging, was completed within 1 min. Embryos were rewarmed by immersing the MVC plate directly into

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**Table 1. Vitrification protocol for feline parthenogenetic and cloned embryos**

<table>
<thead>
<tr>
<th>Stepwise MVC method</th>
<th>MVC method</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Equilibration</strong></td>
<td>4 min</td>
</tr>
<tr>
<td>ES: 7.5% EG, 7.5% DMSO</td>
<td>ES-1: 7.5% EG, 7.5% DMSO</td>
</tr>
<tr>
<td>1 min</td>
<td>ES-2: 7.5% EG, 7.5% DMSO, 0.25M sucrose</td>
</tr>
<tr>
<td>1 min</td>
<td>ES-3: 7.5% EG, 7.5% DMSO, 0.5M sucrose</td>
</tr>
<tr>
<td><strong>Vitrification</strong></td>
<td>1 min</td>
</tr>
<tr>
<td>VS: 15% EG, 15% DMSO, 0.5M sucrose</td>
<td>Plunging into liquid nitrogen</td>
</tr>
<tr>
<td>Plunging into liquid nitrogen</td>
<td></td>
</tr>
<tr>
<td><strong>Thawing</strong></td>
<td>1 min</td>
</tr>
<tr>
<td>TS: 1M sucrose</td>
<td>TS: 1M sucrose</td>
</tr>
<tr>
<td><strong>Dilution</strong></td>
<td>3 min</td>
</tr>
<tr>
<td>DS: 0.5M sucrose</td>
<td>DS-1: 0.5M sucrose</td>
</tr>
<tr>
<td>2 min</td>
<td>DS-2: 0.25M sucrose</td>
</tr>
<tr>
<td><strong>Washing</strong></td>
<td>5 min</td>
</tr>
<tr>
<td>WS-1: basal medium</td>
<td>WS-1: basal medium</td>
</tr>
<tr>
<td>WS-2: basal medium</td>
<td>WS-2: basal medium</td>
</tr>
</tbody>
</table>

rewarming solution containing 1 M sucrose at 37 °C for 1 min. Recovered embryos were transferred to a dilution solution containing 0.5 M sucrose for 3 min. They were transferred to a washing solution for 10 min to remove the cryoprotectant. All solutions, except for the rewarming solution, were maintained at room temperature.

In the stepwise MVC method, embryos were kept for 2 min in an equilibration solution containing 7.5% (v/v) EG and 7.5% (v/v) DMSO. Next, embryos were immersed in a second equilibration solution containing 7.5% EG, 7.5% DMSO and 0.25 M sucrose for 1 min and were then placed in a third equilibration solution containing 7.5% EG, 7.5% DMSO and 0.5 M sucrose for one more minute. Embryos were then immersed in vitrification solution containing 15% EG, 15% DMSO and 0.5 M sucrose. As with the original MVC method, the process from MVC plate loading to LN plunging was completed in less than 1 min. The embryos were rewarmed in a 1-M sucrose solution. Unlike the original MVC method, removal of the cryoprotectant was carried out stepwise. First, the embryos were placed in a dilution solution containing 0.5 M sucrose for 2 min and then in a dilution solution containing 0.25 M sucrose for 2 min. To remove the cryoprotectants, the embryos were placed in washing solution for 10 min. All solutions except for the rewarming solution were maintained at room temperature. Survival of the vitrified blastocysts was assessed by in vitro culture. Blastocysts that re-expanded during culture for 24 h after rewarming were assessed as surviving. On Day 7 or 8, the cell numbers of blastocysts were counted after fixation and staining.

Statistics
Statistical analyses were performed using the SPSS 16.0 software (SPSS, Chicago, IL, USA). Differences between two groups were analyzed using the χ2-test. For comparisons among three groups or more, data were subjected to arcsine transformation and evaluated by one-way analysis of variance (ANOVA) followed by multiple comparisons by Tukey’s test. Differences in blastocyst cell number between groups were analyzed using the Student’s t-test. The level of significance was set at P<0.05.

Results
The rate of cell cycle synchronization for feline preadipocytes in serum starvation culture (0.5% FBS for 1–5 days) is shown in Table 2. After one day in serum starvation conditions, the ratio of cells in the G0/G1 stage was significantly higher than for control cells in the proliferative phase (87.4 and 72.6%, respectively, P<0.05). Furthermore, the rate of cell cycle synchronization at the G0/G1 stage increased significantly after two days (91.6%, P<0.05). However, extending the length of culture to three or five days did not increase the ratio of cells in the G0/G1 stage (92.5–92.9%). Based on these observations, we decided to synchronize the cell cycle of donor cells by culturing for two days under serum starvation conditions.

