

Reproductive and therapeutic cloning: what are the perspectives for medicine?

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Reproduction

In 1981 reproductive cloning in mammals was first reported in rodents by creating adult mice via nuclear transfer from cells of the inner cell mass of blastocysts into enucleated egg cells (Illmensee and Hoppe, 1981). In 1986 cloned sheep were obtained from embryonic cell nuclei transferred into enucleated oocytes, thus establishing the cloning procedures for farm animals (Willadsen, 1986). During the next 10 years, cloning technology has been further developed and extended to pigs (Prather et al., 1989), goats (Zhang et al., 1991), bovines (Sims et al., 1994) and rhesus monkeys (Meng et al., 1997), but always using embryonic cells as nuclear donors for cloning. In 1997 for the first time, adult donor cells have been employed, successfully cloning a sheep by creating "Dolly" from an ewe's udder cell nucleus (Wilmut et al., 1997). Subsequently in 1998, eight calves were cloned from adult cells of a single donor animal (Kato et al., 1998). In 2000 for the first time, cloned calves were produced from adult fibroblast cells after their long-term culture in vitro (Kubota et al., 2000). Several cloned calves of female and male genotype have also been generated from a variety of different adult cell types (Kato et al., 2000).

Concerning primate cloning with adult somatic donor cells, and particularly relevant for the rhesus monkey model, American researchers have recently reported the failure to obtain pregnancy from cloned embryos. From their observations they concluded that primate NT (nuclear transfer) appears to be challenged by stricter molecular requirements for mitotic spindle assembly than in other mammals" (Simerly et al., 2003). Since there is no further detailed information on plausible intrinsic factors responsible for these negative findings on rhesus monkey cloning, reasonable conclusions should not be currently drawn and extrapolated to other primates.

For future applications in reproductive cloning it will be important to advance further in our understanding of how to rejuvenate and reprogram human adult cells by modern molecular bioengineering. In this context, we have recently started a novel approach using adult human ovarian granulosa cells and skin fibroblasts for somatic cell nuclear transfer (SCNT) into enucleated bovine oocytes to test and extend the efficiency of interspecies-specific SCNT with the aim to establish a series of bioassays for different types of human nuclear donor cells. The bovine oocyte is an excellent and highly efficient model as it provides

adequate parameters for SCNT (Wells et al., 1999). Our first results point out that SCNT with enucleated bovine oocytes and somatic human cells are feasible and can yield appreciable success rates. With this interspecies-specific bioassay we present our first attempts to reveal the embryonic capacity of nuclei from adult human cells (Illmensee et al., 2004). Such bioassays will enable us to primarily examine, evaluate and explore the potential development of various human adult somatic cells in their usefulness as nuclear donor cells for reproductive and therapeutic cloning.

The current success rates (between 1 to 8 %) for obtaining adult mammalian clones derived from adult cells for nuclear donor transfer remain rather limited. Several major obstacles, which may contribute to a low success rate in reproductive cloning, are under intense scientific analysis. Some of the key investigations are focusing on nucleocytoplasmic interactions and reprogramming of the transferred somatic cell nucleus by the cytoplasm of the recipient oocyte (Fulka et al., 1996), chromatin remodelling of the somatic nucleus by oocyte factors (Wade and Kikyo, 2002), rebuilding of telomere length of somatic chromosomes (Betts et al., 2001) or cell-cycle co-ordination between nucleus and cytoplasm during the cloning procedure (Liu et al., 1997). Research has also focused on possible biological effects of mitochondrial contributions from both cell types to cloned mammals. In sheep, mtDNA could be detected in cloned offspring only from the recipient oocyte type but not from the donor cell type, leading to mitochondrial homoplasmy (Evans et al., 1999). In cattle, on the other hand, donor cell-specific mtDNA contributions between 0.4 to 4% were found in healthy cloned calves, demonstrating that mitochondrial heteroplasmy does not necessarily prevent normal clonal development (Steinborn et al., 2000). In a recent study using adult cumulus cells as nuclear donors, some cloned calves exhibited considerable mitochondrial heteroplasmy with 4 to 60% mtDNA

from the donor-cell type (Takeda et al., 2003).

