Cell Stem Cell
Review

Interspecies Nuclear Transfer: Implications for Embryonic Stem Cell Biology

Zeki Beyhan,[1] Amy E. Iager,[1] and Jose B. Cibelli[1,2,3,*]
[1]Cellular Reprogramming Laboratory, Department of Animal Science
[2]Department of Physiology
Michigan State University, B270 Anthony Hall, East Lansing, MI 48824, USA
[3]Programa Andaluz de Terapia Celular y Medicina Regenerativa, Avenida Américo Vespucio 5, Bloque 2, 2ª Planta 41092 Isla de la Cartuja, Sevilla, Andalucía, Spain
*Correspondence: cibelli@msu.edu
DOI 10.1016/j.stem.2007.10.009

Accessibility of human oocytes for research poses a serious ethical challenge to society. This fact categorically holds true when pursuing some of the most promising areas of research, such as somatic cell nuclear transfer and embryonic stem cell studies. One approach to overcoming this limitation is to use an oocyte from one species and a somatic cell from another. Recently, several attempts to capture the promises of this approach have met with varying success, ranging from establishing human embryonic stem cells to obtaining live offspring in animals. This review focuses on the challenges and opportunities presented by the formidable task of overcoming biological differences among species.

Introduction

The oocyte is invaluable. This is an unmistakable fact underscored by the scarcity of oocytes available from species where they could be most useful, i.e., human, for biomedical applications, and endangered or extinct species, for their conservation and rescue. One of the possibilities presented by somatic cell nuclear transfer (SCNT) is so-called interspecies cloning, where the recipient ooplast and donor nucleus are derived from different species. Hypothetically, having the means to take advantage of readily available recipient oocytes to reprogram the donor nucleus of a different species holds tremendous promise.

Perhaps the most important prospective application of interspecies SCNT (iSCNT) lies in its potential to facilitate reprogramming of human somatic cells without many of the significant ethical challenges surrounding the use of human oocytes. Ethical considerations aside, the question of availability makes SCNT using human oocytes unfeasible as a long-term solution for cellular reprogramming. While several alternative strategies such as egg sharing and egg donation programs are suggested as the source of human oocytes for assisted reproductive technologies, neither of these approaches has been implemented broadly enough to overcome the shortage of human oocytes facing the research and therapeutic development community. As an alternative approach, producing competent human oocytes from human ESCs has not been realized yet either (Hubner et al., 2003). These constraints draw attention to iSCNT as a workable strategy to address human oocyte shortage for ESC studies. The recent ruling by the Human Fertilization Embryology Authority (HFEA)—the government body in charge of overseeing IVF treatments and research using human embryos in the UK—to allow iSCNT experiments using human somatic cells and nonhuman oocytes offers scientists the long-awaited legal framework to explore the potential of the iSCNT procedure to its full extent. This bold measure taken by the HEFA, and upheld by a large group of scientists, implicitly recognizes the irreplaceable nature of the mammalian oocyte (http://www.hfea.gov.uk/en/1581.html).

The use of nonhuman oocytes for reprogramming could be of immediate value as a tool for the production of human-nuclear-transfer-derived embryonic stem cells (NTESCs) from individuals suffering from such late onset diseases as diabetes, Parkinson's disease, and Alzheimer's disease, among others. In turn, these cells could be used to develop new treatments in vitro.

We reported the first iSCNT experiments shortly after the cloning of a lamb from somatic cells (Wilmut et al., 1997; Dominko et al., 1999). Since then, the feasibility of interspecies cloning has been addressed by several researchers employing various model systems. More than 40 articles have been published in which oocytes and somatic cells from a number of species have been used to generate embryos via interspecies nuclear transfer (Table 1). Live offspring have been obtained by combining closely related species, such as cattle/gaur (*Bos taurus*/*Bos grunensis*) (Lanza et al., 2000) and domestic sheep/argali sheep (*O. aries*/*O. musimon*) (White et al., 1999). In some of the reported experiments, however, genetic distance between donor and recipient species spanned taxonomic classes, such as cattle/chicken (*Bos taurus*/*Gallus gallus*) (Liu et al., 2004) and rabbit/panda (*Oryctolagus cuniculus*/*A. melanoleuca*) (Chen et al., 2002). The majority of these experiments have failed to produce viable embryos. A common limitation in making comparisons between these iSCNT reports is that the definition of experimental endpoints and criteria for

successful reprogramming was often ill-defined, except for those resulting in live offspring. Nevertheless, the potential impact of a successful iSCNT scheme is sufficiently attractive to maintain ample scientific interest in this subject. In this review, we will summarize the recent literature on iSCNT and address a number of technical and theoretical concerns regarding these experiments. We also propose an outline for the evaluation of iSCNT experiments, given that many reports in the literature to date lack a common framework to gauge and compare developmental outcomes.

What Is Interspecies SCNT?

A "species," the basic unit of taxonomic classification, is defined as a group of organisms that share certain phenotypical characteristics, forming a reproductively isolated entity. In practice, however, it is not always easy to draw lines between different species, and researchers have had to define subgroups and transitory groups like subspecies and breeds. For the sake of simplicity, we will define the term species as a group of organisms that could interbreed naturally and produce fertile offspring. We will also use an abridged hierarchy of taxonomic units in our discussions.

As previously mentioned, nuclear transfer (NT) experiments that employ oocytes and donor cells from two different species are defined as interspecies or interspecies nuclear transfers (iSCNT) (Figure 1). The two main assumptions required for iSCNT are that early developmental events and mechanisms are evolutionarily conserved among mammals and that molecules that regulate these events in mammalian oocytes are capable of interacting with nuclei from another species. The validity of these assumptions, however, deserves vigorous scrutiny. Although most mammalian embryos follow a very similar pattern of ontogenic development, significant differences in many aspects of the process do exist among evolutionarily divergent taxonomic groups (Gilbert and Bolker, 2001). Temporal regulation of developmental events—such as cell-cycle progression, embryonic genome activation (EGA), blastocyst formation, implantation, and organogenesis—differs from species to species. One wonders, therefore, how these developmental processes are regulated in an embryo reconstructed using an oocyte and a donor cell from different species. What constituent of this unusual embryo drives the development? Is there crosstalk between the donor nucleus and the recipient cytoplasm? What developmental program is executed—the oocyte's, the donor's, or both? Are the interspecies cybrid cells functional and viable? Is the resulting embryo/fetus viable? Are any live offspring produced, and, if so, are they fertile? These are some of the most important questions that need to be, and can be, addressed by interspecies cloning experiments.

Potential Applications of iSCNT

Theoretically, an iSCNT approach would be beneficial in any situation in which an alternative source of ooplasm is needed, due to either ethical or technical considerations, such as establishing primate ESCs from iSCNT embryos or cloning endangered species, respectively. The ultimate endpoints of these applications are either (1) to generate a preimplantation embryo to be used as a source of ESCs or (2) to produce live offspring in all animals with the exception of human.

Embryos cloned for ESC establishment need not progress through all developmental stages. Instead, a few functional equivalents of inner cell mass (ICM) cells or single blastomeres in an SCNT embryo could be adequate to establish an ESC line (Klimanskaya et al., 2006, 2007; Wakayama et al., 2001). This concept could be applied to iSCNT (Figure 1), assuming that the pattern of gene expression of the interspecies embryos approximates that of same-species SCNTs or of fertilized preimplantation embryos at the same stage of development. iSCNT has been proposed for creating human ESC lines in an effort to avoid the ethically charged issue of soliciting human oocytes for research purposes (Fulka and Mrazek, 2004). While this alternative may alleviate some ethical and practical concerns, the use of such cells for therapeutic purposes is in doubt, due to potential risks of transmission of infectious diseases. Further, ECSs isolated from cybrids may maintain mitochondria derived from the nonhuman recipient oocyte, a result that is likely to have deleterious long-term physiological and immunological consequences to human recipients (Hall et al., 2006). Nonetheless, the ability to produce ESCs from iSCNT embryos could facilitate the creation of new cellular models of human disease and could significantly advance our understanding of basic nuclear-cytoplasmic interactions between a somatic cell and an oocyte.