In order to determine the effects of serum starvation culture on the development of SCNT embryos, a nuclear transfer experiment was conducted using cells that were cultured for two days under serum starvation conditions (0.5% FBS) or cells that were cultured using a conventional method (20% FBS) as nuclear donors (Table 3). In vitro matured oocytes were used as recipient cytoplasts. The fusion rate of serum-starved donor cells to recipient oocytes (66.7%, 70/105) was comparable to that of cells cultured in a conventional manner (67.0%, 59/88). Furthermore, the in vitro development of SCNT embryos reconstructed using donor cells treated with serum starvation (normal cleavage rate of 65.7%, 46/70, and blastocyst formation rate of 20.0%, 14/70) was comparable to that of the controls (52.5%, 31/59, and 20.3%, 12/59).

The in vitro development of SCNT embryos that were recon-

### Table 2. Cell cycle synchronization of feline preadipocytes after serum starvation culture

<table>
<thead>
<tr>
<th>Serum starvation</th>
<th>G0/G1 phase % (mean ± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 days (control)</td>
<td>72.6 ± 0.8a</td>
</tr>
<tr>
<td>1 day</td>
<td>87.4 ± 0.1b</td>
</tr>
<tr>
<td>2 days</td>
<td>91.6 ± 0.2c</td>
</tr>
<tr>
<td>3 days</td>
<td>92.5 ± 0.8e</td>
</tr>
<tr>
<td>5 days</td>
<td>92.9 ± 0.4f</td>
</tr>
</tbody>
</table>

a, b, c Values with different superscripts differ significantly (P<0.05).

### Table 3. Effect of donor-cell cycle synchronization on the development of feline SCNT embryos

<table>
<thead>
<tr>
<th>Cell treatment (day)</th>
<th>No. (%) of fused couplets</th>
<th>No. (%) of reconstructed embryos developed to Two-cell stage</th>
<th>Blastocyst stage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum starvation (2)</td>
<td>70/105 (66.7)</td>
<td>46/70 (65.7)</td>
<td>14/70 (20.0)</td>
</tr>
<tr>
<td>Control (0)</td>
<td>59/88 (67.0)</td>
<td>31/59 (52.5)</td>
<td>12/59 (20.3)</td>
</tr>
</tbody>
</table>

### Table 4. Developmental ability of feline SCNT embryos reconstructed with in vitro or in vivo matured oocytes

<table>
<thead>
<tr>
<th>Oocyte maturation</th>
<th>No. (%) of fused couplets</th>
<th>No. (%) of reconstructed embryos developed to Two-cell stage</th>
<th>Blastocyst stage</th>
</tr>
</thead>
<tbody>
<tr>
<td>In vitro</td>
<td>42/77 (54.5)</td>
<td>18/42 (42.9)</td>
<td>5/42 (11.9)</td>
</tr>
<tr>
<td>In vivo</td>
<td>21/37 (56.8)</td>
<td>11/21 (52.4)</td>
<td>2/21 (9.5)</td>
</tr>
</tbody>
</table>
structured using either in vitro or in vivo matured oocytes is shown in Table 4. Cells treated with serum starvation were used as nuclear donors in this experiment. The rate of fusion to donor cells for in vitro matured oocytes (54.5%, 42/77) was similar to that for in vivo matured oocytes (56.8%, 21/37). In addition, the rates of normal cleavage (42.9%, 18/42) and blastocyst formation (11.9%, 5/42) for SCNT embryos reconstructed using in vitro matured oocytes were comparable to those of embryos reconstructed using in vivo matured oocytes (52.4%, 11/21, and 9.5%, 2/21, respectively).

Feline parthenogenetic blastocysts and SCNT-derived blastocysts were vitrified using either the MVC method or the modified MVC method (stepwise MVC method). A total of 156 control parthenogenetic embryos were cultured for 6–7 days, and 41 embryos (26.3%) developed into blastocysts. On the other hand, 132 SCNT embryos were cultured for 5–6 days, and 38 embryos (28.8%) developed into blastocysts. The parthenogenetic and SCNT-derived blastocysts with a normal appearance were used for the cryopreservation experiment. Due to restrictions in experimental materials, the number of embryos that could be obtained in a single experiment was low (1–7 embryos / experiment). Nonetheless, each cryopreservation experiment was repeated at least three times.

The survival rate of parthenogenetic blastocysts following vitrification by the MVC method was high (93.8%, 30/32). The cell number of the vitrified blastocysts was comparable to that of the nonvitrified blastocysts (n=9; 129.7 ± 11.6 and 116.6 ± 16.9, respectively).

On the other hand, SCNT-derived blastocysts could not survive following vitrification by the MVC method (0%, n=10). However, all SCNT-derived blastocysts vitrified using the stepwise MVC method survived after rewarming (100%, n=11). The mean cell number of these vitrified SCNT blastocysts was similar to that of the control nonvitrified blastocysts (n=3; 264.5 ± 49.8 and 313.67 ± 82.3, respectively, Fig. 1).

