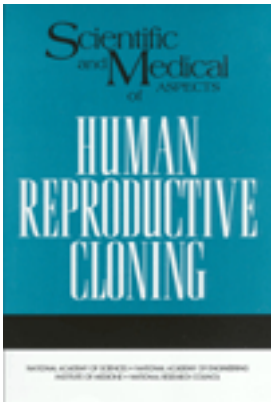

Scientific and Medical Aspects of Human Reproductive Cloning

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Scientific and Medical Aspects of HUMAN REPRODUCTIVE CLONING

Committee on Science, Engineering, and Public Policy
Policy and Global Affairs Division

Board on Life Sciences
Division on Earth and Life Studies

National Academy of Sciences
National Academy of Engineering
Institute of Medicine
National Research Council

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Preface

Advances in animal reproductive cloning methods have encouraged some practitioners to attempt human reproductive cloning to produce newborn humans from a predetermined donor. The decision as to whether the self-proposed practitioners of human reproductive cloning should be allowed to proceed is most properly a societal decision, and likely one that will be made by the government. An informed decision requires two kinds of inputs, medical-scientific and ethical. It is the responsibility of the scientific and medical community to inform society if current methods are scientifically feasible and reproducible, and medically safe; and to provide guidelines to assure that if human reproductive cloning is carried out, the human participants involved are adequately advised and protected. Once society is so informed, it will be in a position to determine whether an attempt to use reproductive cloning methods with humans is acceptable in any circumstance. The scope of this report is limited to informing society by providing an assessment of the medical and scientific aspects of human reproductive cloning.

The public debate on the possible reproductive cloning of humans is often linked to the debate on human embryonic stem (ES) cells. Because one proposed method to establish new human embryonic stem cell lines uses a process very similar to the first steps in the reproductive cloning of complete humans, it is easy to understand how even a scientifically literate society could become confused about these issues. Clarity on these matters is vitally important since these issues involve both medical risk

and opportunity, and the government is considering the use of sanctions on the free inquiry that normally characterizes effective research.

The present panel was charged to consider the biomedical issues surrounding the question of reproductive cloning of human beings, including making clear the distinctions between reproductive cloning and the related methods used to derive new ES cells.[1] As biomedical scientists and physicians it is our job to seek new scientific principles, and from them new therapies to ameliorate the personal tragedies brought on by disease. And we must do so without subjecting patients and society to unwarranted medical experimentation. Medical progress requires clinical experimentation, but that process must go forward with the highest ethical standards—and only when the risks and potential benefits are understood and agreed on by patient, physician, scientist, and participating institution.

Last year, at least three groups declared that they not only were in the process of modifying the methods used first to produce a cloned living lamb (Dolly) in order to apply them to humans, but that they intended to carry out the reproductive cloning of human beings in the near future. In response to the prospect of those medical experiments, the presidents of the National Academies convened a joint panel of the Committee on Science, Engineering, and Public Policy (COSEPUP) and the Board on Life Sciences (BLS) to examine the scientific and medical issues relevant to human reproductive cloning and to consider the ethical issues that apply specifically to the participation of human subjects in cloning research. The purpose of this undertaking is to clarify and provide as much understanding as possible of these issues in order to inform the much broader debate that will be carried out by a larger cross section of society.

The method used to initiate the reproductive cloning procedure is called nuclear transplantation, or somatic cell nuclear transfer (SCNT). It involves replacing the chromosomes of a human egg with the nucleus of a body (somatic) cell from a developed human. In *reproductive cloning*, the egg is then stimulated to undergo the first few divisions to become an aggregate of 64 to 200 cells called a blastocyst. The blastocyst is a preimplantation embryo that contains some cells with the potential to give rise to a fetus and other cells that help to make the placenta. If the blastocyst is placed in a uterus, it can implant and form a fetus. If the blastocyst is instead maintained in the laboratory, cells can be extracted from it and grown on their own. Those cells will grow indefinitely without becoming

[1]Stem cells are the subject of a complementary report from the National Academies entitled *Stem Cells and the Future of Regenerative Medicine*, which was released to the public in September 2001. The full text of that report is available at http://www.nap.edu/catalog/10195.html

specialized, and each blastocyst may give rise to a continuously growing cell line, known as an embryonic stem (ES) cell line. For reasons that are explained in Chapter 2, these cell lines cannot on their own implant or give rise to a fetus. The process of producing ES cell lines by using somatic cell nuclei is called *nuclear transplantation to produce stem cells*.

A potential benefit of *reproductive cloning*—producing a complete human being—is that it offers one solution for complete infertility. The potential benefit of using nuclear transplantation to produce stem cells is that it offers opportunities for medical research, medical discovery, and therapies. Both human reproductive cloning and nuclear transplantation to produce stem cells raise ethical, moral, and legal questions.[2]

The panel that produced this report was chosen to reflect expertise in the relevant scientific and medical disciplines, making it well equipped to explore the scientific literature and identify the current leaders in these fields. We were helped by a superb staff that was deeply experienced in matters of science, science policy, and medical ethics. The entire panel participated in 12 weekly conference calls to identify the key issues that would be the subject of our report and the people who would inform our deliberations, as well as to plan for a workshop wherein experts in the field could address the issues and present us with the appropriate data.

We soon concluded that it was not sufficient to understand the issues only from experiments in the cloning of animals combined with fundamental studies in mammalian embryogenesis. We also needed to inform ourselves concerning the principles and practices used by those clinical entities that provide assisted reproductive technology (ART) services, most often to assist sperm-egg fertilization and test-tube development of an embryo to the stage where it is ready to be placed into the uterus of a biological or surrogate mother. And we also needed to learn about the plans of those who would carry out the reproductive cloning of human beings and, more important, to have them learn, with us, of the scientific and medical results and experiences of those who had cloned animals. We therefore decided to place the three workshop participants who propose to clone humans in a setting where their clinical plans could be scrutinized. Although including them in the workshop provided a platform for the most vocal proponents and opponents, it also provided valuable input to members of our panel.

The report that follows reflects all the data that we have gathered concerning the animal reproductive cloning models used in the years since the cloning of Dolly. We have found that the efficiency of production of a blastocyst from an egg whose own chromosomes have been

[2] *Ibid.*

removed and replaced by the nucleus of another cell is very low. Moreover, the efficiency of further development of such a blastocyst after transfer to a uterus in the same animal species is extremely poor. In view of these findings, it became clear that the number of human eggs needed for a single human reproductive cloning attempt could well reach several hundred. Most importantly, the animal models had an excess of fetal deaths throughout pregnancy. The late fetal deaths could cause excess maternal damage and possibly maternal deaths if the cloned fetus became too large, as was often the case in sheep and cows. And the risk of excess mortality of clones (compared with newborns from normal reproduction) continued in the neonatal and later stages. The experience in reproductive cloning of all animal species tested was of concern and provided powerful evidence of the potential problems with human reproductive cloning. A number of scientific studies on animals pointed to some likely causes of the failures, and these are described extensively in the report.

The panel examined closely the critiques and explanations offered by both those who wish to undertake human reproductive cloning and other participants in the workshop. We determined that the potential tests offered as preconditions to implant a blastocyst by those who wish to undertake human reproductive cloning were incomplete or, in one case, unlikely to be credible. The tests proposed to monitor an implanted fetus were also deemed by the panel to be incomplete and inadequate to protect either the fetus or the woman carrying it. Based on its evaluation of the evidence, the panel supports the proposal that the government enact a legally enforceable ban on the reproductive cloning of humans that remains in place for at least 5 years.

The panel also reviewed the potential of nuclear transplantation to produce stem cells for the development of therapies, for advancing fundamental biomedical knowledge, and for biomedical applications of this research. None of the scientific and medical considerations that led the panel to the above conclusion concerning human reproductive cloning apply to the production of stem cells by nuclear transplantation. The panel supports the conclusion of a recent National Academies report that recommended that biomedical research using nuclear transplantation to produce stem cells be permitted.[3] We encourage a broad national dialogue on the relevant societal, religious, and ethical issues.

Our panel of 11 members has been unanimous in reaching the recommendations and conclusions presented in the Executive Summary and Chapter 6 of this report. In making our decisions, we carefully considered the results of the workshop, some of which have been outlined above. We

[3] *Ibid.*

also read widely and extensively, consulted experts, and took into account the findings of the important recent report from the National Academies entitled *Stem Cells and the Future of Regenerative Medicine*.

This work would not have been possible without the dedication and skill of the lead staff member for this study, Deborah Stine. We are also deeply indebted to Maxine Singer and Corey Goodman, whose many contributions went far beyond those expected for ex-officio members responsible for institutional oversight.

The panel believes that all concerned segments of society should examine and debate the broad ethical issues associated with human cloning. Although we have only examined the scientific and medical aspects, we hope that our report helps to inform this broader consideration by society.

Irving L. Weissman, Chair

Acknowledgments

This report is the product of many people. First, we would like to thank all the speakers who attended our workshop on August 7, 2001. They were (in alphabetical order) Severino Antinori, Brigitte Boisselier, R. Alta Charo, Jose Cibelli, Alan Colman, Jay Cross, Peter Farin, Jonathan Hill, Rudolf Jaenisch, Peter Mombaerts, Virginia Papaioannou, Eugene Pergament, John Robertson, Eric Schon, Alan Trounson, Andre Van Steirteghem, Ian Wilmut, Ryuzo Yanagimachi, and Panayiotis Zavos. Without the input of each of these speakers, this report would not have been possible.

Next, we would like to thank the reviewers of this report. This guide has been reviewed in draft form by persons chosen for their diverse perspectives and technical expertise, in accordance with procedures approved by the National Research Council's Report Review Committee. The purpose of this independent review is to provide candid and critical comments that will assist the institution in making the published report as sound as possible and to ensure that the report meets institutional standards of objectivity, evidence, and responsiveness to the study charge. The review comments and draft manuscript remain confidential to protect the integrity of the deliberative process. We wish to thank the following for their participation in the review of this report:

George J. Annas, Boston University, Massachusetts
Paul Berg, Stanford University, California
Alexander Capron, University of Southern California, Los Angeles
R. Alta Charo, University of Wisconsin, Madison

Neal First, University of Wisconsin, Madison
Fred Gage, The Salk Institute for Biological Studies, San Diego, California
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Lee M. Silver, Princeton University, New Jersey
Alan Trounson, Monash University, Melbourne, Australia
Harold Varmus, Memorial Sloan-Kettering Cancer Center, New York, New York
Bert Vogelstein, The Johns Hopkins University, Baltimore, Maryland

Although the reviewers listed above have provided many constructive comments and suggestions, they were not asked to endorse the conclusions or recommendations nor did they see the final draft of the report before its release. The review of this report was overseen by Robert Frosch and M.R.C. Greenwood, appointed by the NRC's Report Review Committee, who were responsible for making certain that an independent examination of this report was carried out in accordance with institutional procedures and that all review comments were carefully considered. Responsibility for the final content of this report rests entirely with the authoring committee and the institution.

In addition, we would like to thank Maxine Singer and Corey Goodman, the chairs of COSEPUP and BLS, respectively, and Kenneth Shine, president of the Institute of Medicine, who helped greatly with the panel's deliberations.

Finally, we would like to thank the staff for this project, including Deborah Stine, associate director of COSEPUP and study director, who managed the project; William Wells, consultant writer, who worked with the panel to develop the text of the guide; Susan Daniels, who provided research support including the development of the bibliography and the tables and figures in Appendix B, and support in developing the glossary; Robert Cook-Deegan, who provided guidance to the panel on medical policy and ethics; Rebecca Burka, administrative associate, and Kevin Rowan, project assistant, who provided project support; Norman Grossblatt, editor; Fran Sharples, Director of the Board on Life Sciences; and Richard Bissell, executive director of COSEPUP and the Policy and Global Affairs Division.

Contents

Executive Summary

Human reproductive cloning is an assisted reproductive technology that would be carried out with the goal of creating a human being. It is currently the subject of much debate around the world, involving a variety of ethical, religious, societal, scientific, and medical issues. However, this report from the National Academies addresses only the scientific and medical aspects of human reproductive cloning. Consideration of the medical aspects has required the panel 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, if it were found to be scientifically safe, would or would not be acceptable to individuals or society. Instead, the panel defers to others on the fundamental ethical, religious, and societal questions, and presents this report on the scientific and medical aspects to inform the broader debate. Our report differs in this respect from the last major report on the topic in the United States, *Cloning Human Beings*, a 1997 report developed by the National Bioethics Advisory Commission [1].

THE PANEL'S CONCLUSIONS AND RECOMMENDATIONS

The panel has examined and analyzed the scientific, medical, and legal literature on the issues and heard testimony at a workshop from experts in animal cloning, assisted reproductive technologies, and science, technology, and legal policy—including people who, on scientific and medical grounds, either oppose or defend human reproductive clon-

ing. After carefully considering the issues raised, we conclude that the case has not been proved that human reproductive cloning would lead to fewer negative outcomes at this time than reproductive cloning of other mammals. We therefore make the following recommendations:

Human reproductive cloning should not now be practiced. It is dangerous and likely to fail. The panel therefore unanimously supports the proposal that there should be a legally enforceable ban on the practice of human reproductive cloning. For this purpose, we define human reproductive cloning as the placement in a uterus of a human blastocyst derived by the technique that we call nuclear transplantation. In reaching this conclusion, we considered the relevant scientific and medical issues, including the record from cloning of other species, and the standard issues that are associated with evaluating all research involving human participants.

The scientific and medical considerations related to this ban should be reviewed within 5 years. The ban should be reconsidered only if at least two conditions are met: (1) a new scientific and medical review indicates that the procedures are likely to be safe and effective and (2) a broad national dialogue on the societal, religious, and ethical issues suggests that a reconsideration of the ban is warranted.

Finally, the scientific and medical considerations that justify a ban on human reproductive cloning at this time are not applicable to nuclear transplantation to produce stem cells. Because of its considerable potential for developing new medical therapies for life-threatening diseases and advancing fundamental knowledge, the panel supports the conclusion of a recent National Academies report that recommended that biomedical research using nuclear transplantation to produce stem cells be permitted. A broad national dialogue on the societal, religious, and ethical issues is encouraged on this matter.

THE FINDINGS THAT SUPPORT A BAN ON HUMAN REPRODUCTIVE CLONING

It is a serious event when any group that has potential authority over research intercedes to ban it, and the reasons must therefore be compelling. We are convinced that the scientific and medical data concerning the

likely danger to the implanted fetus or the eventual newborn if reproductive cloning of humans is attempted in the near future are compelling.

The panel has based its support for the proposed ban on human reproductive cloning on the following findings:

Finding 1: The scientific and medical criteria used to evaluate the safety of reproductive cloning must be the potential morbidity and death of the woman carrying the clone as a fetus and of the newborn and the risk to women donating the eggs.

Finding 2: Data on the reproductive cloning of animals through the use of nuclear transplantation technology demonstrate that only a small percentage of attempts are successful; that many of the clones die during gestation, even in late stages; that newborn clones are often abnormal or die; and that the procedures may carry serious risks for the mother. In addition, because of the large number of eggs needed for such experiments, many more women would be exposed to the risks inherent in egg donation for a single cloning attempt than for the reproduction of a child by the presently used *in vitro* fertilization (IVF) techniques. These medical and scientific findings lead us to conclude that the procedures are now unsafe for humans.

Finding 3: At least three criteria would have to be fulfilled before the safety of human reproductive cloning could be established:

(1) The procedures for animal reproductive cloning would have to be improved to such an extent that the levels of observed abnormalities in cloned animals, including nonhuman primates, were no more than that seen with existing human assisted reproductive technology (ART) procedures. If that could not be achieved, researchers would have to demonstrate that humans are different from other animals with regard to cloning-related defects. Reproducible data demonstrating that a successful reprogramming of the donor nucleus and proper imprinting can be achieved in animals would be essential, as would an understanding of the mechanisms responsible for such events.

(2) New methods would have to be developed to demonstrate that the human preimplantation embryos produced through the use of nuclear transplantation technology are normal with respect to imprinting and reprogramming. That would best be done by first establishing the normal state of reprogramming and imprinting in nonhuman primates and then documenting that the processes in preimplantation human embryos are substantially similar.

(3) Methods would have to be developed to monitor—effectively

and comprehensively—preimplantation embryos and fetuses in the uterus for cloning-related defects, such as those outlined in Chapter 3; these include alterations in gene expression and imprinting.

Finding 4: The issues of responsible conduct of research raised by the prospect of cloning a person are those of medical ethics—in particular, the protection of the participants (the egg donor, the host mother, and the child produced through cloning) in any human cloning research. Participants in any human cloning research efforts require full protection as human research participants, although it should be noted that, as with fetal surgery, this protection cannot be extended fully to the cloned fetus. Human reproductive cloning has not been performed before, and its introduction, if it ever occurred, would require systematic research. That research would likely entail full review by institutional review boards and other human-subjects protections, including informed consent of donors and recipients of all biological materials.

Finding 5: If any attempts at human reproductive cloning were ever to occur, they would constitute research, not merely innovative therapy. Such research would then be subject to external technical and ethical review by review boards to ensure that the proposed experiments are both technically and ethically sound and that the rights and welfare of all research participants are protected. This institutional review process should be applied equally to both public- and private-sector research and be transparent to the public.

Finding 6: Because medical and scientific findings indicate that cloning procedures are currently not safe for humans, cloning of a human through the use of nuclear transplantation technology is not now appropriate. The panel believes that no responsible scientists or physicians are likely to undertake to clone a human. Nevertheless, no voluntary system that is established to restrict reproductive cloning is likely to be completely effective. Some organizations have already announced their intention to clone humans, and many of the reproductive technologies needed are widely accessible in private fertility clinics that are not subject to federal regulations. The panel therefore concludes that a legally enforceable ban that carries substantial penalties has a much greater potential than a voluntary system or moratorium to deter any attempt to clone a human using these techniques.

Finding 7: If no ban is imposed, it is possible that some organizations will attempt the reproductive cloning of humans. Although such attempts would most likely fail, there is a high probability they would be associ-

ated with serious risks to any possible fetus or newly born child and may harm the woman carrying the developing fetus.

Finding 8: There is concern that legislation or regulation that would ban reproductive human cloning would set a troubling precedent with respect to the restriction of innovative, experimental research and medical procedures. Modern scientific research proceeds rapidly, and its findings are unpredictable and often surprising. It is probable that at least every 5 years there will be significant new information regarding the issues of the safety and applicability of human cloning to medical practice. The above concern can be ameliorated by including in any legislation or regulation a requirement for an updated evaluation of the scientific, medical, and societal issues within 5 years. Such a requirement for periodic reviews would allow for extensive public debate regarding reproductive human cloning and the consideration of modifications to the legislation. Part of that evaluation would include a recommendation as to when the next such evaluation should be conducted.

Finding 9: Two activities will be particularly important for an updated evaluation of human reproductive cloning: a thorough scientific and medical review to evaluate whether the procedures are likely to be safe and effective and a broad national dialogue on the societal, religious, and ethical issues. As part of this process, any persons advocating the practice of human reproductive cloning would need to acknowledge the extent of the abnormalities seen in animal cloning experiments and to demonstrate that these problems—assuming that they persist—are unlikely to occur in humans.

Finding 10: Any future process designed to evaluate the scientific and medical evidence on cloning a person would likely need to involve scientists, physicians, ethicists, and the public. A public debate could be facilitated by a committee that issues regular updates on the state of the science surrounding animal cloning and reaches out to involved constituencies in a systematic manner. Such a body could derive its powers by executive order, by executive action within the Department of Health and Human Services under the Public Health Service Act, or by legislation. Among many other issues, the debate should be structured to inform the public that clones are not precise replicas, but persons with identical genetic material.

Finding 11: The science of cloning is an international one with research conducted throughout the world. Furthermore, the issue of human

reproductive cloning is the subject of worldwide debate. A number of countries and international organizations have prepared reports and issued statements on the issue. Participation by the United States in such international debates about human reproductive cloning will be beneficial to any future process to evaluate the scientific and medical evidence on this issue.

Finding 12: The limited regulation and monitoring of experimental ART procedures in the United States means that important data needed for assessing novel ART procedures are in some cases lacking, in other cases incomplete and hard to find. Because the panel was not charged to investigate ART regulation and did not solicit expert testimony thereon, we make no recommendations regarding oversight of, registration of, or required data collection from ART clinics. But we do believe that a request from Congress or the Executive Branch for a panel of experts to study the matter and report its findings and recommendations publicly would probably be useful. Having such information is likely to be beneficial to any process of evaluating future scientific and medical evidence regarding both reproductive cloning and new ART procedures.

REDUCING CONFUSION CONCERNING THE USE OF THE TERM "HUMAN CLONING"

As we have just discussed, **human reproductive cloning** is an assisted reproductive technology that would be carried out with the goal of creating a human being (see Figure 1). There is a very different procedure, here termed **nuclear transplantation to produce stem cells**—but variously called nonreproductive cloning, therapeutic cloning, research cloning, or somatic cell nuclear transfer (SCNT) to produce stem cells—whose aim is the creation of embryonic stem (ES) cells for clinical and research purposes (see Figure 2).

Unlike reproductive cloning, the creation of embryonic stem cells by nuclear transplantation does not involve implantation of a preimplantation embryo, or blastocyst, in a uterus. For this reason, it cannot produce a complete, live born animal (a "clone"). Some confusion arises because in both cases researchers would use nuclear transplantation, which is an initial step in the successful procedures used to clone animals—beginning with the sheep Dolly and including several other mammals since then. In nuclear transplantation, the nucleus of an egg cell (containing its chromosomes) is removed and replaced with the nucleus of a cell taken from the body of an adult (a "somatic cell"). Thus, nuclear transplantation accurately describes the process.

