Available online at www.sciencedirect.com
ScienceDirect
Theriogenology xxx (2006) xxx–xxx
Theriogenology
www.theriojournal.com

Birth of viable female dogs produced by somatic cell nuclear transfer

G. Jang [a], M.K. Kim [a], H.J. Oh [a,d], M.S. Hossein [a], Y.H. Fibrianto [a], S.G. Hong [a,d], J.E. Park [a,d], J.J. Kim [a], H.J. Kim [a,b], S.K. Kang [a], D.Y. Kim [c,d], B.C. Lee [a,*]

[a] *Department of Theriogenology and Biotechnology, College of Veterinary Medicine, Seoul National University, Seoul 151-742, Republic of Korea*
[b] *Haemaru Small Animal Referral Hospital, Seohyun-Dong, Bundang-Gu, Sung-Nam, Kyung Gi-Do 463-050, Republic of Korea*
[c] *Department of Veterinary Pathology, College of Veterinary Medicine, Seoul National University, Seoul 151-742, Republic of Korea*
[d] *Brain Korean 21 Program for Veterinary Science, College of Veterinary Medicine, Seoul National University, Seoul 151-742, Republic of Korea*

Received 8 October 2006; accepted 3 November 2006

Abstract

Since the only viable cloned offspring born in dogs was a male, the purpose of the present study was to produce female puppies by somatic cell nuclear transfer (SCNT). Adult ear fibroblasts from a 2-month-old female Afghan hound were isolated and used as donor cells. In vivo-matured canine oocytes surgically collected (approximately 72 h after ovulation) from the oviducts of 23 donors were used for SCNT. After removal of the cumulus cells, oocytes were enucleated, microinjected, fused with a donor cell, and activated. A total of 167 reconstructed SCNT embryos were surgically transferred (Day 0) into the oviducts of 12 recipient bitches (average 13.9 embryos/recipient, range 6–22) with spontaneous, synchronous estrous cycles. Three pregnancies were detected by ultrasonography on Day 23, maintained to term, and three healthy female puppies (520, 460, and 520 g), were delivered by Caesarean section on Day 60. These puppies were phenotypically and genotypically identical to the cell donor. In conclusion, we have provided the first demonstration that female dogs can be produced by nuclear transfer of ear fibroblasts into enucleated canine oocytes.

Keywords: Female cloned dogs; SCNT; In vivo oocyte; Embryo transfer; Pregnancy

1. Introduction

Assisted reproduction technologies (ARTs) are less developed in the dog than in domestic livestock. Unique species-specific reproductive characteristics, i.e. monoestrual, polyovulatory and non-seasonal reproductive cycle, combine to increase the degree of difficulty and decrease the level of success. In contrast to most mammals that ovulate mature oocytes at the metaphase II stage, dogs ovulate immature oocytes at the germinal vesicle stage and the oocytes undergo a 48–72 h period of postovulatory maturation in the oviduct. Despite many studies focused on establishing a suitable system for in vitro maturation (IVM) of canine oocytes, efficiency is lower than that of other mammalian species [1–4].

To obtain mature oocytes for somatic cell nuclear transfer (SCNT), the most useful approach currently is the surgical collection of oocytes matured in vivo. However, collection of in vivo canine oocytes by a surgical approach, i.e. salpingectomy or flushing

* Corresponding author. Tel.: +82 2 880 1269; fax: +82 2 873 1269.
E-mail address: bclee@snu.ac.kr (B.C. Lee).

0093-691X/$ – see front matter © 2006 Published by Elsevier Inc.
doi:10.1016/j.theriogenology.2006.11.006

oviducts, is technically difficult because the oviduct wall is thin and the lumen is small. Similarly, laparoscopic oocyte retrieval by follicle aspiration was not feasible due to the presence of the ovarian bursa. Although in vitro maturation techniques can be used in some species to generate large numbers of mature oocytes, the number of in vivo matured canine oocytes that have been collected from oviductal flushing ranged from 7 to 10 oocytes per female [5].

