

# Bovine Oocyte Cytoplasm Supports Development of Embryos Produced by Nuclear Transfer of Somatic Cell Nuclei from Various Mammalian Species<sup>1</sup>

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## ABSTRACT

The transfer of nuclei from one cell to another provides a powerful tool for studying the interactions between the cytoplasm of one cell and the nucleus of another. This study was designed to examine the ability of the bovine metaphase oocyte cytoplasm to support mitotic cell cycles under the direction of differentiated somatic cell nuclei of various mammalian species. Skin fibroblast cells from cows, sheep, pigs, monkeys, and rats were used as sources of donor nuclei. Nuclear transfer units produced by fusion of enucleated bovine oocytes and individual fibroblasts from all species examined underwent transition to interphase accompanied by nuclear swelling, further progression through the cell cycle, and completion of the first mitosis. Regardless of the species of donor fibroblasts used, some cleaving units progressed further and developed to advanced stages, as evidenced by continuation of cell proliferation and formation of a blastocoele cavity at the time appropriate for the donor fibroblast species. Although no pregnancies have been carried to term after transfer of embryos into surrogate animals, these observations suggest that mechanisms regulating early embryonic development may be conserved among mammalian species and that bovine oocyte cytoplasm can support the introduced differentiated nucleus regardless of chromosome number, species, or age of the donor fibroblast.

## INTRODUCTION

Nuclear transfer (NT) methods developed in amphibians [1, 2] and later in mammals [3, 4] offer the opportunity to address fundamental questions about differentiation and its reversibility. After being introduced into an enucleated recipient oocyte, nuclei from various differentiation states have been shown to be reprogrammed and are able to initiate another round of embryonic development. Offspring have been obtained from embryos created by the NT of embryonic blastomeres [4–9], inner cell mass cells [10], cultured embryonic cells [11, 12], embryonic stem cell-derived epithelial cells [13], primordial germ cells [14], fetal fibroblasts [15–17], and adult somatic cells [16, 18]. Development of these embryos to term set the stage for re-examination of the widely accepted hypothesis that during differentiation, a somatic cell nucleus loses its totipotency and therefore cannot be reprogrammed to initiate another round of embryonic development.

Efficiency of the NT procedure, however, remains low despite numerous improvements made during the past decade, and the percentage of live offspring obtained after

transfer of embryos created by NT, regardless of the species, usually does not exceed 3% [16, 18]. A better understanding of cell cycle compatibilities between the recipient cytoplasm and donor cell [19–25], as well as the development of new techniques for the introduction of nuclei and activation of newly created zygotes [18], holds promise for future improvements. One of the many problems that accompany the success of the NT outcome is the availability of species-specific competent recipient cytoplasm. Incomplete understanding of oocyte maturation, limited availability, and high cost of recipient cytoplasts (e.g., monkeys) could be overcome if a common cytoplast donor could be used successfully. Development of such a common model for investigation of interactions between recipient cytoplasm and the introduced diploid nucleus during an NT procedure would greatly benefit ongoing research efforts. In the present paper we examine the ability of bovine oocyte cytoplasm to support proliferation of somatic cell nuclei from several mammalian species. We ask questions about whether chromosome number, donor nucleus species, and age of a differentiated nucleus influence the ability of bovine oocyte cytoplasm to follow the directions of the introduced nucleus. We examine fusion efficiency, rate of the first embryonic cleavage, rate of blastocyst development, and pregnancy initiation of embryos derived by combining rat, pig, monkey, sheep, and bovine fibroblasts with bovine recipient cytoplasm.