Using iSCNT to produce live offspring, a goal of some of the earliest iSCNT experiments, focuses primarily on applications involving the preservation/rescue of endangered species (Dominko et al., 1998). Although the main procedures to construct cloned embryos for any purpose are essentially the same, embryos cloned to produce live offspring require a more comprehensive and complete reprogramming of the somatic genome, since they need to progress through all developmental milestones and survive a rigorous in vivo selection process. A few studies have successfully employed iSCNT in cloning endangered species, such as gaur (Lanza et al., 2000), mouflon (Loi et al., 2001), and African wild cat (Hidalgo et al., 2003), using oocytes from closely related species.

Preimplantation and Postimplantation Development of iSCNT Embryos

The majority of iSCNT experiments published to date have reported the production of at least blastocyst-stage embryos, although with varying degrees of efficiency (Table 1). Development of blastocyst-stage iSCNT embryos was reported even with some crossclass NT experiments in which the donor species exhibits no functional equivalent of the blastocyst stage during normal embryonic development (Liu et al., 2004). Depending on the species and the experiment, the frequency of blastocyst

Table 1. Comprehensive List of Interspecies Cloning Experiments Reported to Date

Taxonomic Relationship	Recipient Oocyte	Donor Cell	Blastocyst	Implantation	Live Offspring	Reference
Interclass	Cow (*B. taurus*)	Chicken (*G. gallus*)	YES	NET	NA	Liu et al., 2004
	Cow (*B. taurus*)	Rat (*R. norvegicus*)	NA[a]	NO	NA	Dominko et al., 1999
	Rabbit (*O. cuniculus*)	Panda (*A. melanoleuca*)	YES	YES	NO	Chen et al., 2002
Interorder	Cow (*B. taurus*)	Whale (*B. acutorostrata*)	NO	NA	NA	Ikumi et al., 2004
	Cow (*B. taurus*)	Dog (*C. familiaris*)	YES	NET	NA	Murakami et al., 2005
	Cow (*B. taurus*)	Human (*H. sapiens*)	YES	NET	NA	Chang et al., 2003; Illmensee et al., 2006
	Cow (*B. taurus*)	Rhesus monkey (*M. mulatta*)	YES	NET	NA	Dominko et al., 1999
	Cow (*B. taurus*)	Mouse (*M. musculus*)	NO	NA	NA	Arat et al., 2003
	Cow (*B. taurus*)	Pig (S. *sucrofa*)	YES	NO	NA	Dominko et al., 1999
	Rabbit (*O. cuniculus*)	Ibex (*C. ibex*)	YES	NET	NA	Jiang et al., 2005
	Rabbit (*O. cuniculus*)	Domestic cat (*F. catus*)	YES	YES	NO	Wen et al., 2005
	Rabbit (*O. cuniculus*)	Marbled cat (*P. marmorata*)	YES	NET	NA	Thongphakdee et al., 2006
	Rabbit (*O. cuniculus*)	Human (*H. sapiens*)	YES	NET	NA	Chen et al., 2003
	Rabbit (*O. cuniculus*)	Rhesus monkey (*M. mulatta*)	YES	NET	NA	Yang et al., 2003
	Rabbit (*O. cuniculus*)	Camel (*C. dromedaries*)	YES	NET	NA	Zhao et al., 2006
	Rabbit (*O. cuniculus*)	Pig (*S. sucrofa*)	YES	NET	NA	Chen et al., 2006
	Rabbit (*O. cuniculus*)	Tbetan antelope (*P. hodgsonii*)	YES	NET	NA	Zhao et al., 2006
	Pig (*S. sucrofa*)	Whale (*B. acutorostrata*)	NO	NA	NA	Ikumi et al., 2004
	Pig (*S. sucrofa*)	Tiger (*P. tigris*)	YES	NET	NA	Hashem et al., 2007
Interfamily	Cow (*B. taurus*)	Takin (*B. taxicolor*)	YES	NET	NA	Li et al., 2006a
	Cow (*B. taurus*)	Sheep (*O. aries*)	YES	YES	NO	Dominko et al., 1999; Hua et al., 2007
	Goat (*C. hirus*)	Tibetan antelope (*P. hodgsonii*)	YES	NET	NA	Zhao et al., 2007
Intergenus	Cow (*B. taurus*)	Buffalo (*B. bubalis*)	YES	NET	NA	Kitiyanant et al., 2001
	Cow (*B. taurus*)	Goral (*N. goral*)	YES	NET	NA	Oh et al., 2006
	Wild cat (*F. silvestris*)	Leopard cat (*P. bengalensis*)	YES	YES	NO	Yin et al., 2006
Interspecies	Cow (*B. taurus*)	Gaur (*B. gaurus*)	YES	YES	YES	Lanza et al., 2000; Mastromonaco et al., 2007
	Cow (*B. taurus*)	Gaur/Cow hybrid	YES	YES	NA	Dindot et al., 2004
	Cow (*B. taurus*)	Yak (*B. grunniens*)	YES	YES	YES	Li et al., 2006a, 2006b
	Cow (*B. taurus*)	Zebu (*B. indicus*)	YES	YES	YES	Meirelles et al., 2001
	Cow (*B. taurus*)	Banteng (Bos javanicus)	YES	YES	NO	Sansinena et al., 2005

Table 1. *Continued*

Taxonomic Relationship	Recipient Oocyte	Donor Cell	Blastocyst	Implantation	Live Offspring	Reference
	Goat (*C. hirus*)	Ibex (C. ibex)	YES	NET	NA	Jiang et al., 2005
	Domestic cat (*F. catus*)	Wild cat (F. silvestris)	YES	YES	YES	Gomez et al., 2003, 2004
	Sheep (*O. aries*)	Muflon (O. orientalis musimon)	YES	YES	YES	Loi et al., 2001

NET, no embryo transfer; NA, not applicable.
[a] Embryos were transferred at two cell stage.

development varied between 4% and 44%. Overall, the ability of an iSCNT embryo to develop to the blastocyst stage decreases as the taxonomic distance between donor and recipient species increases. Several reports—in addition to our own experience with iSCNT embryos—suggest that major barriers to the development of such embryos are first manifested at the time of EGA, i.e., the time when the genome of the zygote—in this case, that of the somatic cell—becomes independent from the maternal transcripts and initiates transcription on its own. These findings reveal that early preimplantation development of iSCNT embryos is controlled by the oocyte and, further, suggest that developmental arrest appears to be imposed just before the time when high-level EGA should take place in bovine embryos (Latham, 2005).

In the majority of experiments, resulting iSCNT blastocysts were not transferred to surrogate animals, and their capacity to implant and develop further was not investigated. As indicated in Table 1, and as discussed previously, in the few instances in which blastocyst implantation and development were assessed, full-term cloned offspring was a rare event and was only observed in iSCNT between closely related species, underscoring the importance of compatibility between donor-cell and recipient-oocyte species.