The first cloned dog, a male named ''Snuppy'', was born from in vivo matured oocytes [6] since the necessary numbers of in vitro-matured oocytes could not be produced [7–10]. Thus far, ''Snuppy'' has not shown morphologically detectable abnormalities. The ultimate assessment of the normality of reproductive traits, such as libido, in cloned male dogs is mating with females produced either by natural mating or by cloning, to determine if pregnancies and live puppies can be produced. Therefore, the objective of the present study was to produce live cloned female dogs derived from SCNT using fibroblasts and enucleated in vivo-matured oocytes.

2. Materials and methods

2.1. Care and use of animals

In this study, a total of 35 mixed-breed female dogs (23 oocyte donors and 12 recipients) from 1 to 5 years of age were used as oocyte donors and embryo transfer recipients. Facilities for dog care and the procedures done met or exceeded the standards established by the Committee for Accreditation of Laboratory Animal Care at Seoul National University. The study was conducted in accordance with recommendations described in ''The Guide for the Care and Use of Laboratory Animals'' published by Seoul National University.

2.2. Chemicals

Unless otherwise indicated, chemicals were purchased from Sigma–Aldrich Corp. (St. Louis, MO, USA).

2.3. Recovery of in vivo matured oocytes

Beginning on the first day of spontaneous estrus, dogs were examined daily for vulvar swelling and serosanguinous discharge, and blood samples (3–5 mL) were collected and serum progesterone concentrations were determined with a DSL-3900 ACTIVE® Progesterone Coated-Tube Radioimmunoassay Kit (Diagnostic Systems Laboratories, Inc., TX, USA). The day of ovulation was considered as the day that serum progesterone concentration reached 4.0–7.5 ng/mL [6]. Approximately 72 h after ovulation, oocytes were retrieved by laparotomy (using aseptic surgical procedures). Anesthesia was induced with 6 mg/kg ketamine HCl and 1 mg/kg xylazine, and general anesthesia was maintained with 2% isoflurane. While in dorsal recumbency, recipients were aseptically prepared for surgery and a mid-line ventral incision was made to expose the reproductive tract. The fimbria of the oviduct was accessed through the bursal slit and cannulated using an inverted flanged bulb steel needle (18 gauge, 7.5 cm; Fig. 1). The needle was held in place by a surgical ligature, which was tied using a quick-release device using 3 cm of plastic tube (diameter, 2 mm) and hemostatic forceps. To improve visualization of the oviductal lumen, digital pressure was applied to the lower portion of the isthmus of the oviduct (near the uterotubal junction), an intravenous catheter (24 gauge) was inserted, and 10 mL of Hepes-buffered TCM-199 (cat no. 11150-059, Invitrogen, Carlsbad, CA, USA) supplemented with 10% FBS, 2 mM $NaHCO_3$ and 5 mg/mL BSA was introduced into the oviduct through the catheter and collected through the needle. In vivo-matured oocytes obtained from oviductal flushing were transported to the laboratory for SCNT within 10 min.

2.4. Donor cell preparation for somatic cell nuclear transfer

Adult fibroblasts were isolated from an ear skin biopsy of a female Afghan hound, 2-month-old. The small pieces of tissue were washed three times in D-PBS, minced with a surgical blade and dissociated in

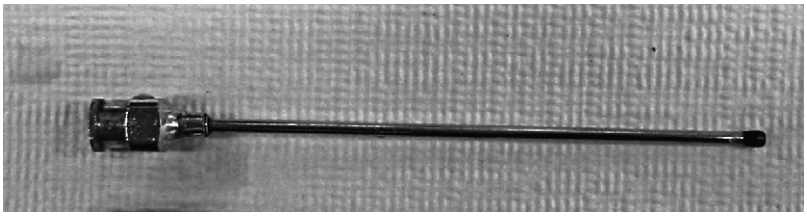

Fig. 1. Inverted flanged bulb steel needle (18 gauge, 7.5 cm) used for recovering canine oocytes from the oviduct.