## MATERIAL AND METHODS

### *Recipient Cytoplasm Preparation*

Recipient bovine oocytes were matured according to procedures previously shown to produce developmentally competent oocytes [26, 27]. Cow oocytes were obtained by aspiration of small antral follicles. Immature cumulus-oocyte complexes were cultured in Tissue Culture Medium 199 supplemented with 10% fetal calf serum (FCS), 0.2 mM pyruvate, 25 µg/ml gentamicin, 0.5 µg/ml LH (NIH, Bethesda, MD), and 1 µg/ml estradiol-17β for 16 h at 39°C with 5% CO<sub>2</sub> in air. Sixteen hours after the start of maturation, cumulus cells were removed by manual pipetting in the presence of 2.5 mg/ml hyaluronidase, and oocytes with extruded first polar body (metaphase II arrest, MII) were selected for enucleation. Early-maturing oocytes that extruded their first polar body by 16 h after the initiation of culture were selected as recipients. The oocytes were labeled with 0.5 µg/ml DNA fluorochrome (Hoechst 33342) for 15 min at room temperature in TALP-Hepes medium (Hepes buffered-Tyrodé's containing lactate, 0.2 mM pyruvate, and 3 mg/ml BSA), washed, and placed in a manipulation drop of TALP-Hepes supplemented with 7.5 µg/ml cytochalasin B. All manipulations were performed on a Nikon Diaphot (Garden City, NY) microscope equipped with Hoffman (Greenvale, NY) optics and Narishige (Tokyo, Japan) micromanipulators. The first polar body and

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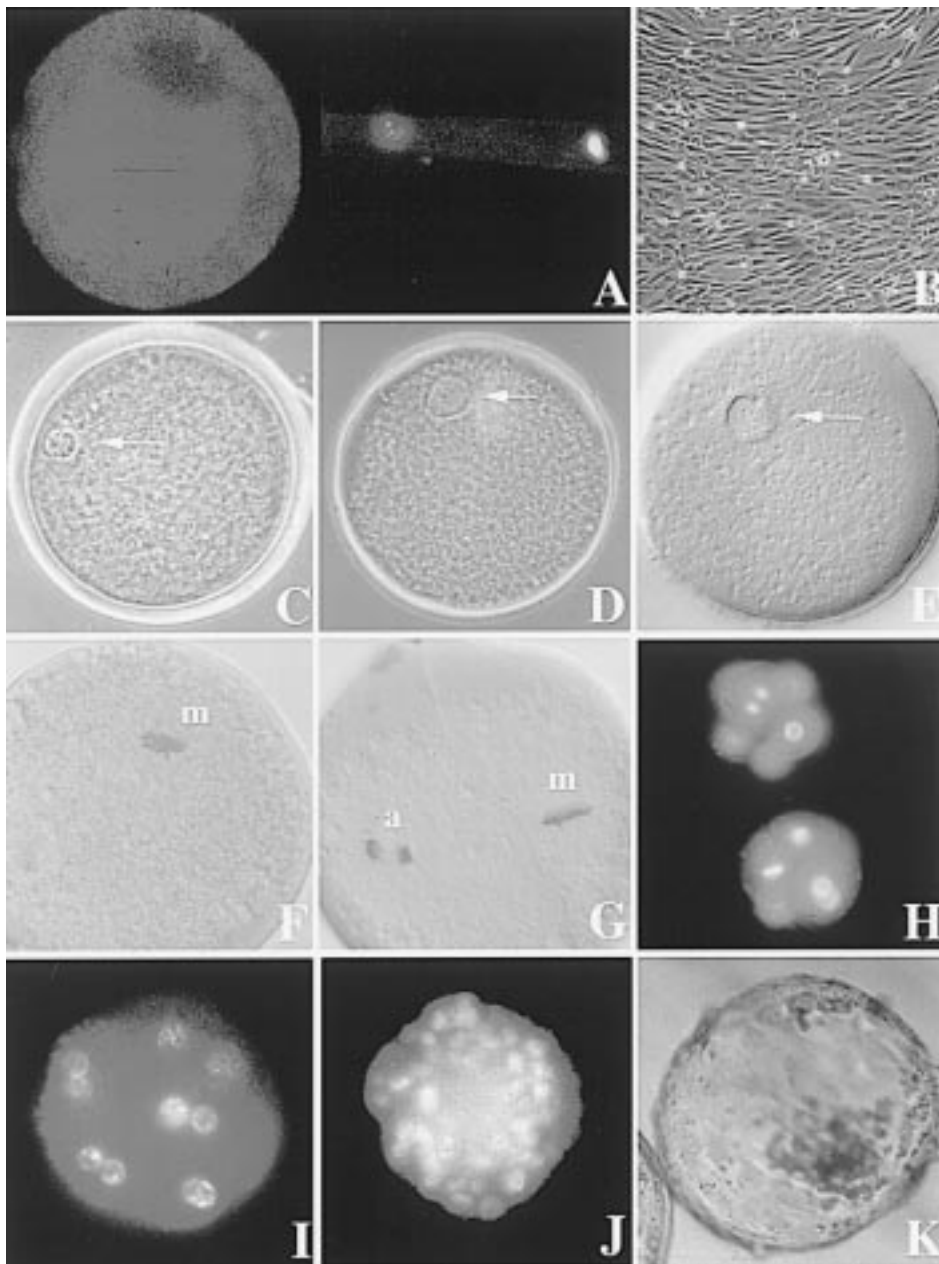