Rabbit Oocytes as Highly Efficient iSCNT Recipients

More than a quarter of iSCNT studies reported were performed using rabbit oocytes. The resulting iSCNT embryos developed to the blastocyst stage with remarkably high efficiency, a phenomenon replicated using donor nuclei from multiple species. Depending on the donor species—these include cat, ibex, panda, camel, antelope,

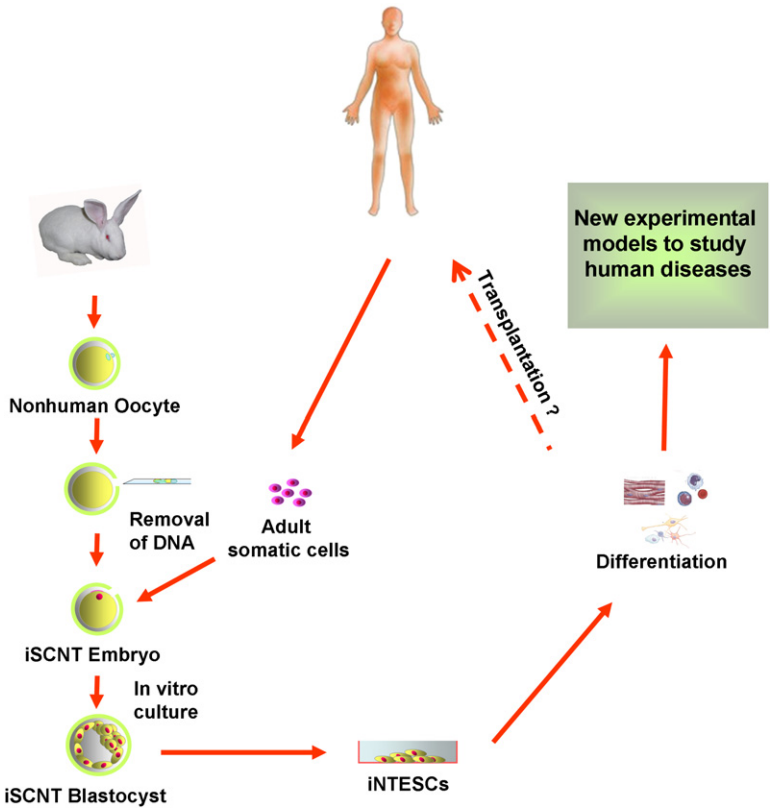

Figure 1. Schematic Representation of Interspecies Nuclear Transfer for Derivation of NTESCs

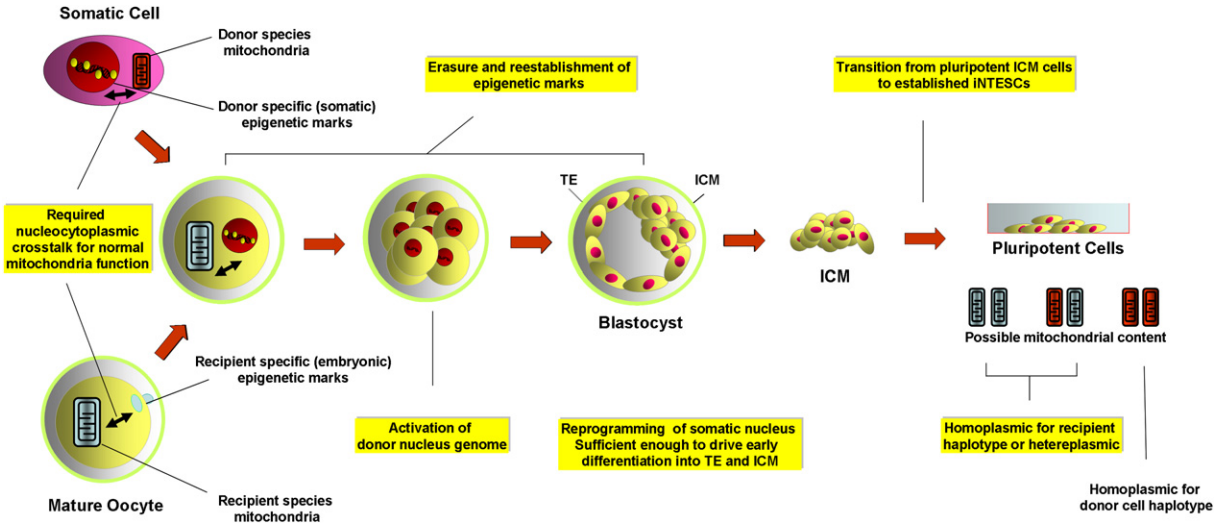

Figure 2. Developmental Milestones Required to Establish Interspecies Nuclear Transfer Embryonic Stem Cells
Yellow text boxes indicate potential problems associated with the model. TE, trophoectoderm; ICM, inner cell mass.

macaque, and human—6%–33% of iSCNT embryos developed into blastocysts (Chen et al., 2003; Wen et al., 2005). These numbers put the efficiency of iSCNT using rabbit oocytes within or above the range of successful intraspecies SCNT blastocyst development frequencies (Dinnyes et al., 2001; Chesne et al., 2002). Assuming that none of the blastocysts reported were of parthenogenetic origin—not determined by the authors—it is intriguing to speculate that the rabbit oocyte may be more efficient at supporting preimplantation development than other species. Unfortunately, none of the reported rabbit iSCNT experiments addressed molecular and physiologic aspects of preimplantation development, leaving the mechanism behind the observed high efficiencies of rabbit iSCNT open to question. One can speculate, however, that the high success rate could be due to intrinsic characteristics of rabbit oocytes that make them more effective in driving early mammalian developmental events. We cannot rule out the possibility that the high efficiency could also be attributed to the technical expertise of the group responsible for the bulk of the rabbit iSCNT experiments. Taken together, a detailed examination of rabbit oocytes at a molecular and functional level in the context of iSCNT may provide interesting insights into somatic cell reprogramming forces that operate during the cloning process.

Challenges Faced by the iSCNT Model

Although the number of interspecies cloning experiments is not large enough to definitively answer fundamental biological questions, studies reported to date imply fairly constricted species barriers that prevent an iSCNT embryo from developing into a viable fetus and offspring. In addition to the failures of iSCNT embryos during preimplantation development, notably around EGA, those that can develop into blastocysts are likely to fail to implant in the uterus. This is reflected in the observation that many different recipient-oocyte/donor-cell combinations were able to develop to the blastocyst stage (albeit at reduced frequencies compared to their intraspecies counterparts) but failed thereafter (Table 1).

The majority of reported iSCNT experiments were designed to address the preimplantation development of reconstructed embryos only at the morphological level and generally did not address physiological aspects of development in great depth. There is, to date, a distinct lack of reports examining interactions and compatibility between nuclei and the cytoplasm. It is not yet clear whether the failure to produce a high percentage of iSCNT-derived blastocysts—as a percentage of fused oocytes—should be attributed to incompatibility between oocyte proteins and the transplanted nuclei, between the mitochondria and the transplanted nuclei, or both (Figure 2). Not surprisingly, due to the few iSCNT embryos that have implanted, attempts to explore fetal-maternal interactions between the embryo and the recipient uterus are simply absent.

The theory of reproductive isolation proposes that the emergence of two species occurs when recombination, through either mutations or hybridizations, renders two populations unable to interbreed and to continue to reproduce. To the extent that a fertilization using sperm from one species and oocytes from another is equivalent to iSCNT in the context of evolutionary biology, the published findings are consistent with this model, in that only closely related species seem capable of generating iSCNT offspring. Although we do not know the identity of the particular genes that cause postzygotic reproductive isolation, it is reasonable to speculate that this pool of genes could contain good candidates for factors that might sabotage the successful development of iSCNT embryos (Orr et al., 2004).

iSCNT for Embryonic Stem Cell Isolation

In addition to providing a great model to study nuclear cytoplasmic interactions, iSCNT embryos can potentially

be used for the isolation of ESCs. While many intraspecies SCNT blastocysts fail to develop into live offspring, a substantial number of ESCs or embryonic stem-like cells have been derived from SCNT embryos in cattle and mouse models (Cibelli et al., 1998; Wakayama et al., 2001, 2006; Wang et al., 2005).

