Cloning of non-human primates: the road "less traveled by"

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ABSTRACT  Early studies on cloning of non-human primates by nuclear transfer utilized embryonic blastomeres from preimplantation embryos which resulted in the reproducible birth of live offspring. Soon after, the focus shifted to employing somatic cells as a source of donor nuclei (somatic cell nuclear transfer, SCNT). However, initial efforts were plagued with inefficient nuclear reprogramming and poor embryonic development when standard SCNT methods were utilized. Implementation of several key SCNT modifications was critical to overcome these problems. In particular, a non-invasive method of visualizing the metaphase chromosomes during enucleation was developed to preserve the reprogramming capacity of monkey oocytes. These modifications dramatically improved the efficiency of SCNT, yielding high blastocyst development in vitro. To date, SCNT has been successfully used to derive pluripotent embryonic stem cells (ESCs) from adult monkey skin fibroblasts. These remarkable advances have the potential for development of human autologous ESCs and cures for many human diseases. Reproductive cloning of nonhuman primates by SCNT has not been achieved yet. We have been able to establish several pregnancies with SCNT embryos which, so far, did not progress to term. In this review, we summarize the approaches, obstacles and accomplishments of SCNT in a non-human primate model.

KEY WORDS: cloning, nuclear transfer, primate, reprogramming

Introduction

Nuclear transfer (NT) efforts involving the nonhuman primate model commenced just prior to the turn of the millennium. By this time, the production of cloned offspring by nuclear transfer had been achieved in rodents, rabbits, and several species of domesticated animals using totipotent embryonic cells as the nuclear donors (McGrath and Solter, 1983; Prather et al., 1987; Sun and Moor, 1995; Willadsen, 1986). These pioneering NT efforts provoked the start of cloning in the primate model, which is the research path that our laboratory chose; the road “less traveled by”. The SCNT approach would enable production of genetically identical or genetically modified animals invaluable for biomedical research and the study of human diseases. During initial attempts to study NT in the rhesus macaque (Macaca mulatta) model, individual blastomeres isolated from in vitro fertilized embryos served as nuclear donor cells (Meng et al., 1997). These blastomeres, isolated from cleavage stage embryos, were transferred into the perivitelline space of enucleated, metaphase-II stage (MII) oocytes collected following controlled ovarian stimulation (Zelinski-Wooten et al., 1995). Oocyte activation was induced immediately prior to membrane fusion by a series of electrical pulses. Resulting embryos were co-cultured on a monolayer of buffalo rat liver cells (Meng et al., 1997; Zhang et al., 1994). When 8- to 16-cell stage blastomeres were utilized as the nuclear donor cells, the results were very promising. Embryonic development to the blastocyst stage was comparable to in vitro fertilized (IVF) control embryos. Transfer of these NT embryos into surrogate animals generated four pregnancies and ultimately culminated in the live births of two healthy infants, Neti and Ditto (Meng et al., 1997). Following STR analysis, used to determine the parentage of each infant, it was definitive that both Neti and Ditto were the result of successful NT technology.

SCNT was pioneered in 1996 with the production of the first live cloned offspring (Campbell et al., 1996; Wilmut et al., 1997). This groundbreaking achievement unveiled the possibility of “resetting” the nuclei of differentiated mammalian cells back to an embryonic state, using factors present in the unfertilized oocyte to

Abbreviations used in this paper: ESC, embryonic stem cell; iPSC, induced pluripotent stem cells; IVF, in vitro fertilized; MII, metaphase-II; MPF, maturation-promoting factor; NT, nuclear transfer; SCNT, somatic cell nuclear transfer; TSA, trichostatin A.