Please cite this article in press as: Jang G, et al., Birth of viable female dogs produced by somatic cell nuclear transfer, Theriogenology (2006), doi:10.1016/j.theriogenology.2006.11.006

Dulbecco's modified Eagle's medium (cat no. 11995-065, DMEM, Invitrogen) supplemented with 0.25% (w/v) trypsin and 1 mM EDTA (Invitrogen) for 1 h at 37 °C. Trypsinized cells were washed once in Ca^{2+}- and Mg^{2+}-free DPBS by centrifugation at 300 × *g* for 2 min and seeded into 60-mm plastic culture dishes (Becton Dickinson, Lincoln Park, NJ, USA). Subsequently, cells were cultured for 6–8 days in DMEM supplemented with 10% (v/v) FBS (Invitrogen), 1 mM glutamine (Invitrogen), 25 mM $NaHCO_3$ and 1% (v/v) minimal essential medium (MEM) nonessential amino acid solution (Invitrogen) at 39 °C in a humidified atmosphere of 5% CO_2 and 95% air. After removal of unattached clumps of cells or explants, attached cells were further cultured to confluence. These cells were subcultured, for intervals of 4–6 days, by trypsinization for 1 min in medium containing 0.1% trypsin/0.02% EDTA, allocated to three new dishes for further passages and then stored in liquid nitrogen at −196 °C. For freezing, cells from early passages (1–3) were frozen in the freezing medium consisted of 80% (v/v) DMEM, 10% (v/v) DMSO and 10% (v/v) FBS. Cells at passages 3–8 were used for SCNT. Prior to SCNT, cells were thawed, cultured for 3–4 days until confluent and retrieved from the monolayer by trypsinization for 1 min.

2.5. Preparation of recipient oocytes for somatic cell nuclear transfer

Cumulus cells were removed from in vivo matured oocytes by repeated pipetting in 0.1% (w/v) hyaluronidase (from bovine testis) in Hepes-buffered Ca^{2+}-free CR2 medium [11] with amino acids (hCR2aa). Oocytes were then stained with 5 μg/mL bisbenzimide (Hoechst 33342) for 5 min and observed under an inverted microscope equipped with epifluorescence at 200× magnification. Using bi-lateral micromanipulators (Nikon-Narishige, Tokyo, Japan), oocytes were held with a holding micropipette (150 μm, inner diameter) in hCR2aa supplemented with 10% (v/v) FBS and 5 μg/mL cytochalasin B. The first polar body and adjacent cytoplasm containing the metaphase-II chromosomes were removed using an aspiration pipette (20 μm, inner diameter) and enucleated oocytes were placed in TCM-199 supplemented with 10% (v/v) FBS to be used for SCNT.

2.6. Microinjection, fusion, activation, and embryo culture

A donor cell was deposited into the perivitelline space of each enucleated oocyte maintained in hCR2aa supplemented with 100 μg/mL phytohemagglutinin. Then, couplets were placed in fusion medium consisting of 0.26 M mannitol, 0.1 mM $MgSO_4$, 0.5 mM Hepes and 0.05% (w/v) BSA and fusion was induced using a needle type electrode. Briefly, the single cell-oocyte couplet was sandwiched between two parallel electrodes attached to micromanipulators (Nikon-Narishige). The contact surface between the cytoplast and the donor cell was parallel to the electrodes and electrical stimulation was delivered with an Electro-Cell Fusion apparatus (NEPA GENE Co., Chiba, Japan). The distance between the electrodes was approximately 180 μm. Two pulses (72 V for 15 μs) were applied. Fusion of the donor cell and the ooplast was evaluated using a stereomicroscope at 1 h after electric stimulation. Fused couplets were selected and cultured for 3 h in modified synthetic oviductal fluid medium (mSOF) as previously described [12]. Chemical activation of reconstructed embryos was induced by incubation for 4 min in mSOF containing 10 μM calcium ionophore at 39 °C. Reconstructed couplets were then washed and further incubated for 4 h in mSOF supplemented with 1.9 mM 6-dimethylaminopurine.