**Discussion**

In this study, we used feline preadipocytes as donor cells in nuclear transfer. Constant development (blastocyst formation: 20%) of the SCNT embryos reconstructed with IVM oocytes was achieved regardless of the cell cycle synchronization for donor cells. This is similar to or better than those obtained by other groups using fetal fibroblasts (4.0–33.0%) [10, 21, 22], cumulus cells (2.6–7.7%) [22, 36], epidermis-derived fibroblasts (6.1–8.5%) [10, 22] or uterus-derived fibroblasts (20.3–30.0%) [37, 38]. While further investigation is needed to determine whether the tissue stem cells or precursor cells can be used in SCNT as advantageous nuclear donor cells, our previous studies have demonstrated that cloned animals can be obtained efficiently by SCNT using porcine preadipocytes or salivary gland-derived progenitor cells as donor cells [23, 24, 33, 34]. Because the fat tissues where preadipocytes are found are near the body surface, these cells can be collected in a less invasive manner compared with stem cells derived from bone marrow and visera [23, 24, 30]. Therefore, preadipocytes may be a new alternative for nuclear donor cells in feline SCNT.

In 1997, Wilmut et al. reported the birth of Dolly, the world’s first cloned animal by SCNT [1]. The cell cycles of the mammary cells used as donor cells were synchronized at the G0 stage by serum starvation. This was believed to be the key for successfully cloning Dolly. Subsequently, the birth of cloned animals from donor cells without cell cycle synchronization has been reported [3]. Cloned cats have also been produced using both cells that were subjected to cell cycle synchronization [8, 10] and cells that were not synchronized [7]. In this study, cell cycle synchronization by serum starvation affected neither the rate of fusion between donor cells and recipient oocytes nor the in vitro development of the SCNT embryos. The induction of apoptosis is one of the known effects of serum starvation culture [39–41]. Therefore, it is important to select nonapoptotic cells when the cell cycles of donor cells are synchronized by serum starvation. In this study, we selected donor cells of the appropriate size and shape as donors for nuclear
transfer. Using this selection standard, it was quite likely that we ended up using nonapoptotic cells in the G0/G1 stage for nuclear transfer both in the serum-starved and nonstarved culture groups.

Compared with in vivo matured oocytes, in vitro matured oocytes can be obtained more conveniently and inexpensively. As a result, they are widely used in livestock animals as recipient oocytes for SCNT [31–34, 42]. On the other hand, studies have shown that the level of cytoplasmic maturation of in vitro matured oocytes is insufficient due to the effects of oxidative stress associated with in vitro culture or the incompleteness of maturation media [43–46]. In fact, SCNT embryos reconstructed with in vitro matured oocytes have a lower rate of development compared with those derived from in vivo matured oocytes [19]. Therefore, we expected the development of feline SCNT embryos reconstructed using in vitro matured oocytes to be lower when compared with embryos derived from in vivo matured oocytes. However, our results did not reveal any differences in development. Gomez et al. also reported no marked difference between in vitro matured oocytes and in vivo matured oocytes as recipient oocytes [21]. Thus, both in vitro and in vivo matured oocytes possess a similar ability to support the development of SCNT embryos. To the best of our knowledge, there have been no reports comparing the suitability of in vivo and in vitro matured oocytes as recipient oocytes based on development into cloned animals in cats. This issue warrants further investigation.

Slow freezing techniques have been used in the past to cryopreserve feline embryos [25, 26]. Favorable results using vitrification were obtained in some animal species, e.g., pigs, where the freezing tolerance of embryos is low and cryopreservation is difficult [27]. Vitrification has also been used in the cryopreservation of human embryos, which requires an even higher survival rate after rewarming [35]. Vitrification is a technique in which embryos are preserved in a noncrystalline solid-phase state by rapid cooling with high concentrations of cryoprotectants [47]. Because slow freezing causes ice crystal formation, vitrification ensures minimal damage to embryos. Other advantages include not needing a programmable freezer and the convenience of the procedure [35]. Feline embryos have been cryopreserved by vitrification, but the number of reports is limited and the rate of development after vitrification has been low [48, 49].

In this study, feline parthenogenetic blastocysts were vitrified using the MVC method, and the results confirmed a high survival rate after rewarming [35]. On the other hand, none of the SCNT blastocysts survived using the conventional MVC method. During the equilibration process, dehydration from embryos and permeation of cryoprotectants into embryos occur, causing the embryonic volume to decrease and then recover. These phenomena were not clearly seen for SCNT embryos, suggesting that the cell membrane of the SCNT embryo is less permeable to water and cryoprotectants. Therefore, we employed the stepwise method for SCNT embryos. To the best of our knowledge, this is the first report of successful vitrification of feline SCNT embryos.

In conclusion, we showed that it is possible to produce feline SCNT embryos that possess a high rate of development using either in vitro or in vivo matured oocytes, irrespective of cell cycle synchronization of donor cells. In vitro matured oocytes collected from ovaries excised during sterilization surgery work satisfactorily as recipient oocytes in SCNT. Furthermore, we showed that it is possible to cryopreserve feline cloned blastocysts using a stepwise vitrification method.

References