FIG. 1. **A)** Enucleation of a mature bovine oocyte. Chromosomes aligned on the second metaphase spindle and the first polar body are removed by micromanipulation. DNA is visualized under UV illumination after vital staining of the oocyte with Hoechst 33342. **B)** Primary fibroblast cell culture from cynomolgus monkey skin. **C–G)** Morphology of the introduced sheep somatic cell nucleus in NT embryos. The embryos were sampled 1 h after fusion (**C**), 1 h after activation (**D**), and 7 h after activation (**E**). The nucleus progressively enlarges (arrows) during the first embryonic interphase. Twenty-four hours after activation, the embryo entered the first mitosis; m, metaphase (**F**). Second mitotic division in an embryo 36 h after activation; m, metaphase; a, anaphase (**G**). **H)** Two NT embryos produced from monkey fibroblasts on Day 3 after activation. **I)** A 10-cell pig-cow embryo after staining with Hoechst 33342. **J)** A 64-cell sheep-cow embryo that failed to undergo compaction and blastocyst formation, stained with Hoechst 33342 and nuclei visualized under UV. **K)** Hatched sheep-cow blastocyst 8 days after activation.

MII plate were removed by aspiration with a 25- $\mu$ m inner diameter enucleation pipette. To ensure that oocyte chromatin was removed, the aspirated cytoplasm was exposed to UV light and examined for the presence of the removed polar body and metaphase plate (Fig. 1A). All chemicals were purchased from Sigma Chemical Company (St. Louis, MO) unless noted otherwise.

#### Culture of Donor Cells

Ear skin samples were obtained by biopsy from an adult cow (7 yr), a pig (4 wk), a ram (5 yr), a cynomolgus monkey (9 yr), and a rat (4 wk), and all the samples were processed identically. Tissue was manually cut into small pieces and enzymatically digested with 0.5% trypsin-EDTA in PBS for 30 min at 30°C with occasional stirring. Digested tissue was washed in PBS, and the procedure was repeated several times. Disaggregated cells were separated from larger pieces of tissue by centrifugation at 100  $\times$  g for 5 min. Supernatants were washed several times in Ca<sup>2+</sup>-

Mg<sup>2+</sup>-free PBS, and the final supernatant was centrifuged at 700  $\times$  g for 10 min to obtain a cell pellet. The pellet was diluted with  $\alpha$ -MEM (Minimum Essential Medium), supplemented with 10% FCS and placed in culture at 37°C with 5% CO<sub>2</sub> in air. After 10–14 days in culture, confluent fibroblast monolayers were obtained (Fig. 1B). Cells were passaged approximately once a week. Samples from progressively growing cell lines that were established were frozen for future studies. Three to ten days prior to NT procedure, fibroblasts were placed into serum-free  $\alpha$ -MEM in order to exit the cell cycle and accumulate in the G<sub>0</sub>/G<sub>1</sub> phase. The cells were detached from the dishes by brief exposure to 0.025% trypsin-EDTA, washed in PBS, and placed into a manipulation drop.