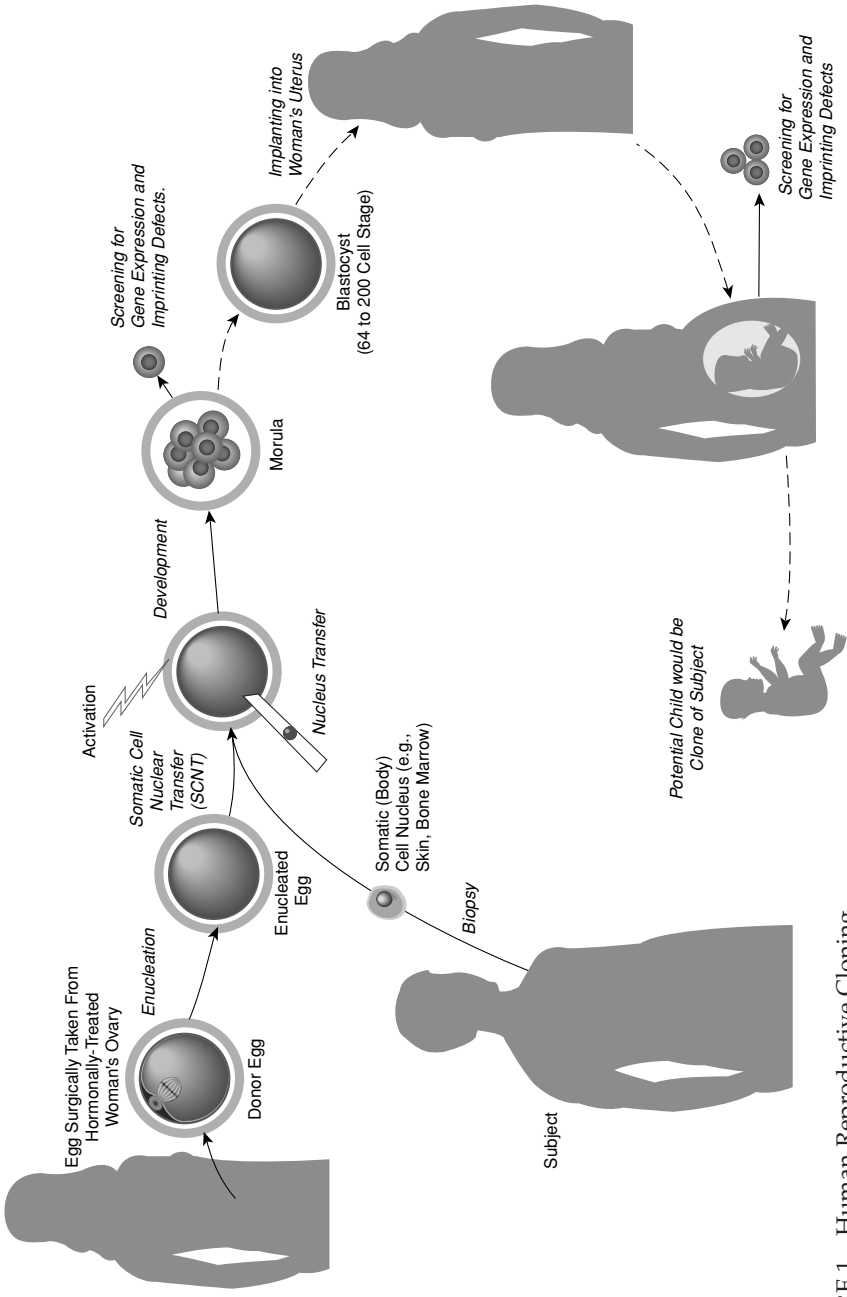

FIGURE 1 Human Reproductive Cloning

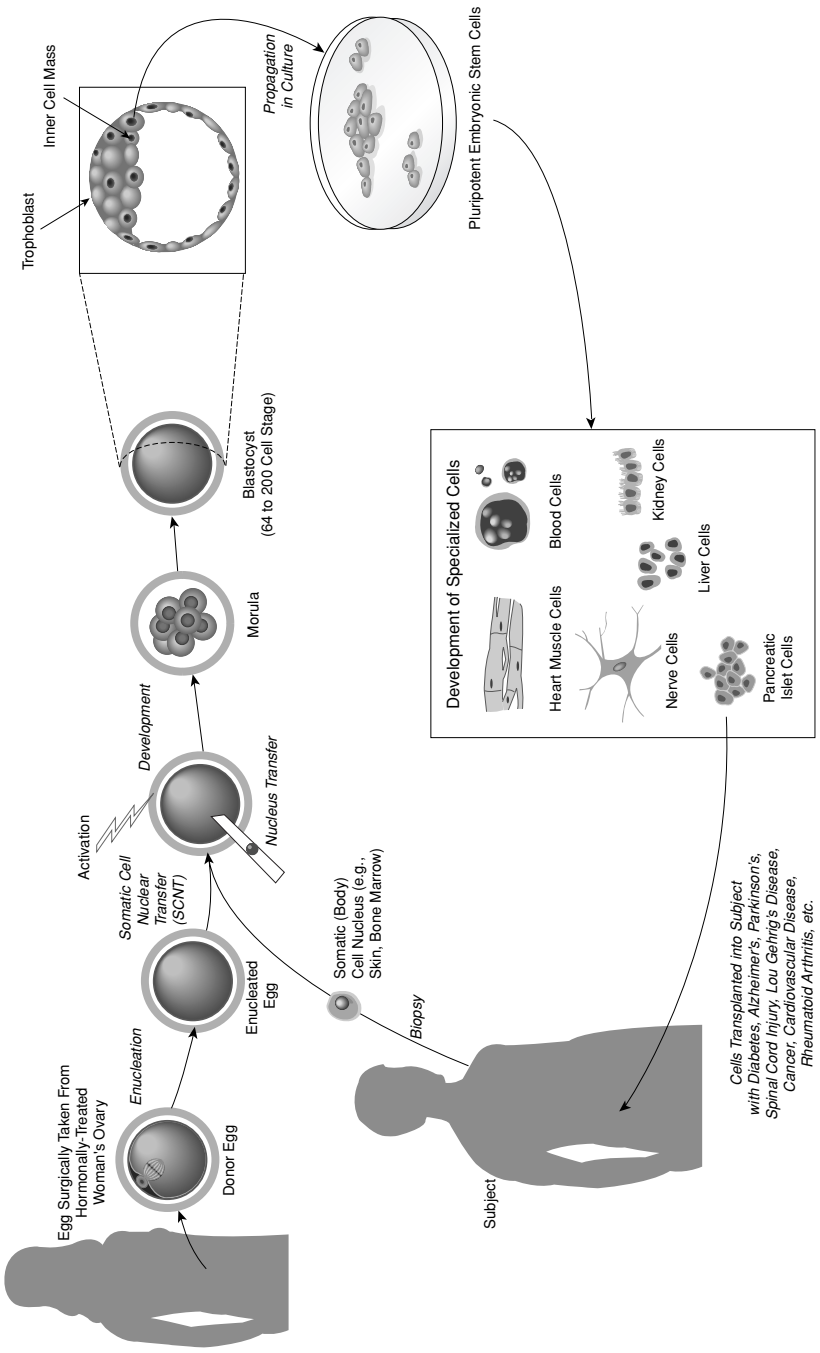

FIGURE 2 Nuclear Transplantation to Produce Stem Cells

For both reproductive cloning and stem cell production, a reconstructed egg cell produced by nuclear transplantation is stimulated to cause it to begin dividing. If that is successful, several sequential cell divisions can give rise to the preimplantation embryo known as a **blastocyst** that is composed of 64-200 cells (see Figure 2).

It is at this stage that the procedures used for reproductive cloning and for nuclear transplantation to produce stem cells become entirely different. In reproductive cloning, a blastocyst formed by the nuclear transplantation procedure is implanted in a uterus, where it begins the process of forming a fetus. Any animals produced in this way will have the same nuclear genes as the adult cells used to produce them, and when the nuclei from several somatic cells from a single animal are transferred to a series of eggs, all the animals born are said to be "clones" of the original adult animal.

Although these clones will be physically very similar, the animals will not be physically or behaviorally identical, because of various factors, including their different uterine and postnatal environments and experiences.

In nuclear transplantation to produce stem cells, cells are isolated from the blastocyst 4-5 days after the procedure, and the cells are used to make a stem cell line for further study and clinical applications. Neither the blastocyst nor the stem cells are ever placed into a uterus. Moreover, as described in Chapter 2, human stem cells do not themselves have the capacity to form a fetus or a newborn animal. Nevertheless, in the popular press and other media, the term "human cloning" has often been misleadingly applied to both this procedure and reproductive cloning whenever either is proposed to be used in a human context.

As part of our panel's charge, we were asked, "Based on the current scientific and medical evidence, should there be a moratorium on the cloning of a person? What are the implications of doing so? Of not doing so?" This raises the question of the implications that a ban on human reproductive cloning could have for the very different process of nuclear transplantation to produce stem cells.

None of the findings summarized in the preceding section that support the panel's conclusions regarding a ban on human reproductive cloning would support a ban on the use of the nuclear transplantation technology to produce stem cells. A recent report prepared by a different committee of the National Academies has emphasized that there is a great potential for studies on stem cells isolated through nuclear transplantation to increase the understanding and potential treatment of various diseases and debilitating disorders, as well as fundamental biomedical knowledge. The diseases and debilitating disorders include "Lou Gehrig's disease" (amyotrophic lateral sclerosis, or ALS), Parkinson's disease, Alz-

heimer's disease, spinal-cord injury, cancer, cardiovascular diseases, diabetes, and rheumatoid arthritis. The necessary research would entail transfer of human somatic cell nuclei into enucleated human eggs for the purpose of deriving blastocysts and embryonic stem cells and stem cell lines; there would be no implantation in a uterus. Some have expressed concern that this research might nevertheless be misdirected to human reproductive cloning. If our recommendation for a legally enforceable ban is adopted, then any attempts at implantation that might lead to the development and birth of a newborn would be criminalized.

The committee that produced the report from the National Academies entitled *Stem Cells and the Future of Regenerative Medicine* considered a wide range of views on the ethical and societal issues involved in the production of human embryonic stem cells—including nuclear transplantation technology [2]. After carefully considering all sides of the issue, that committee produced the following conclusion and recommendation concerning this technology:

> **Conclusion:** Regenerative medicine is likely to involve the implantation of new tissue in patients with damaged or diseased organs. A substantial obstacle to the success of transplantation of any cells, including stem cells and their derivatives, is the immune-mediated rejection of foreign tissue by the recipient's body. In current stem cell transplantation procedures with bone marrow and blood, success hinges on obtaining a close match between donor and recipient tissues and on the use of immunosuppressive drugs, which often have severe and potentially life-threatening side effects. To ensure that stem cell-based therapies can be broadly applicable for many conditions and people, new means of overcoming the problem of tissue rejection must be found. Although ethically controversial, the somatic cell nuclear transfer technique promises to have that advantage. Other options for this purpose include genetic manipulation of the stem cells and the development of a very large bank of ES cell lines [2].
>
> **Recommendation:** In conjunction with research on stem cell biology and the development of potential stem cell therapies, research on approaches that prevent immune rejection of stem cells and stem cell-derived tissues should be actively pursued. These scientific efforts include the use of a number of techniques to manipulate the genetic makeup of stem cells, including somatic cell nuclear transfer.

Our panel includes members who participated in the workshop on stem cells held at the National Academies on June 23, 2001. This work-

shop was convened as part of the data-gathering process for the separate committee that produced the above report focused on stem cells. In our own workshop, held on August 7, 2001, we consulted with many of the world's leaders in nuclear transplantation to produce stem cells—I. Wilmut, R. Jaenisch, R. Yanagimachi, J. Cibelli, P. Mombaerts, and A. Trounson (see Appendix C)—and we have also conducted our own extensive literature review. On the basis of this review and discussion, the panel determined that although there is a clear therapeutic potential for techniques in which stem cells are produced through nuclear transplantation (as in Figure 2), this potential is nascent and needs considerable research. The potential of this research includes developing a broader understanding of how human tissue cells develop normally and how human diseases that have a genetic component are caused at a cellular level.

The panel concludes this executive summary with a review of the scientific subjects that were covered.

ANIMAL CLONING

Since the report in 1997 of the birth of the sheep Dolly, the first successful reproductive clone of a mammal from an adult cell, reproductive cloning has been carried out with several kinds of animals. Five mammalian species have been reproductively-cloned from adult or fetal cells—sheep, mice, pigs, goats, and cattle—and similar attempts are being made, so far without success, in monkeys, dogs, and horses.

The panel reviewed the scientific literature on animal cloning and heard from animal-cloning experts at its workshop. It found that cloning efficiencies in animals remain extremely low despite several years of experimentation. This low efficiency means that any human reproductive cloning attempt would probably require large numbers of eggs. The collection of these eggs would bring with it the risk of ovarian hyperstimulation syndrome in donors, as with all *in vitro* fertilization (IVF). However, in the case of cloning it would probably involve either scores of women for one cloning attempt or a few women being exposed to high levels of hormones.

Furthermore, animal cloning is associated with a wide variety of abnormalities in the fetus and offspring. The abnormalities include a greater than normal size of fetus and placenta (both during gestation and after birth), poor interaction between fetal and maternal components of the placenta, greater early-gestation and late-gestation fetal morbidity and mortality, greater postnatal mortality, and various developmental defects in the immune, cardiovascular, and possibly nervous systems. In addition, it is important to note that subtle behavioral and mental defects that

could create major problems for humans may not be detectable in animal models.

The most likely reasons for the abnormalities thus far observed are failures in genetic reprogramming (the process that changes a cell nucleus from one developmental state to another) and errors in genetic imprinting (the process of establishing, maintaining, and interpreting parent-specific chemical marks on the DNA, which indicate how specific genes should function in specific cells).

On the basis of the animal data, it is also likely that human cloning will be associated with risks to the women involved. Among these risks are increased maternal morbidity and mortality and the risks inherent in the overproduction of oocytes from egg donors. The psychological burden of late-term abortions or the birth of infants with severe defects must also be considered.

HUMAN REPRODUCTIVE CLONING

Those who plan to clone humans have indicated that they will take additional precautionary steps beyond those currently undertaken in animal cloning. The steps include preimplantation testing to detect chromosome defects and errors in imprinting (methylation) at one or more DNA sites, and postimplantation testing of the imprinting (methylation) status at up to 30 DNA sites. All participants would sign an informed-consent form that would outline the risks to both the mother and the child and the low probability of success. Those who have publicly stated their intention to undertake human reproductive cloning are thus far using private funding in a nonuniversity setting, and in some cases they are operating or planning to operate outside the United States.

LESSONS FROM OTHER ASSISTED REPRODUCTIVE TECHNOLOGIES RELEVANT TO HUMAN REPRODUCTIVE CLONING

Assisted reproductive technology (ART) refers to all treatments or procedures for assisting human reproduction that include the laboratory handling of human eggs, sperm, or embryos, including *in vitro* fertilization (IVF). IVF involves the mixing of egg and sperm in the laboratory to generate embryos suitable for transfer to a uterus 2 or 3 days later. ART as currently practiced does not provide a basis for evaluating all the risks inherent in reproductive cloning, because reproductive cloning involves the use of adult somatic nuclei rather than the germ cell (egg and sperm) nuclei used in ART [3]. Germ-cell nuclei are preprogrammed to support early embryonic development and to respond to the egg's regulatory

signals, whereas adult cell nuclei are not and must therefore undergo an extensive reprogramming to be successful in their new environment.

The panel compared the experiences thus far obtained in animal cloning with knowledge of current human ART procedures and found that the reproductive outcomes from cloned blastocysts observed in animals are very low compared with the efficiencies seen with current human IVF—as well as being highly variable. In addition, serious defects and deaths occur in animal cloning, often late in pregnancy and soon after birth, at rates never seen with human or most animal ART procedures.

Existing preimplantation and postimplantation testing methods are inappropriate and inadequate for the needs of human reproductive cloning. Assessing the shape and structure of embryos is of little use in determining the likelihood of successful implantation of a particular embryo, and molecular tests to detect all the possible errors in genetic imprinting and reprogramming do not yet exist. Moreover, such tests, if they become available, would be difficult to adapt to the small amount of material available for preimplantation diagnosis.

Experimental ART procedures have been minimally regulated and monitored in the United States, so there is a shortage of data pertaining to innovative ART procedures. Certification of clinics could allow greater control over any new ART procedures and collection of important information. The UK Human Fertilisation and Embryology Authority might provide a model for certifying ART clinics and clinical and research protocols and procedures, although the terms of the UK legislation would have to be adapted to the federal style of the US government.

USING NUCLEAR TRANSPLANTATION TO PRODUCE EMBRYONIC STEM CELLS

Stem cells are cells that have an extensive ability to self-renew and to differentiate (turn into specialized cells). Embryonic stem cells obtained from blastocysts (5- to 7-day-old preimplantation embryos of about 150 cells each) are particularly important because they can give rise to the widest variety of cells and are immortal. If embryonic stem cells are derived by nuclear transplantation using a nucleus from a patient as the somatic nucleus transferred into the egg, the resulting cells will be immunologically very similar to the patient's cells. However, the nuclear DNA donor and mitochondrial DNA donor will generally be different. Only if the egg donor is the mother of the patient or the patient herself, will the stem cells be genetically identical with the patient's cells—containing not only the same nuclear genome, but also the same mitochondrial DNA. As described in the recent report from the National Academies entitled *Stem Cells and the Future of Regenerative Medicine*, present research with such

cells has the goal of producing cells and tissues for therapeutic transplantation with a reduced risk of rejection [2]. However, mitochondrial gene products that differ can elicit transplant rejection (see Chapter 2).

Current Arguments and Counterarguments Regarding Human Reproductive Cloning

Provided below is a summary of some of the current arguments and counterarguments regarding human reproductive cloning. The panel's analysis of each is based on the scientific and medical literature and on presentations at its workshop.

Argument 1: Animal-safety data do not apply, because humans are very different from the animals under study [4]. In particular, a recent study [5] indicated that an important imprinted gene in mice is not imprinted in humans; therefore, imprinting errors would not be a problem in cloned humans.

Counterargument: Placental function, development, and genetic regulation are similar in humans and animal models, such as mice, so similar SCNT-related defects would be expected [6]. Numerous studies have emphasized that humans and other organisms have the same basic pathways for governing early embryonic and fetal development. Furthermore, widespread defects in all five of the mammalian species that have been reproductively cloned thus far suggest that the defects would affect basic biological functions in humans.

Even if one less gene is imprinted in humans as compared to mice, humans are known to have many imprinted genes (possibly as many as 100), and any number of these are likely to cause problems in reproductively cloned humans.

Argument 2: Frequent failures are seen in normal human reproduction; cloning would be no different [4].

Counterargument: Errors in normal human reproduction occur primarily early in pregnancy; many of the women in question are never aware that they are pregnant. In contrast, many of the defects in reproductively cloned animals arise late in pregnancy or after birth.

Argument 3: Inappropriate culture media for the initial cells cause most cloning-related problems [7; 8]. Culture media for human assisted reproductive technologies have been better optimized [8; 4]. Synchronization between the implanted embryo and the recipient uterus has also been better in human than in animal assisted reproductive technology procedures.

Counterargument: Culture effects appear to account for only some of the defects observed [9; 10]. Many defects in various organ systems are peculiar to reproductive cloning. Expertise in existing human assisted reproductive technologies is not relevant to these problems, because the defects appear to arise from biological rather than purely technical causes [9].

Argument 4: Those who have cloned animals stress the failures, but there are also many successes in animal reproductive cloning [8; 4].

The panel recognizes that a blastocyst derived for scientific purposes by nuclear transplantation could be implanted in a human uterus in violation of a ban on reproductive cloning. But a legally enforceable ban that

Counterargument: The statement is true, but does not necessarily apply to human reproductive cloning. In humans, the likelihood and benefit of success must be weighed against the probability, severity, and lifelong consequences of failure. Failures are all but certain in any human reproductive cloning attempt at this time, based on the experience with animals, and in humans, the consequences could be far more devastating. The likelihood and benefit of possible success must be weighed against the high probability and severe consequences of failure.

Argument 5: Existing preimplantation and postimplantation genetic tests could be used to detect abnormalities, allowing selection of embryos to be implanted and therapeutic abortion in case of any problems. In contrast, there has been no genetic testing and weeding out of animal reproductive clones.

In preimplantation testing, two cells could be removed from an eight-cell morula. One cell could be tested for correctness of the chromosome complement and the other for imprinting errors at one or more DNA sites [11]. It has been claimed that such imprinting tests have been performed with DNA from cells after somatic cell nuclear transfer (SCNT) [4], although no data have been presented. Postimplantation testing could include testing for chromosomal errors, the checking of imprinting status at up to 30 sites, and the measurement of production levels from many genes with DNA chips [12] or reverse-transcription polymerase chain reaction [11].

Counterargument: Many errors would not be detectable until late in pregnancy or after birth, when therapeutic abortion would not be an option. Many of the relevant genetic tests have not yet been developed [8; 9]; existing genetic tests appropriate for single-gene inherited disorders or gross chromosomal rearrangements are insufficient because they are not relevant to the major sources of errors expected in human cloning. Ultrasonographic tests cannot detect the small-scale defects in tissues, such as lung, that have had devastating consequences in newborn animal clones [13;14], and there is insufficient evidence regarding the possible impact of imprinting errors on brain development in humans.

Argument 6: Voluntary informed consent allows potential participants to make their own decisions and elect to take the risks if they so choose.

Counterargument: Our current regulatory system recognizes that when information is lacking it can be difficult or impossible to inform subjects fully. That is the case with respect to human reproductive cloning because the extent of the risks is unknown, and the greatest risk of abnormality, morbidity, and mortality is borne by the cloned fetus/child, who cannot give informed consent. In addition, there are risks borne by the woman donating the eggs and the gestational mother.

When subjects cannot be fully informed, and when a procedure is clearly risky, there is a role for both regulatory agencies and professionals to limit the options available to a subject if the evidence supports such a limitation [14]. Societal concerns can also be taken into account.

criminalizes the implantation step should be sufficient to prevent such proscribed activity. Moreover, because all nuclear transplantation experiments will require the participation of human subjects (the donor of the eggs and the donor of the somatic cell nuclei, who may be the same person or different persons), all this work would necessarily be regulated and controlled by the procedures and rules concerning human-subjects research—subjecting it to close scrutiny.

Stem cells derived directly from an adult's own tissues are an alternative to nuclear transplantation-derived embryonic stem cells as a source of cells for therapies. Two types of adult stem cells—bone marrow and skin stem cells—currently provide the only two stem cell therapies. But, as noted in the above mentioned report, many questions remain before the potential of other adult stem cells can be accurately assessed. Few studies on adult stem cells have sufficiently defined the stem cell by starting from a single isolated cell or defined the necessary cellular environment for correct differentiation or the factors controlling the efficiency with which the cells repopulate an organ. There is a need to show that the cells derived from introduced adult stem cells are contributing directly to tissue function and to improve the ability to maintain adult stem cells in culture without having the cells differentiate. Finally, most of the studies that have garnered so much attention have used mouse rather than human adult stem cells.

The previous report also notes that unlike adult stem cells, it is well established that embryonic stem cells can form multiple tissue types and be maintained in culture for long periods of time. However, embryonic stem cells are not without their own potential problems as a source of cells for transplantation. The growth of human embryonic stem cells in culture now requires a "feeder" layer of mouse cells that may contain viruses, and when allowed to differentiate the embryonic stem cells can form a mixture of cell types at once. Human embryonic stem cells can form benign tumors when introduced into mice, although this potential seems to disappear if the cells are allowed to differentiate before introduction into a recipient.

In addition to possible uses in therapeutic transplantation, embryonic stem cells and cell lines derived by nuclear transplantation could be valuable tools for both fundamental and applied medical and biological research [2]. This research would begin with the transfer of genetically defined donor nuclei from normal and diseased tissues. The resulting cell lines could be used to study how inherited and acquired alterations of genetic components might contribute to disease processes. The properties of the cell lines could be studied directly, or the embryonic stem cells could be studied as they differentiate into other cell types. For example, the way in which cells derived by nuclear transplantation from an Alz-

heimer's disease patient acted while differentiating into brain cells, compared with those derived from a normal patient, might yield new clues about Alzheimer's disease. Such cell lines could also be used to ensure that research covers a more genetically diverse human population than that represented in the blastocysts stored in IVF clinics, promoting studies of the causes and consequences of genetic diseases by allowing researchers to study how embryonic stem cells with different genetic endowments differ in the way that they form cell types and tissues. Finally, studies of genetic reprogramming and genetic imprinting will be substantially enhanced through the use of stem cells derived by nuclear transplantation, compared with studies with stem cells derived from other sources.

SUMMARY

This panel was charged with assessing the scientific and medical issues surrounding human reproductive cloning. Most of the relevant data on reproductive cloning are derived from animal studies. The data reveal high rates of abnormalities in the cloned animals of multiple mammalian species and lead the panel to conclude that reproductive cloning of humans is not now safe. Our present opposition to human reproductive cloning is based on science and medicine, irrespective of broader considerations. The panel stresses, however, that a broad ethical debate must be encouraged, so that the public can be prepared to make decisions if human reproductive cloning is some day considered medically safe for mothers and offspring.

The panel's discussion inevitably included a comparison of the methods used for reproductive cloning and for nuclear transplantation to produce stem cells. The panel is in agreement with the recent report from the National Academies entitled *Stem Cells and the Future of Regenerative Medicine* [2] in affirming the potential of studies on stem cells isolated through nuclear transplantation. The probable benefits include advances in fundamental biomedical knowledge, as well as the understanding and treatment of various diseases and debilitating disorders.

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1

Introduction

Clone is a word that is now commonly used in many contexts in the United States. For example, rather than purchasing a name-brand computer, we might purchase its clone, which provides close to the same benefits but at a lower cost. If we're running out of time, we might say that we wish we had a clone that could help us accomplish all our tasks. When biologists use the word *clone*, they are talking specifically about DNA molecules, cells, or whole plants or animals that have the same genetic makeup.

"Cloning" is achieved commonly in the world of horticulture by, for example, providing a branch or stem of a plant with water and the right environmental conditions and producing a new plant that is a clone, or genetically identical copy, of the original plant. In human reproduction, cloning occurs naturally when identical twins are produced.