Final author corrected PDF published online: 17 February 2011.
help with this reprogramming process. Studies were soon under-
way to examine the feasibility of cloning nonhuman primates by
SCNT. It remains unknown which ooplasmic factors in the oocyte
contribute to nuclear reprogramming during SCNT, but it is
believed that these unidentified factors are present within the
cytoplasm of MII stage oocytes. Initial SCNT studies in the
monkey resulted in low blastocyst yield with a majority of embryos
arresting at the cleavage stage (Mitalipov et al., 2002). Our follow-
up studies revealed incomplete nuclear remodeling, particularly
failure to undergo nuclear envelope breakdown (NEBD) and
premature chromosome condensation (PCC) following fusion of
donor cells with enucleated oocytes (Mitalipov et al., 2002;
Mitalipov et al., 2007). We reasoned that this phenomenon could
be linked to poor subsequent development of SCNT embryos.
Lack of or incomplete NEBD and PCC are generally linked to a
decrease in the activity and level of maturation-promoting factor
(MPF). MPF is a heterodimeric protein made up of cyclin B and
cyclin-dependent kinase, which has been shown to maintain
meiotic and mitotic cell phases by phosphorylating various targets
within the cytoplasm, including several lamins (Peter et al., 1990;
Ward and Kirschner, 1990). When an oocyte is arrested at
metaphase of the second meiotic division, elevated MPF activity
is detectable within the oocyte (Szollosi et al., 1988). During
fertilization, the entry of sperm into the oocyte triggers intracellular
calcium oscillations and ultimately results in MPF degradation
(Szollosi et al., 1988). The decrease in MPF activity releases the
oocyte from meiotic arrest, enabling it to complete meiosis and
initiate mitotic cleavage divisions (Susko-Parrish et al., 1994). We
introduced several modifications to our SCNT procedures de-
signed to preserve MPF activity in monkey oocytes that supported
improved nuclear remodeling immediately upon introduction of
the donor nucleus (Mitalipov et al., 2007). Moreover, SCNT
embryos produced by the modified approach progressed to the
blastocyst stage at much higher rates suggesting a beneficial role
of nuclear remodeling on reprogramming. To date, little is known
as to what role MPF and other unidentified MII ooplasmic factors
may play during nuclear reprogramming of adult somatic cells.
Thus, it is critical to continue investigating various aspects of
SCNT to better understand this seemingly complex cytoplasmic-
mediated reprogramming process. We describe here our current
SCNT steps and recent efforts to produce rhesus embryonic stem
cells and pregnancies from adult somatic cells.

Oocyte production

Controlled ovarian stimulation in non-human primates is rou-
tinely induced by administration of exogenous gonadotropins
(Sotomaru et al., 2009; Zelinski-Wooten et al., 1995). In rhesus
macaques, starting at menses (day 1-4 of their menstrual cycle),
females receive twice-daily injections of recombinant human
follicle stimulating hormone (FSH; Organon; 30 IU, im) for 8 days
and recombinant human luteinizing hormone (LH; Ares Serono;
30 IU, im) on days 7-8 of the stimulation protocol. In addition, the
animals receive a gonadotropin releasing hormone (GnRH) an-
tagonist (Acyline; NIH/NICHD; 0.075 mg/kg body weight, sc) and
human choricron gonadotropin (hCG; Serono; 1,000 IU, im) on
day 7 during the stimulation. Serum estradiol and progesterone
measurements along with ultrasonographic scans are commonly
performed to monitor follicular response. Oocytes are recovered
via laparoscopic follicular aspiration, stripped of cumulus cells,
and examined for determination of developmental stage (Zelinski-
Wooten et al., 1995). Only oocytes that have progressed to the MII
stage of meiosis, evident by the presence of the first polar body
in the perivitelline space, are used for nuclear transfer experi-
ments.

In earlier reports, it was common to collect oocytes 27-32 hours
post-hCG (Wolf et al., 1999). In our current protocols, we recover
oocytes 35-36 hours post-hCG to allow oocyte maturation and
spindle migration away from the polar body, thus making it easier
to visualize and enucleate the meiotic spindle. In the common
marmoset, ovarian stimulation commonly entails a single, daily
injection of 50 IU FSH for 11 days, followed by the administration
of 75 IU hCG on the 12th day of the stimulation protocol (Sotomaru
et al., 2009). Germinal vesicle-stage oocytes, enclosed in cumu-
lus cell-oocyte complexes, are recovered 16 hours post-hCG
injection and placed in maturation medium for approximately 22-
24 hours (Sotomaru et al., 2009).