Livestock, culture conditions have profoundly shown the influence of the development of normal (IVF) and cloned embryos during growth in vitro. After their intrauterine transfer some of them developed into large-sized fetuses giving rise to large offspring (Behboodi et al., 1995; Wilson et al., 1995). This LO syndrome (Young et al., 1998), as well as other organic anomalies and postnatal malformations, have turned out to be of major concern for farm ani-

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mals IVF and cloning (Hyttel et al., 2000). Scottish researchers have reported that sheep fetuses with the LO syndrome when compared to normal fetuses express a low level of IGF2R, a multifunctional protein that amongst other functional tasks is involved in fetal organogenesis. This protein under-expression is concomitantly associated with reduced methylation of the corresponding gene locus (Young et al., 2001). It has been argued that an epigenetic change in methylation (so-called genomic imprinting) is responsible for the observed developmental defects and might be causally influenced by the cultural conditions in vitro. American researchers have recently shown that in artiodactyls (i.e. sheep, goat, cattle, pig) only the maternally inherited allele for

the IGF2R gene locus is expressed, whereas in primates both paternal alleles remain active, most likely as a result of evolutionary processes (Killian et al., 2001). Such species-specific differences in genomic imprinting may explain, to some extent, the observed differences in

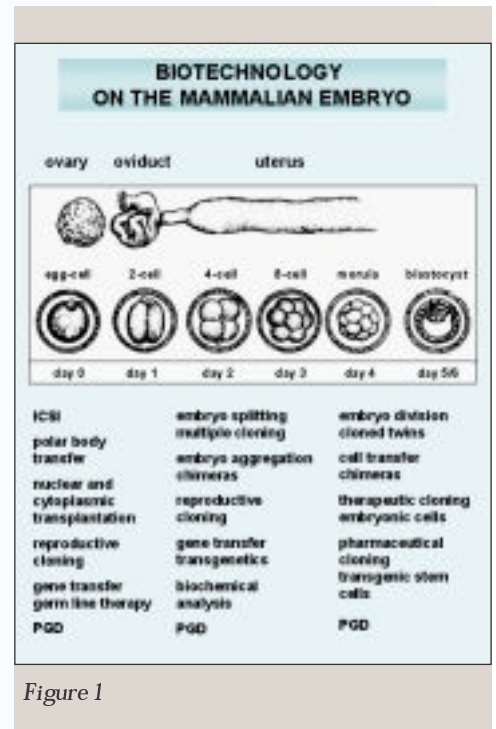


Figure 1

embryo culture between artiodactyls and primates. Whereas in farm animals a large proportion of in vitro- cultured embryos (irrespective of IVF- or clonally- derived) develop into abnormal fetuses and offspring, an elevated percentage of malformations has never been observed world-wide in human assisted reproduction - neither in IVF nor ICSI - when compared to normal conception. Several risk factors for reproductive cloning have been discussed and proposed for intensive investigations (Illmensee, 2001a). Not only epigenetic alterations in methylation of genes, but also point mutations and changes in structure of chromosomes can be envisaged as critical (Santos et al., 2003). Another issue of concern for reproductive cloning pointed out that the short period of time for the somatic donor cell nucleus to be reprogrammed properly in the recipient oocytoplasm may not be sufficient to initiate and

enable normal embryogenesis. Cloning efficiency in goats and cattle can be improved by exposing the donor nucleus from blastomeres of a cloned embryo again to the oocytoplasm during a second recloning step, thus prolongating the exposure time for genomic remodelling (Zhang and Li, 1998; Zakartchenko et al., 1999).

Whether such biotechnology (Fig 1) will be successfully applied to human reproduction depends on scientific progress and social acceptance (Illmensee, 2001b). A recent opinion poll among medical practitioners and members of APART (international association of assisted reproductive technology centers) has revealed that three quarters of them would be willing to provide human cloning for patients in clinically indicated cases (Katayama, 2001). Recently, Zavos and co-workers obtained a human SCNT embryo originating from electric fusion of an enucleated human oocyte with an adult ovarian granulosa cell from a donor. This cloned embryo has developed to the 8- to 10-cell stage and has been subsequently cryopreserved for future purposes (Zavos, 2003). Recently, during a press conference in London, Zavos has announced the first transfer in utero of a cloned human embryo (Laurance, 2004). Currently, reproductive cloning has again sparked a world-wide emotional dispute for and against its application in medicine.