2.7. Embryo transfer and pregnancy diagnosis

Within 4 h after reconstruction, cloned embryos were surgically transferred into the oviduct of naturally synchronized recipient females approximately 72 h after ovulation. The embryos were transferred to recipients (Day 0) using surgical procedures similar to those described in Section 2.3. Reconstructed embryos were placed in the ampullary portion of the oviduct using a 3.5 Fr Tom Cat Catheter (Sherwood, St. Louis, MO, USA). On Day 23, pregnancies were detected with a SONOACE 9900 (Medison Co. Ltd., Seoul, Korea) ultrasound scanner with 7.0 MHz linear-array probe. After the initial diagnosis of pregnancy, ultrasonographic examinations were done every 2 weeks in pregnant recipients.

2.8. DNA extraction and microsatellite analysis for genotyping

Parentage analysis was performed on the nuclear donor fibroblasts, the donor dog, cloned dogs and surrogate recipients to confirm genetic identity. Genomic DNA from cultured fibroblasts and blood of the somatic cell donor, cloned dogs and the surrogate dams was obtained. Genomic DNA from blood and trypsinized donor cells was extracted according to instructions provided with the G-spin™ Genomic DNA

Please cite this article in press as: Jang G, et al., Birth of viable female dogs produced by somatic cell nuclear transfer, Theriogenology (2006), doi:10.1016/j.theriogenology.2006.11.006

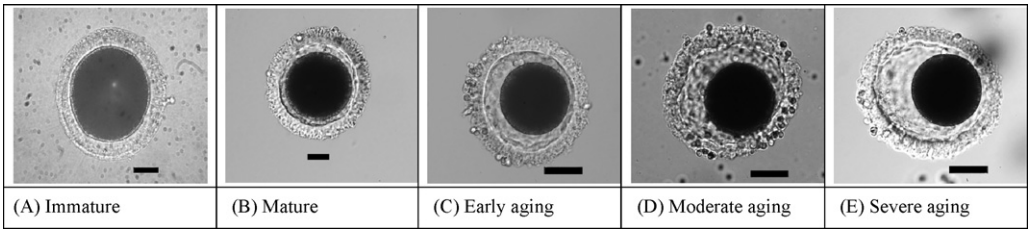

Fig. 2. Various stages of in vivo-matured canine oocytes (scale bar = 40 μm) flushed from oviducts approximately 72 h after ovulation. Cumulus cells from intact oocytes were removed and evaluated. In the absence of perivitelline space (PVS) and first polar body, oocytes were categorized as immature (A). Denuded oocytes with PVS 15, 25, 30, and ≥40 μm were categorized as mature (B), early (C), moderate (D), and severely aged (E).

Table 1
Pregnancy and parturition following transfer of somatic cell nuclear transferred canine embryos; recipients C, J and K each delivered a single viable puppy

Recipient	No. of oocyte donors	Oocyte status	No. of embryos transferred
A	2	Both mature	22
B	2	Mature, early-aging	20
C	2	Both mature	16
D	4	Three: immature, one: moderate-aging	10
E	1	Mature	6
F	1	Immature	8
G	2	Immature, mature	16
H	2	Both early-aging	18
I	1	Severe-aging	8
J	2	Immature, mature	17
K	2	Immature, mature	14
L	2	Early-aging, moderate-aging	12
Total	23		167

Extraction Kit (Intron, Seoul, Korea). The following 14 markers were selected for analysis: PEZ1, PEZ2, PEZ3, PEZ5, PEZ6, PEZ10, PEZ11, PEZ012, PEZ13, PEZ 16, PEZ017, FH2010, FH2054, and FH2079. The isolated genomic DNA samples were dissolved in 50 μL TE and used for microsatellite assay with 14 specific markers originally derived from dogs [6,13]. Length variations were assayed by polymerase chain reaction (PCR) amplification with fluorescently labeled (FAM, HEX, and NED) locus-specific primers and PAGE on an

