

Commentary

Human reproductive cloning: the time is near

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Reproductive cloning today continues to preoccupy the general public and its critics in a very controversial and often misleading manner. We, in the field of scientific and reproductive medicine, realize that our responsibilities are quite numerous and extremely delicate. It was not too long ago that we witnessed the atmosphere at the National Academy of Sciences hearing in Washington, DC (August 2001) on the topic of human reproductive cloning, although not entirely militated against by its concomitant scholarly document (National Academy of Sciences, 2002; Simpson and Edwards, 2003). As one of the invited participants, it was evident from the behaviour of the NAS members and their invited guests that this hearing was scheduled not to discuss the topic of human reproductive cloning, but rather to condemn it.

From the beginning of our efforts, we have never stated that we intended to create the first cloned embryo and the first human being for reproductive purposes by ignoring the public's concerns and the scientific critics. We also never intended to ignore the contradictory results that scientists in the field of animal cloning have obtained during the past years. We merely wanted to learn from all the difficulties that the animal cloning experts encountered, in order to take the criticisms and the public's concerns as seriously as possible and turn them into positive developments. It was quite evident to us from the beginning of this debate that with further elucidation of the molecular mechanisms involved during the processes of embryogenesis, careful tailoring of subsequently developed culture conditions and manipulation strategies, along with appropriate molecular screening methods, we could eventually allow infertile couples to safely have healthy, genetically related children through somatic cell nuclear transfer (SCNT) methods.

Extensive research on nuclear transfer has been performed using the bovine model. It was shown that injecting bovine eggs with granulosa or cumulus cells yielded success rates of 69% (Wells *et al.*, 1999) and 38% (Kato *et al.*, 1998). In other species, the use of similar cell populations showed an efficiency of 61% in the rabbit (Chesné *et al.*, 2002) and 56% in the mouse (Wakayama *et al.*, 1998). Based upon these findings, we have decided to use the bovine model and granulosa cells to test the efficiency of our nuclear transfer techniques. The bovine is an excellent and highly efficient model, as it provides adequate responses when measuring the effects of various treatments and the oocytes are commercially readily available. We have begun to establish a series of bioassays for our cloning procedures by using microsurgically enucleated bovine oocytes and fusing them with human granulosa cells, analogous to previously published interspecies-specific cloning attempts (Dominko *et al.*, 1999).

In the first series of experiments, 11 bovine oocytes (Group A) were microsurgically enucleated and fused with human granulosa cells, and 17 oocytes (group B) served as controls (parthenogenesis). All of the oocytes were electrically stimulated and activated. After 3 days of sequential culture, group A showed embryo development in five of the 11 oocytes (45%), and group B showed activation or parthenogenesis in 10 of the 17 oocytes (59%). In a second series of experiments, we obtained 21% embryo development with human granulosa cells (3 of 14) versus 36% parthenogenesis in the control group (8 of 22). The human granulosa cells used in the second series were cultured for 72 h prior to their use. The decrease in efficiency could be related to possible inter-batch variability of the bovine oocytes. These findings represent preliminary data and further studies are currently underway to establish biological trends using this bovine model as a bioassay. Such bioassays will enable us primarily to examine, evaluate and explore the developmental potential of various adult somatic cells in their usefulness as nuclear donor cells for reproductive and therapeutic cloning.

Recently, our team of scientific and medical experts has created the first human cloned embryo for reproductive purposes. The embryo was the end result of using nine microsurgically enucleated human donor oocytes and fusing them via electrical stimulation and activation with whole human granulosa cells from a patient desiring to have a child via SCNT. The granulosa cells harvested from this patient were previously cryopreserved and thawed and allowed to grow in culture for 1 day prior to the procedure. The resulting cloned embryo was allowed to develop further in culture for 4 days post-SCNT and reached the 8–10-cell stage, which