#### NT, Fusion, and Activation

Fibroblasts that had been cultured without serum for at least 3 days [16] were used as donor cells. Somatic cells were introduced into young bovine MII cytoplasts imme-

diately after removal of bovine nuclear DNA. Manipulation was done in 150- $\mu$ l drops of TALP-Hepes supplemented with 7.5  $\mu$ g/ml cytochalasin B. A single fibroblast was placed into the perivitelline space of an enucleated oocyte. NT was completed by 30 min after enucleation. The couplets were placed into TALP-Hepes supplemented with 3 mg/ml fatty acid-free BSA and kept on a warm plate prior to fusion. Recipient cytoplasts and donor nuclei were fused by a double electric pulse within 30 min of NT. Prior to fusion, NT units were placed into fusion medium (0.25 M sorbitol, 100  $\mu$ M calcium-acetate, and 100  $\mu$ M magnesium-acetate) for 10 min. They were then transferred into a fusion chamber consisting of two wires, 0.5 mm apart, and overlaid with the fusion medium. Two electric pulses (1.8–2.0 KV/cm, 30  $\mu$ sec each) were applied. A 15-min recovery in TALP-Hepes containing 20% FCS followed fusion to allow membranes to return to their normal appearance. NT units were activated between 24 and 28 h after the start of maturation (6–10 h post-NT) using a procedure described by Susko-Parrish et al. [28]. The NT units were washed through  $\text{Ca}^{2+}$ - and  $\text{Mg}^{2+}$ -free TALP-Hepes and then exposed to 5  $\mu$ M ionomycin for 4 min, washed in 30 mg/ml BSA in TALP-Hepes, and incubated in 1.9 mM 6-dimethylaminopurine-6-DMAP [28] in CR1aa [29] for 4 h at 39°C and 5%  $\text{CO}_2$  in air.

#### *Embryo Culture, Transfer, and Pregnancy Monitoring*

After activation, the NT units were washed again and placed into CR1aa for embryo culture. CR1aa was used for culturing of embryos produced from all five species [29]. The embryos were stained with Hoechst 33342 (5  $\mu$ g/ml) to examine fusion efficiency. Some of the zygotes containing DNA were fixed with acid-ethanol (acetic acid:ethanol 1:3) and stained with orcein (1% orcein in 45% acetic acid) to assess chromatin morphology by phase-contrast microscopy. Samples of NT embryos were examined 1 h after fusion and 1, 7, 24, and 36 h after activation. The remaining NT embryos were transferred into 50- $\mu$ l drops of CR1aa medium containing 10% FCS after 3 days. Embryos were examined 24 h after activation for initial cleavage and monitored every 24 h for progression of development through Day 9. At various times throughout embryo culture, a few embryos were selected and stained with Hoechst 33342 to confirm the presence of embryonic nuclei in individual blastomeres. Cow-cow NT blastocysts (a single blastocyst per animal) were transferred nonsurgically into recipients on Day 7 postestrus. NT embryos produced from pig and sheep donor fibroblasts were transferred at the 8- to 16-cell stage into the uterine horns by laparoscopy. Two embryos were transferred into each uterine horn of surrogate sheep on Day 4 after estrus, and 5–6 embryos into each uterine horn of surrogate sows on Day 3 after estrus. Embryos produced from rat fibroblasts were transferred at the 2- to 4-cell stage into rat oviducts. Pregnancies were determined by ultrasound on Day 35 in cows and Day 25 in ewes and sows. Surrogate rats were transabdominally palpated on Day 14 after embryo transfer.

## RESULTS

#### *Donor Chromatin Configuration during the First Embryonic Cell Cycle*

Embryos created from all donor fibroblast species were examined during the first 36 h after fusion. Twenty-three NT embryos were fixed in acetic acid:ethanol (1:3) and

stained with aceto-orcein. Successfully fused NT units contained the somatic cell nucleus at the periphery of the oocyte cytoplasm (Fig. 1C), and the nucleus did not change in size during exposure to MII cytoplasm. The nuclear envelope remained intact, and no premature chromosome condensation was observed. Enlargement of the nucleus was apparent by the time the activation protocol was complete (Fig. 1D) and persisted throughout zygotic interphase (12 h postactivation) in intra- as well as in interspecies NT units. Decondensed chromatin was contained within a nuclear envelope and no chromatin fragmentation was observed at any time point examined (Fig. 1E). First cleavage occurred between 16 and 24 h after activation regardless of the donor nucleus species (Fig. 1F), and the second mitosis could be observed 36 h after activation (Fig. 1G).