Oocyte availability has always been a major limitation in the
nonhuman primate model. To help one understand just how
limited this resource is, in 2009 we subjected 113 females to
ovarian stimulations. Five of these stimulation cycles were can-
celled prior to oocyte retrieval. On average, 43 oocytes were
recovered per female, of which an average of 13 and 14 were
mature MII and metaphase I stage, respectively. Only MII stage
oocytes are suitable for SCNT, so this data indicates that less than
one-third (13/43, 30%) of all oocytes recovered are available for
SCNT. While the overall number of oocytes collected per stimu-
lation is considerably higher compared to our results reported
earlier studies revealed incomplete nuclear remodeling, particularly
in rhesus monkeys resulted in low blastocyst yield with a majority of embryos
arresting at the cleavage stage (Mitalipov et al., 2002). Our follow-
up studies revealed incomplete nuclear remodeling, particularly
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Thus, it is critical to continue investigating various aspects of
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SCNT steps and recent efforts to produce rhesus embryonic stem
cells and pregnancies from adult somatic cells.

Oocyte enucleation

Oocyte enucleation involves the removal of chromosomes,
contained within the meiotic spindle, from unfertilized MII stage
oocytes. Using the naked eye or low magnification stereomicro-
scopes, one cannot see these individual chromosomes or even
the meiotic spindles that hold these chromosomes in place. Initial
attempts to visualize this nuclear DNA utilized fluorochrome
bisbenzimidze (Hoechst 33342) staining and ultraviolet (UV) ex-
sure, the standard enucleation procedure at that time (Mitalipov
et al., 2002). Intact MII stage oocytes were incubated for 5
minutes with 5 μg/ml Hoechst 33342, placed in 30 μl of Telp
HEPES media (Bavister and Yanagimachi, 1977) (TH3; with 3
mg/ml BSA) containing 5 μg/ml cytochalasin B, then incubated for
approximately 10-15 minutes prior to enucleation. During the
enucleation procedure, the first polar body and approximately
10% of the adjacent cytoplasm is removed via aspiration directly
into the enucleation pipette. Confirmation of chromosome re-
moval was accomplished by exposing the enucleation pipette to
UV wavelengths. Low blastocyst development following such
approach caused us to re-evaluate each step of the SCNT
protocol. While Hoechst 33342 is considered to be a live cell DNA
dye, it may adversely affect oocyte and embryo viability (Veillia
et al., 2002). It is likely that Hoechst 33342/UV exposure may hinder
the developmental and reprogramming potential of the primate oocytes by inducing premature oocyte activation and MPF degradation, or by adversely reacting with mitochondrial DNA (Byrne et al., 2007; Mitalipov et al., 2007). Several non-invasive, “blind” enucleation alternatives, including ‘squish’ (Simerly et al., 2004) or ‘one-step micromanipulation’ (OSM) (Zhou et al., 2006) were also tested. The main concern with these approaches is that 100% enucleation efficiency cannot be guaranteed. We tested a new, non-invasive approach, which utilizes the latest spindle imaging technology. The Oosight spindle imaging system (CRI Oosight™) makes use of the birefringence properties of the microtubules, which hold the chromosomes in place within the meiotic spindle. In clinical IVF practice, previous versions of polarized microscope (Polscope) technology have been used to examine digital images of meiotic spindles (Wang et al., 2001). However, the current Oosight system allows real-time microscopy and manipulation during enucleation of meiotic chromosomes in primate oocytes (Byrne et al., 2007; Sparman et al., 2009). Plus, this allows us to consistently achieve 100% efficiency during this spindle removal procedure, without any adverse side-effects. After incorporating spindle imaging technology into our SCNT protocols, a significant increase in blastocyst formation rate (from 1% to 16%) was achieved (Byrne et al., 2007).