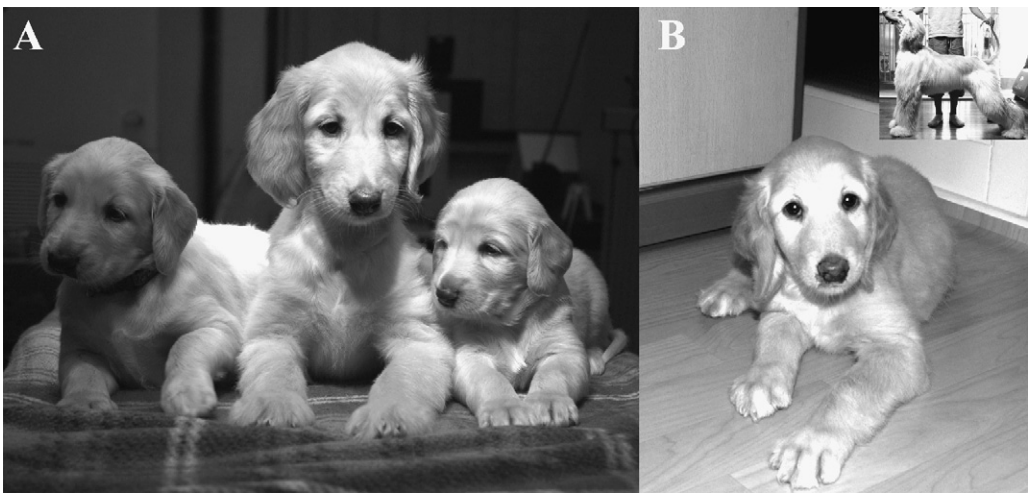

Fig. 3. (A) Three cloned female dogs; (B) somatic cell donor dog (inset is donor dog at maturity).

Please cite this article in press as: Jang G, et al., Birth of viable female dogs produced by somatic cell nuclear transfer, Theriogenology (2006), doi:10.1016/j.theriogenology.2006.11.006

Table 2
Microsatellite genotypes of seven dogs, including three cloned female dogs

	PEZ01	PEZ02	PEZ03	PEZ05	PEZ06	PEZ10	PEZ11	PEZ12	PEZ13	PEZ16	PEZ17	FH2010	FH2054	FH2079
Donor blood	118/118	121/121	127/130	105/113	180/184	264/276	128/146	271/282	174/217	288/300	207/207	227/228	151/155	269/269
Donor cell	118/118	121/121	127/130	105/113	180/184	264/276	128/146	271/282	174/217	288/300	207/207	227/228	151/155	269/269
Cloned-puppy-1	118/118	121/121	127/130	105/113	180/184	264/276	128/146	271/282	174/217	288/300	207/207	227/228	151/155	269/269
Cloned-puppy-2	118/118	121/121	127/130	105/113	180/184	264/276	128/146	271/282	174/217	288/300	207/207	227/228	151/155	269/269
Cloned-puppy-3	118/118	121/121	127/130	105/113	180/184	264/276	128/146	271/282	174/217	288/300	207/207	227/228	151/155	269/269
Puppy-1-surrogate	114/118	117/121	123/124	105/113	177/179	287/287	132/136	271/271	174/221	284/300	203/207	228/236	151/151	273/273
Puppy-2-surrogate	114/118	117/117	123/123	101/101	187/187	280/280	124/128	292/300	174/221	308/308	199/203	227/231	167/167	273/277
Puppy-3-surrogate	114/114	117/125	137/140	101/103	171/181	287/287	125/132	nd/nd[a]	217/221	300/304	199/207	223/231	155/167	273/273

Alleles are named for the total length of the segment amplified.
[a] Allele not determined.

automated DNA sequencer (ABI 373; Applied Biosystems, Foster City, CA, USA). Proprietary software (GeneScan and Genotyper; Applied Biosystems) was used to estimate the PCR product size in nucleotides.

