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Production of Cloned Korean Native Goat (*Capra hircus*) by Somatic Cell Nuclear Transfer

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ABSTRACT : The objectives of the present study were to initiate cloning of Korean native goat by somatic cell nuclear transfer (NT) and to examine whether unovulated (follicular) oocytes can support the same developmental ability of NT embryos as ovulated (oviductal) oocytes after hCG injection in stimulated cycles of the goat. The *in vivo*-matured and immature oocytes were collected from the oviducts and follicles of superovulated does, respectively, and the immature oocytes were maturated *in vitro*. Ear skin fibroblasts derived from a 3-yr-old female Korean native goat were used as the donors of nuclei or karyoplasts. Following fusion, activation and *in vitro* culture to a 2- to 4-cell stage, 49 *in vitro*-derived and 105 *in vivo*-derived embryos were transferred to 6 and 17 recipient does, respectively. One doe and three does of the respective groups were identified as pregnant by ultrasonography on day 30 after embryo transfer. However, only one doe, which had received *in vivo*-derived embryos, delivered a normal female kid of 1.9 kg on d 149. The cloned kid gained more weight than her age-matched females as much as 87% during the first 4 mo after birth (17.7 vs. 9.4±0.8 kg) and reached puberty at 6-mo age a few months earlier than normal female does. The telomere length of the kid, which was similar to that of the donor fibroblast at 2-mo age, decreased 8% between 2- and 7-mo ages. Moreover, at 7-mo age, she had 21% shorter telomere than her age-matched goats. To our knowledge, this is the first case in which a cloned animal born with a normal weight exhibited accelerated growth and development. The unusually rapid growth and development of the cloned goat may have resulted from SCNT-associated epigenetic reprogramming involving telomere shortening. (**Key Words :** Goat, Superovulation, Oocyte, Cloning, Nuclear Transfer, Telomere)

INTRODUCTION

Animal cloning, which makes it possible to propagate a genetically superior individual within a short period of time, is a potential tool in animal reproduction (Vajta and Gjerris, 2006). Moreover, this technique can also be exploited for producing endangered wild life or transgenic animals capable of producing valuable proteins. Up to now, however, production of cloned animals was feasible almost only by transplanting totipotent blastomeres of early embryos into uteri of the surrogates, and accordingly, animal cloning was much more of an experiment than a means of production (Heyman and Renard, 1996). It was only after Wilmut et al. (1997) first reported the production of a cloned sheep by nuclear transfer (NT) that animal cloning from a somatic cell became feasible in cattle (Kato et al., 1998), pigs (Polejaeva et al., 2000), rabbits (Chesne et al., 2002), mice (Wakayama et al., 1999), cats (Shin et al., 2002) and dogs (Lee et al., 2005a). Goat cloning by virtue of NT, the main topic of this study, also has been reported from several groups of workers (Baguisi et al., 1999; Reggio et al., 2001; Keefer et al., 2001, 2002; Zou et al., 2002; Behboodi et al., 2004). However, much more needs to be learned for the cloning of goats and other farm animals to be used as a practical tool in animal production.

Animal cloning by NT begins with the transfer of the nucleus of a donor cell or karyoplast to an enucleated oocyte or cytoplast. The karyoplast-cytoplast couplet is fused, activated and cultured, followed by transfer of the resulting early embryo to the synchronized recipient. Success rate of NT is thus dependent upon a number of

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factors including the source of oocytes. In this connection, Reggio et al. (2001) and Keefer et al. (2001, 2002) have reported that *in vitro*-matured follicular oocytes supported full-term development in goats, whereas in the study of Behoodi et al. (2004), *in vivo*-matured oviductal oocytes, but not *in vitro*-matured oocytes, produced the pregnancy. Obviously, more studies are necessary to elucidate how the origin of the oocyte affects development of the couplet, conceptus and possibly offsprings in this species.

The Korean native goats (KNG), which have been domesticated primarily for meat production throughout the Korean peninsula, have a few advantages as a model animal (Song and Park, 1984; Choe et al., 1986; Park et al., 1991; Park et al., 2004). In addition to its docility and adaptability to various environments and feeds, the small size of this breed also makes it practical for experimental purposes, because both male and female KNG weigh only 21.5 kg at sexual maturity (8.5 months of age) on an average. Moreover, KNG have both black- and white-hair lines, which can be utilized as a phenotypic marker in genetic studies. The present study was therefore undertaken to initiate KNG cloning by somatic cell nuclear transfer (SCNT) and to examine whether unovulated (follicular) oocytes as recipient cytoplasts can support the same developmental ability of NT embryos as ovulated (oviductal) oocytes after hCG injection in stimulated cycles of the goat.