**Donor cell transfer and fusion**

Both the reprogramming efficiency of nuclear donor cells and subsequent embryonic development following SCNT are believed to be affected by several factors, including: the source of donor nuclei, the cell cycle stage of the recipient oocyte and the nuclear donor cell, as well as the oocyte activation method utilized. In the mouse model, the effect of different types of donor cells on SCNT cloning efficiency has been evaluated (Inoue et al., 2003). Primary cultures of fetal and adult fibroblasts are commonly used as nuclear donor cells, although other cell types including myoblasts, neurons, and Sertoli cells have also been tested (Powell et al., 2004). Numerous factors may affect the nuclear donor cell ability to support development following SCNT (e.g. isolation procedures and in vitro culture conditions, age of donor animals, etc.). In addition, it appears that lower passage cells have an advantage over higher passage cells (Powell et al., 2004). Using cynomolgus monkeys, higher blastocyst development was reported following the use of fetal fibroblasts compared to other donor cell types (Okahara-Narita et al., 2007). In the rhesus model, both fetal and adult fibroblast cell lines have been successfully implemented as nuclear donor cells for SCNT and routinely produce blastocysts (Byrne et al., 2007; Mitalipov et al., 2007; Sparman et al., 2009; Zhou et al., 2006). Direct comparison between three different primary cultures of adult, rhesus skin fibroblasts revealed a significant (three-fold) difference in subsequent embryonic development to the blastocyst stage (Sparman et al., 2009). This finding suggests that certain donor cells may possess a higher reprogramming capability and efficiency compared to other cells, although the mechanisms for this difference are not yet clear. It is thought that the differences in epigenetic characteristics amongst different nuclear donor cells may affect the cell’s reprogramming and developmental potential (Humpherys et al., 2001).

During standard primate SCNT (represented in the schematic in Fig. 1), cell fusion is induced using electroporation (Byrne et al., 2007; Mitalipov et al., 2002). In brief, oocyte-donor cell complexes are exposed to two 50 μs DC pulses of 2.7 kV/cm in 0.25 M D-sorbitol buffer containing 0.1 mM calcium acetate, 0.5 mM magnesium acetate, 0.5 mM HEPES and 1 mg/ml fatty acid-free BSA. Approximately 30 minutes following electroporation, fusion is confirmed by the absence of the donor cell in the perivitelline space of the reconstructed embryo. Based on recent results obtained in our laboratory during the spindle-chromosomal complex transfer between MII stage oocytes (Tachibana et al., 2009), we determined that exposure to the electrofusion regimen can trigger premature activation and subsequent resumption of meiosis. In order to sustain higher MPF activity before activation, we incorporated an alternative fusion technique utilizing an extract...
from Sendai virus (SeV; http://www.cosmobio.co.jp) (Tachibana et al., 2009). During the SCNT procedure, the nuclear donor cells (e.g. fibroblasts) are immersed in the SeV extract (30-60 seconds) prior to being transferred into the perivitelline space. Cell membrane fusion between the nuclear donor cell and the oocyte typically occurs within 20-30 minutes. Fusion rates following SeV are similar or higher than what we previously reported following the use of electroporation (Byrne et al., 2007; Sparman et al., 2009).