3. Results

A total of 167 SCNT embryos (range 6–22, average 13.9 embryos/recipient) from 23 oocyte donor dogs were surgically transferred into the oviducts of 12 recipient dogs. The morphological and maturation status of oocytes flushed from the oviducts of donor females are shown (Fig. 2). The numbers of transferred SCNT embryos, pregnancies and puppies delivered are summarized in Table 1. The three pregnancies diagnosed on Day 23 were maintained to term and three healthy female puppies weighing 520, 460, and 520 g, respectively, were delivered by caesarean section on Day 60. The three puppies were of similar phenotype (Fig. 3) and parentage analysis indicated that they were genetically identical with the donor adult fibroblasts (Table 2).

4. Discussion

In our earlier study on canine SCNT [6], we described the production and birth of a viable male puppy. In the present study, we provide further evidence that canine SCNT can be used to produce viable offspring. In particular, multiple pregnancies were produced which resulted in the births of the first viable female dogs from SCNT/ET.

In several species of domestic and laboratory animals, methods for in vitro oocyte maturation are well established and are used to produce mature oocytes in the numbers required for production of SCNT embryos and, subsequently, non-transgenic or transgenic cloned offspring [14–22]. However, the rate of progress made with canine SCNT has been comparatively slow, primarily because of the lack of availability of mature metaphase II oocytes, a prerequisite for in vitro production of embryos, including by SCNT. Thus, the SCNT embryos used to produce the first cloned dog were derived from in vivo matured oocytes collected by oviductal flushing. However, because of the low efficiency of embryo production in the initial study, we did further investigations to improve proficiency in obtaining in vivo matured oocytes and in our SCNT procedures, including fusion, activation, and embryo transfer [23,24]. For example, we found that chemical activation was more beneficial for producing reconstructed embryos than was electrical stimulation.

Moreover, additional surgical experience has enabled us to reduce the time required for collection of oviductal oocytes and for embryo transfer. Thus, the extra experience we have gained, combined with improvements made in methods for generating and transferring SCNT embryos have resulted in higher pregnancy and embryo survival rates, as compared to our first report on the birth of a male cloned puppy [6].

We believe that the higher pregnancy rate achieved in the present study was due to the quality of the oocytes flushed from the oviducts of donors at approximately 72 h after ovulation. Although we used all of the oocytes that were recovered, including immature, mature, and post-mature, the only pregnancies produced were from transferring cloned embryos produced using mature oocytes as cytoplasts.

Somatic cell nuclear transfer in mammals is currently associated with high rates of fetal loss, abnormalities and abortion [25–30]. In the present study, following detection of a single embryonic sac by ultrasonography on Day 23, none of the confirmed pregnancies were subsequently lost. Although signs of impending whelping, including mammary gland swelling and initiation of milk letdown, were observed in recipients at the expected time of delivery date (Day 60), to exclude risk factors associated with natural delivery of a singleton pregnancy (i.e. dystocia related to fetal size), a Caesarean section was done [31,32]. The three cloned puppies were normal in appearance at birth and have grown into active, healthy young dogs (Fig. 3). They were reared by their recipient mothers for 3–5 days. However, due to the effects of Caesarean section on the milk production, volume of milk letdown in recipient dogs was decreased. Thus, the cloned puppies were hand-raised by humans and/or reared by a female that had naturally delivered of puppies. We speculate that the lack of abnormalities, fetal losses and abortions was related to our use of in vivo-matured oocytes for SCNT.

Microsatellite analysis indicated that the cloned puppies were genetically the same as the donor somatic cells. Even though different phenotypes have been seen between the cell donor and cloned offspring in other species (e.g., black and white coat color pattern in cloned calves) [33], the cloned puppies produced in our study had the same phenotype as the donor of the somatic cells.