MATERIALS AND METHODS

Animals

Korean native goats used as a donor of somatic cells as well as donors of oocytes and recipients of cloned embryos were purchased from local farms. After allowing the goats to adapt themselves to the new environment and also to receive general health care at the Jinju National University Farm for at least 2 weeks, only healthy animals were selected for the present experiments. The animal management, including environmental control and feeding, followed the general practice of the university farm.

Blood samples were obtained by jugular venipuncture using heparin-vacutainer from a somatic cell donor, a cloned goat generated in this study, three age-matched companions of the cloned kid, and a doe which delivered the cloned kid. Body weights of the cloned goat and her companions were measured at 1-mo intervals. The entire animal handling procedures including the injections and surgery performed in this study conformed to the guideline for the Care and Use of Animals released by the Ministry of Agriculture and Forestry, Korea.

Preparation of donor cells

Fibroblasts, which were used as donor cells, were prepared from ear skin biopsies taken from a 3-yr-old, white-haired female KNG, as described by Reggio et al. (2001). The biopsies (5 mm×5 mm) were washed in Dulbecco's phosphate-buffered saline (D-PBS), minced and exposed to 0.25% trypsin-EDTA (Sigma Chemical Co., Saint Louis, MO, USA) in D-PBS at 39°C for 10 min. Isolated cells were cultured in TCM-199 medium supplemented with 10% fetal bovine serum (FBS) and 50 μg/ml penicillin-streptomycin (all from Sigma) in 25-cm^2 flasks at 39°C in an humidified atmosphere containing 5% CO_2 for 4 days until a confluent primary fibroblast monolayer was established. The monolayer fibroblasts were harvested by trypsinization and stored in the TCM-199 culture medium containing 10% dimethyl sulfoxide in liquid nitrogen until used.

The frozen fibroblasts were thawed in a 39°C water bath and cultured in the TCM-199 medium containing 10% FBS in 4-well dishes. At confluency, the cells were switched to a low-serum TCM-199 medium containing 0.5% FBS and further incubated for 3 to 5 days until needed. The cells were collected by trypsinization and resuspended in M2 medium containing 10% goat serum (all from Sigma) just before the nuclear transfer.

Superovulation of oocyte donors

Approximately 1-yr-old black KNGs (n = 45) were synchronized following intravaginal insertion of CIDR containing 300 mg progesterone (Eazi Breed, InterAg, New Zealand) as described by Keefer et al. (2001). The does were injected with either a single dose of 1,000 IU PMSG (Folligon; Intervet, Boxmeer, Netherland) on day 8 of CIDR insertion or a total of 70 mg FSH (Folltropin-V®; Vetrepharm Canada Inc., Ontario, Canada) in decreasing doses twice daily on days 8, 9 and 10 (40, 20 and 10 mg, respectively). On day 8, they also received a single intramuscular injection of 10 mg $PGF_2\alpha$ (Lutalyse; Pharmacia, Morris Plains, NJ, USA). On day 10, following removal of CIDR, superovulation was induced by a single intramuscular injection of 400 IU hCG (Chorulon; Intervet, Boxmeer, Netherland).

Collection and preparation of *in vivo*- and *in vitro*-matured oocytes

Does which had received the hormonal treatment for superovulation were fasted 24 h and anesthetized by intramuscular administration of 0.2 mg/kg xylazine (Rompun; Bayer, Ansan, Korea) followed by 11 mg/kg ketamine-HCl (Yuhan, Seoul, Korea). The ovary and oviduct were exteriorized through mid-ventral incision. Oviductal (*in vivo*-matured) oocytes were collected by flushing the oviducts from utero-tubal junction to fimbria with 5- to 10-ml of M2 medium (Sigma). The entire oocyte collection procedure was finished within 29 to 50 h after

hCG injection.