Life scientists conducting research today often clone cells to obtain replicas of the bacterial, animal, or plant cells necessary to perform repeated experiments. They can also develop from a single cell large numbers of identical cells (a "clonal cell line") that can be used for experiments and to test new medicines. Scientists clone DNA ("molecular cloning") so that they have large quantities of identical copies of DNA for scientific experiments.

Cloning of adult animals, known as **reproductive cloning**, has become relatively widespread since the report of the birth of Dolly the sheep in 1997; Dolly was the first clone of a mammal produced from an adult cell. Mammals of five species—sheep, mice, pigs, goats, and cattle—have

now been successfully cloned from adult or fetal cells, and attempts are being made (so far without success) to clone monkeys, dogs, horses, and other animals in the same way. The cloning of mammals involves a process called nuclear transplantation or somatic cell nuclear transfer (SCNT). In biological terminology, clones are not replicas of each other, but contain identical genetic material.

The nuclear transplantation procedure is also used for a purpose distinctly different from cloning whole mammals. Like reproductive cloning, the process of **nuclear transplantation to produce stem cells** (also called "therapeutic cloning, nonreproductive cloning, or research cloning") involves placing the DNA from one mammal into an enucleated egg (an egg from which the chromosomes have been removed). Thereafter, the egg is stimulated to divide. At the blastocyst stage of embryonic development (in humans, a 5-7 day old preimplantation embryo of about 150 cells), its inner cell mass is harvested and grown in culture for subsequent derivation of embryonic stem cells. These cells are then used for scientific and clinical investigations. Neither the cells nor the blastocyst are ever implanted in a uterus, as is required for reproductive cloning and the birth of an animal. Figures 1 and 2 in the Executive Summary illustrate the differences between the techniques of reproductive cloning and nuclear transplantation to produce stem cells.

This report, by a joint panel of the National Academies Committee on Science, Engineering, and Public Policy (COSEPUP) and the National Academies Board on Life Sciences (BLS), focuses on issues raised by the possible application of nuclear transplantation technology to the reproductive cloning of humans.

NATIONAL BIOETHICS ADVISORY COMMISSION

In 1997, after a report announced the cloning experiments that produced Dolly the sheep [1], President Clinton asked that the National Bioethics Advisory Commission (NBAC), chaired by Harold Shapiro, look at the issue of human cloning. The NBAC's report, *Cloning Human Beings* [2], came to various conclusions, including the following (emphasis added):

"**The Commission concludes that at this time it is morally unacceptable for anyone in the public or private sector, whether in a research or clinical setting, to attempt to create a child using somatic cell nuclear transfer cloning.** The Commission reached a consensus on this point because current scientific information indicates that this technique is not safe to use in humans at this point. Indeed, the Commission believes it would violate important ethical obligations were clinicians or researchers to attempt to create a child using these particular technologies, which are

likely to involve unacceptable risks to the fetus and/or potential child. Moreover, in addition to safety concerns, many other serious ethical concerns have been identified, which require much more widespread and careful public deliberation before this technology may be used."

The commission recommended, in part, the following:

- **"A continuation of the current moratorium on the use of federal funding in support of any attempt to create a child by somatic cell nuclear transfer."**
- **"An immediate request to all firms, clinicians, investigators, and professional societies in the private and non-federally funded sectors to comply voluntarily with the intent of the federal moratorium.** Professional and scientific societies should make clear that any attempt to create a child by somatic cell nuclear transfer and implantation into a woman's body would at this time be an irresponsible, unethical, and unprofessional act."
- **"[Enactment of] federal legislation . . . to prohibit anyone from attempting, whether in a research or clinical setting, to create a child through somatic cell nuclear transfer cloning.** It is critical, however, that such legislation include a sunset clause to ensure that Congress will review the issue after a specified time period (three to five years) in order to decide whether the prohibition continues to be needed. If state legislation is enacted, it should also contain such a sunset provision. Any such legislation or associated regulation also ought to require that at some point prior to the expiration of the sunset period, an appropriate oversight body will evaluate and report on the current status of somatic cell nuclear transfer technology and on the ethical and social issues that its potential use to create human beings would raise in light of public understandings at that time."
- **"[Writing of] any regulatory or legislative actions undertaken to effect the foregoing prohibition on creating a child by somatic cell nuclear transfer . . . so as not to interfere with other important areas of scientific research.** In particular, no new regulations are required regarding the cloning of human DNA sequences and cell lines, since neither activity raises the scientific and ethical issues that arise from the attempt to create children through somatic cell nuclear transfer, and these fields of research have already provided important scientific and biomedical advances. Likewise, research on cloning animals by somatic cell nuclear transfer does not raise the issues implicated in attempting to use this technique for human cloning, and its continuation should only be subject to existing regulations regarding the humane use of animals and review by institution-based animal protection committees. "

Other countries are also considering the issues and determining their policies. Different countries are coming to different conclusions about nuclear transplantation to produce stem cells, but they agree with the NBAC advice on reproductive cloning of humans.

CHARGE TO PANEL

The COSEPUP–BLS panel focused on the issue of human reproductive cloning. The National Academies provided the initiative and financial sponsorship for this study.

The time is ripe for a re-examination of cloning-related issues, inasmuch as it has been almost 5 years since the NBAC issued its recommendations. Much has happened scientifically since then. In addition, several organizations have indicated that they plan to clone humans. This report does not address the ethical issues that were the focus of much of the NBAC report. Instead, it provides an analysis focused on the scientific and medical aspects of human cloning.

In this report, the panel responds to the following questions in our task statement:

(1) What does cloning of animals including humans mean? What are its purposes? How does it differ from stem cell research?

(2) What is the state of science on cloning of animals? How does this science apply to cloning of people?

(3) To what extent can our knowledge of assisted reproductive technologies inform the debate on human cloning?

(4) What scientific and medical criteria should be used to evaluate the safety of cloning a person?

(5) What issues of responsible conduct of research are raised by the prospect of cloning a person?

(6) What process should be used to evaluate future scientific and medical evidence regarding cloning a person?

(7) Based on the current scientific and medical evidence, should there be a moratorium on the cloning of a person? What are the implications of doing so? Of not doing so? If a moratorium is enacted, when should the issue be re-evaluated?

In this report, we will be discussing the concepts of bans and moratoriums. The panel uses the following definitions for each (from the unabridged version of *Webster's Third New International Dictionary*).

Ban: "To prohibit by legal means or social pressure the performance, activities, dissemination, or use [of something]; . . . censure or condemna-

tion, especially through public opinion, social pressure, or moral or ethical considerations; severe disapproval [of something]."

Moratorium: "A suspension of activity; a temporary ban on the use or production of something."

METHOD

In developing its responses to those questions, the panel (see Appendix A) gathered and studied a large bibliography of scientific, veterinary, and medical literature (see Appendix B) and held 12 weekly conference calls for discussion. The panel also held a workshop on August 7, 2001, to hear testimony from and question some of the world's foremost experts in embryology, animal cloning, assisted reproductive technologies, and associated public-policy issues (see Appendix C for the workshop agenda). Scientists who are now conducting research concerned with stem cells and those who plan to undertake reproductive cloning to create children also participated in the workshop. A transcript and sound files of the presentations at the meeting are available at the panel's Web site (www.nationalacademies.org/humancloning).

ORGANIZATION OF THIS REPORT

Chapter 2 provides a basic introduction to cloning and its relation to stem cell research. Chapter 3 is an overview of the state of the science of animal cloning and a summary of its possible application to humans. Chapter 4 reviews the panel's understanding of relevant assisted reproductive technologies. Chapter 5 describes the plans of those who wish to clone humans and provides the current policy and regulatory context. Chapter 6 contains the panel's findings and recommendations.

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2

Cloning: Definitions And Applications

In this chapter, we address the following questions in our task statement:

> *What does cloning of animals including humans mean? What are its purposes? How does it differ from stem cell research?*

To organize its response to those questions, the panel developed a series of subquestions, which appear as the section headings in the following text.

WHAT IS MEANT BY REPRODUCTIVE CLONING OF ANIMALS INCLUDING HUMANS?

Reproductive cloning is defined as the deliberate production of genetically identical individuals. Each newly produced individual is a clone of the original. Monozygotic (identical) twins are natural clones. Clones contain identical sets of genetic material in the nucleus—the compartment that contains the chromosomes—of every cell in their bodies. Thus, cells from two clones have the same DNA and the same genes in their nuclei.

All cells, including eggs, also contain some DNA in the energy-generating "factories" called mitochondria. These structures are in the cytoplasm, the region of a cell outside the nucleus. Mitochondria contain their own DNA and reproduce independently. True clones have identical DNA in both the nuclei and mitochondria, although the term *clones* is also used

to refer to individuals that have identical nuclear DNA but different mitochondrial DNA.

HOW IS REPRODUCTIVE CLONING DONE?

Two methods are used to make live-born mammalian clones. Both require implantation of an embryo in a uterus and then a normal period of gestation and birth. However, reproductive human or animal cloning is not defined by the method used to derive the genetically identical embryos suitable for implantation. Techniques not yet developed or described here would nonetheless constitute cloning if they resulted in genetically identical individuals of which at least one were an embryo destined for implantation and birth.

The two methods used for reproductive cloning thus far are as follows:

- *Cloning using somatic cell nuclear transfer (SCNT)* [1]. This procedure starts with the removal of the chromosomes from an egg to create an enucleated egg. The chromosomes are replaced with a nucleus taken from a somatic (body) cell of the individual or embryo to be cloned. This cell could be obtained directly from the individual, from cells grown in culture, or from frozen tissue. The egg is then stimulated, and in some cases it starts to divide. If that happens, a series of sequential cell divisions leads to the formation of a blastocyst, or preimplantation embryo. The blastocyst is then transferred to the uterus of an animal. The successful implantation of the blastocyst in a uterus can result in its further development, culminating sometimes in the birth of an animal. This animal will be a clone of the individual that was the donor of the nucleus. Its nuclear DNA has been inherited from only one genetic parent.

The number of times that a given individual can be cloned is limited theoretically only by the number of eggs that can be obtained to accept the somatic cell nuclei and the number of females available to receive developing embryos. If the egg used in this procedure is derived from the same individual that donates the transferred somatic nucleus, the result will be an embryo that receives *all* its genetic material—nuclear and mitochondrial—from a single individual. That will also be true if the egg comes from the nucleus donor's mother, because mitochondria are inherited maternally. Multiple clones might also be produced by transferring identical nuclei to eggs from a single donor. If the somatic cell nucleus and the egg come from different individuals, they will not be identical to the nuclear donor because the clones will have somewhat different mitochondrial genes [2; 3]

- *Cloning by embryo splitting.* This procedure begins with *in vitro* fertilization (IVF): the union outside the woman's body of a sperm and an

egg to generate a zygote. The zygote (from here onwards also called an embryo) divides into two and then four identical cells. At this stage, the cells can be separated and allowed to develop into separate but identical blastocysts, which can then be implanted in a uterus. The limited developmental potential of the cells means that the procedure cannot be repeated, so embryo splitting can yield only two identical mice and probably no more than four identical humans.

The DNA in embryo splitting is contributed by germ cells from two individuals—the mother who contributed the egg and the father who contributed the sperm. Thus, the embryos, like those formed naturally or by standard IVF, have two parents. Their mitochondrial DNA is identical. Because this method of cloning is identical with the natural formation of monozygotic twins and, in rare cases, even quadruplets, it is not discussed in detail in this report.

WILL CLONES LOOK AND BEHAVE EXACTLY THE SAME?

Even if clones are genetically identical with one another, they will not be identical in physical or behavioral characteristics, because DNA is not the only determinant of these characteristics. A pair of clones will experience different environments and nutritional inputs while in the uterus, and they would be expected to be subject to different inputs from their parents, society, and life experience as they grow up. If clones derived from identical nuclear donors and identical mitocondrial donors are born at different times, as is the case when an adult is the donor of the somatic cell nucleus, the environmental and nutritional differences would be expected to be more pronounced than for monozygotic (identical) twins. And even monozygotic twins are not fully identical genetically or epigenetically because mutations, stochastic developmental variations, and varied imprinting effects (parent-specific chemical marks on the DNA) make different contributions to each twin [3; 4].

Additional differences may occur in clones that do not have identical mitochondria. Such clones arise if one individual contributes the nucleus and another the egg—or if nuclei from a single individual are transferred to eggs from multiple donors. The differences might be expected to show up in parts of the body that have high demands for energy—such as muscle, heart, eye, and brain—or in body systems that use mitochondrial control over cell death to determine cell numbers [5; 6].

WHAT ARE THE PURPOSES OF REPRODUCTIVE CLONING?

Cloning of livestock [1] is a means of replicating an existing favorable combination of traits, such as efficient growth and high milk production,

without the genetic "lottery" and mixing that occur in sexual reproduction. It allows an animal with a particular genetic modification, such as the ability to produce a pharmaceutical in milk, to be replicated more rapidly than does natural mating [7; 8]. Moreover, a genetic modification can be made more easily in cultured cells than in an intact animal, and the modified cell nucleus can be transferred to an enucleated egg to make a clone of the required type. Mammals used in scientific experiments, such as mice, are cloned as part of research aimed at increasing our understanding of fundamental biological mechanisms.

In principle, those people who might wish to produce children through human reproductive cloning [9] include:

- Infertile couples who wish to have a child that is genetically identical with one of them, or with another nucleus donor
- Other individuals who wish to have a child that is genetically identical with them, or with another nucleus donor
- Parents who have lost a child and wish to have another, genetically identical child
- People who need a transplant (for example, of cord blood) to treat their own or their child's disease and who therefore wish to collect genetically identical tissue from a cloned fetus or newborn.

Possible reasons for undertaking human reproductive cloning have been analyzed according to their degree of justification. For example, in reference 10 it is proposed that human reproductive cloning aimed at establishing a genetic link to a gametically infertile parent would be more justifiable than an attempt by a sexually fertile person aimed at choosing a specific genome.

Transplantable tissue may be available without the need for the birth of a child produced by cloning. For example, embryos produced by *in vitro* fertilization (IVF) can be typed for transplant suitability, and in the future stem cells produced by nuclear transplantation may allow the production of transplantable tissue.

The alternatives open to infertile individuals are discussed in Chapter 4.

HOW DOES REPRODUCTIVE CLONING DIFFER FROM STEM CELL RESEARCH?

The recent and current work on stem cells that is briefly summarized below and discussed more fully in a recent report from the National Academies entitled *Stem Cells and the Future of Regenerative Medicine* [11] is not directly related to human reproductive cloning. However, the use of a

common initial step—called either nuclear transplantation or somatic cell nuclear transfer (SCNT)—has led Congress to consider bills that ban not only human reproductive cloning but also certain areas of stem cell research. Stem cells are cells that have the ability to divide repeatedly and give rise to both specialized cells and more stem cells. Some, such as some blood and brain stem cells, can be derived directly from adults [12-19] and others can be obtained from preimplantation embryos. Stem cells derived from embryos are called embryonic stem cells (ES cells). The above-mentioned report from the National Academies provides a detailed account of the current state of stem cell research [11].

ES cells are also called pluripotent stem cells because their progeny include all cell types that can be found in a postimplantation embryo, a fetus, and a fully developed organism. They are derived from the inner cell mass of early embryos (blastocysts) [20-23]. The cells in the inner cell mass of a given blastocyst are genetically identical, and each blastocyst yields only a single ES cell line. Stem cells are rarer [24] and more difficult to find in adults than in preimplantation embryos, and it has proved harder to grow some kinds of adult stem cells into cell lines after isolation [25; 26].

Production of different cells and tissues from ES cells or other stem cells is a subject of current research [11; 27-31]. Production of whole organs other than bone marrow (to be used in bone marrow transplantation) from such cells has not yet been achieved, and its eventual success is uncertain.

Current interest in stem cells arises from their potential for the therapeutic transplantation of particular healthy cells, tissues, and organs into people suffering from a variety of diseases and debilitating disorders. Research with adult stem cells indicates that they may be useful for such purposes, including for tissues other than those from which the cells were derived [12; 14; 17; 18; 25-27; 32-43]. On the basis of current knowledge, it appears unlikely that adults will prove to be a sufficient source of stem cells for all kinds of tissues [11; 44-47]. ES cell lines are of potential interest for transplantation because one cell line can multiply indefinitely and can generate not just one type of specialized cell, but many different types of specialized cells (brain, muscle, and so on) that might be needed for transplants [20; 28; 45; 48; 49]. However, much more research will be needed before the magnitude of the therapeutic potential of either adult stem cells or ES cells will be well understood.

One of the most important questions concerning the therapeutic potential of stem cells is whether the cells, tissues, and perhaps organs derived from them can be transplanted with minimal risk of transplant rejection. Ideally, adult stem cells advantageous for transplantation might be derived from patients themselves. Such cells, or tissues derived from

them, would be genetically identical with the patient's own and not be rejected by the immune system. However, as previously described, the availability of sufficient adult stem cells and their potential to give rise to a full range of cell and tissue types are uncertain. Moreover, in the case of a disorder that has a genetic origin, a patient's own adult stem cells would carry the same defect and would have to be grown and genetically modified before they could be used for therapeutic transplantation.

The application of somatic cell nuclear transfer or nuclear transplantation offers an alternative route to obtaining stem cells that could be used for transplantation therapies with a minimal risk of transplant rejection. This procedure—sometimes called therapeutic cloning, research cloning, or nonreproductive cloning, and referred to here as **nuclear transplantation to produce stem cells**—would be used to generate pluripotent ES cells that are genetically identical with the cells of a transplant recipient [50]. Thus, like adult stem cells, such ES cells should ameliorate the rejection seen with unmatched transplants.

Two types of adult stem cells—stem cells in the blood forming bone marrow and skin stem cells—are the only two stem cell therapies currently in use. But, as noted in the National Academies' report entitled *Stem Cells and the Future of Regenerative Medicine*, many questions remain before the potential of other adult stem cells can be accurately assessed [11]. Few studies on adult stem cells have sufficiently defined the stem cell's potential by starting from a single, isolated cell, or defined the necessary cellular environment for correct differentiation or the factors controlling the efficiency with which the cells repopulate an organ. There is a need to show that the cells derived from introduced adult stem cells are contributing directly to tissue function, and to improve the ability to maintain adult stem cells in culture without the cells differentiating. Finally, most of the studies that have garnered so much attention have used mouse rather than human adult stem cells.

ES cells are not without their own potential problems as a source of cells for transplantation. The growth of human ES cells in culture requires a "feeder" layer of mouse cells that may contain viruses, and when allowed to differentiate the ES cells can form a mixture of cell types at once. Human ES cells can form benign tumors when introduced into mice [20], although this potential seems to disappear if the cells are allowed to differentiate before introduction into a recipient [51]. Studies with mouse ES cells have shown promise for treating diabetes [30], Parkinson's disease [52], and spinal cord injury [53].

The ES cells made with nuclear transplantation would have the advantage over adult stem cells of being able to provide virtually all cell types and of being able to be maintained in culture for long periods of time. Current knowledge is, however, uncertain, and research on both

adult stem cells and stem cells made with nuclear transplantation is required to understand their therapeutic potentials. (This point is stated clearly in Finding and Recommendation 2 of *Stem Cells and the Future of Regenerative Medicine* [11] which states, in part, that "studies of both embryonic and adult human stem cells will be required to most efficiently advance the scientific and therapeutic potential of regenerative medicine.") It is likely that the ES cells will initially be used to generate single cell types for transplantation, such as nerve cells or muscle cells. In the future, because of their ability to give rise to many cell types, they might be used to generate tissues and, theoretically, complex organs for transplantation. But this will require the perfection of techniques for directing their specialization into each of the component cell types and then the assembly of these cells in the correct proportion and spatial organization for an organ. That might be reasonably straightforward for a simple structure, such as a pancreatic islet that produces insulin, but it is more challenging for tissues as complex as that from lung, kidney, or liver [54; 55].

The experimental procedures required to produce stem cells through nuclear transplantation would consist of the transfer of a somatic cell nucleus from a patient into an enucleated egg, the *in vitro* culture of the embryo to the blastocyst stage, and the derivation of a pluripotent ES cell line from the inner cell mass of this blastocyst. Such stem cell lines would then be used to derive specialized cells (and, if possible, tissues and organs) in laboratory culture for therapeutic transplantation. Such a procedure, if successful, can avoid a major cause of transplant rejection. However, there are several possible drawbacks to this proposal. Experiments with animal models suggest that the presence of divergent mitochondrial proteins in cells may create "minor" transplantation antigens [56; 57] that can cause rejection [58-63]; this would not be a problem if the egg were donated by the mother of the transplant recipient or the recipient herself. For some autoimmune diseases, transplantation of cells cloned from the patient's own cells may be inappropriate, in that these cells can be targets for the ongoing destructive process. And, as with the use of adult stem cells, in the case of a disorder that has a genetic origin, ES cells derived by nuclear transplantation from the patient's own cells would carry the same defect and would have to be grown and genetically modified before they could be used for therapeutic transplantation. Using another source of stem cells is more likely to be feasible (although immunosuppression would be required) than the challenging task of correcting the one or more genes that are involved in the disease in adult stem cells or in a nuclear transplantation-derived stem cell line initiated with a nucleus from the patient.

In addition to nuclear transplantation, there are two other methods by which researchers might be able to derive ES cells with reduced likeli-

hood for rejection. A bank of ES cell lines covering many possible genetic makeups is one possibility, although the National Academies report entitled *Stem Cells and the Future of Regenerative Medicine* rated this as "difficult to conceive" [11]. Alternatively, embryonic stem cells might be engineered to eliminate or introduce certain cell-surface proteins, thus making the cells invisible to the recipient's immune system. As with the proposed use of many types of adult stem cells in transplantation, neither of these approaches carries anything close to a promise of success at the moment.

The preparation of embryonic stem cells by nuclear transplantation differs from reproductive cloning in that nothing is implanted in a uterus. The issue of whether ES cells alone can give rise to a complete embryo can easily be misinterpreted. The titles of some reports suggest that mouse embryos can be derived from ES cells alone [64-72]. In all cases, however, the ES cells need to be surrounded by cells derived from a host embryo, in particular trophoblast and primitive endoderm. In addition to forming part of the placenta, trophoblast cells of the blastocyst provide essential patterning cues or signals to the embryo that are required to determine the orientation of its future head and rump (anterior-posterior) axis. This positional information is not genetically determined but is acquired by the trophoblast cells from events initiated soon after fertilization or egg activation. Moreover, it is critical that the positional cues be imparted to the inner cells of the blastocyst during a specific time window of development [73-76]. Isolated inner cell masses of mouse blastocysts do not implant by themselves, but will do so if combined with trophoblast vesicles from another embryo [77]. By contrast, isolated clumps of mouse ES cells introduced into trophoblast vesicles never give rise to anything remotely resembling a postimplantation embryo, as opposed to a disorganized mass of trophoblast. In other words, the only way to get mouse ES cells to participate in normal development is to provide them with host embryonic cells, even if these cells do not remain viable throughout gestation (Richard Gardner, personal communication). It has been reported that human [20] and primate [78-79] ES cells can give rise to trophoblast cells in culture. However, these trophoblast cells would presumably lack the positional cues normally acquired during the development of a blastocyst from an egg. In the light of the experimental results with mouse ES cells described above, it is very unlikely that clumps of human ES cells placed in a uterus would implant and develop into a fetus. It has been reported that clumps of human ES cells in culture, like clumps of mouse ES cells, give rise to disorganized aggregates known as embryoid bodies [80].

Besides their uses for therapeutic transplantation, ES cells obtained by nuclear transplantation could be used in laboratories for several types of studies that are important for clinical medicine and for fundamental research in human developmental biology. Such studies could not be

carried out with mouse or monkey ES cells and are not likely to be feasible with ES cells prepared from normally fertilized blastocysts. For example, ES cells derived from humans with genetic diseases could be prepared through nuclear transplantation and would permit analysis of the role of the mutated genes in both cell and tissue development and in adult cells difficult to study otherwise, such as nerve cells of the brain. This work has the disadvantage that it would require the use of donor eggs. But for the study of many cell types there may be no alternative to the use of ES cells; for these cell types the derivation of primary cell lines from human tissues is not yet possible.