#### *Timing of Embryonic Cleavage Divisions and Developmental Potential of Interspecies Embryos*

The biggest challenge in producing NT units using fibroblasts proved to be fusion by electroporation, the current method of choice in NT. The small size of fibroblasts used as nuclear donors (12–20  $\mu$ m) provided less extensive contact area between the oocyte cytoplasm and the donor plasma membrane (in comparison to embryonic blastomeres), making fusion rates low. The removal of a portion of the oocyte cytoplasm during enucleation increased the size of the perivitelline space—size that could not be compensated for by a small somatic cell. Even though the donor cell was wedged between the zona and the oocyte plasma membrane after insertion, it was often displaced by the time the units were being fused. Absence of a close contact between the membranes made fusion unsuccessful in numerous attempts to create NT units (Table 1). However, from fused units, proportions of those that completed the first cell cycle were 55.8%, 66%, 52.2%, 85.7%, and 90.2% for cow, sheep, pig, monkey, and rat NT embryos, respectively. Timing of the first two cleavage divisions corresponded more closely to the timing of cleavage observed in bovine in vitro-produced embryos regardless of the donor nucleus species. However, with every progressive cleavage division the embryos started approaching the donor species-specific timing of development (Fig. 2). Embryonic nuclei were visualized by Hoechst 33342 fluorochrome staining under UV illumination [30, 31] to exclude the possibility of cytoplasm fragmentation in the absence of DNA (Fig. 1, H and I). We further observed that a number of embryos failed to undergo compaction. In these noncompacting embryos, numerous well-defined small blastomeres occupied the center of the intrazonal space, leaving a substantial gap between the embryo and the zona pellucida (Fig. 1J). No flattening of the outer cells was observed, and cells remained distributed evenly throughout the embryo. The embryos failed to undergo blastocoele formation after prolonged culture even though they remained viable and the cell number was increasing. The embryos that underwent compaction, however, started forming a blastocoele cavity (Fig. 1K). Proportions of interspecies embryos that developed to the blastocyst stage were not significantly different from those for intraspecies NT embryos (Table 1). With progression of embryo culture, expansion of the blastocoele cavity, as well as flattening of outer cells on one pole and concentration of inner cells at the other pole, was observed in these embryos (Fig. 1K). The time of the onset of blastocoele cavity formation corresponded more closely with the time of this event in the species of the donor nucleus. Sheep and pig

TABLE 1. In vitro development of intra- and interspecies NT embryos produced in bovine oocyte cytoplasm.

Donor nucleus species	NT units produced	Fused (%) <sup>a</sup>	2-Cell stage (% of fused)	>16 Cells (% of 2-cell)	Blastocyst (% of 2-cell)
<i>Bos taurus</i> (cow)	220	145 (65.9) <sup>a</sup>	81 (55.8) <sup>a</sup>	22 (27.2) <sup>a</sup>	14 (17.3) <sup>a</sup>
<i>Ovis aries</i> (sheep)	327	182 (55.6) <sup>b</sup>	129 (66.0) <sup>b</sup>	38 (31.6) <sup>a</sup>	18 (13.9) <sup>a</sup>
<i>Sus scrofa</i> (pig)	175	76 (43.4) <sup>b</sup>	42 (52.2) <sup>a</sup>	18 (42.8) <sup>b</sup>	6 (14.3) <sup>a</sup>
<i>Macaca fascicularis</i> (monkey)	30	28 (93.3) <sup>c</sup>	24 (85.7) <sup>c</sup>	14 (58.3) <sup>b</sup>	4 (16.6) <sup>a</sup>
<i>Rattus rattus</i> (rat)	108	37 (34.2) <sup>b</sup>	33 (90.2) <sup>c</sup>	NA <sup>**</sup>	NA <sup>**</sup>

<sup>a</sup> Fusion was determined after staining with Hoechst 33342.

