Scientific Aspects of Human and Animal Cloning

President's Commission on Bioethics

Staff Working Paper[1]

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Introduction
The following brief review of some scientific aspects of human and animal cloning is based on scientific research published through the end of December 2001. However, the scientific fields involved in cloning are being very actively investigated, and significant new developments are being published frequently. Publication of new results could change some of the interpretations and emphases in this review.

Use of unfamiliar technical terms and jargon has been avoided wherever possible. Scientific names and terms whose use was required are described and defined in the Glossary of Terms below. In cases where there is disagreement about definitions, this has been noted.

Some Basic Facts about Human Cell Biology and Sexual Reproduction
These elementary facts about human cells, germ cells (egg and sperm), and early embryogenesis will provide the background for understanding the mechanism of cloning and the differences between sexual and asexual reproduction.

Adult human cells with nuclei contain 46 chromosomes, 22 pairs plus two X chromosomes if the adult is female, or 22 pairs plus one X and one Y chromosome if the adult is male. These chromosomes contain the bulk of the cell's DNA, and therefore the genes of the cell. During formation of sperm cells, specialized cell division (meiosis) produces mature sperm cells containing 23 chromosomes (22 chromosomes plus either X or Y). During fertilization of eggs (or oocytes) by sperm, a pronucleus containing half the egg chromosomes is ejected from the cell. Fusion of egg and sperm cells and their nuclei (the defining acts of all sexual reproduction) produces a zygote that again contains a nucleus with the adult cell complement of 46 chromosomes, half from each parent [See Figure 1]. The zygote then begins the gradual process of cell division, growth, and differentiation. After several days in an appropriate nutritive environment, the zygote attains the 100-200-cell (blastocyst) stage. In normal reproduction, the blastocyst implants into the uterine wall, where, suitably nourished, it continues the process of coordinated cell, tissue, and organic differentiation that eventually produce the organized, articulated, and integrated whole that is the new-born infant.

Not quite all the genetic material of a cell resides in its nucleus. Both egg and sperm cells contain small, energy-producing organelles called mitochondria. Mitochondria contain a small piece of DNA that contains the genetic information for making several essential mitochondrial proteins. When additional mitochondria are produced in the cell, the mitochondrial DNA is replicated, and a copy is passed along to the new mitochondria that are formed. During fertilization, sperm mitochondria are selectively tagged and subsequently degraded inside the cell. Thus, the developing embryo inherits solely or principally mitochondria (and mitochondrial DNA) from the egg.

Human reproduction, in cases hampered by one or another cause of infertility, has been accomplished with the help of in vitro fertilization of egg by sperm (IVF) and the subsequent transfer of the early embryo to a woman for gestation and birth. Though such union of egg and sperm requires laboratory assistance and takes place outside of the body, IVF reproduction is still sexual in the biological sense: the new human being arises from two biological parents through the union of egg and sperm.

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Egg and sperm cells combined in vitro have also been used to start the processes of animal embryonic development. Implantation of the resulting blastocysts into the uterus of a female of the appropriate animal species is widely used in animal husbandry.

"Reproductive" Cloning (Asexual Reproduction) of Mammals
The startling 1997 announcement that "Dolly" the sheep had been produced by "reproductive" cloning (Wilmut et al, 1997) indicated that it was possible to produce live mammalian offspring via asexual reproduction through cloning with adult donor cell nuclei.1

In outline form, the steps used to produce live offspring in the animal species that have been cloned to date are (see Figure 1):

1. Obtain an egg cell from a female of the animal species.

2. Remove the nuclear DNA from the egg cell to produce an enucleated egg.

3. Insert the nucleus of a donor adult cell into the enucleated egg to produce a reconstructed egg.

4. Activate the reconstructed egg with chemicals or electric current in vitro to stimulate the reconstructed egg to commence cell division.

5. Initiate development of the activated, reconstructed egg (zygote) to a suitable stage of early embryonic development in vitro, then transfer the embryo to the uterus of a female host that has been suitably prepared to receive it.

6. A cloned animal is born that is genetically virtually identical to the animal that donated the nucleus. However, the mitochondria and mitochondrial DNA of such a cloned animal would most likely be derived from those of the egg.