If the differentiation of ES cells into specialized cell types can be understood and controlled, the use of nuclear transplantation to obtain genetically defined human ES cell lines would allow the generation of genetically diverse cell lines that are not readily obtainable from embryos that have been frozen or that are in excess of clinical need in IVF clinics. The latter do not reflect the diversity of the general population and are skewed toward genomes from couples in which the female is older than the period of maximal fertility or one partner is infertile. In addition, it might be important to produce stem cells by nuclear transplantation from individuals who have diseases associated with both simple [81] and complex (multiple-gene) heritable genetic predilections. For example, some people have mutations that predispose them to "Lou Gehrig's disease" (amyotrophic lateral sclerosis, or ALS); however, only some of these individuals become ill, presumably because of the influence of additional genes. Many common genetic predilections to diseases have similarly complex etiologies; it is likely that more such diseases will become apparent as the information generated by the Human Genome Project is applied. It would be possible, by using ES cells prepared with nuclear transplantation from patients and healthy people, to compare the development of such cells and to study the fundamental processes that modulate predilections to diseases.

Neither the work with ES cells, nor the work leading to the formation of cells and tissues for transplantation, involves the placement of blastocysts in a uterus. Thus, there is no embryonic development beyond the 64 to 200 cell stage, and no fetal development.

FINDINGS

2-1. Reproductive cloning involves the creation of individuals that contain identical sets of nuclear genetic material (DNA). To have complete genetic identity, clones must have not only the same nuclear genes, but also the same mitochondrial genes.

2-2. Cloned mammalian animals can be made by replacing the chromosomes of an egg cell with a nucleus from the individual to be cloned, followed by stimulation of cell division and implantation of the resulting embryo.

2-3. Cloned individuals, whether born at the same or different times, will not be physically or behaviorally identical with each other at comparable ages.

2-4. Stem cells are cells that have an extensive ability to self-renew and differentiate, and they are therefore important as a potential source of cells for therapeutic transplantation. Embryonic stem cells derived through nuclear transplantation into eggs are a potential source of pluripotent (embryonic) stem cell lines that are immunologically similar to a patient's cells. Research with such cells has the goal of producing cells and tissues for therapeutic transplantation with minimal chance of rejection.

2-5. Embryonic stem cells and cell lines derived through nuclear transplantation could be valuable for uses other than organ transplantation. Such cell lines could be used to study the heritable genetic components associated with predilections to a variety of complex genetic diseases and test therapies for such diseases when they affect cells that are hard to study in isolation in adults.

2-6. The process of obtaining embryonic stem cells through nuclear transplantation does not involve the placement of an embryo in a uterus, and it cannot produce a new individual.

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3

Animal Cloning

In this chapter, we address the following questions in our task statement:

> *What is the state of science on cloning of animals? How does this science apply to cloning of people?*

To organize its response to those questions, the panel developed a series of subquestions, which appear as the section headings in the following text. For a general overview of the history and current status of animal cloning, see Solter (2000) [1] and Lewis et al. (2001) [2].

WHICH MAMMALIAN SPECIES HAVE BEEN CLONED, AND HOW EFFICIENT ARE THE REPRODUCTIVE CLONING PROCEDURES?

The animals that have been reproductively cloned through transfer of postembryonic nuclei are sheep [3-5], cattle [6-18], goats [19; 20], pigs [21; 22], and mice [23-29]. Similar attempts have been made in rhesus macaques, but the only success has been in experiments with nuclei from preimplantation embryos rather than postembryonic cells [30; 31]. In addition, reproductive cloning efforts in rabbits, rats, cats, dogs, and horses are ongoing [32].

The cloning efficiencies for various species are listed in Table 1 (developed by the panel) and Tables 3 and 4 (developed by Lewis et al., 2001[2]) in Appendix B. These efficiencies vary greatly—in general they

are low, whether looked at in terms of live births per embryo produced in the laboratory or live births per embryo transferred to the uterus (see Table 1). Note that the two highest percentages are derived from one experiment and are outliers; in this experiment, the numbers are small and half the newborns (four of eight) died soon after birth [7]. In monkeys, reproductive cloning with adult nuclei has not been successful, but cloning with nuclei from the individual cells of several eight-cell embryos yielded 53 embryos for transfer; these resulted in four pregnancies, two of which gave normal offspring and two of which were lost [30; 31].

The results summarized in Table 1 and the cloning literature can be looked at from several points of view. It is clear that many healthy, apparently normal, clones have been born and have survived to fertile adulthood (for example, see [21; 27; 28; 33]). Dolly has given birth to lambs [34-36], and in the case of mice, six generations of clones have been produced serially, although the efficiency declined with succeeding generations [25]. While some cloned mice may die soon after birth [23], one detailed follow-up of five surviving cloned mice revealed no serious problems, and the weight gain seen after several weeks might have been caused by non-cloning-related genetic effects [37]. On the negative side, however, it is quite clear that across multiple species there are far more failures in the development of cloned fetuses than there are live normal births.

This low efficiency of cloning reflects, among other causes, a high rate of fetal loss after embryo transfer and implantation. Spontaneous abortion is also common in natural pregnancies, but there is a major difference in the timing of fetal and neonatal loss between animal reproduction based on reproductive cloning and reproduction based on *in vitro* fertilization (IVF). Whereas most fetal losses in conventional zygotic pregnancies occur in the first trimester, with reproductive cloning, fetuses are lost throughout pregnancy and in the early neonatal period [6; 8; 9; 13; 23; 24; 29; 32; 38; 39].

In humans, late gestational fetal loss causes increased maternal morbidity and mortality. Cloning studies in animals have shown that a high proportion of pregnancies involving cloned fetuses have abnormalities, including abnormal placentation, pregnancy toxemia, and hydroallantois—excessive fluid accumulation in the uterus often associated with fetal abnormality [14; 33; 43; 100; 101; 115]. Those pregnancy complications can cause fetal loss and risk maternal health. For example, in the cow-cloning study by Hill et al. (1999)[8], four of the 13 pregnant mothers and their fetuses died because of complications late in pregnancy. Results of animal studies suggest that reproductive cloning of humans would similarly pose a high risk to the health of both fetus or infant and mother and lead to associated psychological risks for the mother as a consequence

of late spontaneous abortions or the birth of a stillborn child or a child with severe health problems.

WHAT DEFECTS HAVE BEEN OBSERVED IN CLONED ANIMALS?

A wide array of abnormalities and defects have been observed in reproductively cloned animals, both before and after birth [4; 6; 8-10; 13; 16; 20; 23; 24; 29; 32; 38-45]. However, these abnormalities have not always been studied in detail, possibly because most reproductive animal cloning has been done for commercial purposes and there is less interest in the failures than in the successes. The panel was told that funding for studies to catalog and understand the basis of the abnormalities is sorely needed [39].

The reported defects in cloned animals are summarized in Table 1 and detailed in Table 2. The most notable defects are increased birth size, placental defects, and lung, kidney, and cardiovascular problems [39; 46]. Other problems have included liver, joint, and brain defects, immune dysfunction, and postnatal weight gain. Thus, a wide variety of tissues and organs can fail to develop properly in cloned animals, and some of the reported defects (such as aberrant growth and development of lung tissue and the immune system) cannot be diagnosed or prevented with current technology, such as prenatal screening with ultrasonography.

Many of the defects seen in cloned cattle and sheep (for example, high birth weight, abnormal placentation, fluid accumulation associated with maternal and fetal distress, and cardiovascular abnormalities) are the same as those described for "large offspring syndrome" (LOS). This is frequently seen in uncloned offspring produced after *in vitro* fertilization and embryo manipulation in these species (but not in others, including humans [47]) and is attributed to, among other things, the exposure of eggs and embryos to suboptimal culture conditions in the laboratory [41; 47-49]. In spite of much work to identify the causative factors (given the economic benefits that could come from efficient embryo manipulation in cattle), the etiology and species specificity of LOS are not understood. All that can be said is that it probably results from abnormal gene expression in the early embryo, including the misexpression of imprinted genes (see later) [41; 47]. As will be discussed again below, this highlights the fact that perturbations in gene expression during the preimplantation period can have serious consequences for later development. For the purposes of this report, it is important to stress two other things: some of the postnatal defects described in cloned cattle have not so far been associated with LOS (for example, [13]); and species that do not show LOS after normal

embryo manipulation or IVF (for example, mouse, goat and pig) still have a very low reproductive cloning efficiency, with prenatal and early postnatal losses [19-23; 29; 50]. Moreover, until the molecular basis of LOS is known, it is not possible to say that the syndrome would not occur in human reproductive cloning attempts.

Animal cloning can also result in danger to the mother of any cloned offspring. Increased maternal morbidity and mortality can result from late gestational fetal loss, increased size of the fetus, abnormal placentation, pregnancy toxemia, and, most notably, hydroallantois and/or hydramnios (excessive fluid accumulation in the uterus often associated with fetal abnormality and maternal distress) [6; 8-11; 16]. These effects have been seen most prominently in studies with cattle and sheep. For example, in the cattle cloning study by Hill et al. (1999) [8], four of the 13 pregnant cows and their fetuses died because of complications late in pregnancy. Tim King and Ian Wilmut (pers. comm.) have noted that hydroallantois can affect up to 5% of established sheep pregnancies involving cloned offspring, although this condition is "extremely rare" in normal pregnancies. Documentation of these and related maternal problems appears to be relatively sparse in the literature, possibly because the focus of research has been on the cloned offspring rather than the pregnant cows.

In conclusion, if results from animal reproductive cloning studies are extrapolated to humans, they suggest that reproductive cloning of humans could carry a very high risk to the health of both fetus or infant and mother and lead to associated psychological risks for the parents as a consequence of late spontaneous abortions or the birth of a stillborn child or a child with severe health problems. Moreover, if the cloned human fetus or placenta grew abnormally large, this could cause problems before a cesarean section would be an option, particularly if multiple embryos are placed in the uterus, which is the procedure in most IVF clinics in the United States. There is no reason, at this time, to expect the efficiency of implantation to be better for reproductive cloning than IVF.

WHAT ARE SOME POSSIBLE REASONS FOR THE DEFECTS?

Failures in several aspects of mammalian development are likely to contribute to the defects observed in cloned animals, and probably no one cause is responsible for all the problems. Some of the processes that are likely to be suboptimal have been enumerated [1; 2] and are outlined in the final sections of this chapter. Two processes, reprogramming and imprinting, are thought to be especially problematic [32; 38; 51].

FAILURES IN REPROGRAMMING

What is reprogramming, and why is it necessary?

Reprogramming is the process by which DNA and associated proteins in the nucleus transplanted from the somatic cell are reset so that the genes are ready to coordinate early developmental processes and make products required for growth of the early embryo [1; 52]. When researchers place animal somatic cell nuclei into enucleated eggs, they expect to "coerce" the adult cell nucleus into responding to egg cytoplasm as though it were the nucleus of a zygote. The nucleus should switch off many of the genes that were active in the adult cells and "restart" the genes needed to support the growth of embryonic tissues. Reprogramming must be completed in a relatively short time—within a few days in most mammals—so that the gene products that are normally supplied by the zygote nucleus can be delivered to the developing embryo [1].

In sexual reproduction, the process of reprogramming is not necessary, because the chromosomes come from germ cells, not somatic cells. The DNA in the egg and sperm are preprogrammed during the long processes of egg and sperm development and continue to be programmed through early development [53].

Does reprogramming fail during cloning?

After nuclear transplantation, there is probably insufficient time to accomplish reprogramming before the embryo begins to develop into a blastocyst. Incomplete or incorrect reprogramming is likely to result in the embryos making products in an inappropriate and uncoordinated manner. However, gene expression in embryos after nuclear transplantation has not been surveyed extensively or systematically except in one case, when errors were found [54]. Other studies are under way with mice, and it will be possible to compare the resulting data with the extensive available information about gene expression in normal early embryos of this species [1]. Abnormalities in the methylation of a DNA region were seen in cloned bovine blastocysts compared with embryos derived by IVF [55]. Additional investigations into the molecular events of reprogramming (such as the identification of proteins that enter or leave the transferred nucleus) have also just begun [52; 56].

It is important to note that reprogramming errors could involve any genes. Those who wish to assess the safety of human reproductive cloning would have to survey a large fraction of or perhaps all genes at various times to check the integrity of a cloned embryo. Moreover, they would

have to examine the quality and quantity of gene activity and whether it is appropriate for the particular cell type. Furthermore, some errors can be manifest only in particular tissues and only later in development.

FAILURES IN GENOMIC IMPRINTING

What is imprinting?

Imprinted genes usually have a "mark" imposed on or near them in the egg or the sperm, so the copy of a gene inherited from the mother behaves differently from the copy inherited from the father [57-59]. In the embryo and resulting offspring, the mark controls whether the gene is expressed. The best characterized of these marks is a methyl chemical group, which is added to some segments of the DNA in regions near the imprinted genes that are termed imprint control regions. Methylation is a mark that can be measured; other marks will probably be found in the future, but for now they are unknown.

For normal development to occur, an embryo needs one set of chromosomes with the imprints imposed by the father and another set with imprints imposed by the mother. In experimental studies with mice, embryos that inherit both copies of their chromosomes from the mother's germ cells can be generated; they inherit two versions of the mother's imprint. (Similarly, mouse embryos that inherit two copies of the father's chromosomes can be made.) Such genome-wide imprinting errors in mice result in fetal abnormalities and death [60-64]. Moreover, the size of the fetus and placenta may be abnormal. In humans, mutations that perturb or inactivate one copy of an imprinted region can result in the development of tumors in children or adults [65] or several well-known genetic disorders in children [66-68]. Three such disorders are Prader-Willi syndrome, Angelman syndrome, and Beckwith-Weidemann syndrome; these are characterized by various combinations of mental retardation and congenital abnormalities [67].

When are imprinting patterns established?

Imprints are first erased and then re-established in a purely maternal or paternal pattern during the early development of the germ cells in the ovary or testis [58]. Further modifications occur in some genes during or after fertilization [69-74]. Maternal and paternal imprints are retained in somatic cells, although changes occur later in life in some tissues. Methylated regions are usually faithfully replicated in cell division, but errors occur [38], and some marks can be erased as cells multiply and develop

into various cell types. In this case the missing marks cannot be added back again if the cell divides and replicates.

Do imprinting errors happen in reproductive cloning?

Many of the imprinting errors that have been studied through genetic manipulation of mice result in too much or too little fetal or placental growth. Similar effects seen in some animal reproductive cloning experiments lead scientists to suspect a common cause. Although a direct link has not yet been demonstrated in most cases, mice cloned using ES cells as nucleus donors show widespread, unpredictable and aberrant regulation of their imprinted genes, as well as developmental abnormalities [75]. ES cells are essentially embryonic cells, and it is not yet known whether the same imprinting errors will be seen in the genes of animals cloned with adult nuclei [76]. However, mouse reproductive cloning experiments with adult nuclei have revealed errors in methylation in about 0.5% of some 1000 normally methylated DNA segments studied (but not necessarily associated with imprinted genes) [77; 78]. In addition, studies on bovine blastocysts obtained by cloning from fetal fibroblasts showed abnormal DNA methylation compared with blastocysts obtained by IVF [55].

Understanding the relationship between imprinting and increased offspring size in animal reproductive cloning experiments is complicated by the fact that, as discussed and referenced earlier, overgrowth, or LOS, can occur in cattle [79] and sheep [80] simply as a result of the culturing of normal cleavage-stage embryos before implantation, as is done in IVF procedures. Although the mechanisms underlying LOS are not known, changes in the expression of genes that are imprinted in other species may occur during *in vitro* culture of sheep and cattle embryos [41; 47; 81]. In addition, aberrant regulation of imprinted genes has been reported after culturing mouse ES cells [82] and preimplantation mouse embryos [83], although, in the latter case, the embryos apparently develop normally [84]. Thus, abnormal development of cloned animals may result in part from the culturing of embryos in the laboratory in association with the SCNT technique. However, the presence of cloning-specific defects and a study in mice [50] suggest that at least some of the errors arise as a result of the nuclear transplantation procedure itself. Further work is needed to understand how external conditions can perturb the expression of imprinted and non-imprinted genes in the preimplantation embryos of different species, and to understand the relationship between these changes and those shown to be specifically associated with the technique of transplantation of somatic cell nuclei.

How widespread are imprinting effects?

In addition to the growth effects mentioned above, imprinting errors are known to affect brain development and mental function [85-88] and placental function [89].

One hundred or more genes might be imprinted in humans [58; 90]. They seem to be mostly genes that are important and turned on differentially early in development. The expression of each gene varies according to the time, the tissue, the species, and the parent of origin.

How might imprinting go awry in reproductively cloned animals?

There are several ways in which reproductive cloning might result in the abnormal expression of imprinted genes:

- Imprints and methylation marks may not be maintained in all cells during adult life, and random errors may occur. If nuclei from these cells are used for reproductive cloning, the errors cannot be repaired in the embryo. It is therefore important in the future to examine the possibility that the rare cases when reproductive cloning is successful involve a small subpopulation of cells that have kept their imprints unaltered.
- The pattern of imprints from the nucleus donor's parents might not be maintained or copied correctly as the chromosomes from the donor nucleus replicate in the preimplantation embryo. This problem might be exacerbated by the culture of embryos before implantation.
- Even if the imprinting marks are copied correctly, incorrect reprogramming might result in the imprinted genes not being read correctly in the embryonic tissues.
- There is evidence that imprinting of some genes is modified in the preimplantation embryo [69]. This process might work properly only if the cellular machinery is faced with two distinct sets of DNA from a sperm and an egg [70]. Nuclear transplantation, however, presents the egg cytoplasm with two sets of DNA from a single somatic cell.

Could imprinting errors cause problems for the human mother?

Incorrectly imprinted cells could cause problems for the mother, as well as the child. Three lines of evidence support that possibility:

- Some imprinting problems—for example, after sheep preimplantation embryo culture [41]—are associated with excessive growth of the fetus or placenta [49]. If LOS occurs in humans, it could be serious because humans have an extended growing time in the mother and are

already close to the maximal size that will allow for safe birth. In addition, if multiple embryos are implanted, as in nearly all IVF procedures in the United States, the risk to the mother would be higher.

- Incorrectly imprinted cells can be malignant [91]. An example is seen in complete hydatidiform moles. These form when an egg that lacks a nucleus is fertilized by a sperm, so that all the DNA is contributed by the sperm. An embryo does not develop, but, possibly as a result of imprinting problems [92], a potentially malignant growth (mole) forms inside the uterus.
- A few human fetal cells normally circulate in the mother's blood during and after pregnancy [93], and it has been speculated that they might be implicated in the development of some skin, autoimmune, and muscle diseases [94-98]. If incorrectly imprinted fetal cells have a growth advantage, it is theoretically possible that they could lodge in the mother's tissues and grow into a tumor.

Errors in processes other than reprogramming and imprinting are also possibilities. Some of these possibilities are listed below.

MITOCHONDRIAL HETEROPLASMY AND CONFLICT

What is mitochondrial heteroplasmy?

Normally, mitochondria are inherited from the mother. In mitochondrial heteroplasmy, a mix of mitochondria is present in a single cell. That can happen naturally [99; 100] and has been induced in humans with ooplasmic transfer ([101]; see Chapter 4). When the SCNT procedure involves fusion of a somatic cell and an egg from two different individuals, mitochondrial heteroplasmy can result [102]. However, the relatively small number of incoming mitochondria will probably be swamped by the vast excess of egg mitochondria [103; 104] and might in any case be subject to elimination by the egg [105-107].

Could a transferred nucleus conflict with egg-derived mitochondria?

When the SCNT procedure is used, the incoming nuclear DNA will encounter a foreign set of egg-derived mitochondrial DNA. That has the potential to cause problems because, for example, there are natural variants of both nuclear and mitochondrial genes, and some pair combinations work less efficiently than others [108-110].

Mitochondria are inherited almost exclusively from the mother [106]. In the mother, previous natural selection might have eliminated potentially deleterious conflicts between nuclear and mitochondrial genomes

[111] particularly by eliminating unfit oocytes [112; 113]. In sexual reproduction, the father's nuclear DNA therefore encounters a "foreign" set of mitochondrial genes from the mother, but in this case products encoded by the mother's nuclear DNA may compensate for any potential conflict between products encoded by the father's nuclear DNA and the mother's mitochondria. But when SCNT is performed, such compensation might no longer be present. In mice, a conflict between transplanted nuclei and foreign egg cytoplasm (which includes mitochondria) has been shown to cause growth deficiency and misregulation of some genes [114].

TELOMERE SHORTENING

Could shortened telomeres result in prematurely "old" clones?

Telomeres, the caps on the ends of chromosomes, shorten during aging in somatic cells. In germ cells, the caps are rebuilt by an enzyme called telomerase. Thus, there is a potential for cloned embryos, with their chromosomes from somatic cells, to have shortened telomeres. That could result in prematurely "old" cells in a clone and the misproduction of proteins from genes near the telomeres [115].

The possibility does not seem to be a major concern. Any shortening of telomeres in cloned sheep appears to be minor and can be minimized by judicious choice of the cell type used as a nucleus donor [116]. No sign of telomere shortening or aging was seen in mice cloned serially for six generations [25], and telomeres in cattle are rebuilt in cloned embryos [117-119] and can eventually be longer than [18; 120] or the same size as [117] those in age-matched control animals. Human blastocysts have high levels of telomerase activity [121]; this suggests that they might be able to rebuild telomeres after reproductive cloning.

MUTATIONS

Could adult-donor nuclei carry more mutations than do gamete nuclei?

The source of a nucleus for reproductive cloning would have to be chosen very carefully. Sun-exposed skin cells, for example, might be a bad source of nuclei, because their DNA could have many mutations induced by the sun's ultraviolet radiation. Cells that have been grown in culture dishes for a considerable time might also make poor nucleus donors, because growth in culture favors the accumulation of growth-promoting mutations that are often associated with cancer development. However, it should be noted that normal calves were born from cloning

experiments in which nuclei were derived from cells obtained from a 17-year-old bull and then cultured for 3 months in the laboratory [17]. In this study, six healthy calves were born from a total of 15 pregnancies involving nine abortions and 54 embryos transferred. The overall efficiency of live births (11% of embryos transferred) was thus not significantly lower than with nuclei from younger animals and less extensive cell culture (see Table 1 in Appendix B).

X-CHROMOSOME INACTIVATION

Can cloned female animals shut off one of their X chromosomes?

Females and males differ in their complement of sex chromosomes: females have two X chromosomes, and males have one X chromosome and one Y chromosome. Females reduce the production from X-chromosome genes to the level seen in males by shutting down almost an entire X chromosome. Experiments in mice [122] suggest that cloned embryos can successfully recapitulate that process, so failure of X inactivation is unlikely to be a source of defects in cloned animals.

Can reprogramming and imprinting errors be understood and controlled, and can cloning efficiencies be improved?

Our survey of the literature on animal cloning, as well as presentations at the workshop, revealed great variability in its efficiency (Table 1). Moreover, it is clear that although healthy clones can in some cases be produced, success is not a reproducible phenomenon, and the precise molecular mechanisms responsible for the high failure rate are almost entirely unknown. The optimal method for animal reproductive cloning cannot be determined from current studies, because the number of variables makes direct comparisons between multiple studies difficult or impossible. Studies often differ in species used, method of nuclear transplantation (fusion or injection, and single transfer or serial transfer), method of egg activation, expertise of the investigators, and condition of cells used as nucleus donor (for example, different cell type, cell cycle stage, and time of growth in culture before nuclear transplantation).

In the sections above, several of the most likely problems, including defects in genetic reprogramming and defects in imprinting, have been outlined and discussed. Several other potential sources of error have been summarized elsewhere with pertinent references [1]. For example, one problem may lie in the methods now used to activate the egg after nuclear transplantation. Immediately after normal fertilization, waves of increased calcium concentration pass through the egg in an orderly way, and this

may impart some organization on the egg cytoplasm or membrane important for gene activation and later development [123]. When the egg is activated by an electric shock or by chemicals, as is the case in animal cloning, these calcium waves do not occur in an orderly way. Another example is that problems may arise if the donor cell is replicating its DNA at the time the nucleus is taken for nuclear transplantation [6].