<sup>\*\*</sup> NA, not available; NT embryos were used for embryo transfer.

<sup>a-c</sup> Different superscripts within the same column are statistically different ( $p < 0.05$ ); percentages were transformed using arcsine transformation, analyzed by ANOVA and means compared by protected LSD.

blastocysts were observed as early as Day 5, and bovine and monkey blastocysts not before Day 6 and 8 after NT, respectively (Fig. 2). All of the early embryos (2- to 4-cell stage) produced from rat fibroblasts were transferred into uterine horns of surrogate rats, and therefore further developmental results were not available.

*Embryo Transfer and Detection of Pregnancy*

Pregnancy results are presented in Table 2. None of the embryos transferred to surrogates progressed to a developmental stage when heartbeats should have been detected by transabdominal (Day 25 after transfer in sheep and pigs) or transvaginal (Day 35 after transfer in cows) ultrasonography. No fetuses were detected by palpation on Day 14 after transfer in rats. Ten surrogate cows displayed extended estrous cycles (35–48 days after embryo transfer). Two surrogate sheep displayed sacks of fluid within uterine horns and were hysterectomized shortly after ultrasonography. Both uterine horns in both animals had distinctly developed caruncles; however, no fetal membranes or fetal remnants were found.

**DISCUSSION**

Events occurring just after fusion between recipient cytoplasm and a donor nucleus are poorly understood. The

extent of remodeling that enables the introduced genome to recall its embryonic characteristics depends, among other factors, on the cell cycle stage of the recipient cytoplasm and donor cell [19–25]. Which combination is optimal for induction of a new round of embryonic development, able to support development to term consistently, remains to be determined. In an attempt to improve the interaction between the recipient cytoplasm and donor nucleus, a novel protocol was employed. We hypothesized that the interaction between a differentiated nucleus and MII cytoplasm for an extended period of time would be beneficial for modification of the somatic nucleus. Donor nuclei remained in this environment for at least 6 h prior to activation. It has been observed that morphological changes of the introduced nuclei after transfer into an enucleated MII oocyte depend on whether or not the recipient cytoplasm has been activated prior to the introduction of a new nucleus [25, 32–37]. Nuclear envelope breakdown (NEBD) followed by premature chromosome condensation (PCC), disappearance of nucleoli, reformation of the nuclear envelope, and nuclear swelling can frequently be observed when MII cytoplasm is used [20, 25, 32]. In the present study we did not observe NEBD and PCC when nuclei from fibroblasts presumably arrested in G0/G1 were introduced into MII cytoplasm; these results are similar to those of Kubiak et al.