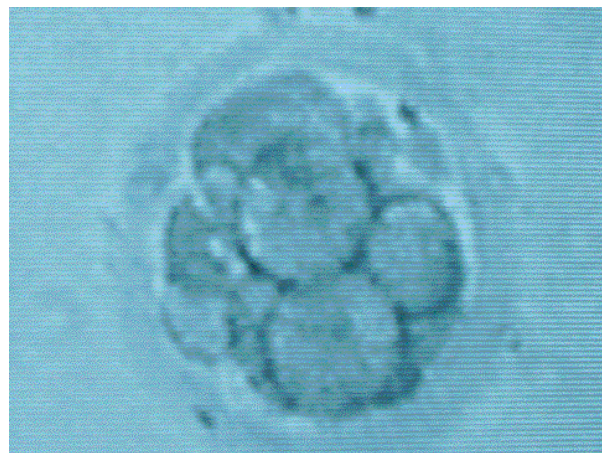


Figure 1. An 8–10-cell human embryo derived from somatic cell nuclear transfer (SCNT) of granulosa at 92 h.

showed a rate of development equivalent to that of normal IVF embryos (**Figure 1**). Its development was observed and recorded, and the embryo was cryopreserved for future molecular analysis and other observations. Full documentation of the data of all of the accomplished results depicted herein will be described in detail in peer-reviewed journals. It is important to note that the above scientific work was performed outside the United States and not at any of the above mentioned institutions that the author is affiliated with.

This is yet another new development in assisted reproductive technologies for the world to consider. Will the intense objections made against IVF and the original researchers be repeated with the same criticisms and misjudgements in the 1970s to 1990s (Edwards, 2001)? Will not the acceptance earned by IVF repeat itself through clinical successes of recently developed technologies such as reproductive and therapeutic cloning? Professor Robert Edwards, who helped create the world's first test-tube baby in 1978, recently stated and predicted in a newspaper interview that 'cloning, too, will probably come to be accepted as a reproductive tool if it is carefully controlled' (Schmickle, 2001). In this context, recent scientific and technological progress very clearly demonstrates and documents significant improvements in cloning procedures, similar to IVF and other assisted reproductive technologies (Illmensee, 2001). As we witness these new successes in this area, the public opinion may, on the one hand, be reassured about the sincerity of our efforts and, on the other hand, may lead to a more susceptible and positive point of view towards reproductive and therapeutic cloning.

References

- Chesné P, Adenot PG, Viglietta C *et al.* 2002 Cloned rabbits produced by nuclear transfer from adult somatic cells. *Nature Biotechnology* **20**, 366–369.
- Dominko T, Mitalipova M, Haley B *et al.* 1999 Bovine oocyte cytoplasm supports development of embryos produced by nuclear transfer of somatic cell nuclei from various mammalian species. *Biology of Reproduction* **60**, 1496–1502.
- Edwards RG 2001 Is scientific history cloning itself? Comment on the Washington conference. *Reproductive BioMedicine Online* **3**, 136–137.
- Illmensee K 2001 Cloning in reproductive medicine. *Journal of Assisted Reproduction and Genetics* **18**, 451–477.
- Kato Y, Tani T, Sotomara Y *et al.* 1998 Eight calves cloned from somatic cells of a single adult. *Science* **282**, 2095.
- National Academy of Sciences 2002 *Scientific and Medical Aspects of Human Reproductive Cloning*. National Academy Press, Washington DC, 272 pp.
- Schmickle S 2001 Human cloning researcher has scientific roots in 'U' animal science lab. *Star Tribune*, October 28.
- Simpson JL, Edwards RG 2003 Public objections to designer babies and cloning in USA: not quite what was expected. *Reproductive BioMedicine Online* **6**, 147–148.
- Wakayama T, Perry AC, Zuccotti M *et al.* 1998 Full-term development of mice from enucleated oocytes injected with cumulus cell nuclei. *Nature* **394**, 369–374.
- Wells DN, Misica PM, Tervit HR 1999 Production of cloned calves following nuclear transfer with cultured adult mural granulosa cells. *Biology of Reproduction* **60**, 996–1005.

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