Oocyte activation

Mammalian oocytes naturally go through a process called “activation” following fertilization to help the egg prepare for subsequent development. Successful oocyte activation promotes an increase in metabolic activity and releases the oocyte from meiotic arrest, thus enabling the resumption of the mitotic cell cycle (Susko-Parrish et al., 1994). During the SCNT procedure, as no sperm come into contact with the oocyte, an artificial activation method is required. Such artificial oocyte activation can be achieved by the use of chemical treatments which successfully induce calcium oscillations, much like those detected following normal fertilization with sperm. Examples of several proven methods of oocyte activation in the monkey model include treatment with ionomycin/6-dimethylaminopurine (DMAP), electroporation/cycloheximide/cytchalasin B, and ionomycin/roscovitine/cytchalasin B (Mitalipov et al., 2001). In our lab, reconstructed oocytes are typically activated 2 hours post-fusion, to allow nuclear remodelling to occur (Byrne et al., 2007; Sparman et al., 2009). Following the standard SCNT activation protocol (Mitalipov et al., 2007), all reconstructed oocytes are exposed first to 5 μM ionomycin for 5 minutes followed by a 5 hour incubation in 2 mM 6-dimethylaminopurine (DMAP). Treatment with DMAP prevents completion of meiosis and extrusion of the second polar body (Mitalipov et al., 2001). We refer to this type of activation procedure as “regular” activation. One recent change to our SCNT activation protocol has been the testing of an “instant” activation technique, in which the 2 hour incubation period after fusion (prior to the start of activation) has been completely eliminated. In such a case, fused embryos are immediately activated with DMAP and placed in culture. Our recent comparison of blastocyst development following the “regular” versus “instant” activation demonstrated the beneficial effect of the latter treatment (Table 1).

**Histone deacetylase inhibitor treatment**

Recent studies indicate that cloning efficiency after SCNT in the mouse, pig and bovine model could be improved with trichostatin A (TSA) treatment, a histone deacetylase inhibitor (Ding et al., 2008; Kishigami et al., 2006; Li et al., 2008). Histone deacetylation is one of the commonly studied epigenetic modifications. Inefficient nuclear reprogramming of the donor cell during SCNT may be attributed to abnormal epigenetic modification. It has been shown that treatment with TSA reduces the activity of histone deacetylase, thus resulting in an increased level of histone acetylation and enhanced regulation of DNA methylation (Yoshida et al., 1990). In theory, enhancing epigenetic regulation may positively affect the reprogramming of the somatic genome (Li et al., 2008). We tested the effect of TSA exposure during the SCNT procedure in the primate model. Fused SCNT embryos were first incubated with 37.5 nM TSA for 5 hours during DMAP treatment. Activated embryos were then transferred into embryo culture media containing 37.5 nM TSA for an additional 17-19 hours. In total, SCNT embryos were exposed to TSA for 22-24 hours. Based on our recent results, TSA treatment significantly improved the percentage of SCNT embryos that reached the blastocyst stage (Table 2). Moreover, the subsequent in vivo development following transfer of SCNT blastocysts into recipients was also dramatically improved in the TSA group (Table 3). While further testing could be required to optimize the proper concentration and timing of TSA supplementation, we have modified our current SCNT procedures to include the TSA.

### Table 1

<table>
<thead>
<tr>
<th>Activation</th>
<th># of replicates</th>
<th># of oocytes</th>
<th>Fusion (%)</th>
<th>Pronuclei (%)</th>
<th>8-cell (%)</th>
<th>Morulae (%)</th>
<th>Blastocyst (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Instant</td>
<td>7</td>
<td>45</td>
<td>85 (94%)</td>
<td>45 (100%)</td>
<td>40 (89%)</td>
<td>22 (49%)</td>
<td>9 (20%)</td>
</tr>
<tr>
<td>Regular</td>
<td>7</td>
<td>42</td>
<td>42 (100%)</td>
<td>42 (100%)</td>
<td>39 (93%)</td>
<td>16 (38%)</td>
<td>5 (12%)</td>
</tr>
</tbody>
</table>

*Percentages are calculated based on the number of embryos forming pronuclei.

### Table 2

<table>
<thead>
<tr>
<th>TSA</th>
<th># of replicates</th>
<th># of oocytes</th>
<th>Fusion (%)</th>
<th>Pronuclei (%)</th>
<th>8-cell (%)</th>
<th>Morulae (%)</th>
<th>Blastocyst (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>9</td>
<td>90</td>
<td>85 (94%)</td>
<td>84 (99%)</td>
<td>81 (96%)</td>
<td>47 (56%)</td>
<td>4 (5%)</td>
</tr>
<tr>
<td>37.5 nM TSA</td>
<td>32</td>
<td>243</td>
<td>242 (99%)</td>
<td>242 (100%)</td>
<td>229 (95%)</td>
<td>148 (61%)</td>
<td>43 (18%)</td>
</tr>
</tbody>
</table>

**Different superscripts indicate significant difference (p<0.05).** Percentages are calculated based on the number of embryos forming pronuclei. Both groups were activated by the “regular” activation protocol.