"Reproductive" cloning carries with it several possibilities not available through sexual reproduction. Because the number of presumably identical donor cells is very large, a very large number of genetically virtually identical individuals could be produced by this process, limited only by the supply of eggs and female animals that could bear the young. In principle, any animal, male or female, newborn or adult, could be cloned, and in any quantity. Because mammalian cells can be frozen and stored for prolonged periods at low temperature and grown again for use as donor cells in cloning, one may even clone individuals who have died. In theory, a clone could be cloned again, on and on, without limit.

Since the report by Wilmut et al. (1997), attempts have been made to clone several other mammalian species. As described in more detail below, live offspring have been produced in a low percentage of cloning attempts with sheep, cattle, goats, mice and pigs. According to a recent press report (Kolata, 2001, see Appendix A), attempts to clone rabbits, rats, cats, dogs and primates using adult cell DNA have not yet yielded live offspring. While a variety of health problems have been reported in some cloned animals, surviving cloned cattle appear physiologically similar to their uncloned counterparts, and two cloned cows have given birth to their own offspring (Lanza et al., 2001). Several aspects of the data from the five species that have been cloned are compared and summarized in Table 1.

Human "Reproductive" Cloning
At this time it is unknown whether any experiments involving human cloning for reproductive purposes have been carried out. Although statements have appeared in the press that this was underway (Weiss, 2001), no published scientific report of any such experiments has been made as of the end of December 2001. However, the steps in such an experiment would probably be similar to those described for animal "reproductive" cloning [see References to Table 1]. A recently reported first attempt at human "research" cloning has followed the first four steps (Cibelli, et al, 2001, see Appendix B).

Human "Research" Cloning
In vitro production of human embryos through cloning could also be used by scientists interested in studying the early embryonic forms of human life, including those produced by cloning. Cloning would make available embryos that are genetically virtually identical; this would greatly facilitate comparison of results between different experiments. Specific genes could be introduced into developing cloned embryos to study the role(s) of these genes on early human development. Research on cloned embryos is also being considered as a source of stem cells potentially useful in developing treatments for degenerative diseases.

One such attempt at human "research" cloning has been published in the scientific literature (Cibelli, et al., 2001) as of the end of December 2001. It involved the following steps (see Figure 1):

1. Obtaining human eggs from informed and consenting female volunteers.

2. Removing the nuclear DNA from the egg cell to produce an enucleated egg.

3. Inserting the nucleus of a cell from an informed and consenting adult donor into the enucleated egg produce a reconstructed egg.

4. Activating the reconstructed egg with chemicals to stimulate the reconstructed egg to commence cell division in vitro.

5. Using a microscope to follow the early cell divisions of the reconstructed egg.

6. In this experiment, 3 of 19 reconstructed embryos underwent cell division, but none progressed beyond the six-cell stage.

In the "research" cloning experiments described by Cibelli et al (2001) the stated intent was to create embryos that would progress to the 100-200 cell (blastocyst) stage, at which point the embryo would be taken apart, stem cells from the inner cell mass would be isolated, and an attempt would be made to grow and preserve "individualized" human stem cells (see Figure 2) for the possible future medical benefit of the donor (NRC/IOM Report). Since the embryos stopped dividing at the six-cell stage, no stem cells were isolated in these experiments. Although the steps Cibelli et al (2001) followed up to the blastocyst stage were the same as those that would be used by those attempting human "reproductive" cloning, Cibelli et al (2001) distinguished their intent from "reproductive" cloning by stating:

Strict guidelines for the conduct of this research have been established by Advanced Cell Technology's independent Ethics Advisory Board (EAB). In order to prevent any possibility of reproductive cloning, the EAB requires careful accounting of all eggs and embryos used in the research. No embryo created by means of NT [nuclear transfer] technology may be maintained beyond 14 days of development.

Parthenogenesis
It is also possible, using chemical or electrical stimuli, to stimulate human eggs to undergo several rounds of cell division, as if they had been fertilized (see Figure 1). In this case, the egg retains all 46 egg cell chromosomes and egg cell mitochondria. In some animal species this asexual reproduction process, known as parthenogenesis, has produced live offspring that contain the same nuclear DNA as the egg. These offspring are all necessarily female.