Follicular (*in vitro*-matured) oocytes were harvested by direct aspiration from the remaining follicles (2 to 5 mm) of both ovaries after the oviductal oocytes had been recovered. Cumulus-oocyte complexes (COCs) containing immature oocytes were aspirated from ovarian follicles using a 22-gauge needle attached to a 5-ml syringe. After extensive washing in M2 medium containing 5% goat serum, COCs exhibiting the compact cumulus cell layers and homogeneous ooplasms were selected for *in vitro* culture. Fifteen to 20 COCs were transferred into 0.5-ml of maturation medium (TCM-199 supplemented with 25-mM Hepes, 10% goat serum, 10 μg/ml LH, 1 μg/ml estradiol 17-β, 5 μg/ml FSH and 50 IU/ml penicillin-streptomycin] in 4-well dishes which had been prewarmed at 39°C for 12 h in an humidified atmosphere of 5% CO_2. The COCs were incubated for 20 to 22 h to induce maturation of the oocytes.

Nuclear transfer

Following *in vitro* culture of COCs, cumulus cells surrounding the *in vitro*-matured oocytes were removed by immersing the COCs in 0.3% hyaluronidase (Sigma) in D-PBS for 3 to 5 min. Isolated oocytes were washed three times in D-PBS/10% FBS, after which only good-looking oocytes exhibiting homogeneous ooplasms and also clearly visible second polar bodies were selected in D-PBS/10% FBS.

Nuclear transfer began with making a hole on the zona pellucida (ZP) of the oocyte on the stage of an inverted microscope equipped with a micromanipulator (Narishige, Tokyo, Japan) and fluorescent illumination. The ZP was partially drilled to a 60 to 80% thickness by projecting a laser beam (MTM, Montreux, Switzerland) on the parietal surface of the oocyte for 20 to 40 μsec once or twice in a 60- to 80-μl droplet of M2 medium containing 7.5 μg/ml cytochalasin B overlaid with mineral oil. The nucleus and nearby polar body were removed by suction through the partially drilled hole using a 20- to 30-μm (outer diameter) micropipette. A single fibroblast, which had been cultured and loaded within the micropipette in advance, was next inserted into the perivitelline space of the ZP route which had been pierced for enuncleation.

Fusion, activation and *in vitro* culture of the karyoplast-cytoplast couplet

The enulcleated oocyte (cytoplast)-fibroblast (karyoplast) couplet was fused by the electrical pulse using a BTX Electrocell Manipulator (Genetronics, San Diego, CA, USA) as described (Keefer et al., 2001). The couplet was placed within the gap fusion chamber filled with a 0.5-mM Hepes buffer containing 0.05-mM $CaCl_2$, 0.1-mM $MgSO_4$ and 0.3-M mannitol (all from Sigma) in such a way that the cytoplast and karyoplast were directed to the negative and positive electrodes, respectively. The couplet was induced to fuse by applying a single DC pulse of 2.4 kV/cm for 30 μsec. Non-fused couplets after the initial pulse received the second and third repulses under the same conditions until fused 60 and 30 min after the first and second pulses, respectively.

The fused couplets were activated sequentially in M16 medium/10% goat serum for 3 h, in M16 medium/5-μM ionomycin for 5 min and in M16 medium/2-mM 6-DMAP for 4 h. The activated couplets, now entitled embryos, were transferred to SOF medium containing 0.8% BSA and incubated at 39°C in 5% CO_2/5% O_2/90% N_2 for 12 to 20 h up to a 2- to 4-cell stage until transferred to the recipient.

Embryo transfer

A total of 23 primiparous or biparous surrogate does (1 or 2-yr-old) were synchronized by the same hormonal treatment used for superovulation, except that FSH was not used. Two to 16 embryos were surgically transferred into the oviducts of a total of 23 recipients which had been fasted for 24 h. Pregnancy and fetal development were examined by unltrasonography using a 6.5 MHz Convex scanner (Medison, Seoul, Korea) on 30 and 60 days after embryo transfer.