### Table 3

<table>
<thead>
<tr>
<th>Treatment</th>
<th># of recipients</th>
<th># of embryos transferred</th>
<th># of pregnancies (# of clinical pregnancies)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>12</td>
<td>55</td>
<td>0 (0)</td>
</tr>
<tr>
<td>With TSA</td>
<td>10</td>
<td>67</td>
<td>6 (5)</td>
</tr>
</tbody>
</table>

Recovered oocytes were divided into two groups and both groups were activated by the “regular” activation protocol. One biochemical pregnancy was confirmed by the continual rise in E2 and P4 levels.

**ES cell derivation and embryo transfer**

In the mid-1990’s, Dr. James Thomson of the Wisconsin National Primate Research Center reported the isolation of the first embryonic stem cell (ESC) lines from rhesus macaque blastocysts (Thomson et al., 1995). These primate ESCs were maintained in an undifferentiated state while cultured on feeder
layers but retained the potential to differentiate into cells repre-
senting all three germ cell layers (endoderm, mesoderm, and
ectoderm) (Thomson et al., 1995; Thomson et al., 1996). Prior to
the establishment of rhesus ESCs, only mouse ESCs were
available (Martin, 1981). Primate ESCs provided an exciting
model for better understanding human development and disease
in vitro and in vivo. As one would expect, following protocols and
markers developed for monkey ESCs, studies were soon carried
out with human embryos that culminated in the successful deriva-
tion of human ESCs (Thomson et al., 1998).

Coupling of ESC research with SCNT technology also cata-
lyzed the development of new strategies for reprogramming
somatic cells and deriving patient-matched pluripotent cells (Byrne
et al., 2007). The ability to derive pluripotent cells that are
genetically identical to individual patients, holds tremendous
potential to produce cells that will not be rejected by the patient’s
immune system and that may help prevent numerous degenera-
tive diseases (Drukker and Benvenisty, 2004; McKay, 2000). We
employed a modified SCNT approach to produce blastocysts
from adult rhesus macaque skin fibroblasts and established two
ESC lines from these cloned embryos, designated as CRES-1
and CRES-2 (Byrne et al., 2007). These CRES cell lines are
genetically identical to each other in terms of nuclear DNA, since
nuclear donor cells employed were from the same adult male.
However, these cell lines contain different mitochondrial ge-
nomes since oocytes from two different females were used for
SCNT. Characterization of these novel SCNT-derived ESC lines
confirmed their pluripotency and origin from somatic cells (Byrne
et al., 2007).

As for the efficiency of this approach, in our initial study we
used a total of 304 oocytes to derive two CRES cell lines; less than
a 1% derivation efficiency rate. While the low efficiency rates still
leave room for improvement, these results suggest that nuclear
reprogramming by SCNT can support derivation of ESCs in
higher primates, including humans. In a more recent study, we
used adult rhesus female skin fibroblasts for SCNT and derived
two additional ESC lines (CRES-3 and CRES-4) (Sparman et al.,
2009). Further optimizations and improvements in SCNT resulted
in a nearly three-fold higher blastocyst development rate (43%)
and ESC derivation rate (29%) as compared to the previous
outcomes (Byrne et al., 2007). Of particular interest, the oocytes
that gave rise to CRES-3 and -4 were all recovered from only one rhesus female,
subjected to a single controlled ovarian stimulation. These findings reveal that it
is now possible to derive a primate stem cell line from as few as 10 oocytes, or
less, and that it may be economically and technically feasible to derive patient-
matched ESCs for tissue replacement therapy.