Earlier reports indicated that the functional characteristics of ESCs derived by NT (ntESCs) may not be compromised by the aberrations observed in cloned fetuses and embryos, including chromosomal, genetic, and epigenetic abnormalities (Renard et al., 1999; Hill et al., 2000; Humpherys et al., 2001, 2002). Live, healthy offspring have been obtained by using ntESCs as nuclear donors in a second round of NT, indicating that at least some of the ntESCs are competent to support full-term development (Wakayama et al., 2005b). In addition, mouse chimeras generated with ntESCs resulted in germline transmission of the injected cells, strongly supporting the idea that they are functionally similar to conventional ESCs (Wakayama et al., 2005a, 2005b, 2005c). A recent study by Wakayama et al., employing 150 mouse ntESC lines, reported that these ntESCs are comparable to their in vivo-derived counterparts in terms of their differentiation capacity, pluripotency marker expression profile, global gene expression profile, and select methylation characteristics (Wakayama et al., 2006). These observations are not only limited to the mouse. Recently, rabbit ESCs have also been established from fertilized, parthenogenetic and NT embryos with high efficiency (Fang et al., 2006). Conventional and NT rabbit ESCs exhibit similar characteristics in their ESC marker expression and in their in vivo and in vitro differentiation abilities (Fang et al., 2006). Taken together, these data demonstrate that the developmental problems observed for SCNT embryos do not seem to affect their ability to form ESCs, reinforcing the fact that iSCNT could be used to address questions related to interspecies nucleocytoplasmic compatibility and even to provide another source of cytoplasm for isolating human ESCs, albeit with the limitations outlined previously.

To date, only one report (Chen et al., 2003) has established ESCs from rabbit/human iSCNT blastocysts. The authors produced 158 blastocysts, employing rabbit oocytes and human fibroblasts from four individuals ranging in age from 5 to 60 years old. They isolated NTESCs from 14 iSCNT blastocysts, 4 of which were expanded for more than 25 passages (Chen et al., 2003). The reported frequency of blastocyst development (14.5%) and the frequency of ESC establishment (13% and 4% up to passage 10 and passage 25, respectively) are very encouraging, considering the fact that many more intraspecies SCNT attempts have produced only a handful of blastocysts and no human embryonic stem cell line. In this report, the ESC lines produced by iSCNT were positive for various protein markers known to be expressed by human ESC lines, such as alkaline phosphatase, SSEA-3, SSEA-4, TRA-1-10, and TRA-1-85. Upon differentiation, embryoid bodies and outgrowths expressed marker proteins specific for several somatic cell lineages—such as myoglobin, α-fetoprotein, α-1-antitrypsin, VEGF receptor-2, Tie-2, nestin, and MyoD1—consistent with their presumed pluripotency. These ntESCs phenotypically resembled human ESCs isolated from fertilized embryos. While the report indicates that the ESCs carried both human and rabbit mitochondrial DNA, quantitative and functional analyses testing the compatibility of the donor nuclei and recipient mitochondria were not performed. However, the successful, prolonged culture of ntESCs derived in this way suggests at least some degree of compatibility. This study is interesting, since it implies that rabbit oocytes could enable human somatic nuclei to go through not only several landmarks of preimplantation development, including EGA and compaction, but also could overcome the possible nucleocytoplasmic conflict during establishment and differentiation of ESCs. These results have yet to be replicated by an independent group; nonetheless, in the event that the results are consistent in other hands, future iSCNT studies using rabbit oocytes are warranted.

Is SCNT the only way to obtain bona fide ESCs from adult individuals? Recently, three different independent groups reported that mouse somatic cells can be forced to dedifferentiate by exogenously inserting four genes, Oct4, Klf4, c-*myc*, and Sox2 (Okita et al., 2007; Takahashi and Yamanaka, 2006; Wernig et al., 2007; Maherali et al., 2007). These findings imply that the requirement for oocytes to reprogram somatic cells could be overcome one day. While true, considerable additional research must be conducted to understand and subsequently mimic the epigenetic mechanisms that take place during early embryonic development. Among the limitations of these induced pluripotent stem cells (iPS) is the low efficiency of their derivation. As reported by Takahashi and Yamanaka (Takahashi and Yamanaka, 2006), in order to obtain one pluripotent colony of cells, 5000 cells must be transfected, although this efficiency has been improved, at least to some degree (Maherali et al., 2007). Nevertheless, murine iPS cell generation remains 100–500 times less efficient than NTESC derivation (Wakayama et al., 2005c). Furthermore, two of the four genes required to obtain iPS are proto-oncogenes, casting serious doubts about the safety of the cells if they are ever produced in human for therapeutic purposes. Nonetheless, it would be unwise not to recognize the intrinsic value of iPS cells as a biological tool, reinforcing the notion that all avenues should be explored when trying to understand the gene reprogramming processes necessary to dedifferentiate somatic cells, including iSCNT. Indeed, the authors of these studies have collaborated to contribute a Correspondence article in the October issue of *Cell Stem Cell* (Hyun et al., 2007) underscoring the necessity of pursuing all avenues toward the generation of human pluripotent cells.

Theoretical Considerations for iSCNT Models

SCNT is an inefficient technique, even in the context of intraspecies cloning. The best frequencies reported for offspring born after embryos are transferred into surrogate

mothers hover around 5%. It is generally accepted that the litmus test for functional genome reprogramming is having produced healthy and fertile adult offspring. For SCNT embryos, failing this test is the norm. These failures have been associated with early embryonic losses and various developmental abnormalities, including an enlarged, less-vascularized placenta; pulmonary immaturity; and liver, renal, and endocrine failure (Chavatte-Palmer et al., 2002; Hill, 2002; Hill et al., 1999, 2000, 2002). Numerous factors have been investigated as the potential culprits of failed reprogramming in SCNT embryos. Failed epigenetic reorganization of the genome has emerged as one likely source of problems associated with SCNT. These problems include deregulation of chromatin structure through alterations in DNA and histone methylation/acetylation patterns (Chung et al., 2003; Santos et al., 2003) and aberrant expression of imprinted genes (Humpherys et al., 2001; Mann et al., 2003). Unfortunately, our understanding of the regulation of epigenetic changes is still limited, and as a consequence, we are at a disadvantage when trying to discover their role in the context of SCNT (see reviews, Hochedlinger and Jaenisch, 2003; Jaenisch et al., 2005; Kishigami et al., 2006).

Nuclear-Mitochondrial Compatibility

The fact that iSCNT experiments yield live offspring only when the oocyte and donor-cell sources used are derived from related species may be the result of incompatibilities in mitochondrial physiology.

Earlier studies using mouse blastomere NTs have suggested that the typical pattern of maternal inheritance observed in many mammalian species does not apply in SCNT, and varying degrees of heteroplasmy were observed in most of the resulting embryos, fetuses, and live offspring (Hiendleder et al., 2003). Moreover, even tissue-specific selection of certain haplotypes was observed in cloned mice. With few exceptions, neutral segregation of mtDNA in most bovine SCNT embryos, fetuses, and animals appears to be the dominant pattern of inheritance in which the amount of donor-cell mtDNA is not more than the original amount contributed during reconstruction of the embryo (i.e., the donor-cell mitochondria are not selectively replicated over the recipient mitochondria) (Hiendleder et al., 2003).

Mitochondria are semiautonomous organelles, with their own genomes and transcriptional machinery. This compact genome encodes 13 protein subunits of oxidative phosphorylation, 22 tRNAs, and 2 rRNAs. To be operational, however, the mitochondria also rely on ''imported'' proteins encoded in the nucleus. Hence, close coordination between the nuclear and mitochondrial genomes is essential (Brenner et al., 2004; St John et al., 2004). Coevolution of nuclear and mitochondrial genomes and the transfer of genetic information from mitochondria to the nucleus have resulted in a very specific and unique complementation of mitochondrial and nuclear function within an individual species. This specific interaction has also been proposed to contribute to the speciation process (Herrmann et al., 2003). This assumption would lead us to conclude that the compatibility of nuclear and mitochondrial genomes may be of paramount importance to iSCNT experiments.

In all eukaryotic cells, mitochondria play a major role in many biological processes. They are known to be involved in ATP production, regulation of intracellular calcium levels, apoptosis, and cellular aging. During mammalian gametogenesis, fertilization, and embryogenesis, mitochondria have an unusual morphology and pattern of transmission from one generation to another. Mitochondria in oocytes and preimplantation embryos are spherical in shape and bear fewer, less-prominent cristae. Moreover, a subset of the mitochondrion pool in primordial germ cells serves as the founders of the mitochondria population in the oocyte and in the offspring. This genetic bottleneck is thought to ensure mitochondrial homoplasmy (defined as having mitochondria derived from a single source—in this case, the oocyte), which is important to the maintenance of proper mitochondrial function.