Cibelli et al (2001) activated eggs obtained from informed and consenting human donors by parthenogenesis, and obtained multiple cell divisions up to the early blastocyst stage in 6 out of 22 attempts. Although there was no report that stem cells were isolated in these experiments, it is possible that parthenogenesis of human eggs could induce them to develop to a stage where embryonic stem cells could be isolated.

Epigenetic Modification and Reprogramming
During differentiation of embryonic cells to produce the cells of the adult, specific chromosomal DNA segments are selectively repressed through processes called epigenetic modification (see Glossary). In adult cells, genes whose expression is required only during early embryonic development are shut down. During the formation of egg and sperm cells, "epigenetic reprogramming" of genes whose products will be needed during early embryonic development is required to make these genes active once more. In cloning using donor nuclei from adult cells, a similar "epigenetic reprogramming" is required.

Based on the data on "reproductive" cloning experiments in animals in Table 1 and the results of Cibelli et al (2001), most cloned embryos die either in vitro or after transfer to the uterus. Why is production of live cloned mammalian offspring a relatively rare event? Several factors may play a role. Enucleation of the egg may (variably from one attempt to the next) remove or damage its "epigenetic reprogramming" capabilities. An optimal in vitro nutritive environment for the development of cloned zygotes may not yet have been determined. One interpretation (Rideout et al, 2001) attributes the early death of many cloned embryos to complete failure or incompleteness of "epigenetic reprogramming."

At this early stage of experimental work with cloned human embryos, it is perhaps not surprising that 16 of 19 of the reconstructed eggs did not undergo cell division and none of the other three reconstructed eggs

divided beyond the six-cell stage (Cibelli et al, 2001). A more detailed discussion of epigenetic modification and reprogramming as they relate to cloning will be developed as a component of a broader analysis of "research" cloning.

References

Cibelli, J.B. et al., "Somatic Cell Nuclear Transfer in Humans: Pronuclear and Early Embryonic Development," e-biomed: The Journal of Regenerative Medicine, 2: 25-31 (2001) [see Appendix B]

Kolata, G. "In Cloning, Failure Far Exceeds Success," New York Times, December 11, 2001, page D1 [see Appendix A]

Lanza, R.P. et al., "Cloned Cattle Can Be Healthy and Normal," Science, 294: 1893-4 (2001)

NRC/IOM Report - "Stem Cells and the Future of Regenerative Medicine"

Rideout III, W.M. et al., "Nuclear Cloning and Epigenetic Reprogramming of the Genome," Science, 293, 1093-1098 (2001)

Weiss, R. "Human cloning bid stirs experts' anger; problems in animal cases noted," Washington Post, April 11, 2001, page A1

Wilmut, I. et al., "Viable offspring derived from fetal and adult mammalian cells" Nature, 385: 810-813 (1997)

Glossary of Terms

In writing Working Papers for the January 2002 Council meeting, members of the Council staff have attempted to use terms consistently as they are defined below in this glossary.

Asexual reproduction

The term "asexual reproduction" means reproduction not initiated by the union of oocyte and sperm.

Blastocyst

An early stage in the development of mammalian embryos, when the embryo is a spherical body comprising an inner cell mass that will become the fetus and an outer ring of cells that will become part of the placenta.

Biologically related children

Children are "biologically related" to the individuals who are the sources of their genetic endowment, in the case of sexual reproduction, to one man and one woman who are the sources of sperm and egg (the "biological" parents), in the case of cloning, to the source of the donor nucleus.

Cloning

"Reproductive" cloning -- 1) Obtaining a human or animal egg cell and removing its DNA. 2) Inserting a nucleus or a cell from a donor human or animal in order to produce a reconstructed egg that is genetically very similar to the donor. 3) Implanting the reconstructed egg in a uterus and delivering the resulting baby or newborn animal.

"Research" cloning -- 1) Obtaining a human or animal egg cell and removing its DNA. 2) Inserting a nucleus or a cell from a donor human or animal to produce a reconstructed egg that is genetically very similar to the donor. 3a) In some cases, termed "therapeutic" cloning or "cell replacement through nuclear transfer" (CRNT), the next steps include growing the reconstructed egg to the 100-200 cell embryo (blastocyst) stage, then taking the embryo apart to isolate and preserve "individualized" stem cells for immediate or future use in cell transplantation therapies. 3b) In other cases, the next steps comprise various scientific experiments designed to better understand the earliest stages of human embryonic development.