The nonhuman primate is clearly an attractive research model for SCNT be-
cause of its remarkable similarity to hu-
mans, genetically and from a reproduc-
tive standpoint. The benefits of producing
rhesus monkeys by reproductive cloning
for biomedical research are tremendous.
For instance, the production of geneti-
cally identical monkeys would significantly reduce the number of
animals utilized in biomedical research. Another advantage is that we
can carry out a variety of genetic manipulations with cultured
nuclear donor cells, including gene targeting. Reproductive clon-
ing with such cells would allow for the production of genetically
modified primates, including gene knock-out models, to study
gene function and human diseases.

At present, the production of live primate offspring following
SCNT has yet to be accomplished (Mitalipov et al., 2002; Simerly
et al., 2003). We summarize here our recent unpublished efforts
in embryo transfer using rhesus blastocysts produced by SCNT
with adult monkey skin cells expressing GFP (Table 3). A total of
5 pregnancies were established following transfer of 67 embryos
into 10 recipients (Tables 3 and 4). Only one pregnancy resulted
in a live fetus that possessed a fetal heartbeat, detected by
ultrasonographic scans, while other pregnancies contained sacs
without a fetus (Fig. 2). Unfortunately, this pregnancy failed to go
to term and was aborted at day 81 of gestation. To determine if
there was an abnormal phenotype in the aborted cloned preg-
nancy, we recovered fetal tissues and carried out histological and
molecular analyses. The SCNT origin of the fetus was confirmed
by both GFP-specific PCR and direct observation of GFP expres-

**TABLE 4**

<table>
<thead>
<tr>
<th>Recipient</th>
<th># of embryos transferred</th>
<th>Embryonic age (stage)*</th>
<th>Recipient stage</th>
<th># of GS</th>
<th># of fetuses</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>8</td>
<td>D4 (M/CM)</td>
<td>D2</td>
<td>1</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>8</td>
<td>D4 (M/CM)</td>
<td>D2</td>
<td>1</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>D13 (Blastocyst)</td>
<td>D3</td>
<td>1</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>8</td>
<td>D4 (16-cell/M)</td>
<td>D2</td>
<td>1</td>
<td>1</td>
<td>aborted at 81 days of gestation</td>
</tr>
<tr>
<td>5</td>
<td>7</td>
<td>D6 (M/CM)</td>
<td>D3</td>
<td>1</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

*Embryonic age, measures the actual age of the embryo, in days, from the time of fertilization (e.g. D4 = day 4 post-fertilization). *Stage, refers to the embryo stage of development at the time of the embryo transfer (e.g. M = Morula; CM = Compact morula). **Recipient stage, measures the stage of the recipient’s uterus, in days, post-ovulation (D0 = day of ovulation, so D2 = day 2 post-ovulation). GS, Gestational sac
In conclusion, we provide here an overview of the current state of SCNT research in the rhesus macaque model. Remarkable progress has been made in adapting and optimizing SCNT protocols to produce embryos and ESCs from adult monkey somatic cells. In contrast, the in vivo development of monkey SCNT embryos following transfer into recipients is still limited, thus necessitating further studies to help us better understand the mechanisms of reprogramming in primates. With this, we would like to conclude with a few lines from the famous The Road Not Taken poem by Robert Frost:

Two roads diverged in a wood,
And I—
I took the one less traveled by,
And that has made all the difference.

Acknowledgements

The authors would like to recognize the expertise and services provided by the Division of Animal Resources, Assisted Reproductive Technology & ESC Core, Surgical Team, Endocrine Services Core, Imaging & Morphology Core, and Molecular & Cellular Biology Core at the Oregon National Primate Research Center (ONPRC). We are also grateful to Tomita Tatsuo for assisting with the microscopic analysis of the recovered SCNT fetal tissue, as well as Organon International and Ares Advanced Technology, Inc. (Ares Serono) for their generous donation of the recombinant human gonadotropins used in this study. This work was largely supported by funds from ONPRC, Oregon Stem Cell Center and by grants from the National Institutes of Health HD057121, HD059946, HD063276, RR000163, HD047675, HD018185, and HD047721.
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