During SCNT, a relatively small number of the donor-cell mitochondria are inserted into the reconstructed embryo, resulting in mtDNA heteroplasmy. Studies on ovine, mouse, and bovine SCNT embryos indicate a high degree of variability in mitochondrial distribution, with some animals displaying complete homoplasmy (Evans et al., 1999; Hua et al., 2007; Meirelles et al., 2001) and others displaying heteroplasmy to varying degrees (Han et al., 2003; Hiendleder et al., 2003; Inoue et al., 2004; Takeda et al., 2003). In heteroplasmic animals, the level of contribution from donor-cell mitochondria is also highly variable between subjects and within tissues of the same subject. It has been reported that the level of heteroplasmy increases when iSCNT is performed (St John et al., 2004). Although healthy, live offspring have been obtained by both intraspecies and iSCNT, possible negative effects of heteroplasmy introduced in these animals may be responsible, in part, for many of the failures of iSCNT. For example, incompatibility of mitochondrial and nuclear genomes could impair mitochondrial function, leading to suboptimal respiration (St John et al., 2004). Most of the available SCNT studies have not provided information about the mitochondrial DNA and the metabolic status of SCNT embryos and animals.

The study by Chen et al. (2003) describes the presence of rabbit mitochondria in human cells. However, it is unknown whether those mitochondria were able to fully restore functional nucleocytoplasmic crosstalk. In human-monkey cybrids, for instance, mitochondria from common chimpanzee, pigmy chimpanzee, and gorilla were deficient in mitochondrial complex I activity, and the cybrids displayed reduced cellular respiration (Barrientos et al., 1998; McKenzie et al., 2003).

To date, NT studies addressing mitochondrial transmission have limited their scope to the detection of mtDNA and have provided no information about indicators of mitochonrial function. Two recent studies have investigated the amount of mtDNA, ATP production, and gene expression in bovine SCNT embryos with different haplotypes (Jiao et al., 2007) and mtRNA expression in sheep goat

SCNT embryos (Ma et al., 2007). The results of these studies suggest that the haplotype of recipient oocyte affects the ATP output, and developmental competence of SCNT embryos and that of the donor cell's mitochondria are selectively eliminated in iSCNT embryos during preimplantation development. Although metabolic pathways are well conserved among mammals, the proper activity of respiratory chain complexes (i.e., involving nuclei-mitochondrial compatibility, as discussed above) has never been directly studied in iSCNT embryos, leaving a significant gap in our understanding of the potential role that metabolic insufficiencies may play in the success of both iSCNT and SCNT developmental competency.

Taken together, these studies suggest that more data are necessary to determine whether or not cell-donor/recipient-oocyte mitochondrial incompatibilities are the cause of the problems in iSCNT.

Activation of the Embryonic Genome

Early development in mammals is controlled by proteins and mRNAs stored in the oocyte during oogenesis. After fertilization, these factors direct early cleavage divisions and are gradually depleted. Depletion of these molecules seems to coincide with the activation of the embryonic genome. Experiments using RNA polymerase II inhibitors have established that the embryo can develop up to the two cell, four cell, eight cell, and 16 cell stages in mouse, pig, human, rabbit, and sheep and cattle embryos, respectively, using maternal transcripts. These experiments demonstrated that the stage at which the embryo gains full control of transcription is a species-specific occurrence (Latham and Schultz, 2001; Schultz, 2002). However, recent studies have established that this process is more dynamic than previously thought. In fact, transcription can start as early as the one cell stage and can gradually increase until the embryonic genome gains control, reaching the "tipping point" at the stages previously described in the RNA polymerase II inhibitor experiments. Since timely and orderly expression of developmental genes is very critical for embryos to develop properly (Latham, 2005), the process of EGA plays a major role by providing control over spatial and temporal patterns of gene expression during preimplantation development. Considering that live offspring have been obtained using SCNT, it is easy to assume that donor somatic-genome-initiated transcription can either adapt to or be adapted to a developmentally compatible program, even though resulting gene expression patterns are reportedly altered in some of these animals (Latham, 2005; Latham and Schultz, 2001). In the context of SCNT, regulation of EGA deserves special attention, because the depletion of maternal messages stored in the ooplasm and chromatin modification appear to be concurrent events and could affect the timing of transcriptional initiation. For an iSCNT embryo to develop successfully into a blastocyst and beyond, it needs to coordinate both the donor and recipient components of EGA.

Data on reactivation of endogenous genes from the donor cell are scarce. Only one study directly addresses questions of EGA in iSCNT embryos; in this study, bovine/mouse iSCNT embryos reactivated a stable transgene (EGFP under CMV promoter) by the eight cell stage, while several selected endogenous genes failed to be transcribed by the same stage (Arat et al., 2003). In this study, no bovine/mouse iSCNT embryos developed further than the eight cell stage. Despite these limited data in gene expression, morphologic and developmental observations provide indirect evidence suggesting that, in the case of closely related species, at least some iSCNT embryos are able to activate the donor-cell genome, develop into blastocysts, and complete fetal development. Our experience with iSCNT between distant species, however, suggests that the majority of failed embryos experience a developmental block when the genome of the recipient oocyte is required to undergo a major activation event. Collectively, these results suggest that EGA could manifest itself as the culprit in the failure to overcome one of the species-specific developmental blocks and also deserves more rigorous attention in the context of iSCNT.

Minimal Standards for Future iSCNT Studies

In light of the arguments we have made, there are only two sound approaches for assessing whether reprogramming has occurred in iSCNT embryos: (1) establishing pluripotent embryonic cells that can be later analyzed in detail or (2) obtaining live offspring in all animals with the exception of human. The majority of published studies using iSCNT are difficult to interpret, in part due to a lack of compelling evidence showing that cells can or cannot be reprogrammed by a given oocyte. We would like to put forward specific methodological approaches that may yield more meaningful results: (1) PCR-based methods should be used to identify genomic and mitochondrial DNA, using primers specific to both species. (2) Greater emphasis should be placed on quantification, and not just the detection, of mtDNA. (3) If enough cells are generated, karyotyping should be performed to determine the relative stability of resulting cell genomes and to confirm their origin. (4) Endpoints for reprogramming should be either the establishment of pluripotent embryonic cell lines or in vivo development. (5) If preimplantation development is the focus of the study, basic physiological aspects of embryo biology should be addressed, such as timing of EGA, expression of the donor cell's specific genes, and embryo metabolism.

Although studies focusing on preimplantation stages of development in iSCNT embryos can and have uncovered many interesting facts about developmental physiology, future research in this field will undoubtedly shift from purely descriptive reports to more complex quantitative analyses that seek to better define the relationship between molecular events and developmental success.

Conclusions

With few exceptions, available iSCNT data suggest that species-specific barriers stand in the way of reprogramming somatic cells into embryonic stem cells. These

barriers appear to be overcome, or disappear, only when the two species are sufficiently closely related as to be able to crossbreed. In the event that studies aimed at the isolation of human ESCs using iSCNT prove successful, they could present an invaluable experimental model to investigate causes and potential treatments for late-onset diseases in human. Meanwhile, SCNT preimplantation embryos could be used to provide information about physiological problems associated with iSCNT, such as cell-cycle kinetics, EGA, and embryo metabolism. A detailed understanding of the limitations imposed on these embryos by species differences might lead to our being able to manipulate the limiting mechanisms and to exploit the iSCNT model in more practical ways. Although it presents a challenging task, the application of iSCNT could offer benefits not only for practical purposes but also for understanding fundamental aspects of biology.

ACKNOWLEDGMENTS

The authors thank Steve Suhr for critically reading the manuscript. Funding was provided by the Naylor Family Foundation, the Michigan State University Foundation, and the Michigan Agriculture Experiment Station.

REFERENCES

Arat, S., Rzucidlo, J., and Stice, S. (2003). Gene expression and in vitro development of inter-species nuclear transfer embryos. Mol. Reprod. Dev. *66*, 334–342.