A number of different strategies have been used by different groups to try to overcome those and other problems and so increase the efficiency of cloning. In some cases, progress has been made, but no clear picture has emerged, particularly when nuclear transplantation from adult rather than embryonic cells is used.

Studies were undertaken to determine whether inbreeding may be important in the poor efficiency of cloning in mice, since many mouse strains commonly used in the laboratory are inbred. Inbred mice are generally less fertile than hybrid or outbred mice and their embryos may be more difficult to culture in the laboratory [124]. In two cloning studies [26; 50], researchers did find that inbred animals showed much poorer cloning success than outbred animals, but even in outbred strains, cloning efficiency was low 0.36-1.8% of the hybrid cloned embryos produced from nuclei of hybrid cells resulted in live births). That suggests that inbreeding, although it plays a role, cannot by itself account for the poor efficiency of cloning in mice.

One of the first approaches to overcoming reprogramming problems involved culturing the donor cells in the laboratory under conditions in which the cells become quiescent and shut down the activity of their genes [3]. However, this strategy was not the solution to low cloning efficiency (see, for example, [6]). Nevertheless, it is possible that in the future some particularly quiescent cells in an adult tissue (for example, stem cells) will be found to be better nucleus donors than others.

Another early approach to improve reproductive cloning efficiency involved delaying the activation of the egg after nuclear transplantation; theoretically, this should allow more time for the regulatory proteins to be stripped off the incoming DNA and for cytoplasmic proteins to bind to the DNA [23]. Again, this strategy has not led to a solution to low cloning efficiencies. In the future, new and improved methods of activation might allow the process to be controlled more precisely [1].

Finally, several groups have tried the technique of serial nuclear transfer or recloning in an attempt to overcome both reprogramming and egg-activation problems. The strategy here is to carry out nuclear transplantation in the usual way, by transferring a nucleus into an enucleated egg, then activating the egg and allowing the embryo to develop to the two-cell stage. Nuclei are then taken from this embryo and transferred into the

cytoplasm of an unfertilized egg from which the chromosomes have been removed, or a normally fertilized egg from which both the male and female nuclei have been removed (for diagram, see [1]). The embryo then continues to develop into a blastocyst for transfer. This serial transfer does two things: it allows the nucleus more time to be exposed to egg cytoplasm for possible reprogramming, and in some cases it uses an egg that has been activated normally by fertilization. The first use of this technique in mice gave high cloning efficiency [125], but the original nuclei came from embryos, not adults; when it was used with adult nuclei, there was no improvement in cloning efficiency [23], or only a very low efficiency was obtained with fetal losses [29]. In experiments with pigs, a relatively high cloning efficiency was also achieved [21], but the effect was not repeated in cattle [10]. It should be noted that if this procedure were applied to human cloning, it would involve not only donation of large numbers of unfertilized eggs, but also large numbers of *fertilized* eggs, or zygotes.

In conclusion, research into the science of genetic reprogramming and animal cloning is in its infancy, and much more information is needed. It is unlikely that the poor outcomes of cloning are the result of only one defect arising from the nuclear transplantation procedure. More likely, they arise from the accumulated effects of sometimes unpredictable and stochastic (random) errors in several complex and interdependent biological processes.

HOW DOES THE SCIENCE OF ANIMAL REPRODUCTIVE CLONING APPLY TO THE CLONING OF HUMANS?

Theoretically, it should be possible to use animal-cloning techniques for reproductive cloning of humans. Reproductive cloning with nuclear transplantation from adult cells has not yet been performed successfully in nonhuman primates, so no data on the efficiency or safety of the procedure in primates are available. Such data might be helpful in assessing the possible results of a human reproductive cloning attempt, given the close evolutionary relationship and reproductive similarities of humans and nonhuman primates.

It cannot be ruled out that the abnormalities observed in cloned animals would occur in humans produced with reproductive cloning [51], especially given the widespread conservation of basic developmental mechanisms between different mammalian species and the impressive level of conservation—for example, between mice and humans—of placental anatomy and the genes controlling placental function [126]. Nevertheless, differences do exist in the developmental programs of various

mammals, including humans, and at the present time, we do not know whether attempts at human cloning would reveal fewer, more, or different abnormalities.

FINDINGS

3-1. In general, the efficiency of reproductive cloning in animals remains extremely low despite several years of experimentation.

3-2. Animal cloning results in a wide variety of abnormalities, including greater than normal size (both during gestation and after birth), greater early- and late-gestation fetal morbidity and mortality, greater postnatal mortality, and various developmental defects in the immune, cardiovascular, and possibly nervous systems. (Subtle behavioral and mental defects might be undetectable in animal models.) In addition to the risks inherent in the overproduction of oocytes from egg donors, increased maternal morbidity and mortality are to be expected.

3-3. The most likely reasons for the abnormalities are failures in reprogramming in the adult nucleus used for reproductive cloning, so that it fails to turn on all the appropriate embryo-specific genes at the right times, and errors in imprinting.

3-4. Before human reproductive cloning is feasible, a great deal more research is necessary, including studies of cloning in nonhuman primates. Research focused on gaining an understanding of all aspects of reprogramming and imprinting, determining which steps in the reproductive cloning technique contribute to the overall low efficiency, and determining how these problems can be overcome would be most useful.

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4

Assisted Reproductive Technology

In this chapter, we address the following question in our task statement:

> *To what extent can our knowledge of assisted reproductive technologies inform the debate on human cloning?*

To organize its response to that question, the panel developed a series of subquestions, which appear as the section headings in the following text.

WHAT IS ASSISTED REPRODUCTIVE TECHNOLOGY?

Assisted reproductive technology (ART) refers to any treatment or procedure for assisting reproduction that includes the handling of human eggs, sperm or embryos, such as *in vitro* fertilization (IVF).

HOW EFFICIENT IS IN VITRO FERTILIZATION? HOW DOES IT COMPARE IN EFFICIENCY WITH ANIMAL CLONING?

IVF involves the mixing of egg and sperm in the laboratory to generate embryos suitable for transfer to a uterus 2 or 3 days later. An IVF cycle in humans usually involves the transfer of at least two embryos at a time. In the United States in 1998, 20% of human IVF transfers involved one or two embryos, 33% involved three embryos, 28% involved four embryos, and 19% involved five or more embryos [1].

Of all the reported IVF cycles in the United States in 1998 using fresh eggs and embryos derived from the patient, 30.5% resulted in pregnan-

cies, and 82% of these pregnancies (25% of all cycles) resulted in live births [1]. Although efficiencies are not usually reported as the fraction of successful pregnancies per embryo transferred, 12% of embryos transferred in one study after preimplantation genetic diagnosis (PGD) implanted successfully (yielding a success rate of 19.9% when measured in the usual terms of pregnancy per cycle) [2].

Clinical characteristics of the male and female partners play a major role in determining the success rate of IVF treatment. For example, in 1994, the highest success was reported for couples in which the female partner was younger than 40 years old and the male had a normal semen analysis (24.5% live births per cycle). The lowest success was reported for women older than 40 years old with a male partner with a normal semen analysis (9% live births per cycle) or abnormal semen analysis (8.5% live births per cycle) [3].

The success rate of IVF may be constrained by the relatively high rate of pregnancy loss in humans. In unassisted reproduction, many pregnancies are lost before there is any clinical sign of their existence ("occult pregnancies"), and additional pregnancies are lost after they are detectable with hormone measurements but before they are detectable with ultrasonography ("chemical pregnancies"). According to one source [4], "more than 80% of [spontaneous] abortions occur in the first twelve weeks, and the rate decreases rapidly thereafter." This contrasts with the frequent loss of cloned animal fetuses late in gestation.

IVF procedures involve the collection of eggs for fertilization. Any human reproductive cloning attempt would also involve this procedure, and the low efficiency of animal cloning suggests that a large number of eggs would have to be collected. The collection of these eggs would bring with it the risk of ovarian hyperstimulation syndrome in donors. The incidence of moderate and severe cases of this syndrome in studies in which more than 1000 IVF cycles were evaluated ranges from 0.8% [5] to 1.95% [6]. Maternal death resulting from the syndrome is rare enough that it is the subject of occasional case reports.

In the United States, multiple embryos are frequently implanted during an IVF cycle to increase the chances of a successful pregnancy [1]. That often results in multiple births, which are associated with risks of morbidity and mortality for the mother and, because of prematurity and low birth weight, for the children.

When IVF was first adopted in humans, no increase in the frequency of major malformations had been seen in IVF experiments in mice relative to normal animal reproduction [7]. That situation is in contrast with the data on animal cloning discussed in Chapter 3; cloned animals have markedly more problems, particularly severe abnormalities throughout gestation, than those animals produced by normal reproduction.

WHAT OTHER ART PROCEDURES ARE RELEVANT TO HUMAN REPRODUCTIVE CLONING? WHAT IS THEIR RELEVANCE?

- *Blastocyst culture and transfer* involve the growth of preimplantation embryos for 5 or 6 days before transfer to a uterus [8]. People who wish to clone humans might take advantage of this technique for two reasons: to try to extend the time available for carrying out preimplantation genetic diagnosis without freezing the embryos and to improve implantation rates.
- *Intracytoplasmic sperm injection (ICSI)* is a method in which a single sperm or sperm-precursor cell is injected directly into an unfertilized egg. It is used in cases of severe male factor infertility. The possibility has been raised that sperm will not set up or maintain all necessary male imprints before being injected in ICSI [9; 10]; this is a concern particularly if the sperm are isolated at an early stage of development (from testes rather than ejaculate) [11]. There have been reports of more frequent congenital defects [12] and delayed mental development [13] in some children conceived through ICSI, although both reports have been contested [14; 15]. Other clinicians, after controlling for the effects of multiple births and parental age, have observed no increased risks after ICSI relative to other ART procedures when they scored for congenital malformations [16] (except an increased risk of a genital malformation termed hypospadias possibly related to paternal subfertility [16]) [16-18], obstetric outcome [19; 20] or neurodevelopment [21]. Furthermore, a small study of one particular DNA location did not reveal any imprinting defects after ICSI [22]. Additional research is needed, however, to assess imprinting at multiple genomic sites and to determine the relevance to pregnancy outcome of imprinting status at these sites. If ICSI does lead to imprinting problems, it would suggest that human eggs are incapable of ensuring that the correct pattern of sperm-derived imprints are established or maintained. Similar failures in imprinting after cloning could result in birth defects.

  ICSI does cause a minor increase in the frequency of sex-chromosome abnormalities [23; 24], but this is probably a result primarily of genetic defects inherited from the infertile father [25-28] and unrelated to concerns about imprinting.
- *Ooplasmic transfer* involves the transfer of a small amount of cytoplasm from a fresh donor egg (one that has never been frozen) into a recipient egg that for some reason (such as age or mitochondrial abnormalities) is defective for fertilization or postfertilization development. The success of this technique in producing a live human birth [29; 30] suggests that the mixing of cytoplasm from two different cells, as occurs in reproductive cloning, does not necessarily cause problems. It is important to note, however, that the donor cytoplasm in ooplasmic transfer comes

from another egg, whereas the cytoplasm that might come along with the donor nucleus in nuclear transplantation is derived from a somatic cell.

- *Oocyte nuclear transplantation* involves the transfer of an egg nucleus into a fresh egg that lacks its own nucleus. It differs from cloning in that the nucleus is derived from a normal egg rather than a diploid somatic cell, and the procedure is followed by fertilization by a normal haploid sperm. If oocyte nuclear transplantation were successful, however, it would suggest that a nuclear transplantation step *itself*, and the associated manipulations—such as embryo culture, nuclear extraction, and nuclear injection—do not preclude the birth of healthy babies. Oocyte nuclear transplantation has resulted in live births in mice, although the mice have shown growth deficiencies [31]. The procedure has also been carried out in humans, but the resulting blastocyst was terminated [32], and further experimentation was prohibited by the Food and Drug Administration (FDA) [33].
- *Embryo assessment* is the process by which embryos are graded visually for their rate of cell division and degree of "intactness" and therefore likelihood of successful implantation [34; 35]. Those who wish to attempt reproductive cloning might want to take advantage of similar techniques to reduce the number of failed transfers. However, it is not possible to predict which of the embryos deemed intact by embryo assessment will implant successfully [36], so this method will be of limited use to those attempting human reproductive cloning, as is the case for IVF.

People who wish to clone humans with any of those approaches might want to implant multiple embryos, as is frequently done in IVF, to increase the chances of a successful pregnancy. As in IVF, the resulting increase in multiple births would be expected to cause considerable risks of morbidity and death for the child (because of prematurity and low birth weight) and the mother. The risk to the mother might be increased by the possibility of multiple overweight fetuses.

CAN CURRENT ART PROCEDURES BE USED TO ASSESS POSSIBLE RISKS ASSOCIATED WITH CLONING?

No current ART procedure mimics identically the risks inherent in cloning, because current ART procedures all deal with some form of combining sperm and egg and therefore do not give rise to the widespread problems with reprogramming or imprinting that are expected in cloning [37].

The first successful live human birth after IVF was in 1978 [38]. ART procedures, such as IVF, are still new enough that possible long-term effects (for example, adult disorders among the offspring, or disorders in

the children born to IVF children) remain unknown. Studies have not turned up major problems if such factors as the mother's age and the occurrence of multiple pregnancies are taken into account [39], except for an approximately three-fold increase in the frequencies of three very rare conditions (neural tube defects, alimentary atresia and omphalocele) [39].

DOES CLONING PROVIDE BENEFITS NOT PROVIDED BY CURRENT ART PROCEDURES?

With current ART procedures, many people are capable of having a child to whom they have at least some genetic link. Exceptions include people who lack any germ cells because of severe infertility. Human reproductive cloning would provide an alternative for these people.

Future options for those who lack any germ cells may include the use of artificial gametes, where a diploid adult nucleus is reduced to a haploid state before combination with an oocyte haploid genome (although this may result in the same abnormalities seen in animal cloning procedures), and the transfer of male germ cells from donors to testes of sterile men.

CAN THE SCREENING METHODS USED IN ART PROCEDURES BE USED TO PREVENT POTENTIAL SEVERE DEFECTS IN REPRODUCTIVELY CLONED HUMANS?

Preimplantation genetic diagnosis

Preimplantation genetic diagnosis is performed 2-4 days after fertilization on one or two cells removed from the developing preimplantation embryo [40-45]. Whole-genome amplification [46] can be used as an initial step to increase the amount of DNA available for analysis. Chromosomal abnormalities and specific, preidentified mutations can be detected before implantation of a normal embryo. At least in a research context [46], it is possible to start from a single cell's worth of DNA and get sufficient amplification to allow for accurate quantification with comparative genomic hybridization. Researchers have projected that this technique can be abbreviated to make it compatible with the limited time available for preimplantation genetic diagnosis [43], and the same could be true for related techniques that use RNA as a starting material. However, technical challenges must be overcome and accuracy and utility demonstrated.

A similar analysis could be performed on reproductively cloned embryos, but the emphasis would be on detecting errors caused by defective reprogramming or imprinting. (It is important to recognize that any genetic defect present in the nucleus donor, such as a mutation in a gene required for fertility, would be reproduced in the cloned offspring.) Tests

for defective reprogramming or imprinting have not been reported in connection with current preimplantation genetic diagnosis, so the appropriate method would have to be developed first. (At the meeting on August 7, the panel was told that such methods had been developed and applied, but no details were provided [47].) Furthermore, the probable location of the errors would not be known ahead of time. Most genes important for placental function are not active in the morula [48], the only stage when cells can be taken for preimplantation genetic diagnosis, so the functioning of these genes could not be tested with these procedures. For genes that are active in the morula, two tests would be important:

- Expression levels. The amount of RNA or protein product made by each gene should be tested in screens that are capable of assaying for thousands of genes or proteins. The levels should match those seen in normally fertilized embryos. To allow detection of gene transcripts present in low abundance in the embryo, the RNA molecules would first have to be amplified, but this amplification step could be unequal for different RNAs (because of variation in the efficiency of primer hybridization and other factors) and therefore introduce errors [49-51].
- Imprinting levels. This test will be especially difficult in the context of preimplantation genetic diagnosis because the methods used to increase the tiny amounts of DNA available from single embryo cells are currently a challenge for imprinting tests. The location of many imprinted areas in the human genome and the total number of imprinted genes remain unknown [52]. In addition, the observation that imprinting can occur later in development and at dissimilar times in different tissues suggests that examination of imprinting in early embryos might not provide adequate information.

Early embryos often have a mixture of cells, of which some have defects and some do not. Thus, if a given cell is found to lack reprogramming and imprinting errors, it does not guarantee that other cells in the embryo will not have problems.

Postimplantation screening

Screening after implantation is done by acquiring cells through amniocentesis, chorionic villus sampling (CVS), or recovery from maternal blood [53-55]. As with preimplantation genetic diagnosis, cloned embryos would need to be screened for expression levels and imprinting defects. The technical challenges here would be reduced in that more cells would be available for analysis, but they would be complicated because imprinting patterns differ between the embryo and the placenta.

Testing of fetal cells would have to be done with a sample from amniocentesis or maternal blood rather than CVS, because CVS samples placental cells. But testing of placental tissue with CVS might also be important. If human embryonic cells develop a problem, they often become incorporated preferentially into the placenta [56]. The presence of such defective cells in the placenta can be an indicator that a rarer subset of cells in the embryo proper is defective. Placental defects might become apparent at many times during gestation, but in current clinical practice CVS is used only during a narrow time period. (CVS is not used earlier, for fear of causing problems with the pregnancy; and it is not used later, because of a desire to induce any necessary abortion as early as possible in the pregnancy.)

The errors in reprogramming seen in cloned cattle and mouse embryos [57-59] suggest that few cloned embryos will have a perfect expression profile. It is not clear how the "best" embryos would be selected from such an imperfect pool. Errors in the methylation of genes have been seen in both the placenta and tissues of cloned mice [60; 61]. These errors, which involved only about 0.5% of over 1000 DNA regions screened, varied from mouse to mouse and appeared to be random. However, it is not known whether the errors are associated with specific abnormalities [60; 61].

Modifications of imprinting occur in some specific tissues (such as the brain) later in development [62; 63]. It might be impossible to test for the correct occurrence of these modifications, and others occur too late for abortion to be considered. Some cloned animals have developed additional problems (such as late-onset obesity and immune problems; see also Chapter 3) as they have been observed longer.

TO WHAT EXTENT ARE ART PROCEDURES REGULATED IN THE UNITED STATES?

Reproductive cloning can be considered an assisted reproductive technique and thus may be subject to any regulations that cover existing ART procedures. In the United States, ART procedures have generally been subject to minimal oversight and regulation [64-66]. The reasons include a lack of federal funding (and thus lack of institutional review board activity), a lack of FDA review, noncoverage of ART procedures by health-insurance companies, and a paucity of medical malpractice litigation because some level of failure is expected in ART procedures.

Unlike some countries, the United States does not have a structure for evaluating experimental ARTs as they are developed. Nor is information publicly available on the total number of eggs retrieved, the number of embryos donated for research in IVF clinics, or what studies are per-

formed on them. The United Kingdom, in contrast, licenses research and clinical services involving IVF [67] via the Human Fertilisation and Embryo Authority [68].

The Fertility Clinic Success Rate and Certification Act of 1992 provides the only means for national oversight of ART procedures in the United States. That federal legislation requires ART clinics and embryo laboratories to report their pregnancy success rates and follow good laboratory practices [69]. These and other data covering United States ART clinics are published yearly under peer review in *Fertility and Sterility* [3; 70-75] and form the basis of a database that was established in 1987 by the Society of Assisted Reproductive Technologies (SART), an affiliated society of the American Society for Reproductive Medicine (ASRM). Since 1995, SART has collected the data annually from the 373 IVF programs (of about 400 total programs in the United States) that are SART members. These data are provided to the Centers for Disease Control and Prevention (CDC), which analyzes and publishes them, making them available on its website [1].

HAVE ANY ART PROCEDURES EVER BEEN PROHIBITED OR THREATENED WITH PROHIBITION?

In the past, ART procedures have frequently faced opposition and bans that were later lifted. In the 1950s and early 1960s, state bills were introduced to ban, and in some cases criminalize, donor insemination. Similar opposition occurred when IVF was introduced in the 1970s. Both are common procedures today. The concept of surrogate motherhood was introduced in the 1980s, and some state laws ban surrogacy contracts [67].

FINDINGS

4-1. Reproductive cloning efficiencies observed in animals are variable and extremely low compared with efficiencies seen with current human IVF.

4-2. Current techniques for embryo assessment are of limited use in determining the likelihood of successful implantation of a particular embryo.

4-3. No current ART procedure mimics the risks inherent in reproductive cloning, because reproductive cloning involves the use of somatic rather than germ-cell nuclei.

4-4. Tests to detect all the possible errors in imprinting and reprogramming do not exist. Such tests would be difficult to adapt to the small

amount of material and the short period available for preimplantation diagnosis.

4-5. ART procedures have been minimally regulated in the United States, and the lack of regulation has resulted in a shortage of data pertaining to experimental ART procedures and the number of eggs obtained, embryos donated for research, and the studies for which they were used.

4-6. Certification of clinics could allow greater control over any new ART procedures. The United Kingdom might be a model for certifying ART clinics, although the terms of the legislation would have to be adapted to the US federal style of government.

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5

Human Reproductive Cloning: Proposed Activities and Regulatory Context

In this chapter, we describe our understanding of the processes that would be used if anyone conducted human reproductive cloning now or in the near future and the regulatory context in which they would or could operate.

WHAT METHODS ARE LIKELY TO BE USED IF ANYONE CARRIES OUT HUMAN REPRODUCTIVE CLONING NOW OR IN THE NEAR FUTURE?

The methods that might be used now to clone a human would follow the general scheme used to clone other animals. These would be modified according to information peculiar to human biology obtained through research and the observations made while using assisted reproductive technology (ART) procedures. Current technology would be applied for assessing the quality and potential of an embryo before implantation and the health of the fetus during development in a uterus. For preimplantation tests, one or more cells from the preimplantation embryo would be removed and used to test for the quality and integrity of the 46 human chromosomes and for the presence of imprinting errors in one or more genes.

All aspects of such undertakings are open to scientific and clinical questions and uncertainties. The questions and uncertainties were illustrated by the testimony given at the workshop by three people representing organizations that have publicly indicated an intention to carry out reproductive cloning of humans in the near future [1-4]. Their work is

supported by private funds in nonuniversity settings and is likely to be performed outside the United States.

The following table provides a summary of the current arguments and counterarguments regarding human reproductive cloning. Responses are based on the literature (see especially [5; 6] and references cited in Chapter 3) and the testimony of other scientists.

WHAT PROTECTIONS SHOULD BE PROVIDED TO HUMAN SUBJECTS WHO PARTICIPATE IN HUMAN CLONING?

Any participant in human reproductive cloning would require at least the same protection afforded to a participant in any other kind of research. Two overarching international codes provide the basic principles for protecting humans who participate in experiments. The *Nuremberg Code* [50], was articulated in 1947 by the U.S. Military Tribunal No. 1 at the "Doctor's Trial."

The *Nuremberg Code* indicates the following (italics added for emphasis):

> "1. The voluntary consent of the human subject is absolutely essential. This means that the person involved should have legal capacity to give consent; should be situated as to be able to exercise free power of choice, without the intervention of any element of force, fraud, deceit, duress, over-reaching, or other ulterior form of constraint or coercion, and should have sufficient knowledge and comprehension of the elements of the subject matter involved as to enable him to make an understanding and enlightened decision. This latter element requires that before the acceptance of an affirmative decision by the experimental subject there should be made known to him the nature, duration, and purpose of the experiment; the method and means by which it is to be conducted; all inconveniences and hazards reasonably to be expected; and the effects upon his health or person which may possibly come from his participation in the experiment.
>
> The duty and responsibility for ascertaining the quality of the consent rests upon each individual who initiates, directs or engages in the experiment. It is a personal duty and responsibility which may not be delegated to another with impunity.
>
> 2. The experiment should be such as to yield fruitful results for the good of society, unprocurable by other methods or means of study, and not random and unnecessary in nature.
>
> *3. The experiment should be so designed and based on the results of animal experimentation and a knowledge of the natural history of the disease or other problem under study that the anticipated results will justify the performance of the experiment.*

Current Arguments and Counterarguments Regarding Human Reproductive Cloning

Provided below is a summary of some of the current arguments and counterarguments regarding human reproductive cloning. The panel's analysis of each is based on the scientific and medical literature and on presentations at its workshop.