Analysis of blood cells and plasma: genotpying, karyotyping, quantitation of telomere content and IGF-I

Genomic DNAs were extracted from donor fibroblasts and blood lymphocytes of the cloned goat and her dam of the present study using a DNA extraction kit (Qiagen, Hilden, Germany). Genotypes of the goat major histocompatibility complex (MHC) class II DRB gene were determined by the polymerase chain reaction-single strand conformational polymorphism (PCR-SSCP) as described by Keefer et al. (2001). Briefly, a 286-bp exon 2 fragment of the gene was amplified using a pair of primers: ACB0445, 5'-TATCCCGTCTCTCTGCAGCACATTTC-3'; ACB0446, 5'-ATCGCCGCTGCACACTGAAACTC-3'. After denaturing the PCR product at 80°C for 5 min and immediate chilling on ice for 2 min, resulting single-strand DNAs were resolved by 10% polyacrylamide gel electrophoresis and ethidium bromide staining. The PCR-SSCP results were further confirmed by sequencing the PCR products using an automated DNA sequencer.

The karyotype of the cloned goat was analyzed after GTG-banding on chromosome spreads prepared from peripheral blood lymphocytes following *in vitro* culture as previously described (Sohn et al., 2002a). The ratio of telomeric DNA to whole genome in cultured donor fibroblasts and blood lymphocytes was determined by the quantitative fluorescence *in situ* hybridization (Sohn et al., 2002b) using the telomeric repeat (TTAGGG)n probe and MetaMorph Imaging System (Universal Imaging Co.,

Table 1. Comparison between the two sources of oocytes in the rate of the cytoplast-karyoplast couplet fusion and subsequent cloning efficiency

Source of oocytes[a]	Couplets pulsed	Couplets fused	Embryos transferred	Recipient does	Pregnancy[b]		Parturition
					D30	D60	
Ovulated (*In vivo*)	169	123 (72.8%)**	105	17	3	3	1
Unovulated (*In vitro*)	115	61 (53.0%)	49	6	1	0	0

Data denote numbers unless indicated otherwise.

[a] Mature and immature oocytes were obtained from the infundibulum and ovary, respectively. Ovarian oocytes were maturated *in vitro* prior to nuclear transfer.

[b] Judged by ultrasonography at indicated days after embryo transfer.

** $p<0.01$ by the chi-square test.

Figure 1. A cloned female Korean Native Goat "Jinsoonny." Jinsoonny was cloned from an ear skin fibroblast derived from a 3-yr-old white doe via nuclear transfer. Note Jinsoonny's white hair vs. black hair of the doe which gave a birth to Jinsoonny.

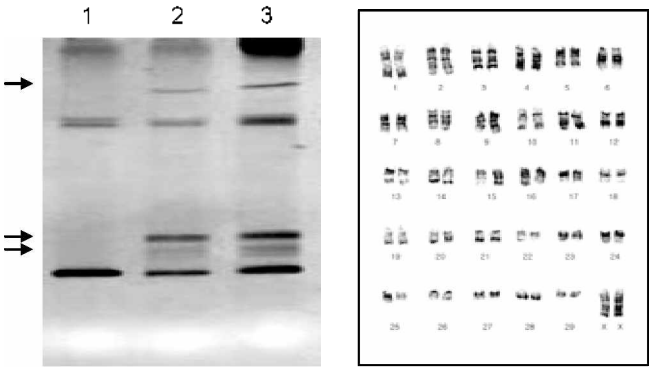

Figure 2. Genetic identification of the cloned female kid Jinsoonny. Left: MHC class II DRB genotyping by polymerase chain reaction-single stranded conformational polymorphism (PCR-SSCP). A 286-bp exon 2 fragment of the gene was amplified by PCR using genomic DNAs extracted from donor fibroblasts and blood lymphocytes as templates. After denaturing the PCR product at 80°C for 5 min, resulting single-strand DNAs were resolved by 10% polyacrylamide gel electrophoresis and ethidium bromide staining. Lane 1, lymphocytes of the surrogate doe; lane 2, donor fibroblasts; lane 3, Jinsoonny's lymphocytes. Note the identity of the bands of the SSCP band patterns between lanes 2 and 3 as well as the absence of the bands indicated by *arrows* in lane 1. Right: GTG-banded karyotype of Jinsoonny. GTG-banding was performed on chromosome spreads prepared from cultured blood lymphocytes of Jinsoonny.

Downingtown, PA, USA). Plasma insulin-like growth factor (IGF)-I concentration was determined by double-antibody radioimmunoassay after removal of IGF-binding proteins by the acid-ethanol extraction procedure as previously described (Lee et al., 2005b). The sensitivity of the assay and the intra-assay CV were 31.25 ng/ml and 5%, respectively.