Barrientos, A., Kenyon, L., and Moraes, C.T. (1998). Human xenomitochondrial cybrids. Cellular models of mitochondrial complex I deficiency. J. Biol. Chem. *273*, 14210–14217.

Brenner, C.A., Kubisch, H.M., and Pierce, K.E. (2004). Role of the mitochondrial genome in assisted reproductive technologies and embryonic stem cell-based therapeutic cloning. Reprod. Fertil. Dev. *16*, 743–751.

Chang, K.H., Lim, J.M., Kang, S.K., Lee, B.C., Moon, S.Y., and Hwang, W.S. (2003). Blastocyst formation, karyotype, and mitochondrial DNA of interspecies embryos derived from nuclear transfer of human cord fibroblasts into enucleated bovine oocytes. Fertil. Steril. *80*, 1380–1387.

Chavatte-Palmer, P., LeBourhis, D., Camous, S., Vignon, X., Renard, J.P., and Enright, B.P. (2002). Reproductive characteristics of cloned heifers derived from adult somatic cells. Biol. Reprod. *66*, 6–13.

Chen, D.Y., Wen, D.C., Zhang, Y.P., Sun, Q.Y., Han, Z.M., Liu, Z.H., Shi, P., Li, J.S., Xiangyu, J.G., Lian, L., et al. (2002). Interspecies implantation and mitochondria fate of panda-rabbit cloned embryos. Biol. Reprod. *67*, 637–642.

Chen, Y., He, Z.X., Liu, A., Wang, K., Mao, W.W., Chu, J.X., Lu, Y., Fang, Z.F., Shi, Y.T., Yang, Q.Z., et al. (2003). Embryonic stem cells generated by nuclear transfer of human somatic nuclei into rabbit oocytes. Cell Res. *13*, 251–263.

Chen, T., Zhang, Y.L., Jiang, Y., Liu, J.H., Schatten, H., Chen, D.Y., and Sun, Q.Y. (2006). Interspecies nuclear transfer reveals that demethylation of specific repetitive sequences is determined by recipient ooplasm but not by donor intrinsic property in cloned embryos. Mol. Reprod. Dev. *73*, 313–317.

Chesne, P., Adenot, P.G., Viglietta, C., Baratte, M., Boulanger, L., and Renard, J.P. (2002). Cloned rabbits produced by nuclear transfer from adult somatic cells. Nat. Biotechnol. *20*, 366–369.

Chung, Y.G., Ratnam, S., Chaillet, J.R., and Latham, K.E. (2003). Abnormal regulation of DNA methyltransferase expression in cloned mouse embryos. Biol. Reprod. *69*, 146–153.

Cibelli, J.B., Stice, S.L., Golueke, P.J., Kane, J.J., Jerry, J., Blackwell, C., Ponce de Leon, F.A., and Robl, J.M. (1998). Transgenic bovine chimeric offspring produced from somatic cell-derived stem-like cells. Nat. Biotechnol. *16*, 642–646.

Dindot, S.V., Farin, P.W., Farin, C.E., Romano, J., Walker, S., Long, C., and Piedrahita, J.A. (2004). Epigenetic and genomic imprinting analysis in nuclear transfer derived Bos gaurus/Bos taurus hybrid fetuses. Biol. Reprod. *71*, 470–478.

Dinnyes, A., Dal, Y.P., Barber, M., Liu, L., Xu, J., Zhou, P.L., and Yang, X.Z. (2001). Development of cloned embryos from adult rabbit fibroblasts: effect of activation treatment and donor cell preparation. Biol. Reprod. *64*, 257–263.

Dominko, T., Mitalipova, M., Haley, B., Beyhan, Z., Memili, E., and First, N. (1998). Bovine oocyte as a universal recipient cytoplasm in mammalian nuclear transfer. Theriogenology *49*, 385.

Dominko, T., Mitalipova, M., Haley, B., Beyhan, Z., Memili, E., McKusick, B., and First, N.L. (1999). Bovine oocyte cytoplasm supports development of embryos produced by nuclear transfer of somatic cell nuclei from various mammalian species. Biol. Reprod. *60*, 1496–1502.

Evans, M.J., Gurer, C., Loike, J.D., Wilmut, I., Schnieke, A.E., and Schon, E.A. (1999). Mitochondrial DNA genotypes in nuclear transfer-derived cloned sheep. Nat. Genet. *23*, 90–93.

Fang, Z.F., Gai, H., Huang, Y.Z., Li, S.G., Chen, X.J., Shi, J.J., Wu, L., Liu, A., Xu, P., and Sheng, H.Z. (2006). Rabbit embryonic stem cell lines derived from fertilized, parthenogenetic or somatic cell nuclear transfer embryos. Exp. Cell Res. *312*, 3669–3682.

Fulka, J., Jr., and Mrazek, M. (2004). Cloning of mammals-biological aspects. Cas. Lek. Cesk. *143*, 295–298.

Gilbert, S.F., and Bolker, J.A. (2001). Homologies of process and modular elements of embryonic construction. J. Exp. Zool. *291*, 1–12.

Gomez, M.C., Jenkins, J.A., Giraldo, A., Harris, R.F., King, A., Dresser, B.L., and Pope, C.E. (2003). Nuclear transfer of synchronized african wild cat somatic cells into enucleated domestic cat oocytes. Biol. Reprod. *69*, 1032–1041.

Gomez, M.C., Pope, C.E., Giraldo, A., Lyons, L.A., Harris, R.F., King, A.L., Cole, A., Godke, R.A., and Dresser, B.L. (2004). Birth of African Wildcat cloned kittens born from domestic cats. Cloning Stem Cells *6*, 247–258.

Hall, V.J., Stojkovic, P., and Stojkovic, M. (2006). Using therapeutic cloning to fight human disease: A conundrum or reality? Stem Cells *24*, 1628–1637.

Han, Z.M., Chen, D.Y., Li, J.S., Sun, Q.Y., Wan, Q.H., Kou, Z.H., Rao, G., Lei, L., Liu, Z.H., and Fang, S.G. (2003). Mitochondrial DNA heteroplasmy in calves cloned by using adult somatic cell. Mol. Reprod. Dev. *67*, 207–214.

Hashem, M.A., Bhandari, D.P., Kang, S.K., and Lee, B.C. (2007). Cell cycle analysis and interspecies nuclear transfer of in vitro cultured skin fibroblasts of the Siberian tiger (Panthera tigris Altaica). Mol. Reprod. Dev. *74*, 403–411.

Herrmann, R.G., Maier, R.M., and Schmitz-Linneweber, C. (2003). Eukaryotic genome evolution: rearrangement and coevolution of compartmentalized genetic information. Philos. Trans. R. Soc. Lond. B Biol. Sci. *358*, 87–97.

Hidalgo, C.O., Diez, C., Duque, P., Facal, N., and Gomez, E. (2003). Pregnancies and improved early embryonic development with bovine oocytes matured in vitro with 9-cis-retinoic acid. Reproduction *125*, 409–416.

Hiendleder, S., Zakhartchenko, V., Wenigerkind, H., Reichenbach, H.D., Bruggerhoff, K., Prelle, K., Brem, G., Stojkovic, M., and Wolf, E. (2003). Heteroplasmy in bovine fetuses produced by intra- and inter-subspecific somatic cell nuclear transfer: neutral segregation of nuclear donor mitochondrial DNA in various tissues and evidence for recipient cow mitochondria in fetal blood. Biol. Reprod. *68*, 159–166.

Hill, J.R. (2002). Abnormal in utero development of cloned animals: implications for human cloning. Differentiation *69*, 174–178.

Hill, J.R., Roussel, A.J., Cibelli, J.B., Edwards, J.F., Hooper, N.L., Miller, M.W., Thompson, J.A., Looney, C.R., Westhusin, M.E., Robl, J.M., et al. (1999). Clinical and pathologic features of cloned transgenic calves and fetuses (13 case studies). Theriogenology *51*, 1451–1465.