Argument 1: Animal-safety data do not apply, because humans are very different from the animals under study [3]. In particular, a recent study [7] indicated that an important imprinted gene in mice is not imprinted in humans; therefore, imprinting errors would not be a problem in cloned humans.

Counterargument: Placental function, development, and genetic regulation are similar in humans and animal models, such as mice, so similar nuclear transplantation-related defects would be expected [8]. Numerous studies have emphasized that humans and other organisms have the same basic pathways for governing early embryonic and fetal development. Furthermore, widespread defects in all five of the mammalian species that have been reproductively cloned thus far suggest that the defects would affect basic biological functions in humans.

Even if one less gene is imprinted in humans as compared to mice, humans are known to have many imprinted genes (possibly as many as 100), and any number of these are likely to cause problems in reproductively cloned humans.

Argument 2: Frequent failures are seen in normal human reproduction; cloning would be no different [3].

Counterargument: Errors in normal human reproduction occur primarily early in pregnancy; many of the women in question are never aware that they are pregnant. In contrast, many of the defects in reproductively-cloned animals arise late in pregnancy or after birth.

Argument 3: Inappropriate culture media for the initial cells cause most cloning-related problems [1; 2]. Culture media for human assisted reproductive technologies have been better optimized [2; 3]. Synchronization between the implanted embryo and the recipient uterus has also been better in human than in animal assisted reproductive technology procedures.

Counterargument: Culture effects appear to account for only some of the defects observed [9; 10]. Many defects in various organ systems are peculiar to reproductive cloning. Expertise in existing human assisted reproductive technologies is not relevant to these problems, because the defects appear to arise from biological rather than purely technical causes [9].

Argument 4: Those who have cloned animals stress the failures, but there are also many successes in animal reproductive cloning [2; 3].

4. The experiment should be so conducted as to avoid all unnecessary physical and mental suffering and injury.

5. *No experiment should be conducted where there is an a priori reason to believe that death or disabling injury will occur;* except, perhaps, in those experiments where the experimental physicians also serve as subjects.

Counterargument: The statement is true, but does not necessarily apply to human reproductive cloning. In humans, the likelihood and benefit of success must be weighed against the probability, severity, and lifelong consequences of failure. Failures are all but certain in any human reproductive cloning attempt at this time, based on the experience with animals, and in humans, the consequences could be far more devastating. The likelihood and benefit of possible success must be weighed against the high probability and severe consequences of failure.

Argument 5: Existing preimplantation and postimplantation genetic tests could be used to detect abnormalities, allowing selection of embryos to be implanted and therapeutic abortion in case of any problems. In contrast, there has been no genetic testing and weeding out of animal reproductive clones.

In preimplantation testing, two cells could be removed from an eight-cell morula. One cell could be tested for correctness of the chromosome complement and the other for imprinting errors at one or more DNA sites [4]. It has been claimed that such imprinting tests have been performed with DNA from cells after somatic cell nuclear transfer (SCNT) [3], although no data have been presented. Postimplantation testing could include testing for chromosomal errors, the checking of imprinting status at up to 30 sites, and the measurement of production levels from many genes with DNA chips [11] or reverse-transcription polymerase chain reaction [4].

Counterargument: Many errors would not be detectable until late in pregnancy or after birth, when therapeutic abortion would not be an option. Many of the relevant genetic tests have not yet been developed [2; 9]; existing genetic tests appropriate for single-gene inherited disorders or gross chromosomal rearrangements are insufficient because they are not relevant to the major sources of errors expected in human cloning. Ultrasonographic tests cannot detect the small-scale defects in tissues, such as lung, that have had devastating consequences in newborn animal clones [12; 13], and there is insufficient evidence regarding the possible impact of imprinting errors on brain development in humans.

Argument 6: Voluntary informed consent allows potential participants to make their own decisions and elect to take the risks if they so choose.

Counterargument: Our current regulatory system recognizes that when information is lacking it can be difficult or impossible to inform subjects fully. That is the case with respect to human reproductive cloning because the extent of the risks is unknown, and the greatest risk of abnormality, morbidity, and mortality is borne by the cloned fetus/child, who cannot give informed consent. In addition, there are risks borne by the woman donating the eggs and the gestational mother.

When subjects cannot be fully informed, and when a procedure is clearly risky, there is a role for both regulatory agencies and professionals to limit the options available to a subject if the evidence supports such a limitation [14]. Societal concerns can also be taken into account.

6. The degree of risk to be taken should never exceed that determined by the humanitarian importance of the problem to be solved by the experiment.

7. Proper preparations should be made and adequate facilities provided to protect the experimental subject against even remote possibilities of injury, disability, or death.

> 8. The experiment should be conducted only by scientifically qualified persons. The highest degree of skill and care should be required through all stages of the experiment of those who conduct or engage in the experiment.
>
> 9. During the course of the experiment the human subject should be at liberty to bring the experiment to an end if he has reached the physical or mental state where continuation of the experiment seems to him to be impossible.
>
> 10. During the course of the experiment the scientist in charge must be prepared to terminate the experiment at any stage, if he has probable cause to believe, in the exercise of the good faith, superior skill and careful judgement required by him that a continuation of the experiment is likely to result in injury, disability, or death to the experimental subject."

Private physician groups have also adopted codes of conduct, including the World Medical Association *Declaration of Helsinki: Recommendations Guiding Medical Doctors in Biomedical Research Involving Human Subjects* which was adopted initially in 1964 and revised as recently as October 2000 [51-53].

In the United States, the National Institutes of Health (NIH) established policies for the protection of human participants in 1966, which subsequently became regulations in 1974. The National Commission for the Protection of Human Subjects of Biomedical and Behavorial Research met from 1974 to 1978. Its report, called *The Belmont Report*, set forth basic ethical principles for the conduct of biomedical and behavorial research involving human participants. These principles are:

- *Respect for Persons* involves a recognition of the personal dignity and autonomy of individuals and special protection of those persons with diminished autonomy.
- *Beneficence* entails an obligation to protect persons from harm by maximizing anticipated benefits and minimizing risks.
- *Justice* requires that the benefits and burdens of research be distributed fairly.

The Federal Policy for the Protection of Human Subjects was adopted in 1991. This is sometimes called the "Common Rule" as it provides a uniform human subject protection system for most relevant federal agencies and departments [54]. In addition, the Department of Health and Human Services has adopted additional protections for various populations. One section is particularly relevant to human reproductive cloning research—Subpart B "Additional Protections Pertaining to Research, Development, and Related Activities Involving Fetuses, Pregnant Women,

and Human In Vitro Fertilization." The Food and Drug Administration has a separate set of regulations, but they closely parallel the common rule and differ in detail to accommodate FDA's statutory responsibilities to regulate food, drugs, devices, and biologics. The Common Rule provides more specific procedures than the general codes described above, including the use of Institutional Review Boards (IRBs) (IRB Guidebook 1993; NBAC, 2001).

An Institute of Medicine (IOM) report, *Preserving Public Trust: Accreditation and Human Participant Protection Programs* (2001) [15], states that human-subjects protection should:

- Ensure that the research design is sound and that a study's promise for augmenting knowledge justifies the involvement of human participants.
- Assess the risks and benefits independently of the investigators who carry out the research.
- Ensure that participation is voluntary and informed.
- Ensure that participants are recruited equitably and that risks and benefits are fairly distributed.

All participating subjects must give informed consent if it is possible, and experimentation involving vulnerable subjects should receive special review and heightened human-subjects protection procedures. An infertile man who wishes to be cloned suffers no risk other than the risk of losing a substantial amount of money. A woman impregnated with a clone faces risks, and the greatest risks of abnormality, morbidity, and death will be borne by the newborn, or older clone, who is in no position to give informed consent.

HOW ARE HUMAN-SUBJECTS OF RESEARCH PROTECTED?

The current system for ensuring the ethical conduct of research with humans in the United States is centered on review of the proposed research by Institutional Review Boards (IRBs). IRB review of research that involves human subjects, such as experiments in human reproductive cloning, is mandatory under federal regulations under either of two conditions:

(1) If the research involves a drug, device, or biologic subject to Food and Drug Administration (FDA) approval, it falls under FDA human-subjects regulations and must be approved by an IRB.

(2) If the research is carried out at an institution that accepts federal funds or has an "assurance" agreement with the federal government, it is covered by the federal Common Rule. This requires IRB approval when

the research involves human subjects, with a few exceptions not relevant to human reproductive cloning experiments.

Many institutions have signed agreements stating that they will extend IRB review to cover all research, whether funded by the federal government or not. Such an extension of the standard assurance is not required, however, and some institutions—including many ART clinics—either have signed only the minimal agreement covering federally funded research or receive no federal research funds and have no assurance document with the federal government.

In both cases listed above, work is subject to IRB review only if it is classed as "research" under the regulations, which define research as "a systematic investigation, including research development, testing and evaluation, designed to develop or contribute to generalizable knowledge" (45 *CFR* 46.102(d)) [16].

Those proposing human reproductive cloning experiments could claim they are conducting "innovative therapy," and not "research," so that their work would fall outside the human-subjects regulatory framework. As described below, however, human reproductive cloning experiments should be intended, at least in part, to contribute to development, testing, and evaluation of a technique that has never been tried in humans. While it might indeed by considered "innovative therapy," it would also constitute research.

IN THE ABSENCE OF A CLONING BAN IN THE UNITED STATES, HOW WOULD HUMAN REPRODUCTIVE CLONING BE REGULATED, IF AT ALL?

How does the federal government regulate medical care?

In general, the federal government does not have specific powers under the Constitution to regulate medical care, but there are several means by which it regulates medical research and clinical practice via its powers over taxation, spending, and interstate commerce [17]. Funding of a person or organization can be made contingent on that person's or organization's following regulations, such as human-subjects regulations that cover federally funded research. Similarly, the federal government can require states to take actions as a prerequisite for receiving funds in a related field, such as the requirement that states regulate in vitro fertilization if they are to receive funding in connection with the Aid to Families with Dependent Children program [17].

Most infertility clinics do not receive federal funds, so it would not be possible for the federal government to regulate them directly on the basis

of funding. Some have suggested that clinics could be regulated by the federal government, "whether or not they receive federal funds, if patients travel across state lines to use them, if supplies come from out of state, and if the doctors attend conferences in other states" [18]. Thus, the federal government potentially could either require the states to regulate any human reproductive cloning attempts as a condition of their receiving healthcare-related federal funds or regulate it directly, under its power to regulate interstate commerce (similar to the way it regulates organ transplantation) [17]. In addition, states under their inherent police powers can regulate the licensing of medical personnel and medical facilities.

Can institutional review boards regulate human reproductive cloning research?

IRB's can regulate human reproductive cloning research, under some conditions; however, some human reproductive cloning research may fall outside federal oversight.

Individuals carrying out new ART procedures can avoid IRB oversight either by claiming that the investigations do not constitute research, and instead characterizing their work as "innovative therapy" or a clinical service, or by avoiding federal funds for the research and conducting the work at institutions that do not have a federal assurance agreement that covers their work.

Attempts at human reproductive cloning should be construed as research because, as described under the regulations covering human-subjects protections (45 *CFR* 46.102) [16], they should be carried out as "systematic investigation, including research development, testing and evaluation, designed to develop or contribute to generalizable knowledge."

Can the Food and Drug Administration regulate human reproductive cloning?

Although the FDA does not have authority to regulate the practice of medicine, it does have the authority over entities trying to create drugs or biological treatments. In a 1998 "Dear Colleague" letter, FDA asserted that it had regulatory jurisdiction "over clinical research using cloning technology to create a human being" under the Public Health Service Act and the Federal Food, Drug, and Cosmetic Act (FFDCA) [19]. The assertion was restated in a July 6, 2001, letter. Three reasons have been provided for FDA's reasoning [22]:

- Designation of cloning materials as "biological products."
- Designation of such products as "drugs."
- Regulation of cloning procedures as involving "medical devices."

In its 1997 proposal, FDA argued that reproductive human cloning involves a "biological product" in that the human egg cell must undergo more than minimal manipulation, which occurs when a procedure "alters the biological characteristics (and potentially the functional integrity) of those cells or tissues, or when adequate information does not exist to determine whether the processing will alter the biological characteristics of the cell or tissue" [23]. That proposal has since been clarified by a final rule promulgated on January 19, 2001 [24].

Concerns have been expressed, most recently at a congressional hearing on human reproductive cloning, that such jurisdiction stretches FDA authority too far [20; 21].

FDA has asserted its jurisdiction, so unless it is successfully challenged human cloning will require premarket approval of any cellular or tissue-based products. Some suggest that FDA's jurisdiction would not be recognized by the courts, because the courts do not consider pregnancy to be a disease and the intended use of cloning products would be to create a new life, not to treat, diagnose, or prevent a pregnancy [22; 25]. FDA has until recently refrained from attempting to regulate ART, which involves many of the same techniques as would be used in human reproductive cloning.

In contrast, another commentator recently stated that "as a practical matter . . . if FDA says it has authority, it does have authority until somebody challenges it and a court says it does not, and courts generally are quite deferential to regulatory agencies who are interpreting their own enabling language" [14].

The initial stages of FDA review are generally confidential. Open FDA advisory committees early in the process have been rare and strongly opposed by industry. Thus, any FDA review of human reproductive cloning research is unlikely to occur in public early in the process without a change in the FDA statute (FFDCA). Furthermore, current FDA review procedures concentrate on safety and efficacy and cannot take ethical issues into account.

Can the Recombinant DNA Advisory Committee regulate human reproductive cloning?

The Recombinant DNA Advisory Committee [26] at NIH has reviewed experiments involving recombinant DNA and human gene transfer since 1977. Its formal authority is restricted to review of federally funded research, although medical researchers in the private sector have generally also submitted their work for review [26; 27]. The RAC's authority is to provide advice to federal agencies that can terminate or suspend federal grants and contracts.

A similar body could be established to regulate cloning; but unless its terms of establishment differed from those governing the RAC, the new body's authority would not extend beyond federally funded research.

What other policy mechanisms could be used to regulate human reproductive cloning?

New legislation or executive action would be required to set up a review system if the system is to cover both public and private sectors and be open to the public. A review body could probably be created under the National Institutes of Health (NIH) authorization statute (although this might not be binding for research that is not federally funded), using the model of human-subjects regulations and recombinant-DNA guidelines of the 1970s. Without explicit legislation, however, the review body's authority might not extend to privately funded research at institutions that do not have a signed assurance with the federal government. Such authority would have to be established by federal legislation that granted additional powers to NIH, the Office of Human Research Protections, FDA, or a new regulatory body created for the purpose.

Another mechanism for restricting human reproductive cloning activities is the tort system, using either the existing negligence standard for medical malpractice or a revised strict liability standard for medical malpractice (proposed by Charo [14]). Especially under the latter system, the threat of litigation would act as a strong deterrent to the practice of any procedure that has a great likelihood of failure.

Yet another mechanism would be a regulatory body similar to that in place in the United Kingdom. The UK body—the Human Fertilisation and Embryology Authority—was enacted by Parliament in 1990 and operated voluntarily before then. It oversees and licenses all ART procedures in both the private and public sectors.

HOW DOES A MORATORIUM COMPARE WITH OTHER POTENTIAL POLICY INTERVENTIONS RELATED TO HUMAN REPRODUCTIVE CLONING?

A moratorium is similar to a ban in its immediate consequences, but as "a suspension of activity pending further analysis or action" [28] it implies that the issue will be revisited later. In contrast, a ban would prohibit by federal statute efforts to clone human beings. The penalties could be criminal or civil.

Have others suggested a human reproductive cloning moratorium?

The National Bioethics Advisory Commission (NBAC) report on human cloning, issued in 1997 [29], concluded that human reproductive

cloning was not safe, in that it imposed unacceptable risks on the life and health of the fetus and the surrogate mother. The NBAC recommended a moratorium on the use of federal funding to support human reproductive cloning. It also suggested that there be a voluntary moratorium in the private sector and that the federal government cooperate with other nations and international organizations to enforce any common aspects of their policies on human reproductive cloning. (The NBAC also recommended federal legislation to prohibit human reproductive cloning.) The Clinton administration's moratorium restricted the use of federal funding for human reproductive cloning. Those who desire to carry out reproductive cloning in humans, however, are not planning to use federal funds.

Voluntary moratoriums have been proposed by various industrial and professional associations, such as the Federation of American Societies for Experimental Biology [30], the American Medical Association [31; 32], the Association of American Medical Colleges [31] and the American Society for Reproductive Medicine [33].

WHAT TYPES OF LEGISLATION ARE UNDER CONSIDERATION WITH RESPECT TO HUMAN REPRODUCTIVE CLONING?

US federal laws

A number of bills that would regulate human reproductive cloning have been introduced in Congress. In general, they are in two categories. The first set of bills would ban both human reproductive cloning and nuclear transplantation to produce stem cells. The second set would ban only human reproductive cloning.

While the present report was being developed, a bill introduced by Representative Dave Weldon was passed by the House of Representatives. It would outlaw, with criminal penalties, the production of a reproductively-cloned human embryo and would also outlaw nuclear transplantation to produce human embryonic stem cell lines. It would also prohibit the importation of any medical treatments from abroad that were created from such activity. Alternatives to that bill, such as the bill of Representative James Greenwood, would ban human reproductive cloning but would permit the use of nuclear transplantation to produce stem cells; the House defeated an amendment to the Weldon bill proposing this alternative. Similar bills are under discussion in the Senate [20].

US state laws

California, Michigan, Louisiana, Virginia, and Rhode Island have banned human cloning [34]. Legislation has been proposed in Illinois,

Massachusetts, and New York. A number of states (including California and New York) have laws that apply the federal research regulations to research with human beings conducted within the state that is not otherwise covered by the federal rules (because it is not sponsored by a federal agency).

Human reproductive cloning in the California statute is defined as "the practice of creating or attempting to create a human being by transferring the nucleus from a human cell from whatever source into a human egg cell from which the nucleus has been removed for the purpose of, or to implant, the resulting product to initiate a pregnancy that could result in the birth of a human being" (California Health and Safety Code 24187 as cited in [35]). Most state laws have difficulties with respect to implementation. For example, if a cow egg were used instead of a human egg, the California law would not apply [35].

A blanket ban on nuclear transplantation could have unintended consequences, such as an inability to use the process for preimplantation genetic diagnosis or the treatment of some mitochondrial diseases.

International treaties and laws

Several other countries have instituted human reproductive cloning bans. The Council of Europe[1] [36], in a protocol signed by 19 nations, banned human reproductive cloning research defined as "any intervention seeking to create a human being genetically identical to another human being, whether living or dead." The explanatory memorandum to the protocol specifies that *human being* is to be interpreted according to domestic law; so nuclear transplantation to produce stem cells might be banned in some countries but not others.

Germany and the United Kingdom have not signed the protocol, because they are not signatories to the underlying Bioethics Convention. Germany forbids all research on human embryos. In the United Kingdom, human reproductive cloning is now banned by law, but nuclear

[1]The Council of Europe is an intergovernmental organization focused on human rights and other issues. Although The Council of Europe is an intergovernmental organization which aims to (1) protect human rights, pluralist democracy and the rule of law; (2) promote awareness and encourage the development of Europe's cultural identity and diversity; (3) seek solutions to problems facing European society (discrimination against minorities, xenophobia, intolerance, environmental protection, human cloning, AIDS, drugs, organized crime, etc.); (4) help consolidate democratic stability in Europe by backing political, legislative and constitutional reform. The Council of Europe should not be confused with the European Union. The two organizations are quite distinct. The 15 European states, however, are all members of the Council of Europe.

transplantation to produce cells and tissues for research or experimental treatment is not prohibited.

The foreign ministers of France and Germany intend to launch a joint UN initiative "on the question of human cloning in order to establish its unacceptability as a practice contrary to human dignity, and to enshrine its prohibition in a universal legal instrument" [37]. More up-to-date information about the constantly-changing laws from around the world dealing with human reproductive cloning are collected by the Association of Global Lawyers and Physicians (http://www.glphr.org).

WOULD A MORATORIUM ON HUMAN REPRODUCTIVE CLONING HOLD?

A voluntary moratorium has worked in the past to delay scientific research. The moratorium leading up to a meeting at Asilomar, California, in 1975 successfully delayed recombinant-DNA research until proper guidelines could be put into place [38-44]. The moratorium was conceived by the molecular biology community and imposed on itself, and it was eventually supplanted by a federally sanctioned set of guidelines and a prospective group review process [28]. The moratorium and guidelines succeeded in part for two reasons that do *not* pertain to human reproductive cloning today. First, there was a strong consensus on the value of observing the moratorium among the practicing scientists most capable of doing the work, both in the United States and elsewhere. Second, when the Recombinant DNA Advisory Committee was established and its guidelines put into place, the vast majority of research biologists in the United States were funded by NIH or the National Science Foundation, so the sanction—loss of federal grants—was a strong disincentive.

A voluntary moratorium is unlikely to work for human reproductive cloning, because reproductive technology is widely accessible in numerous private fertility clinics that are not subject to federal research regulations. Several groups have already signaled their intention to forge ahead despite scientific consensus that the techniques are not ready for human application.

Would a ban on human reproductive cloning be legal in the United States?

A number of legal scholars believe that a ban on human reproductive cloning would not be considered constitutional in that it might contravene both a right of privacy (specifically, a perceived right to procreative liberty [45; 46]) and a right of scientific inquiry. In addition, it is possible

that the courts would rule that an egg or nucleus donor has the right to control what happens to the embryos created.

At this time however, there is no reason to expect that the Supreme Court will expand the right to privacy to include human reproductive cloning. In the case of a right of privacy, the Supreme Court has recognized the right of persons to decide whether to "bear or beget a child" [47]. The Supreme Court has not considered whether ART procedures—particularly an asexual procedure, such as reproductive cloning—are accorded the same considerations. Some, however, do not believe that human reproductive cloning should be treated in the same way as other ART procedures with regard to reproductive rights, because it departs too much from sexual reproduction. Difficulty in assigning parentage might, for example, be a competing state interest in relation to the national authority promoting a right of privacy [48].

In the case of a right of scientific inquiry, scientific research is viewed as a means of exercising free speech. This right, although implicit in many Supreme Court cases, has never been explicitly defined [49]. The existence of state and federal restrictions on research with human subjects suggests that there is a difference between research that poses no threat to others and research that may harm human beings or other important interests.

FINDINGS

5-1. Those who wish to undertake human reproductive cloning lack the fundamental biological knowledge, demonstration of safety in animals, and testing methods to make it a safe course of action. The panel believes that any such effort would contravene international ethics codes for research on human subjects, such as Article 5 of the *Nuremberg Code* [50], which states in part that "no experiment should be conducted where there is an a priori reason to believe that death or disabling injury will occur."

5-2. If human reproductive cloning is ever to be undertaken responsibly, it would need to be done systematically with the intention of creating reliable knowledge. Any responsible efforts toward human reproductive cloning would therefore conform to the federal definition of research. As such, whether the source of funding is public or private, the research would be subject to a review by a review board independent of the investigators conducting the research, such as the Institutional Review Board. (Those who wish to reproductively clone humans are more interested in being the first to be successful with human cloning than in collecting reliable knowledge. This "first of its kind" venture, without systematic

data collection, is not considered "research" under current federal regulation.) If responsible research on human reproductive cloning were undertaken, it may be considered "innovative therapy," but that does not escape the need to protect the rights and interests of those participating in the research or the need for independent external review.