In summary, we have demonstrated that female cloned dogs can be derived by SCNT. Based on the present results, it seems that our efficiency in producing cloned embryos and pregnancies was increased by the use of good quality in vivo matured oocytes. We will continue to monitor the growth and health of the three female cloned dogs and evaluate their reproductive capability by natural breeding with a male cloned dog.

Acknowledgements

This study was financially supported by KOSEF (grant #M10625030005-06N250300510). We thank K.S. Kim, owner of the female Afghan hound for donated donor cells and Y.H. Kim (NICEM) for assistance regarding microsatellite analysis. The authors are grateful for a graduate fellowship provided by the Korean MOE, through the BK21 program for Veterinary Science, SNU.

References

[1] Hewitt DA, Watson PF, England GC. Nuclear staining and culture requirements for in vitro maturation of domestic bitch oocytes. Theriogenology 1998;49:1083–101.
[2] Otoi T, Fujii M, Tanaka M, Ooka A, Suzuki T. Effect of serum on the in vitro maturation of canine oocytes. Reprod Fertil Dev 1999;11:387–90.
[3] Farstad W. Assisted reproductive technology in canid species. Theriogenology 2000;53:175–86.
[4] Kim MK, Fibrianto YH, Oh HJ, Jang G, Kim HJ, Lee KS, et al. Effects of estradiol-17beta and progesterone supplementation on in vitro nuclear maturation of canine oocytes. Theriogenology 2005;63:1342–53.
[5] Hossein MS, Kim MK, Jang G, Fibrianto HY, Oh HJ, Kim HJ, Kang SK, Lee BC. Influence of season and parity on the recovery of in vivo canine oocytes by flushing fallopian tubes. Anim Reprod Sci; in press.
[6] Lee BC, Kim MK, Jang G, Oh HJ, Yuda F, Kim HJ, et al. Dogs cloned from adult somatic cells. Nature 2005;436:641.
[7] Westhusin ME, Burghardt RC, Ruglia JN, Willingham LA, Liu L, Shin T, et al. Potential for cloning dogs. J Reprod Fertil Suppl 2001;57:287–93.
[8] Yamada S, Shimazu Y, Kawaji H, Nakazawa M, Naito K, Toyoda Y. Maturation, fertilization, and development of dog oocytes in vitro. Biol Reprod 1992;46:853–8.
[9] Rodrigues Bde A, dos Santos LC, Rodrigues JL. Embryonic development of in vitro matured and in vitro fertilized dog oocytes. Mol Reprod Dev 2004;67:215–23.
[10] Otoi T, Murakami M, Fujii M, Tanaka M, Ooka A, Une S, et al. Development of canine oocytes matured and fertilised in vitro. Vet Rec 2000;146:52–3.
[11] Rosenkrans Jr CF, Zeng GQGTMC, Schoff PK, First NL. Development of bovine embryos in vitro as affected by energy substrates. Biol Reprod 1993;49:459–62.
[12] Jang G, Lee BC, Kang SK, Hwang WS. Effect of glycosaminoglycans on the preimplantation development of embryos derived from in vitro fertilization and somatic cell nuclear transfer. Reprod Fertil Dev 2003;15:179–85.
[13] Parker HG, Kruglyak L, Ostrander EA. Molecular genetics: DNA analysis of a putative dog clone. Nature 2006;440:E1–2.
[14] Jang G, Bhuiyan MM, Jeon HY, Ko KH, Park HJ, Kim MK, et al. An approach for producing transgenic cloned cows by nuclear transfer of cells transfected with human alpha 1-antitrypsin gene. Theriogenology 2006;65:1800–12.