Hill, J.R., Burghardt, R.C., Jones, K., Long, C.R., Looney, C.R., Shin, T., Spencer, T.E., Thompson, J.A., Winger, Q.A., and Westhusin, M.E. (2000). Evidence for placental abnormality as the major cause of mortality in first-trimester somatic cell cloned bovine fetuses. Biol. Reprod. *63*, 1787–1794.

Hill, J.R., Schlafer, D.H., Fisher, P.J., and Davies, C.J. (2002). Abnormal expression of trophoblast major histocompatibility complex class I antigens in cloned bovine pregnancies is associated with a pronounced endometrial lymphocytic response. Biol. Reprod. *67*, 55–63.

Hochedlinger, K., and Jaenisch, R. (2003). Nuclear transplantation, embryonic stem cells, and the potential for cell therapy. N. Engl. J. Med. *349*, 275–286.

Hua, S., Zhang, Y., Song, K., Song, J., Zhang, Z., Zhang, L., Zhang, C., Cao, J., and Ma, L. (2007). Development of bovine-ovine interspecies cloned embryos and mitochondria segregation in blastomeres during preimplantation. Anim. Reprod. Sci. Published online March 6, 2007. 10.1016/j.anireprosci.2007.03.002.

Hubner, K., Fuhrmann, G., Christenson, L.K., Kehler, J., Reinbold, R., De La Fuente, R., Wood, J., Strauss, J.F., III, Boiani, M., and Scholer, H.R. (2003). Derivation of oocytes from mouse embryonic stem cells. Science *300*, 1251–1256.

Humpherys, D., Eggan, K., Akutsu, H., Hochedlinger, K., Rideout, W.M., 3rd, Biniszkiewicz, D., Yanagimachi, R., and Jaenisch, R. (2001). Epigenetic instability in ES cells and cloned mice. Science *293*, 95–97.

Humpherys, D., Eggan, K., Akutsu, H., Friedman, A., Hochedlinger, K., Yanagimachi, R., Lander, E.S., Golub, T.R., and Jaenisch, R. (2002). Abnormal gene expression in cloned mice derived from embryonic stem cell and cumulus cell nuclei. Proc. Natl. Acad. Sci. USA *99*, 12889–12894.

Hyun, I., Hochedlinger, K., Jaenisch, R., and Yamanaka, S. (2007). New advances in iPS cell research do not obviate the need for human embryonic stem cells. Cell Stem Cell *1*, 367–368.

Ikumi, S., Sawai, K., Takeuchi, Y., Iwayama, H., Ishikawa, H., Ohsumi, S., and Fukui, Y. (2004). Interspecies somatic cell nuclear transfer for in vitro production of Antarctic minke whale (Balaenoptera bonaerensis) embryos. Cloning Stem Cells *6*, 284–293.

Illmensee, K., Levanduski, M., and Zavos, P.M. (2006). Evaluation of the embryonic preimplantation potential of human adult somatic cells via an embryo interspecies bioassay using bovine oocytes. Fertil. Steril. *85* (*Suppl 1*), 1248–1260.

Inoue, K., Ogonuki, N., Yamamoto, Y., Takano, K., Miki, H., Mochida, K., and Ogura, A. (2004). Tissue-specific distribution of donor mitochondrial DNA in cloned mice produced by somatic cell nuclear transfer. Genesis *39*, 79–83.

Jaenisch, R., Hochedlinger, K., and Eggan, K. (2005). Nuclear cloning, epigenetic reprogramming and cellular differentiation. Novartis Found. Symp. *265*, 107–118.

Jiang, Y., Chen, T., Nan, C.L., Ouyang, Y.C., Sun, Q.Y., and Chen, D.Y. (2005). In vitro culture and mtDNA fate of ibex-rabbit nuclear transfer embryos. Zygote *13*, 233–240.

Jiao, F., Yan, J.B., Yang, X.Y., Li, H., Wang, Q., Huang, S.Z., Zeng, F., and Zeng, Y.T. (2007). Effect of oocyte mitochondrial DNA haplotype on bovine somatic cell nuclear transfer efficiency. Mol. Reprod. Dev. *74*, 1278–1286.

Kishigami, S., Wakayama, S., van Thuan, N., and Wakayama, T. (2006). Cloned mice and embryonic stem cell establishment from adult somatic cell. Hum. Cell *19*, 2–10.

Kitiyanant, Y., Saikhun, J., Chaisalee, B., White, K.L., and Pavasuthipaisit, K. (2001). Somatic cell cloning in Buffalo (Bubalus bubalis): effects of interspecies cytoplasmic recipients and activation procedures. Cloning Stem Cells *3*, 97–104.

Klimanskaya, I., Chung, Y., Becker, S., Lu, S.J., and Lanza, R. (2006). Human embryonic stem cell lines derived from single blastomeres. Nature *444*, 481–485.

Klimanskaya, I., Chung, Y., Becker, S., Lu, S.J., and Lanza, R. (2007). Derivation of human embryonic stem cells from single blastomeres. Nat. Protoc. *2*, 1963–1972.

Lanza, R.P., Cibelli, J.B., Diaz, F., Moraes, C.T., Farin, P.W., Farin, C.E., Hammer, C.J., West, M.D., and Damiani, P. (2000). Cloning of an endangered species (Bos gaurus) using interspecies nuclear transfer. Cloning *2*, 79–90.

Latham, K.E. (2005). Early and delayed aspects of nuclear reprogramming during cloning. Biol. Cell *97*, 119–132.

Latham, K.E., and Schultz, R.M. (2001). Embryonic genome activation. Front. Biosci. *6*, D748–D759.

Li, Y., Dai, Y., Du, W., Zhao, C., Wang, H., Wang, L., Li, R., Liu, Y., Wan, R., and Li, N. (2006a). Cloned endangered species takin (Budorcas taxicolor) by inter-species nuclear transfer and comparison of the blastocyst development with yak (Bos grunniens) and bovine. Mol. Reprod. Dev. *73*, 189–195.

Li, Y., Dai, Y., Du, W., Zhao, C., Wang, L., Wang, H., Liu, Y., Li, R., and Li, N. (2006b). In vitro development of yak (Bos grunniens) embryos generated by interspecies nuclear transfer. Anim. Reprod. Sci. *101*, 45–59.

Liu, S.Z., Zhou, Z.M., Chen, T., Zhang, Y.L., Wen, D.C., Kou, Z.H., Li, Z.D., Sun, Q.Y., and Chen, D.Y. (2004). Blastocysts produced by nuclear transfer between chicken blastodermal cells and rabbit oocytes. Mol. Reprod. Dev. *69*, 296–302.

Loi, P., Ptak, G., Barboni, B., Fulka, J., Jr., Cappai, P., and Clinton, M. (2001). Genetic rescue of an endangered mammal by cross-species nuclear transfer using post-mortem somatic cells. Nat. Biotechnol. *19*, 962–964.

Ma, L.B., Yang, L., Zhang, Y., Cao, J.W., Hua, S., and Li, J.X. (2007). Quantitative analysis of mitochondrial RNA in goat-sheep cloned embryos. Mol. Reprod. Dev. *75*, 33–39. Published online June 14, 2007. 10.1002/mrd.20736.

Maherali, N., Sridharan, R., Xie, W., Utikal, J., Eminli, S., Arnold, K., Stadtfeld, M., Yachechko, R., Tchieu, J., Jaenisch, R., et al. (2007). Directly reprogrammed fibroblasts show global epigenetic remodeling and widespread tissue contribution. Cell Stem Cell *1*, 55–70.

Mann, M.R.W., Chung, Y.G., Nolen, L.D., Verona, R.I., Latham, K.E., and Bartolomei, M.S. (2003). Disruption of imprinted gene methylation and expression in cloned preimplantation stage mouse embryos. Biol. Reprod. *69*, 902–914.

Mastromonaco, G.F., Favetta, L.A., Smith, L.C., Filion, F., and King, W.A. (2007). The influence of nuclear content on developmental competence of gaur x cattle hybrid in vitro fertilized and somatic cell nuclear transfer embryos. Biol. Reprod. *76*, 514–523.