Therapeutics

A few years ago, American and Australian researchers successfully managed to culture embryonic cells from 5 to 6 days-old human preimplantation embryos and primordial germ cells from 5 to 9 weeks-old human embryos in suitable nutritional media (Thomson et al., 1998; Shambloott et al., 1998). Embryonic stem (ES) cells and primordial germ (PG) cells remain diploid and maintain their embryonic and proliferative characteristics over many cell divisions in culture. ES cells are derived from the inner cell mass (ICM) of cultured human blastocysts produced by in

vitro fertilization for clinical purposes in assisted reproduction and donated by patients. Human ES cell lines can be established by electing and expanding individual ICM colonies of uniform and undifferentiated morphology.

Human embryo-derived ES cells may be employed for future therapeutic purposes to reconstitute or repair defective organ systems since they are capable of differentiating into a variety of tissues and possess a large regenerative potential (Asahara et al., 2000). Therapeutic cloning, with the aid of assisted reproductive technologies for the extraction and expansion of human ES cells, will open up novel and quite controversial applications for transplantation medicine. Therapeutic cloning may be performed by utilizing donated preimplantation embryos from IVF and ICSI programmes or by creating cloned embryos via nuclear transfer from patients' somatic cells into enucleated oocytes (Fig 2). The latter concept would offer a unique opportunity to establish patient-specific stem cells for regenerative therapy. In future applications it may also be envisaged that early embryo, after splitting them into twin embryos, will provide a unique opportunity to implant one embryo in utero and to establish and cryopreserve stem cells from the monozygotic twin embryo. Should there be a successful pregnancy giving rise to a child, it may profit in the future from its own cryopreserved genetically embryonic stem cells (Illmensee, 2001b).

In a public intensified press release, Cibelli and co-workers reported somatic cell nuclear transfer (SCNT) in humans in the context of future therapeutic cloning. Using fibroblasts and cumulus cells as nuclear donor types, they obtained two abnormal embryos that have already stopped development respectively at the 4-cell and 6-cell stage (Cibelli et al., 2001). However, at this early embryonic stage, stem cells can not yet be isolated under current biotechnology. Embryos have to be capable of developing in order to advance preimplantation stages, which is necessary prerequisite for stem cell isolation.

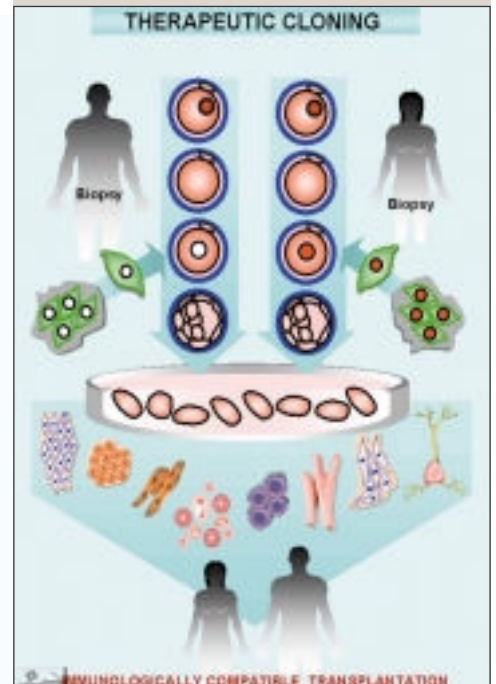


Figure 2

Recently, Korean researchers have published strong evidence for establishing human ES cells derived from a cloned human blastocyst (Hwang et al., 2004). Analogously, Chinese researchers have recently reported successful SCNT using enucleated rabbit oocytes fused with human adult fibroblast cells. From the developing cloned embryos they have established in vitro cultures of interspecies-derived ES cells (Chen et al., 2003). Such interspecies embryos may serve as a potential source for stem cell research in therapeutic cloning. Future extensive investigations will be required to discover the embryonic capacity of human adult somatic cells and their ability to create embryonic stem cells via cloning for therapeutic purposes.

With respect to human therapeutic cloning by means of developing cloned embryos in assisted reproduction, a novel discipline of biotechnology, dedicated to stem cell therapy in regenerative medicine for a variety of human diseases, will emerge for the benefit of patients' health. For the future, our society will have to set up the proper scientific, legal and ethical rules concerning human cloning with its multiple applications in medicine.

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