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The Future of human cloning: can human embryos resulting from somatic cell nuclear transfers (SCNT) be used to treat human infertility?

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Introduction

It is well established that a variety of adult cell types have been successfully employed for somatic cell nuclear transfer (SCNT) in farm animal cloning technology.[1,2] So far, follicular granulosa cells and skin fibroblast cells have been most efficiently and extensively utilized as cell donor source in reproductive cloning.[3,4] Despite of multiple attempts and worldwide efforts to improve the cloning efficiency for live-born progeny, the success rates for obtaining mammalian clones from adult somatic cells employed for SCNT remain still limited.[5]

Several risk factors for reproductive cloning in animals that may be responsible for the rather low survival rates have been discussed and proposed for further investigations. Not only epigenetic alterations in methylation of genes,[6–8] but also changes in structure of chromosomes[9–11] can be envisaged as critical for the cloned offspring. A number of researchers in the cloning field have pointed out that the short period of time for somatic donor cell nuclei to be properly reprogrammed in the cytoplasm of recipient oocytes may not be sufficient enough to initiate and sustain normal clonal embryogenesis.[12–14] Also, the volume ratio of karyoplast (from the donor cell) and cytoplast (from the enucleated oocyte) in SCNT procedures has shown to affect the developmental potential of cloned embryos.[15] In addition, cloning efficiency can be influenced by cell-cycle heterogeneity of the cultured somatic cells and may be improved by increasing cell-cycle uniformity for somatic donor cells used in SCNT.[16–18]

For future advancements in reproductive cloning, it will be important to further increase our experience and knowledge about how to rejuvenate and reprogram human adult cells by modern molecular and cellular biotechnology.[19] In this context, we have started a novel approach using two different types of adult human cells (granulosa cells from female patients enrolled in IVF programs and fibroblast cells from infertile male patients) for SCNT into enucleated bovine oocytes.[20,21] Development of such a cloning biological model enables us to utilize it as a bioassay to test and compare the efficiency of different human adult somatic cells for their ability to promote embryonic development via interspecies SCNT.

In several attempts, we have documented and proven via PCR analysis and DNA sequencing that fibroblast cells from azoospermic patients when fused with enucleated bovine oocytes could promote development of interspecies embryos.[21] In the current report, we have summarized our interspecies SCNT using fibroblast cells from four azoospermic patients devoid of germ cells. In parallel but not concurrently, we have carried out human SCNT utilizing these cultured fibroblasts and oocytes from the infertile patients' wives in order to create human cloned embryos for reproductive purposes. We recently reported on the first transfer of a cloned human embryo, using fibroblast cells from an azoospermic patient for SCNT as a possible modality for treatment of severe male infertility.[22] Via our various efforts we have described and documented our continuous efforts of transferring cloned human embryos in-utero for the purpose of establishing a cloned pregnancy and the birth of the first healthy cloned baby.

It attempting to do just that we have found out that during the past years, several interspecies bioassays have been developed to test the embryonic potential of various adult cells. Bovine oocytes have been successfully employed in interspecies SCNT to test adult cells from pig, sheep, rat and rhesus monkey for their embryonic potential *in-vitro*.[23] Karyotyping results and molecular data using PCR and DNA technology have been published on interspecies embryos that were derived from SCNT using adult human cord fibroblast cells fused with enucleated bovine oocytes.[24]

We have employed such interspecies bioassays to test the embryonic *in-vitro* potential of human adult fibroblast cells from infertile male patients via SCNT in the bovine system. Our results have shown that these somatic cells are able to promote development leading to interspecies embryos.[21] We have also shown via PCR amplification and DNA sequencing that these interspecies embryos created via SCNT contained human genomic and mitochondrial (mt) DNA which was identical to the human donor cell source. In addition, these interspecies SCNT embryos contained bovine mtDNA of oocyte origin. Heteroplasmy of mtDNA has been revealed in cloned animals and has been discussed in the context of possible implications for the cloned offspring.[25,26]

Furthermore, we have examined the embryonic potential of fibroblast cells derived from another infertile male patient suffering from azoospermia and cryptorchidism using the bovine bioassay model.[22] From the resulting interspecies embryos, PCR amplification and DNA sequencing unequivocally documented that these embryos were composed of the human genomic DNA specific for the patient's fibroblast donor cell source and contained both human and bovine mtDNA. Due to these previous extensive and positive investigations and results on interspecies SCNT embryos,[21,22] We have established very clearly that such elaborate molecular analysis was not essential

and necessary and, therefore, has not been included in the present clinical report.

With regards to human SCNT, it was recently reported that human oocytes were only promoting optimal development when they were enucleated and further processed for SCNT within 1hr post-retrieval.[27] Furthermore, when using failed fertilized oocytes from IVF programs about 24hrs post-retrieval, they were not able to initiate proper and regular embryonic development and therefore, turned out to be inefficient for SCNT purposes.[28] It therefore seems crucial to utilize mature human oocytes rather quickly after retrieval for successful SCNT. We have also established that immature oocytes, on the other hand, need further culture for maturation to metaphase II (polar body extrusion) before their use in SCNT (unpublished data).

In 2003, the creation of the first human cloned embryo for reproductive purposes was reported using human enucleated eggs and heterologous human granulosa cells for SCNT.[29] In 2006, we published results on the first intrauterine transfer of a human SCNT-derived embryo but without achieving a pregnancy. In our most recent efforts, fibroblast cells from an infertile male devoid of germ cells were employed for successful SCNT.[22] Concerning these attempts and future developments in the field of reproductive cloning, it was stated that a wide perspective must be maintained on this type of work.[30] In the context of ethical and medical considerations, critical safety issues concerning risk factors for malformations have also been emphasized for human cloning.[31,32]

In a more recent clinical report, we have presented and summarized further evidence for our attempts in creating SCNT human embryos for intrauterine transfer.[33] Even though no pregnancies were established in the four attempted cases so far, we have shown that human reproduction via SCNT and the creation and transfer of human cloned embryos may eventually be applicable in the future for patients that suffer from severe infertility and may have no other viable alternative options for procreating their own biological offspring. The World recognizes that human reproductive cloning is another form of assisted reproductive technology that would be carried out with the goal of creating a human being. It has been and remains the subject of much debate around the world, involving a variety of ethical, religious, societal, scientific, and medical issues. However, it is the intend of this team's objective to addresses only the scientific and medical aspects of human reproductive cloning. Consideration of the medical aspects has required for this team to examine issues of scientific conduct and human-subjects protection. But we have not attempted to address the issue of whether producing a new individual by reproductive cloning and use of SCNT technology, if it were found to be scientifically safe, would or would not be acceptable to individuals or society. Instead, this team defers to others on the fundamental ethical, religious, and societal questions, and only focuses on the scientific and medical aspects and to inform the broader debate.

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Conflict of interest

The author declares no conflict of interest.

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