RESULTS

Comparison of *in vivo*- vs. *in vitro*-matured oocytes in couplet fusion and subsequent development

Cloning of the KNG by virtue of nuclear transfer was attempted in the present study using a total of 284 enucleated oocytes and 23 recipient does. There was no difference in oocyte grade by the collection time (12.0 to 24.2% of Grade I oocytes; data not shown). As shown in Table 1, the rate of fusion of the enucleated oocyte (cytoplast) and the donor fibroblast (karyoplast) following the electrical pulse was greater ($p<0.01$) for *in vivo*-matured oocytes than for *in vitro*-matured ones (72.8 vs. 53.0%). A total of 105 *in vivo*-derived embryos were transferred to a total of 17 recipient does, three of which were judged pregnant by ultrasonography on days 30 and 60 after embryo transfer. However, only one doe delivered a normal female kid of 1.9 kg on day 149, indicating that the other two miscarried after day 60. The other 49 *in vitro*-derived embryos were transferred to 6 recipients, one of which conceived but miscarried between days 30 and 60 as judged by ultrasonography.

Genetic identification of the cloned kid

The cloned female kid, which was named "Jinsoonny," was first identified by her white hair vs. black of her dam at birth (Figure 1), because Jinsoonny was cloned from a white-colored doe. Consistent with this, Jinsoonny's major histocompatibility complex class II DRB genotype was

Figure 3. Growth curve of the cloned goat. Indicated in this figure are body weight (BW) of the cloned female goat Jinsoonny and the mean±SD of BW of three age (day)-matched female goats at each month during 7-mo postnatal development.

identical with that of the donor fibroblast (karyoplast), but not with that of the surrogate doe's lymphocyte, as analyzed by the polymerase chain reaction-single stranded conformation polymorphism (Figure 2, left panel). Genotypes of these cells were further confirmed by sequencing the PCR products (data not shown).

The karyotype of Jinsoonny was also analyzed after GTG-banding on chromosome spreads prepared from her peripheral blood lymphocyte at 2 mo of age. As shown in the right panel of Figure 2, she had 60, XX normal chromosomes whose GTG-banded karyotype was identical with the ISCNDB2000 (2001) standard for the goat.

Growth and development and associated variables of the cloned kid

Jinsoonny was born with a normal weight (1.9 kg), which was close to the average of her three female companions (2.05±0.10 (SD) kg) born on the day of Jinsoonny's birth. However, Jinsoonny grew much faster than her age-matched companions (Figure 3). She outweighed her companions at 2 and 4 mo of age by 56% (12.48 vs. 8.0±0.96 kg) and 70% (19.58 vs. 11.49±0.80 kg), respectively. Jinsoonny's accelerated growth rate tended to decrease after 4 mo of age, but, nevertheless, the weight difference between her and the others was greater than 2 SD up to 7 mo of age. By contrast, plasma IGF-I concentration of Jinsoonny at 7-mo age was not different from that of her companions (126 vs. 131±20 ng/ml).

Jinsoonny exhibited a faster development than her age-matched companions as well. She showed physiological and behavioral signs of estrus at 6 mo of age. It was a few months earlier than that of normal kid does. She mated with a white-colored buck at her second natural estrus. Jinsoonny's pregnancy was confirmed on day 45 by ultrasonography.