5-3. Any future attempt at human reproductive cloning would constitute human-subjects research. As such, it would best be regulated according to the following conditions:

- The review process would be applied equally to both public- and private-sector research.
- The review process would be made open to the public. That would not be the case if review were restricted to FDA unless FDA took special measures, such as those recently taken to make data relevant to the safety of gene-transfer trials and transplantation of animal organs public.
- The review process would (1) decide the criteria that should be used to judge whether protocols are ready for human experimentation (that is, set the rules) and (2) review the protocols involving human experiments to see that they satisfy these criteria (that is, apply the rules). Those two functions could be carried out by a single body or by two distinct bodies.
- The review process would have to take into account ethical issues beyond clinical safety and efficacy (see, for example, the NBAC report [29]). FDA review does not cover such issues, so FDA review by itself would be incomplete.

New legislation or executive action would be required to set up a review system so that it would cover both public and private sectors and be open to the public.

5-4. A voluntary ban or moratorium is unlikely to work, given that reproductive technology is widely accessible in numerous private fertility clinics that are not subject to federal research regulations. A ban enforced by legislation would probably need to carry substantial civil or criminal penalties to have an impact on such activities within the United States.

5-5. If a ban on research in human reproductive cloning is reassessed, participants in any such research efforts would need to be afforded human-subjects protection as described in the Nuremberg and Helsinki codes, US law, and the IOM report *Preserving Public Trust: Accreditation and Human Participant Protection Programs* (2001) [15]. Such protections include external technical and ethical review by review boards to ensure that proposed experiments are technically and ethically sound. The review boards should be independent of the investigators conducting the research.

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6

Findings and Recommendations

Human reproductive cloning is currently the subject of much debate around the world, involving a variety of ethical, religious, societal, scientific, and medical issues. This report from the National Academies addresses only the scientific and medical aspects of human cloning. Consideration of the medical aspects has required the panel to examine issues of scientific conduct and human-subjects protection. But we have not attempted to address the issue of whether cloning, if it were found to be scientifically safe, would or would not be acceptable to individuals or society. Instead, the panel defers to others on the fundamental ethical, religious, and societal questions, and presents this report on the scientific and medical aspects to inform the broader debate. This report differs in this respect from the last major report on the topic in the United States, *Cloning Human Beings*, a 1997 report developed by the National Bioethics Advisory Commission (NBAC) [1].

Four of the questions in our statement of task remain for the panel to answer:

- What scientific and medical criteria should be used to evaluate the safety of cloning a person?
- What issues of responsible conduct of research are raised by the prospect of cloning a person?
- What process should be used to evaluate future scientific and medical evidence regarding cloning a person?
- Based on the current scientific and medical evidence, should there

be a moratorium on the cloning of a person? What are the implications of doing so? Of not doing so? If a moratorium is enacted, when should the issue be re-evaluated?

The panel's findings with respect to these questions are presented here and are followed by our recommendations based on them.

THE FINDINGS THAT SUPPORT A BAN ON HUMAN REPRODUCTIVE CLONING

It is a serious event when any group that has potential authority over research intercedes to ban it, and the reasons must therefore be compelling. We are convinced that the scientific and medical data concerning the likely danger to the implanted fetus or the eventual newborn if reproductive cloning of humans is attempted in the near future are very compelling.

The panel has based its support for the proposed ban on human reproductive cloning on the following findings:

Finding 1: The scientific and medical criteria used to evaluate the safety of reproductive cloning must be the potential morbidity and death of the woman carrying the clone as a fetus and of the newborn and the risk to women donating the eggs.

Finding 2: Data on the reproductive cloning of animals through the use of nuclear transplantation technology demonstrate that only a small percentage of attempts are successful; that many of the clones die during gestation, even at late stages; that newborn clones are often abnormal or die; and that the procedures may carry serious risks for the mother. In addition, because of the large number of eggs needed for such experiments, many more women would be exposed to the risks inherent in egg donation for a single cloning attempt than for the reproduction of a child by the presently used *in vitro* fertilization (IVF) techniques. These medical and scientific findings lead us to conclude that the procedures are now unsafe for humans.

Finding 3: At least three criteria would have to be fulfilled before the safety of human reproductive cloning could be established:

(1) The procedures for animal reproductive cloning would have to be improved to such an extent that the levels of observed abnormalities in cloned animals, including nonhuman primates, were no more than that seen with existing human assisted reproductive technology (ART) procedures. If that could not be achieved, researchers would have to demonstrate that humans are different from other

animals with regard to cloning-related defects. Reproducible data demonstrating that a successful reprogramming of the donor nucleus and proper imprinting can be achieved in animals would be essential, as would an understanding of the mechanisms responsible for such events.

(2) New methods would have to be developed to demonstrate that the human preimplantation embryos produced through the use of nuclear transplantation technology are normal with respect to imprinting and reprogramming. That would best be done by first establishing the normal state of reprogramming and imprinting in nonhuman primates and then documenting that the processes in preimplantation human embryos are substantially similar.

(3) Methods would have to be developed to monitor—effectively and comprehensively—preimplantation embryos and fetuses in the uterus for cloning-related defects, such as those outlined in Chapter 3; these include alterations in gene expression and imprinting.

Finding 4: The issues of responsible conduct of research raised by the prospect of cloning a person are those of medical ethics—in particular, the protection of the participants (the egg donor, the host mother, and the child produced through cloning) in any human cloning research. Participants in any human cloning research efforts require full protection as human research participants, although it should be noted that, as with fetal surgery, this protection cannot be extended fully to the cloned fetus. Human reproductive cloning has not been performed before, and its introduction, if it ever occurred, would require systematic research. That research would likely entail full review by institutional review boards and other human-subjects protections, including informed consent of donors and recipients of all biological materials.

Finding 5: If any attempts at human reproductive cloning were ever to occur, they would constitute research, not merely innovative therapy. Such research could then be subject to external technical and ethical review by review boards to ensure that the proposed experiments are both technically and ethically sound and that the rights and welfare of all research participants are protected. This institutional review process should be applied equally to both public- and private-sector research and be transparent to the public.

Finding 6: Because medical and scientific findings indicate that cloning procedures are currently not safe for humans, cloning of a human through the use of nuclear transplantation technology is not now appropriate. The panel believes that no responsible scientists or physicians are

likely to undertake to clone a human. Nevertheless, no voluntary system that is established to restrict reproductive cloning is likely to be completely effective. Some organizations have already announced their intention to clone humans, and many of the reproductive technologies needed are widely accessible in private fertility clinics that are not subject to federal regulations. The panel therefore concludes that a legally enforceable ban that carries substantial penalties has a much greater potential than a voluntary system or moratorium to deter any attempt to clone a human using these techniques.

Finding 7: If no ban is imposed, it is possible that some organizations will attempt the reproductive cloning of humans. Although such attempts would most likely fail, there is a high probability they would be associated with serious risks to any possible fetus or newly born child and may harm the woman carrying the developing fetus.

Finding 8: There is concern that legislation or regulation that would ban reproductive human cloning would set a troubling precedent with respect to the restriction of innovative, experimental research and medical procedures. Modern scientific research proceeds rapidly, and its findings are unpredictable and often surprising. It is probable that at least every 5 years there will be significant new information regarding the issues of the safety and applicability of human cloning to medical practice. The above concern can be ameliorated by including in any legislation or regulation a requirement for an updated evaluation of the scientific, medical, and societal issues within 5 years. Such a requirement for periodic reviews would allow for extensive public debate regarding reproductive human cloning and the consideration of modifications to the legislation. Part of that evaluation would include a recommendation as to when the next such evaluation should be conducted.

Finding 9: Two activities will be particularly important for an updated evaluation of human reproductive cloning: a thorough scientific and medical review to evaluate whether the procedures are likely to be safe and effective, and a broad national dialogue on the societal, religious, and ethical issues. As part of this process, any persons advocating the practice of human reproductive cloning would need to acknowledge the extent of the abnormalities seen in animal cloning experiments and to demonstrate that these problems—assuming that they still persist—are unlikely to occur in humans.

Finding 10: Any future process designed to evaluate the scientific and medical evidence on cloning a person would likely need to involve

scientists, physicians, ethicists, and the public. A public debate could be facilitated by a committee that issues regular updates on the state of the science surrounding animal cloning and reaches out to involved constituencies in a systematic manner. Such a body could derive its powers by executive order, by executive action within the Department of Health and Human Services under the Public Health Service Act, or by legislation. Among many other issues, the debate should be structured to inform the public that clones are not precise replicas, but persons with identical genetic material.

Finding 11: The science of cloning is an international one with research conducted throughout the world. Furthermore, the issue of human reproductive cloning is the subject of worldwide debate. A number of countries and international organizations have prepared reports and issued statements on the issue. Participation by the United States in such international debates about human reproductive cloning will be beneficial to any future process to evaluate the scientific and medical evidence on this issue.

Finding 12: The limited regulation and monitoring of experimental ART procedures in the United States means that important data needed for assessing novel ART procedures are in some cases lacking, in other cases incomplete and hard to find. Because the panel was not charged to investigate ART regulation and did not solicit expert testimony thereon, we make no recommendations regarding oversight of, registration of, or required data collection from ART clinics. But we do believe that a request from Congress or the Executive Branch for a panel of experts to study the matter and report its findings and recommendations publicly would probably be useful. Having such information is likely to be beneficial to any process of evaluating future scientific and medical evidence regarding both reproductive cloning and new ART procedures.

IMPLICATIONS OF THE PROPOSED BAN ON REPRODUCTIVE CLONING FOR NUCLEAR TRANSPLANTATION TO PRODUCE STEM CELLS

As part of our panel's charge, we were asked: "Based on the current scientific and medical evidence, should there be a moratorium on the cloning of a person? What are the implications of doing so? Of not doing so?" This raises the question of the implications of a ban on human reproductive cloning for the very different process of nuclear transplantation to produce stem cells.

None of the findings summarized in the preceding section that sup-

port the panel's conclusions regarding a ban on human reproductive cloning would support a ban on the use of the nuclear transplantation technology to produce stem cells. An independent recent report from the National Academies has emphasized that there is a great potential for studies on stem cells isolated through nuclear transplantation to increase the understanding and potential treatment of various diseases and debilitating disorders, as well as fundamental biomedical knowledge. The diseases and debilitating disorders include "Lou Gehrig's disease" (amyotrophic lateral sclerosis, or ALS), Parkinson's disease, Alzheimer's disease, spinal-cord injury, cancer, cardiovascular diseases, diabetes, and rheumatoid arthritis. The necessary research would entail transfer of human somatic cell nuclei into enucleated human eggs for the purpose of deriving blastocysts and embryonic stem cells and stem cell lines; there would be no implantation in a uterus. Some have expressed concern that this research might nevertheless be misdirected to human reproductive cloning. If our recommendation is adopted, the development and birth of a newborn would be criminalized by a legally-enforceable ban on any such attempts at implantation.

The committee that produced the report from the National Academies entitled *Stem Cells and the Future of Regenerative Medicine* considered a wide range of views on the ethical and societal issues involved in the production of human embryonic stem cells—including nuclear transplantation technology [2]. After carefully considering all sides of the issue, that committee produced the following conclusion and recommendation concerning this technology:

> **Conclusion:** Regenerative medicine is likely to involve the implantation of new tissue in patients with damaged or diseased organs. A substantial obstacle to the success of transplantation of any cells, including stem cells and their derivatives, is the immune-mediated rejection of foreign tissue by the recipient's body. In current stem cell transplantation procedures with bone marrow and blood, success hinges on obtaining a close match between donor and recipient tissues and on the use of immunosuppressive drugs, which often have severe and potentially life-threatening side effects. To ensure that stem cell-based therapies can be broadly applicable for many conditions and people, new means of overcoming the problem of tissue rejection must be found. Although ethically controversial, the somatic cell nuclear transfer technique promises to have that advantage. Other options for this purpose include genetic manipulation of the stem cells and the development of a very large bank of ES cell lines [2].

> **Recommendation:** In conjunction with research on stem cell biology and the development of potential stem cell therapies, research on approaches that prevent immune rejection of stem cells and stem cell-derived tissues should be actively pursued. These scientific efforts include the use of a number of techniques to manipulate the genetic makeup of stem cells, including somatic cell nuclear transfer [2].

Our panel includes members who participated in the workshop held at the National Academies on June 23, 2001. This workshop was convened as part of the data-gathering process for the separate committee that produced the above report focused on stem cells. We have also conducted our own extensive literature review and consulted with many of the world's leaders in nuclear transplantation to produce stem cells in our own workshop held on August 7, 2001 — including I. Wilmut, R. Jaenisch, R. Yanagimachi, J. Cibelli, P. Mombaerts, and A. Trounson (see Appendix C). Based on this review and discussion, the panel determined that although there is a clear therapeutic potential for techniques in which stem cells are produced through nuclear transplantation (as in Figure 2), this potential is nascent and needs considerable research. As described in Chapter 2, the potential of this research also includes developing a broader understanding of how human tissue cells develop normally, and how human diseases that have a genetic component are caused at a cellular level.

THE PANEL'S CONCLUSIONS AND RECOMMENDATIONS

The panel has examined and analyzed the scientific, medical, and legal literature on the issue, and heard testimony at a workshop from experts in animal cloning, assisted reproductive technologies, and science, technology, and legal policy—including people who, on scientific and medical grounds, either oppose or defend human cloning. After carefully considering the issues raised, we conclude that the case has not been proven that human reproductive cloning would lead to fewer negative outcomes at this time than reproductive cloning in other mammals, and we make the following recommendations:

> **Human reproductive cloning should not now be practiced. It is dangerous and likely to fail. The panel therefore unanimously supports the proposal that there should be a legally enforceable ban on the practice of human reproductive cloning. For this purpose, we define human reproductive cloning as the placement in a uterus of a human blastocyst derived by the technique that we call nuclear transplantation. In reaching this con-**

clusion, we considered the relevant scientific and medical issues, including the record from cloning other species, and the standard issues that are specifically associated with evaluating all research involving human participants.

The scientific and medical considerations related to this ban should be reviewed within 5 years. The ban should be reconsidered only if at least two conditions are met: (1) a new scientific and medical review indicates that the procedures are likely to be safe and effective and (2) a broad national dialogue on the societal, religious, and ethical issues suggests that a reconsideration of the ban is warranted.

Finally, the scientific and medical considerations that justify a ban on human reproductive cloning at this time are not applicable to nuclear transplantation to produce stem cells. Because of its considerable potential for developing new medical therapies for life-threatening diseases and advancing fundamental knowledge, the panel supports the conclusion of a recent National Academies report that recommended that biomedical research using nuclear transplantation to produce stem cells be permitted. A broad national dialogue on the societal, religious, and ethical issues is encouraged on this matter.

SUMMARY

This panel was charged with assessing the scientific and medical evidence surrounding human reproductive cloning. Most of the relevant data on reproductive cloning are derived from animal studies. The data reveal high rates of abnormalities in the cloned animals of multiple mammalian species and lead the panel to conclude that reproductive cloning of humans is not now safe. Our present opposition to human reproductive cloning is based on science and medicine, irrespective of broader considerations. The panel stresses, however, that a broad ethical debate must be encouraged, so that the public can be prepared to make decisions if human reproductive cloning is some day considered medically safe for mothers and offspring.

The panel's discussion inevitably included a comparison of the methods used for reproductive cloning and nuclear transplantation to produce stem cells. The panel is in agreement with the recent report from the National Academies entitled *Stem Cells and the Future of Regenerative Medicine* [2] in affirming the potential for studies on stem cells isolated through nuclear transplantation. The probable benefits include advances in funda-

mental biomedical knowledge, as well as the understanding and treatment of various diseases and debilitating disorders.

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2. COMMITTEE ON STEM CELLS AND THE FUTURE OF REGENERATIVE MEDICINE, BOARD ON LIFE SCIENCES AND BOARD ON NEUROSCIENCE AND BEHAVIORAL HEALTH. **Stem Cells and the Future of Regenerative Medicine. Report of the National Academy of Sciences and the Institute of Medicine.** 2001 Sep.

Appendixes

A

Panel and Staff Biographical Information

PANEL

Provided below is biographical information for the members of the Panel on Scientific and Medical Aspects of Human Cloning. Most panel members receive funding from federal agencies, particularly the National Institutes of Health and the National Science Foundation, and the Department of Energy, to support their research. In addition, many belong to professional scientific disciplinary societies. As is the case with many such scientific organizations, these societies frequently take public positions in favor of increased government funding for research. No panel members are involved with corporations or personally conduct research in reproductive cloning or in the use of nuclear transplantation to produce embryonic stem cells.

Irving L. Weissman (chair) is Karel and Avice Beekhuis Professor of Cancer Biology and professor of pathology and developmental biology at Stanford University. Dr. Weissman was a member of the Scientific Advisory Board of Amgen (1981-1989), DNAX (1981-1992), and T-Cell Sciences (1988-1992). He was a cofounder of SyStemix and was chairman of its Scientific Advisory Board and a member of its Board of Directors in 1988-1997. He also cofounded StemCells, Inc. and is a director and chair of its Scientific Advisory Board. His main research interests are hematopoietic stem cells, lymphocyte differentiation, and phylogeny of the immune system. He is past president (1994) of the American Association of Immu-

nologists. Dr. Weissman is a member of the National Academy of Sciences and the recipient of several awards, including the Leukemia Society of America de Villier's International Achievement Award, and the E. Donnall Thomas Prize of the American Society of Hematology.

Arthur L. Beaudet is Henry and Emma Meyer Professor and Chair of the Department of Molecular and Human Genetics and professor in the Department of Molecular and Human Genetics, Department of Pediatrics, and Department of Cell Biology at the Baylor College of Medicine in Houston, Texas. He received his BA from Holy Cross and his MD from Yale University School of Medicine. His research interests include the molecular abnormalities that cause Prader Willi syndrome and Angelman syndrome. Dr. Beaudet is a member of the Institute of Medicine.

Patricia K. Donahoe is chief of Surgical Pediatric Services and director of Pediatric Surgical Research Laboratories at Massachusetts General Hospital, where she has worked virtually her entire career. She is the Marshall K. Bartlett Professor of Surgery and a member of the biochemical and biological sciences graduate program at the Harvard Medical School. She is chair of the Scientific Advisory Board of St. Jude's Medical Center and has been a member of the Scientific Advisory Board of Memorial Sloan-Kettering Cancer Center and of the National Institute of Child Health and Human Development National Advisory Council. Dr. Donahoe received her MD from Columbia University and her BS from Boston University, where she now serves on the Board of Trustees. She is a member of the National Academy of Sciences and of the Institute of Medicine.

David J. Galas is vice president, chief academic officer and Norris Professor of Applied Life Science at Keck Graduate Institute of Applied Life Sciences (KGI). Before helping to found and develop KGI, a research and educational institution in the applied life sciences, Dr. Galas served as president and chief scientific officer of Seattle-based Chiroscience R & D Inc., a genomics and drug-discovery company formed through the acquisition of Darwin Molecular Corporation, which Dr. Galas helped to start in 1993. Before his involvement in biotechnology, Dr. Galas served as director for health and environmental research at the US Department of Energy (DOE) Office of Energy Research, where he headed DOE's Human Genome Project from 1990 to 1993. He was professor of biological sciences at the University of Southern California from 1981 to 1993. He is on the board of directors of Impath Inc. and the scientific advisory boards of several companies (none of which are engaged in cloning research of any kind). He received his PhD in physics from the University of California, Davis-Livermore and his undergraduate degree in physics from the

University of California, Berkeley. He has held positions at the University of Geneva, Switzerland, and the University of California's Lawrence Livermore Laboratory.

Judith G. Hall is a clinical geneticist and pediatrician. She trained at Wellesley College, the University of Washington School of Medicine, and Johns Hopkins Hospital. She is professor of pediatrics and medical genetics at the University of British Columbia based at Children's & Women's Health Centre of British Columbia in Vancouver, BC Canada. Her research interests are human congenital anomalies, including neural tube defects; the genetics of short stature; newly recognized mechanisms of disease, such as mosaicism and imprinting; the natural history of genetic disorders; the genetics of connective tissue disorders, such as arthrogryposis; dwarfism; and monozygotic twins. She has described numerous new syndromes and defined the natural history of many disorders. The book she coedited on human malformations received the Association of American Publishers Award for best medical book published in 1993. Dr. Hall is a member of many professional organizations, editorial boards and councils. Most recently, she has been president of the American Society of Human Genetics and the American Pediatric Society. She is an officer of the Order of Canada.

Brigid L.M. Hogan is an investigator with the Howard Hughes Medical Institute and Hortense B. Ingram Professor in the Department of Cell Biology at Vanderbilt University School of Medicine. She obtained her PhD from Cambridge University, England, and carried out postdoctoral training at the Massachusetts Institute of Technology. Before moving to the United States, she was head of the Laboratory of Molecular Embryology, first at the Imperial Cancer Research Fund and then at the National Institute of Medical Research in London. Dr. Hogan is a member of the European Molecular Biology Organization and the Institute of Medicine. She is also a Fellow of the American Academy of Arts and Sciences and a Fellow of The Royal Society of London.

Robert B. Jaffe is Fred Gellert Professor of Reproductive Medicine and Biology and director of the Center for Reproductive Sciences, Department of Obstetrics, Gynecology, and Reproductive Sciences at the University of California, San Francisco School of Medicine. He received his MD from the University of Michigan, Ann Arbor and an MS in endrocrinology from the University of Colorado, Denver. His expertise and research interests are in endocrinology and metabolism and in obstetrics and gynecology. He is a Fellow of the Royal College of Obstetricians and Gynaecologists and a member of the Institute of Medicine.

Edward R.B. McCabe serves as professor and executive chair of the Department of Pediatrics at the UCLA School of Medicine. He is responsible for establishing the UCLA Children's Hospital (renamed the Mattel Children's Hospital at UCLA), where he serves as physician-in-chief. He is the director of the UCLA Center for Society, the Individual and Genetics. He is chair of the Secretary's Advisory Committee on Genetic Testing. His memberships include the American College of Medical Genetics (president, 2001-2002) and the American Board of Medical Genetics (president, 1995-1996). For the American Academy of Pediatrics (AAP), he was the chair of the Committee on Genetics (1987-1991), and co-founder (1990) and chair of the executive committee (1993-1995) of the Section on Genetics and Birth Defects. He also co-chaired the Newborn Screening Taskforce (1999) which was sponsored by the AAP and the Health Resources and Service Administration. He is a member of the American Society for Biochemistry and Molecular Biology, and the American Society of Human Genetics. Dr. McCabe received his BS in biology from Johns Hopkins University and his MD and PhD from the University of Southern California. His research focuses on developmental molecular genetics. He is a member of the Institute of Medicine.

Anne McLaren is principal research associate of The Wellcome Trust and Research Campaign, Institute of Cancer and Developmental Biology, at the University of Cambridge. She did her undergraduate and postgraduate studies at Oxford University. She was director of the Medical Research Council's Mammalian Development Unit in London for 18 years, until 1992. For the previous 15 years, she worked for the Agriculture Research Council, in C.H. Waddington's Institute of Animal Genetics in Edinburgh. She was a member of the UK government's Warnock Committee on Human Fertilisation and Embryology, served on the Voluntary (later Interim) Licensing Authority for human in vitro fertilization and embryology, and is now a member of the UK Human Fertilisation and Embryology Authority that regulates in vitro fertilization and human embryo research in the UK. She chaired the scientific and Technical Advisory Group of the World Health Organization's Human Reproduction Programme and has been a member of the Nuffield Foundation's Bioethics Council. She is a member of the European Group on Ethics that advises the European Commission on social and ethical implications of new technologies.

Gerald M. Rubin is vice president for biomedical research at the Howard Hughes Medical Institute. He is also professor of genetics at the University of California, Berkeley and adjunct professor of biochemistry and biophysics at the University of California, San Francisco, School of Medi-

cine. He received his BS in biology from the Massachusetts Institute of Technology and his PhD in molecular biology from the University of Cambridge, England. Dr. Rubin's postdoctoral work was done at Stanford University with David Hogness. He has held faculty positions at Harvard Medical School and the Carnegie Institution of Washington. Dr. Rubin is a member of the National Academy of Sciences and counts among his honors the American Chemical Society Eli Lilly Award in biological chemistry.