[15] Hyun S, Lee G, Kim D, Kim H, Lee S, Nam D, et al. Production of nuclear transfer-derived piglets using porcine fetal fibroblasts transfected with the enhanced green fluorescent protein. Biol Reprod 2003;69:1060–8.
[16] Schnieke AE, Kind AJ, Ritchie WA, Mycock K, Scott AR, Ritchie M, et al. Human factor IX transgenic sheep produced by transfer of nuclei from transfected fetal fibroblasts. Science 1997;278:2130–3.
[17] Lai L, Kolber-Simonds D, Park KW, Cheong HT, Greenstein JL, Im GS, et al. Production of alpha-1,3-galactosyltransferase knockout pigs by nuclear transfer cloning. Science 2002;295: 1089–92.
[18] Lai L, Park KW, Cheong HT, Kuhholzer B, Samuel M, Bonk A, et al. Transgenic pig expressing the enhanced green fluorescent protein produced by nuclear transfer using colchicine-treated fibroblasts as donor cells. Mol Reprod Dev 2002;62:300–6.
[19] Wilmut I, Schnieke AE, McWhir J, Kind AJ, Campbell KH. Viable offspring derived from fetal and adult mammalian cells. Nature 1997;385:810–3.
[20] Wakayama T, Perry AC, Zuccotti M, Johnson KR, Yanagimachi R. Full-term development of mice from enucleated oocytes injected with cumulus cell nuclei. Nature 1998;394:369–74.
[21] Wakayama T, Rodriguez I, Perry AC, Yanagimachi R, Mombaerts P. Mice cloned from embryonic stem cells. Proc Natl Acad Sci USA 1999;96:14984–9.
[22] Eggan K, Baldwin K, Tackett M, Osborne J, Gogos J, Chess A, et al. Mice cloned from olfactory sensory neurons. Nature 2004;428:44–9.
[23] Kim MK, Oh HJ, Fibrianto YH, Jang G, Kim HJ, Lee KN, et al. A surgical method for collecting canine oocytes of in vivo matured from uterine tube. Korean J Embryo Transfer 2004; 19:147–53.
[24] Jang G, Kim MK, Oh HJ, Fibrianto HY, Hossein MS, Kim HJ, et al. Study on the establishment of somatic cell nuclear transfer using in vivo matured oocytes in dogs. Reprod Fertil Dev 2006;18:129.
[25] Hill JR, Roussel AJ, Cibelli JB, Edwards JF, Hooper NL, Miller MW, et al. Clinical and pathologic features of cloned transgenic calves and fetuses (13 case studies). Theriogenology 1999; 51:1451–65.
[26] Fecteau ME, Palmer JE, Wilkins PA. Neonatal care of high-risk cloned and transgenic calves. Vet Clin North Am Food Anim Pract 2005;21:637–53.
[27] Gomez MC, Pope CE, Giraldo A, Lyons LA, Harris RF, King AL, et al. Birth of African Wildcat cloned kittens born from domestic cats. Cloning Stem Cells 2004;6:247–58.
[28] Lee GS, Kim HS, Lee SH, Kim DY, Seo KM, Hyun SH, et al. Successful surgical correction of anal atresia in a transgenic cloned piglet. J Vet Sci 2005;6:243–5.
[29] Li S, Li Y, Du W, Zhang L, Yu S, Dai Y, et al. Aberrant gene expression in organs of bovine clones that die within two days after birth. Biol Reprod 2005;72:258–65.
[30] Jouneau A, Zhou Q, Camus A, Brochard V, Maulny L, Collignon J, et al. Developmental abnormalities of NT mouse embryos appear early after implantation. Development 2006;133:1597–607.
[31] Simpson GM, England GCW, Harvey M. Bsava manual of small animal reproduction and neonatology, 1st ed., Iowa State Press; 1988. pp. 127–142.
[32] Johnston SD, Kustritz MVR, Olson PNS. Canine and feline theriogenology, 1st ed., Saunders; 2001.
[33] Wells DN, Misica PM, Tervit HR. Production of cloned calves following nuclear transfer with cultured adult mural granulosa cells. Biol Reprod 1999;60:996–1005.

Please cite this article in press as: Jang G, et al., Birth of viable female dogs produced by somatic cell nuclear transfer, Theriogenology (2006), doi:10.1016/j.theriogenology.2006.11.006