McKenzie, M., Chiotis, M., Pinkert, C.A., and Trounce, I.A. (2003). Functional respiratory chain analyses in murid xenomitochondrial cybrids expose coevolutionary constraints of cytochrome b and nuclear subunits of complex III. Mol. Biol. Evol. *20*, 1117–1124.

Meirelles, F.V., Bordignon, V., Watanabe, Y., Watanabe, M., Dayan, A., Lobo, R.B., Garcia, J.M., and Smith, L.C. (2001). Complete replacement of the mitochondrial genotype in a Bos indicus calf reconstructed by nuclear transfer to a Bos taurus oocyte. Genetics *158*, 351–356.

Murakami, M., Otoi, T., Wongsrikeao, P., Agung, B., Sambuu, R., and Suzuki, T. (2005). Development of interspecies cloned embryos in yak and dog. Cloning Stem Cells *7*, 77–81.

Oh, B.C., Kim, J.T., Shin, N.S., Kwon, S.W., Kang, S.K., Lee, B.C., and Hwang, W.S. (2006). Production of blastocysts after intergeneric nuclear transfer of goral (Naemorhedus goral) somatic cells into bovine oocytes. J. Vet. Med. Sci. *68*, 1167–1171.

Okita, K., Ichisaka, T., and Yamanaka, S. (2007). Generation of germline-competent induced pluripotent stem cells. Nature *448*, 313–317.

Orr, H.A., Masly, J.P., and Presgraves, D.C. (2004). Speciation genes. Curr. Opin. Genet. Dev. *14*, 675–679.

Renard, J.P., Chastant, S., Chesne, P., Richard, C., Marchal, J., Cordonnier, N., Chavatte, P., and Vignon, X. (1999). Lymphoid hypoplasia and somatic cloning. Lancet *353*, 1489–1491.

Sansinena, M.J., Hylan, D., Hebert, K., Denniston, R.S., and Godke, R.A. (2005). Banteng (Bos javanicus) embryos and pregnancies produced by interspecies nuclear transfer. Theriogenology *63*, 1081–1091.

Santos, F., Zakhartchenko, V., Stojkovic, M., Peters, A., Jenuwein, T., Wolf, E., Reik, W., and Dean, W. (2003). Epigenetic marking correlates with developmental potential in cloned bovine preimplantation embryos. Curr. Biol. *13*, 1116–1121.

Schultz, R.M. (2002). The molecular foundations of the maternal to zygotic transition in the preimplantation embryo. Hum. Reprod. Update *8*, 323–331.

St John, J.C., Lloyd, R.E., Bowles, E.J., Thomas, E.C., and El Shourbagy, S. (2004). The consequences of nuclear transfer for mammalian foetal development and offspring survival. A mitochondrial DNA perspective. Reproduction *127*, 631–641.

Takahashi, K., and Yamanaka, S. (2006). Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell *126*, 663–676.

Takeda, K., Akagi, S., Kaneyama, K., Kojima, T., Takahashi, S., Imai, H., Yamanaka, M., Onishi, A., and Hanada, H. (2003). Proliferation of donor mitochondrial DNA in nuclear transfer calves (Bos taurus) derived from cumulus cells. Mol. Reprod. Dev. *64*, 429–437.

Thongphakdee, A., Numchaisrika, P., Omsongkram, S., Chatdarong, K., Kamolnorranath, S., Dumnui, S., and Techakumphu, M. (2006). In vitro development of marbled cat embryos derived from interspecies somatic cell nuclear transfer. Reprod. Domest. Anim. *41*, 219–226.

Wakayama, T., Tabar, V., Rodriguez, I., Perry, A.C., Studer, L., and Mombaerts, P. (2001). Differentiation of embryonic stem cell lines generated from adult somatic cells by nuclear transfer. Science *292*, 740–743.

Wakayama, S., Kishigami, S., Van Thuan, N., Ohta, H., Hikichi, T., Mizutani, E., Yanagimachi, R., and Wakayama, T. (2005a). Propagation of an infertile hermaphrodite mouse lacking germ cells by using nuclear transfer and embryonic stem cell technology. Proc. Natl. Acad. Sci. USA *102*, 29–33.

Wakayama, S., Mizutani, E., Kishigami, S., Thuan, N.V., Ohta, H., Hikichi, T., Bui, H.T., Miyake, M., and Wakayama, T. (2005b). Mice cloned by nuclear transfer from somatic and ntES cells derived from the same individuals. J. Reprod. Dev. *51*, 765–772.

Wakayama, S., Ohta, H., Kishigami, S., Thuan, N.V., Hikichi, T., Mizutani, E., Miyake, M., and Wakayama, T. (2005c). Establishment of male and female nuclear transfer embryonic stem cell lines from different mouse strains and tissues. Biol. Reprod. *72*, 932–936.

Wakayama, S., Jakt, M.L., Suzuki, M., Araki, R., Hikichi, T., Kishigami, S., Ohta, H., Van Thuan, N., Mizutani, E., Sakaide, Y., et al. (2006). Equivalency of nuclear transfer-derived embryonic stem cells to those derived from fertilized mouse blastocyst. Stem Cells *24*, 2023–2033.

Wang, L., Duan, E., Sung, L.Y., Jeong, B.S., Yang, X., and Tian, X.C. (2005). Generation and characterization of pluripotent stem cells from cloned bovine embryos. Biol. Reprod. *73*, 149–155.

Wen, D.C., Bi, C.M., Xu, Y., Yang, C.X., Zhu, Z.Y., Sun, Q.Y., and Chen, D.Y. (2005). Hybrid embryos produced by transferring panda or cat somatic nuclei into rabbit MII oocytes can develop to blastocyst in vitro. J. Exp. Zoolog. A Comp. Exp. Biol. *303*, 689–697.

Wernig, M., Meissner, A., Foreman, R., Brambrink, T., Ku, M., Hochedlinger, K., Bernstein, B.E., and Jaenisch, R. (2007). In vitro reprogramming of fibroblasts into a pluripotent ES-cell-like state. Nature *448*, 318–324.

White, K.L., Bunch, T.D., Mitalipov, S., and Reed, W.A. (1999). Establishment of pregnancy after the transfer of nuclear transfer embryos produced from the fusion of argali (Ovis ammon) nuclei into domestic sheep (Ovis aries) enucleated oocytes. Cloning *1*, 47–54.

Wilmut, I., Schnieke, A.E., McWhir, J., Kind, A.J., and Campbell, K.H.S. (1997). Viable offspring derived from fetal and adult mammalian cells. Nature *385*, 810–813.

Yang, C.X., Han, Z.M., Wen, D.C., Sun, Q.Y., Zhang, K.Y., Zhang, L.S., Wu, Y.Q., Kou, Z.H., and Chen, D.Y. (2003). In vitro development and mitochondrial fate of macaca-rabbit cloned embryos. Mol. Reprod. Dev. *65*, 396–401.

Yin, X., Lee, Y., Lee, H., Kim, N., Kim, L., Shin, H., and Kong, I. (2006). In vitro production and initiation of pregnancies in inter-genus nuclear transfer embryos derived from leopard cat (Prionailurus bengalensis) nuclei fused with domestic cat (Felis silverstris catus) enucleated oocytes. Theriogenology *66*, 275–282.

Zhao, Z.J., Ouyang, Y.C., Nan, C.L., Lei, Z.L., Song, X.F., Sun, Q.Y., and Chen, D.Y. (2006). Rabbit oocyte cytoplasm supports development of nuclear transfer embryos derived from the somatic cells of the camel and Tibetan antelope. J. Reprod. Dev. *52*, 449–459.

Zhao, Z.J., Li, R.C., Cao, H.H., Zhang, Q.J., Jiang, M.X., Ouyang, Y.C., Nan, C.L., Lei, Z.L., Song, X.F., Sun, Q.Y., and Chen, D.Y. (2007). Interspecies nuclear transfer of Tibetan antelope using caprine oocyte as recipient. Mol. Reprod. Dev. *74*, 412–419.