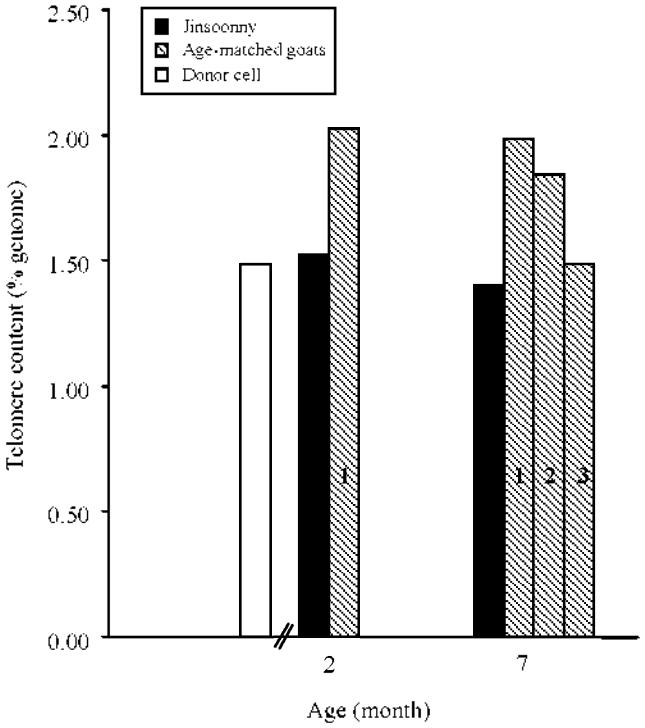

Figure 4. Telomere lengths of donor fibroblasts and peripheral blood lymphocytes of the cloned female goat Jinsoonny and her age-matched companions. Telomere length or content, expressed as percent genome, was determined by quantitative fluorescence *in situ* hybridization. Numbers within the bars of the three age-matched goats indicate their identities of which telomere length was measured at both 2- and 7-mo ages only in #1 animal.

The ratio of telomeric DNA to total genome representing telomere length was measured by Q-FISH using a telomeric DNA repeat probe. Jinsoonny's telomere length at 2-mo age was similar to that of the donor fibroblast (Figure 4). The telomere length of Jinsoonny decreased 8% between 2- and 7-mo ages, while it decreased only 2% during the same period in one of her age-matched companions. Furthermore, at 7-mo age, Jinsoonny's telomere length was 21% shorter than the average of those of her three age-matched companions (1.40% vs. 1.77±0.26% (SD)).

DISCUSSION

One cloned female KNG originated from an ear skin fibroblast of a 3-yr-old female was generated out of 154 NT embryos which had been transferred to 23 surrogates in the present study. The identity of the cloned kid was confirmed by her hair color and major histocompatibility complex class II DRB genotype at birth which were identical with those of the somatic cell donor. The overall cloning efficiency (no. of offsprings/no. of transferred embryos) of 0.65% (1/154) obtained in the present study was lower than previously reported values of 2.7% to 4.7% in this species

(Baguisi et al., 1999; Keefer et al., 2001; Reggio et al., 2001; Behboodi et al., 2004). One possible explanation for the low cloning efficiency in the present study could be the low pregnancy rate (17.4%) on day 30 after embryo transfer compared with previously reported values ranging from 21.7% to 65.8% (Baguisi et al., 1999; Keefer et al., 2001; Reggio et al., 2001; Behboodi et al., 2004). In other species, a wide range of overall cloning efficiency from 0.85% to 10% has been reported (Campbell et al., 1996; Wells et al., 1997; Wakayama et al., 1998; Wells et al., 1998; Wilmut and Campbell, 1998; Dominiko et al., 1999). Another reason for the low efficiency might be pregnancy loss. There are several reports supporting our speculation on the pregnancy loss during early pregnancy, especially between days 30 and 60 (Wells et al., 1998; Hill et al., 2000; Edwards et al., 2001). In goats, Baguisi et al. (1999) reported that the failure of early embryonic development and resorption of embryonic structures were observed by day 55 to 70 after transfer of NT embryos.

It is well known that many factors related to NT procedures, culture conditions, and season for superovulation are involved in obtaining normal pregnancies and offsprings by NT (Edwards et al., 2003; Chang et al., 2006). Of them, the site of oocyte maturation, i.e. *in vivo* vs. *in vitro*, is one of major factors affecting the cloning efficiency (Wakayama et al., 1998; Wells et al., 1998; Baguisi et al., 1999). In farm animal species including goats, it has been reported that NT embryos derived from *in vitro*-matured oocytes can cause high prenatal and postnatal losses as well as poor embryo survival and pregnancy rates compared with NT embryos derived from *in vivo*-matured oocytes (Wilmut et al., 1997; Hill et al., 1999, 2000; Zakhartchenko et al., 1999; Keefer et al., 2001; Reggio et al., 2001; Rideout et al., 2001). However, there are some cases where cloned goats were produced from *in vitro*-matured oocytes (Baguisi et al., 1999; Keefer et al., 2001, 2002; Reggio et al., 2001). Oocyte maturation consists of nuclear and cytoplasmic maturation. The synchronization of nuclear and cytoplasmic events is critical for further development of embryos (Eppig, 1996; Thomas et al., 2004). In this study, we used a method of different from others for harvesting follicular oocytes. As mentioned above, follicular (*in vitro*-matured) oocytes were collected by aspirating the follicles on the ovaries just after recovery of oviductal (*in vivo*-matured) ones. The oocytes in small antral follicles on both ovaries had already been influenced by exogenous hCG when the dominant follicles were ovulated. As a result, the oocytes from these follicles might have been aged or lost their developmental potential. Kumar et al. (1991) observed that injection of hCG induced premature condensation of chromatin without germinal vesicle breakdown (GVBD) in goat oocytes. In this respect, the failure of obtaining any cloned kid in the *in vitro*-matured oocyte group in the present study, which was consistent with a previous report (Behboodi et al., 2004), may have resulted from an inferior quality of *in vitro*-matured oocytes to that of *in vivo*-matured ones. In addition, the 3 non-viable pregnancies in our study might have resulted from a lack of developmental ability of the oocytes and/or early pregnancy loss. However, it still remains to be elucidated how embryos derived from *in vivo*-matured oviductal oocytes can support further development and fetal growth better than those derived from immature follicular oocytes in cloning by NT.