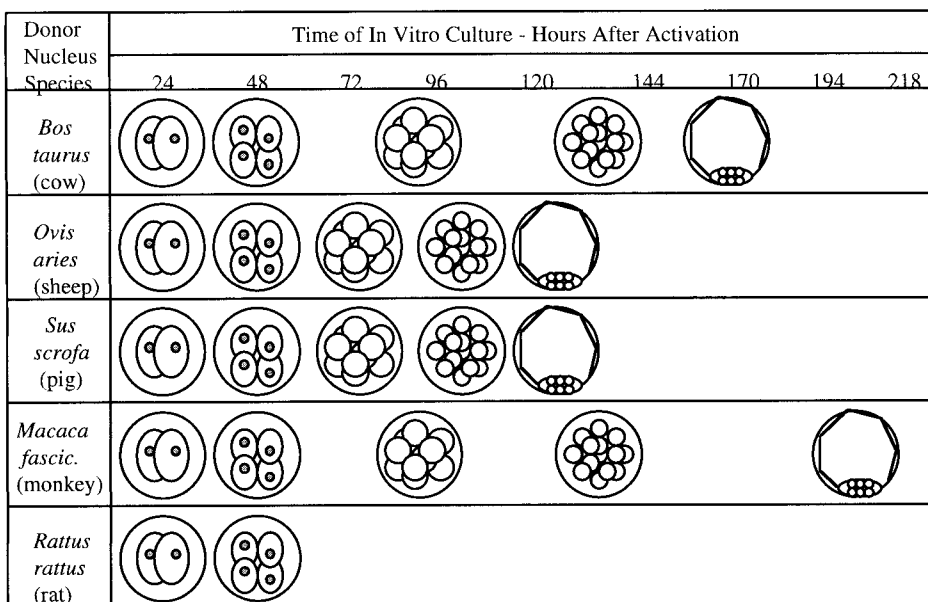


FIG. 2. Average timing of early embryonic development in intra- and interspecies NT embryos.

TABLE 2. Embryo transfer of intra- and interspecies NT embryos into surrogate animals.

Donor nucleus species	Embryos transferred (n)	Recipients (n)	Pregnant (n)	Offspring (n)
<i>Bos taurus</i> (cow)	19	19	10*	0
<i>Ovis aries</i> (sheep)	14	8	2**	0
<i>Sus scrofa</i> (pig)	25	3	0	0
<i>Macaca fascicularis</i> (monkey)	0	NA	NA	NA
<i>Rattus rattus</i> (rat)	32	2	0	NA

\* Surrogate animals had extended estrous cycles (35–45 days).

\*\* Fluid-filled sack was present in uterine horns of surrogate sheep on Day 32 after embryo transfer, however, no heart beats were detected; both animals were slaughtered and their uteri examined for fetuses and fetal membranes.

[38] and Fulka et al. [39]. Two electrical pulses used to fuse the donor cells with MII cytoplasts in our experiments may have been sufficient to decrease the remaining maturation-promoting factor activity and prevent NEBD and PCC from occurring. They were not, however, sufficient to cause the transition to zygotic interphase. The fact that high percentages of zygotes in this study completed several cleavage divisions leads us to believe that the combination of an MII cytoplast with G0/G1 somatic nucleus and their interaction for an extended period of time was beneficial for later development. Similar observations have been recently reported by Wakayama et al. [18]. Studies unequivocally showing which cell cycle stage of the recipient cytoplasm is the most suitable for reprogramming remain to be performed.

Fusion rates were lower than those observed after intraspecies fusion, due at least in part to the smaller size of fibroblasts of various species. Efficient fusion depends on not only healthy cell membranes but also on the extent of the contact between the donor cell plasma membrane and the oocyte plasma membrane. In addition, cell membrane properties and their compatibility with bovine oocyte plasma membrane may be determining the optimal time and field strength of electrical pulsing. Nevertheless, fused NT units initiated cleavage divisions and progressed to the 16-cell stage at high rates. The earliest stages of embryogenesis in normal embryos are regulated by maternally inherited gene products stored within the oocyte cytoplasm. Progression of development becomes dependent on embryonic gene activation at a species-specific developmental stage (reviewed in [40]). This occurs at the 2- to 4-cell stage in rats [41], late 4-cell stage in cattle [42] and pig [43], and 8-cell stage in sheep [44]. To our knowledge the timing of the maternal-to-embryonic transition in nonhuman primate embryos has not been reported; in human embryos it occurs at the 4- to 8-cell stage [45]. One of the morphological features that characterize the time of the transition is a developmental block in nonpermissive in vitro culture conditions [44]. In our study, high proportions of NT units progressed beyond the transcription-requiring, species-specific developmental stage. We can propose at least two scenarios explaining our results. Continuation of development could be a consequence of efficient reprogramming of the donor nucleus, regardless of the species, followed by now embryonic gene expression. Alternatively, it could indicate incompatibilities between the new components synthesized

by the donor nucleus and the components left over from the recipient cytoplasm. In this case, the introduced fibroblast nucleus would be directing cell proliferation, and the resulting multicellular structure would have few or no embryonic characteristics. Our observations of embryo-like structures containing a high number of cells that would not undergo compaction support the latter scenario. Expression of specific genes [46, 47] has been shown to be required for compaction and cavitation in developing embryos [48]. Absence of compaction in some embryos in this study would suggest lack of or impairment in transcription of genes required for these differentiation events to occur [48].