Gene (molecular) cloning -- Using carrier pieces of DNA (called vectors) to isolate and characterize DNA segments coding for proteins.

"Human cloning" -- The term 'human cloning' means the asexual reproduction of a new human organism that is genetically virtually identical to an already existing, or previously existing, human being. Operationally, it is currently accomplished by introducing the nuclear material of a human somatic cell into an oocyte whose own nuclear material has been removed or inactivated, to produce a living organism -- at whatever stage of development -- that has a human (or predominantly human) genetic constitution.

Chromosomes
Structures inside the nucleus of a cell, made up of long pieces of DNA coated with specialized cell proteins, that are duplicated at each cell division. Chromosomes thus transmit the genes of the organism from one generation to the next.

Embryo
1. An organism in the early stages of development.

2. In humans, the developing organism from conception until approximately the end of the second month; developing stages from this time to birth are commonly designated as fetal.

Epigenetic modification
Turning genes encoded by chromosomal DNA on and off during cell differentiation through changes in a) DNA methylation, b) the assembly of histone proteins into nucleosomes, and c) remodeling of chromosome-associated proteins such as linker histones.

Epigenetic reprogramming
The process of removing epigenetic modifications of chromosomal DNA, so that genes whose expression was turned off during embryonic development and cell differentiation become active again. Epigenetic reprogramming of the donor cell DNA is believed to be an essential process in generating live offspring through "reproductive" cloning using adult donor cells and nuclei.

Eugenics
Activity seeking to alter (with the aim of improving) the genetic constitution of future generations.

Infertility
The inability to conceive a child through sexual intercourse.

Mitochondria
Small energy-producing organelles inside of cells. Mitochondria give rise to other mitochondria by copying their small piece of mitochondrial DNA and passing one copy of the DNA along to each of the two resulting mitochondria.

Nuclear transfer
Transferring the nucleus with its chromosomal DNA from one (donor) cell to another (recipient) cell. In cloning, the recipient is a human egg cell and the donor cell can be any one of a number of different adult tissue cells.

Oocyte
As used here, the term oocyte is synonymous with egg.

Parthenogenesis
A form of nonsexual reproduction . . . in which the female reproduces its kind without fecundation by the male.

Parthenogenote
An embryo that has been produced by parthenogenesis.

Somatic cell (human)
The term "somatic cell" means a diploid cell (having a complete set of 46 chromosomes) obtained or derived from a living or deceased human body at any stage of development.

Stem cells
"The definition of 'stem cells' is still a debated issue (Morrison et al., 1997; Watt and Hogan, 2000).

According to the currently prevailing view (Watt and Hogan, 2000) a stem cell can be defined on the basis of the following two features: (1) it has an unlimited or prolonged self-renewal capacity (i.e. the capability to maintain a pool of undifferentiated stem cells besides giving rise to differentiated daughter cells); (2) it has uni/multipotency, i.e. the potential to produce one or more differentiated descendent cell types. . . .

Uncertainty that can derive from the terminological ambivalence of the term 'stem cell' is increased by the misuse of this term, which is often inappropriately employed (or perhaps voluntarily 'manipulated') in the popular press to sustain scientific and/or ethical positions for political ends." (Guena, S., et al, 2001)

In this paper, we use the Watt and Hogan (2000) definition given above.

Zygote
The diploid cell that results from the fusion of a sperm cell and an egg cell.

Glossary References
Guena, S., et al., "Adult Stem Cells and Neurogenesis: Historical Roots and State of the Art"

The Anatomical Record (New Anat.), 265: 132-141 (2001)
Morrison, S.J., et al., "Regulatory mechanisms in stem cell biology" Cell, 88: 287-298 (1997)

Watt F.M. and Hogan B.L.M. "Out of Eden: Stem cells and their niches" Science, 287: 1441-1446 (2000)

1. Previous experiments dating from the 1950s had shown that it was possible to clone amphibians. Earlier experiments had also produced clones of animals using embryonic donor cells. What made the report of Dolly's birth stand out was the use of adult donor cells and the fact that a mammal had been cloned.