An interesting feature of the cloned female goat, Jinsoonny, was her accelerated growth and development. Even though she was born with a normal range (1.9 to 2.1 kg) of body weight (1.9 kg), she outgrew her age-matched females during the first 4 mo of postnatal life by as much as 87% and reached puberty by 6 mo of age. It was a few months earlier than that of normal kid does. It is well known, however, that over-sized fetuses and large offsprings can appear following various manipulations of the embryos in ruminants (Walker et al., 1996; Vignon et al., 1998; Young et al., 1998).

Insulin-like growth factor-I (IGF-I) is well known to be associated with postnatal growth rate in domestic animals. Thus, we speculated that circulating IGF-I concentration may vary in accordance with the growth rate. However, we found no difference in plasma IGF-I concentration between Jinsoonny and her age-matched companions at 7 months of age. In cattle, decreased plasma IGF-I level was found in cloned calves compared with age-matched controls (Govoni et al., 2002; Matsuzaki and Shiga, 2002). In contrast to this, another study showed that IGF-I level was not different between cloned calves and control ones (Chavatte-Palmer et al., 2002). In goats, Landry et al. (2005) reported that circulating IGF-I level was less variable in cloned does than in naturally mated does, but that was within a normal range in the former. Research has suggested that IGF-I level may depend on temperature, feeding, stress and other factors (Buonomo and Baile., 1991; Carroll et al., 1998; Landry et al., 2005). Taken together, there may not exist any direct correlation between IGF-I concentration and growth rate in cloned goats.

There have been lots of reports focusing on telomere length of cloned animals by SCNT. Some authors reported that telomere length of the cloned animals was shorter than that of age-matched controls (Shiels et al., 1999a; Miyashita et al., 2002). In contrast, others reported it was not different (Tian et al., 2000; Betts et al., 2001; Miyashita et al., 2002) or even longer (Lanza et al., 2000; Miyashita et al., 2002; Jeon et al., 2005). Accordingly, telomere lengths of Jinsoonny and her age-matched companions at different ages were measured. Jinsoonny's telomere length, which was similar to that of the somatic cell donor at 2 mo of age.

decreased 8% during a 5-mo period up to 7-mo of age at which time her telomere was 21% shorter than the average of her companions. These results are interpreted to suggest that Jinsoonny's fast growth is likely to have resulted from NT-associated epigenetic reprogramming involving telomere shortening, although she had the normal 60, XX karyotype. Similarly, "Dolly" cloned from a 6-yr-old ewe (Wilmut et al., 997) also displayed 20% shortened telomere at 1 yr of age (Shiels et al., 1999b) compared with age-matched sheep, although she did not grow faster than the others. However, it also needs to be noted that telomere lengths of cloned animals by SCNT were variable depending on donor cell types (Miyashita et al., 2002; Betts et al., 2005).

In conclusion, our results showed that the *in vivo*-matured oocytes as recipient cytoplasts could support full development of a healthy cloned goat by SCNT. To our knowledge, this is the first case in which a cloned animal born with a normal weight exhibited accelerated growth and development. As the shorten telomeres are not solely responsible for a postnatal rapid growth, more studies are needed to find out the factors which cause the fast growth of the cloned goat.

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