The embryos that underwent compaction started forming a blastocoele cavity. Since bovine oocyte DNA had been removed and its removal confirmed by UV illumination, it is reasonable to assume that development in interspecies embryos was directed by the introduced fibroblast nucleus. As expected, disparity in the number of chromosomes between the species used for NT (60 in cattle, 54 in sheep, 38 in pigs, 42 in monkeys, 42 in rats) does not seem to be limiting for this developmental success. Even though embryonic development seemed “normal,” possible ploidy problems have to be considered [25, 36]. High proportions of cleaving embryos, regardless of the donor nucleus species, developed to advanced stages in CR1aa medium, the medium designed for bovine embryo culture [29]. It is well established that embryos from different mammalian species require species-specific embryo culture conditions (reviewed in [49]). The ability of CR1aa to support embryonic development of interspecies units may be attributable to the fact that this development is driven by bovine cytoplasm alone. Since bovine oocyte cytoplasm supports the first three embryonic cell cycles in the absence of embryonic transcription, this seems like a reasonable possibility. Alternatively, CR1aa may be supporting embryo development of embryos of other species as successfully as it supports bovine embryos.

Timing of the first two cleavage divisions was not different in NT embryos produced regardless of the donor nucleus species. These results are not surprising, since the first cleavages occur in several mammalian species embryos at very similar times (reviewed in [50]). As development progresses, the length of embryonic cell cycles begins to vary between species. There is a considerable species variation in the length of preimplantation period as well as time of blastocyst formation [50], but the overall structural and morphological characteristics of the developing embryos and ultimately blastocysts are similar in most mammals. Cow-cow NT units that successfully fused and activated started cleaving and developed to specific embryonic stages in accordance with the timing of these events observed in in vitro-fertilized bovine embryos. Timing of cleavage divisions in the sheep-cow embryos was accelerated as compared to that in bovine-bovine counterparts. Sheep-cow embryos started forming blastocyst-like structures on Day 5 after activation, similar to in vitro fertilization-produced sheep embryos [51]. Similarly, timing for NT embryos produced from nuclei of other species resembled timing of development in vitro similar to that for in vitro fertilization-produced embryos: units produced from pig fibroblasts started forming blastocoele cavity 5 days after activation [52], and those produced from monkey fibroblasts 8 days after activation [53]. This would argue for an active role of the donor nucleus during development that was donor species specific.

At present we do not have sufficient data to attribute our

developmental observations to the nuclear reprogramming of the fibroblast nucleus. Despite a number of studies that have addressed reprogramming after NT [5, 6, 20, 23, 54–57], molecular descriptors of successful reprogramming have yet to be described. The only criterion that when satisfied proves dedifferentiation, is a pregnancy carried to term. Our results show that the cytoplasm of a mature cow oocyte has the ability to support several mitotic cell cycles directed by newly introduced nuclear DNA. Whether this introduced differentiated DNA is reprogrammed, is modified, or simply remains unchanged is currently under investigation.

Before the usefulness of interspecies NT can be judged, the extent and faithfulness of nuclear reprogramming, as well as compatibilities between the somatic cell's and the recipient's cytoplasmic components, such as mitochondrial DNA, have to be examined. Nevertheless, the genome of a differentiated somatic cell from a variety of mammalian species can sustain development and form a blastocyst-like structure with distinct blastocyst morphology (inner cell mass, trophectoderm, and blastocoele cavity) when placed into bovine oocyte cytoplasm. With respect to this ability, bovine oocyte competency does not discriminate among the species investigated, chromosome number, or the age of the animals donating somatic nuclei. If safety and efficiency are proven, a common mammalian oocyte cytoplasm may be a suitable host for dedifferentiation of somatic nuclei of various mammals, including humans. Much more work is required to evaluate long- and short-term effects of mixing of nuclear and cytoplasmic components of various species. With increasing success of embryonic stem cell technology [58, 59], the embryonic cell lines grown from these interspecies embryos could be used to address some of the concerns.

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