Mark Siegler is the Lindy Bergman Distinguished Service Professor at the University of Chicago, professor in the Department of Medicine, and director of the MacLean Center for Clinical Medical Ethics. An honors graduate of Princeton University, he received his MD in 1967 from the University of Chicago. In 1984, the University of Chicago established the Center for Clinical Medical Ethics, one of the first centers in the nation devoted to this clinical specialty, and appointed Dr. Siegler as its director. He has practiced general medicine for more than 30 years and is one of the few physicians who combines expertise in medical ethics with active medical practice. Dr. Siegler currently serves on the ethics committee of the American College of Surgeons, on the advisory board of the Spanish Bioethics Institute (Madrid), and is a member of the Association of American Physicians.

INSTITUTIONAL OVERSIGHT

President, Institute of Medicine

Kenneth I. Shine is professor of medicine emeritus at the University of California, Los Angeles, School of Medicine. He is the school's immediate past dean and provost for medical services and he was director of the Coronary Care Unit, chief of the Cardiology Division, and chair of the Department of Medicine. Dr. Shine has served as chairman of the Council of Deans of the Association of American Medical Colleges and as president of the American Heart Association. His research interests include metabolic events in the heart muscle, the relation of behavior to heart disease, and emergency medicine.

Chair, Committee on Science, Engineering, and Public Policy

Maxine F. Singer is president of the Carnegie Institution of Washington (Washington, DC) and is a biochemist whose wide-ranging research on RNA and DNA has greatly advanced scientific understanding of viral and human genes. Dr. Singer received her bachelor's degree from Swarth-

more College (1952) and her PhD from Yale University (1957). She worked at the National Institutes of Health as a research biochemist in the National Institute of Arthritis and Metabolic Diseases until 1975, studying the synthesis and structure of RNA. In 1975, she moved to the National Cancer Institute. She received the Distinguished Presidential Rank Award, the highest honor given to a civil servant, and the National Medal of Science in 1991. Dr. Singer is also director of Perlegen Sciences, Inc. (a biotechnology startup) and is on the Board of Directors at Johnson & Johnson.

Chair, Board on Life Sciences

Corey Goodman, PhD, is President and CEO of Renovis, Inc., a neuroscience biotechnology company. He is also Professor of Neurobiology in the Department of Molecular and Cell Biology, and the Wills Neuroscience Institute, at the University of California, Berkeley. He served formerly as Howard Hughes Medical Institute Investigator, and co-founder and Director of the Wills Neuroscience Institute. His expertise is in developmental neurobiology for which he is recognized for his use of genetic analysis to elucidate the molecular mechanisms that control the wiring of the brain. Dr. Goodman was elected a member of the National Academy of Sciences in 1995, and in January of 2001 he became chair of the Board on Life Sciences of the National Research Council. His many honors include the Alan T. Waterman Award in 1983, the Gairdner Award in 1997, and the March-of-Dimes Prize in Developmental Biology in 2001. He also serves as president of the McKnight Endowment Fund for neuroscience. He is cofounder of two biotechnology companies—Exelixis and Renovis. He received his BS in biology from Stanford University and his PhD in developmental neurobiology from the University of California, Berkeley.

PROFESSIONAL STAFF

Deborah D. Stine (Study Director) is associate director of the Committee on Science, Engineering, and Public Policy (COSEPUP) and director of the Office of Special Projects. She has worked on various projects in the National Academies since 1989. She received a National Research Council group award for her first study for COSEPUP, on policy implications of greenhouse warming, and a Commission on Life Sciences staff citation for her work in risk assessment and management. Other studies have addressed international benchmarking of US research fields, graduate and postdoctoral education, responsible conduct of research, careers in science and engineering, and many environmental topics. She holds a bachelor's degree in mechanical and environmental engineering from the Uni-

versity of California, Irvine; a master's degree in business administration; and a PhD in public administration, specializing in policy analysis, from the American University. Before coming to the National Academies, she was a mathematician for the US Air Force, an air-pollution engineer for the state of Texas, and an air-issues manager for the Chemical Manufacturers Association.

Robert Cook-Deegan is a senior program officer for the National Cancer Policy Board of the Institute of Medicine (IOM) and the Division on Earth and Life Studies and for IOM's Health Sciences Policy Board. He is also a Robert Wood Johnson Health Policy Investigator at the Kennedy Institute of Ethics, Georgetown University, where he is writing a primer on how national policy decisions are made about health research, and a seminar leader for the Stanford-in-Washington program, for which he recently directed a world survey of genomics research.

William Wells is a consultant science writer for the project. He received a BS from the University of Adelaide, Australia, in 1989, and a PhD for work on cell-cycle checkpoints from the University of California, San Francisco in 1995. He then worked at Current Biology Ltd. in San Francisco as an in-house editor for *Chemistry & Biology*. When the journal moved to London a year later, he began 5 years of full-time freelancing. He is now the news editor for the *Journal of Cell Biology* in New York.

Susan Chandra Daniels is a science-research consultant for the project. She received a bachelor's degree in biology and French from Wheaton College (IL) in 1993 and a PhD in molecular and cell biology from Brandeis University in 2000. She has done research on fertilization and early embryogenesis in the sea urchin and reprogramming of somatic cell nuclei in the frog *Xenopus laevis*. Her PhD thesis research focused on the molecular genetics of the sensory nervous system of a soil nematode. In 2001, she served as a Christine Mirzayan Science Policy Intern at the National Academy of Sciences, where she worked with the Board on Life Sciences on several projects related to agricultural biotechnology policy.

Frances E. Sharples has served as the director of the Board on Life Sciences since October 2000. Immediately before that, she was a senior policy analyst for the Environment Division of the White House Office of Science and Technology Policy (OSTP) for 4 years. Dr. Sharples went to OSTP from the Oak Ridge National Laboratory, where she served in various positions in the Environmental Sciences Division between 1978 and 1996, most recently as a Research and Development Section head. Dr. Sharples received her BA in biology from Barnard College and her MA

and PhD in zoology from the University of California, Davis. She served as an American Association for the Advancement of Science (AAAS) Environmental Science and Engineering Fellow at the Environmental Protection Agency during the summer of 1981 and as an AAAS Congressional Science and Engineering Fellow in the office of Senator Albert Gore in 1984-1985. She was a member of the National Institutes of Health's Recombinant DNA Advisory Committee in the middle 1980s and was elected a Fellow of AAAS in 1992.

Richard E. Bissell is executive director of the Policy and Global Affairs Division of the National Research Council and director of the Committee on Science, Engineering, and Public Policy. He took up his positions in 1998, having served as coordinator of the Interim Secretariat of the World Commission on Dams (1997-1998) and as a member and chairman of the Inspection Panel at the World Bank (1994-1997). He worked closely with the National Academy of Sciences during his tenure in senior positions at the US Agency for International Development (1986-1993) as head of the Bureau of Science and Technology and head of the Bureau of Program and Policy Coordination. He has published widely in political economy, and he taught at Georgetown University and the University of Pennsylvania. He received his BA from Stanford University (1968) and his MA and PhD from Tufts University (in 1970 and 1973).

B

Animal Reproductive Cloning Data Tables on Reproductive Cloning Efficiency and Defects

The purpose of these tables is to provide an overview of the data from animal cloning experiments done to date (August 2001). Table 1 describes the success/failure rates of reproductive cloning in animals, and Table 2 provides details of the defects or lack of observable defects in reproductively cloned animals. These data were obtained through a comprehensive review of the publications cited in the "Reference" column of each table. Only experiments that yielded live-born cloned offspring were included in the table.

Tables 1 and 2 developed by the panel are supplemented by Tables 3 and 4 developed by Lewis et al., 2001. Note that Tables 3 and 4 use the term "cytoplast" for what the panel calls "enucleated egg."

How to read Table 1:

Example: The first line from the table can be read as following:

In the experiments described in the paper published by Campbell in 1996 (Column 12), 244 sheep embryos were created using somatic cell nuclear transplantation techniques. The donor nuclei were taken from epithelial-like cells grown from a culture of embryonic stem cells (Column 2). Of these 244 embryos, only 34, or 14%, went on to develop into the morula or blastocyst embryos that are used in the embryo transfer procedure (Column 4). All 34 of those developing embryos were transferred into the wombs of female sheep (as we can tell from Column 8, which indicates

number of embryos transferred). Of those 34 embryos, only 8 individual pregnancies resulted (Column 5). Of those 8 pregnancies, 3, or 38%, ended in miscarriage, and 5, or 63%, went on to produce live offspring (Columns 6 and 7, respectively). Of the five lambs that were born alive, only 2 (40%) survived until the time of publication. In all, 2% of the 244 embryos created resulted in live offspring (Column 9), and 12.5% of the 34 embryos transferred into recipient female sheep resulted in live offspring (Column 8).

How to read Table 2:

Any given line in Table 2 gives an overview of the clinical outcomes of each animal reproductive cloning experiment. For example, in line 1, in the sheep nuclear transplantation experiments published by Campbell in 1996 (Column 7), no information was given concerning the defects seen in miscarried fetuses (Column 3) or about the characteristics of placentas from these pregnancies (Column 6). However, Columns 4 and 5 indicate that 2/5 of the cloned lambs produced in this experiment were healthy and normal, whereas 3/5 died of unknown causes.

Note about Figures 1, 2, and 3

Figures 1, 2, and 3 were generated based on data presented in Table 1. Certain experiments whose results are displayed in Table 1 were omitted from the graphs due to incomplete data for all categories displayed in the graphs. Data from reproductive cloning experiments using embryonic, fetal and adult cells as nucleus donors were included in these graphs.

Tables and Figures

TABLE 1 Rates of Success/Failure of Somatic Cell Nuclear Transfer in Mammals

1	2	3	4	5	6	7
Species[a]	Cell type[b]	# Embryos produced[c]	# Embryos developed into morula/ blastocyst (%)[d]	# Fetuses after embryo transfer[e]	# Fetuses miscarried (%)[f]	# Live births/ Total # fetuses (%
Sheep	Embryo-derived epithelial-like	244	34 (14)	8	3 (38)	5/8 (63
	Adult mammary gland	277	29 (12)	1	0 (0)	1/1 (100
	Fetal fibroblast	172	47 (27)	5	2 (40)	3/5 (60
	Embryo-derived epithelial-like	385	126 (33)	15	11 (73)	4/15 (27
	Fetal fibroblast	507	69 (13.6)	14	7 (50)	7/14 (50
	ES cell line-derived epithelial-like	128	31 (24.2)	>9	>7 (~78)	2/>9 (<22
	ES cell line-derived epithelial-like	258	44 (17)	>11	>10 (~91)	1?>11 (<9
	ES cell line-derived epithelial-like	423	75 (18)	8	5 (63)	3/8 (38
	ES cell line-derived fibroblast-like	158	39 (31)	10	7 (70)	3/10 (30
	ES cell line-derived fibroblast-like	187	51 (27)	15	8 (53)	7/15 (47
	Fetal fibroblast	417	80 (19)	20	6 (30)	14/20 (70
Cattle	Blastomere (embryonic)	641	152 (24)	>13	>4 (~31)	N/A
	Blastomere (embryonic)	132	84 (64)	N/A	N/A	N/A
	Embryonic stem cell	239	42 (18)	N/A	N/A	N/A
	Fetal fibroblast	276	33 (12)	6	2 (33)	4/6 (67
	Adult mural granulosa from 13 yr old cow	621	259 (42)	28	26 (93)	2/28 (7.1

	7	8	9	10	11	12
uses arried (%)[f]	# Live births/ Total # fetuses (%)[g]	# Live births/ # Embryos transferred to uterus(%)[h]	# Live births/ # Embryos produced (%)[i]	# Offspring alive or healthy at time of publication/ # Live births (%)[j]	Phenotypes observed[k]	Reference[L]
3 (38)	5/8 (63)	5/34 (12.5)	5/244 (2.0)	2/5 (40)	#	Campbell 1996
0 (0)	1/1 (100)	1/29 (3.4)	1/277 (.36)	1/1 (100)	#	Wilmut 1997
2 (40)	3/5 (60)	3/40 (7.5)	3/172 (1.7)	2/3 (67)	E#	Wilmut 1997
11 (73)	4/15 (27)	4/87 (4.6)	4/385 (1.0)	4/4 (100)	#	Wilmut 1997
7 (50)	7/14 (50)	7/67 (10.4)	7/507 (1.3)	5/7 (71)	BC#	Schnieke 1997
7 (~78)	2/>9 (<22)	2/31 (6.5)	2/128(1.6)	2/2 (100)	CE#	Wells 1997 in vivo-matured oocytes
0 (~91)	1?>11 (<9)	1/44 (2.3)	1/258 (.39)	0/1 (0)	BEF	Wells 1997 in vitro-matured ooctyes
5 (63)	3/8 (38)	3/75 (4.0)	3/423 (.7)	2/3 (67)	B#	Wells 1998[n] experiment 1
7 (70)	3/10 (30)	3/39 (7.7)	3/158 (1.9)	1/3 (33)	B#	Wells 1998[n] experiment 2
8 (53)	7/15 (47)	7/44 (16)	7/187 (3.7)	2/7 (29)	BE#	Wells 1998[n] experiment 3
6 (30)	14/20 (70)	14/80 (17.5)	14/417 (3.4)	3/14 (21)	E#	McCreath 2000
4 (~31)	N/A	9/59 (15)	9/641 (1.4)	N/A	N/A	Chesne 1993
N/A	N/A	19/78 (24)	19/132 (14)	N/A	N/A	Cheong 1993
N/A	N/A	4/34 (12)	4/239 (1.7)	N/A	N/A	Sims 1994
2 (33)	4/6 (67)	4/28 (14.3)	4/276 (1.4)	3/4 (75)	ABCF#	Cibelli 1998
26 (93)	2/28 (7.1)	2/74 (2.7)	2/621 (.32)	1/2 (50)	CD#	Wells 1998[o]

continues

TABLE 1 Continued

1	2	3	4	5	6	7
Species[a]	Cell type[b]	# Embryos produced[c]	# Embryos developed into morula/ blastocyst (%)[d]	# Fetuses after embryo transfer[e]	# Fetuses miscarried (%)[f]	# Live births/ Total # fetuses (%
	Adult cumulus	47	18 (38)	5	0 (0)	5/5 (100
	Adult oviduct epithelial	94	20 (21)	3	0 (0)	3/3 (100
	Adult mural granulosa	552	383 (69)	45	35 (78)	10/45 (22
	Adult mammary gland epithelium	140	36 (26)	>2	>1	1/>2 (<50
	Adult ear skin fibroblast	82	49 (60)	>5	>1	1/>5 (<20
	Fetal germ cell	279	85 (30)	>17	>16	1/>17 (<6
	Fetal fibroblast	174	35 (20)	>3	>1	2/>3 (<67
	Adult skin cell from ES cell clone	175	N/A	1	0 (0)	1/1 (100
	Adult muscle	346	73 (21)	8	4 (50)	4/8 (50
	Fetal fibroblast	876	>110? (>13)	>36	>28 (~78)	8/36 (22
	Adult senescent fibroblast	1896	87 (4.6)	>18	>11 (~61), 1 induced[m]	6/>18 (<33
	Adult fibroblast from 17 yr old bull	338	103 (30)	12	6 (50)	6/12 (50
	Many adult and fetal types	1502	596 (40)	>50	>26 (~52)	24/>5((<48
	Adult and fetal fibroblast	N/A	N/A	>54	>50 (~92)	4/>5- (<7.4
	Adult fibroblast from 21 yr old bull	190	53 (28)	6	1 induced[m]	1/6 (17
Mice	Adult cumulus	2468	1385 (56)	N/A	N/A	N/A
	Embryonic stem cell	36	23 (64)	N/A	N/A	N/A
	Mural trophectoderm	26	16 (62)	N/A	N/A	N/A
	Adult fibroblast	463	377 (81)	N/A	N/A	N/A
	Immature adult Sertoli cell	1846	436 (24)	235	219 (93)	16/23 (6.8

	7	8	9	10	11	12
uses arried (%)[f]	# Live births/ Total # fetuses (%)[g]	# Live births/ # Embryos transferred to uterus(%)[h]	# Live births/ # Embryos produced (%)[i]	# Offspring alive or healthy at time of publication/ # Live births (%)[j]	Phenotypes observed[k]	Reference[L]
0 (0)	5/5 (100)	5/6 (83)	5/47 (11)	2/5 (40)	#	Kato 1998
0 (0)	3/3 (100)	3/4 (75)	3/94 (3)	2/3 (67)	#	Kato 1998
35 (78)	10/45 (22)	10/100 (10)	10/552 (1.8)	10/10 (100)	ABC#	Wells 1999
>1	1/>2 (<50)	1/4 (25)	1/140 (.7)	1/1 (1)	#	Zakhartchenko 1999[p]
>1	1/>5 (<20)	1/16 (6.3)	1/82 (1.2)	0/1 (0)	AG	Zakhartchenko 1999[p]
>16	1/>17 (<6)	1/32 (3.1)	1/279 (.36)	0/1 (0)	N/A	Zakhartchenko 1999[q]
>1	2/>3 (<67)	2/7 (29)	2/174 (1.1)	1/2 (50)	AB#	Zakhartchenko 1999[r]
0 (0)	1/1 (100)	1/6 (16)	1/175 (.57)	0/1 (0)	CD	Renard 1999
4 (50)	4/8 (50)	4/26 (15)	4/346 (1.2)	1/4 (25)	ABG#	Shiga 1999
8 (~78)	8/36 (22)	8/110 (7.2)	8/876 (.9)	6/8 (75)	BCF#	Hill 1999
(~61), uced[m]	6/>18 (<33)	6/79 (7.6)	6/1896 (.32)	6/6 (100)	ABD#	Lanza 2000[s]
6 (50)	6/12 (50)	6/54 (11)	6/338 (1.2)	4/6 (67)	AD#	Kubota 2000
6 (~52)	24/>50 (<48)	24/172 (14)	24/1502 (1.6)	13/24 (54)	ADEG#	Kato 2000
0 (~92)	4/>54 (<7.4)	4/243 (1.6)	4/?	1/4 (25)	BCDF#	Hill 2000[t]
uced[m]	1/6 (17)	1/26 (3.8)	1/190 (.52)	1/1 (100)	BD#	Hill 2000[u]
N/A	N/A	31/1385 (2.2)	31/2468 (1.3)	22/31 (71)	#	Wakayama 1998
N/A	N/A	2/18 (11)	2/36 (5.6)	N/A	N/A	Tsunoda 1998
N/A	N/A	2/25 (8)	2/26 (7.7)	N/A	N/A	Tsunoda 1998
N/A	N/A	3/274 (1.1)	3/463 (.6)	1/3 (33)	BF#	Wakayama 1999
19 (93)	16/235 (6.8)	16/436 (3.7)	16/1846 (.87)	15/16 (94)	GF#	Ogura 2000[v]

continues

TABLE 1 Continued

1	2	3	4	5	6	7
Species[a]	Cell type[b]	# Embryos produced[c]	# Embryos developed into morula/ blastocyst (%)[d]	# Fetuses after embryo transfer[e]	# Fetuses miscarried (%)[f]	# Live births/ Total # fetuses (%
	Tail tip fibroblast	753	260 (41)	126	119 (94)	7/126 (5.6
	Adult cumulus	3920	N/A	N/A	N/A	N/A
	Fetal fibroblast	938	278 (30)	45	40 (89)	5/45 (11
	Adult cumulus (from hybrid strains)	4326	2583 (60)	N/A	N/A	N/A
	Embryonic gonadal cell	179	114 (64)	N/A	N/A	N/A
	Embryonic stem cell (from hybrid strain)	783	169 (22)	N/A	N/A	N/A
Goat	Blastomere (embryonic)	354	96	N/A	N/A	45/
	Fetal fibroblast	230	89 (39)	20	17 (85)	3/20 (15
	Fetal fibroblast	198	157 (79)	>6?	N/A	N/A
Pig	Adult granulosa	>401	401 (?)	9	4 (44)	5/9 (55
	Fetal fibroblast	210	188 (90)	N/A	N/A	N/A
	Fetal body cell	143	N/A	N/A	N/A	N/A
	Fetal genital ridge	340	N/A	N/A	N/A	N/A
Monkey	Blastomere (embryonic)	78	59 (76)	3	1/3 (33)	2/3 (67

A = High birth weight
B = Pulmonary problems
C = Cardiovascular abnormalities
D = Immune system abnormalities/infection
E = Kidney and/or liver abnormalities
F = Placental abnormalities
G = Joint malformations or other gross deformities
# = Healthy offspring produced

NOTE: "N/A" indicates that no data were available in the cited publication.
NOTE: ES cell = embryonic stem cell.
NOTE: (~) indicates percentages extrapolated from data available, as shown in other columns.

[a]The species of animal used in the experiment.
[b]The cell type used as the source of the donor nucleus for the nuclear transfer.

	7	8	9	10	11	12
uses arried (%)[f]	# Live births/ Total # fetuses (%)[g]	# Live births/ # Embryos transferred to uterus(%)[h]	# Live births/ # Embryos produced (%)[i]	# Offspring alive or healthy at time of publication/ # Live births (%)[j]	Phenotypes observed[k]	Reference[L]
19 (94)	7/126 (5.6)	7/280 (2.5)	7/753 (.93)	7/7 (100)	#	Ogura 2000[w]
N/A	N/A	35/? (>.9%?)	35/3920 (.89)	34/35? (97?)	#	Wakayama 2000
40 (89)	5/45 (11)	5/272 (1.8)	5/938 (.53)	3/5 (60)	BGF#	Ono 2001
N/A	N/A	80/2573 (3.1)	80/4326 (1.8)	N/A	#	Wakayama 2001
N/A	N/A	6/114 (5.2)	6/179 (3.4)	5/6 (83)	#	Wakayama 2001
N/A	N/A	28/? (>16.6?)	28/783 (.36)	22/28 (79)	ABF#	Eggan 2001
N/A	45/?	45/141 (32)	45/354 (13)	N/A	#	Yong 1998
17 (85)	3/20 (15)	3/85 (3.5)	3/230 (1.3)	3/3 (100)	#	Baguisi 1999
N/A	N/A	6/97 (6.1)	6/198 (3.0)	3/6 (50)	#D	Keefer 2001
4 (44)	5/9 (55)	5/401 (1.2)	5/>401 (<1.2)	5/5 (100)	#	Polejaeva 2000
N/A	N/A	1/110 (.9)	1/210 (.5)	1/1 (100)	#	Onishi 2000
N/A	N/A	2/143 (1.4)	2/143 (1.4)	N/A	N/A	Betthauser 2000
N/A	N/A	2/164 (1.2)	2/340 (.59)	N/A	N/A	Betthauser 2000
/3 (33)	2/3 (67)	2/29 (6.9)	2/78 (2.6)	2/2 (100)	#	Meng 1997

continues

TABLE 1 Continued

[c]The number of embryos that were successfully formed after the nuclear transfer (cloning) procedure (in the literature usually referred to as # "fused" or # "reconstituted").
In cases where this number was not available, the total number of oocytes injected with nuclei was used (including both successful and failed attempts to produce embryos).
In cases of double (serial) nuclear transfer, numbers of successfully reconstructed embryos from the second transfer were used.
[d]The number and percentage of cloned embryos that continued to develop past the one-cell stage into multicellular embryos called morulae or blastocysts.
[f]The number of fetuses that were spontaneously aborted at any time during the pregnancy.
[g]The proportion of pregnancies that were carried to term (comparison of # live births to total # pregnancies).
[I]The proportion of cloned embryos that went on to become live offspring (comparison of live births to the number of cloned embryos created).
[j]The survival rate of live born clones after birth (comparison of live born offspring to the number still alive at the time of publication of the reference from which the data were obtained).
[k]The letters indicate categories of characteristics observed in cloned animals (miscarried, live born or those that died after birth). Categories are provided above, in a key located below the table.
[L]The peer reviewed scientific article in which data for any given experiment were published. Full references can be found in the bibliography.
[m]Fetal miscarriage (abortion) was induced by researchers for medical or research reasons.
[n]Wells et al. Cloning sheep from cultured embryonic cells. *Reprod. Fertil. Dev.* 1998; 10:615-626.
[o]Wells et al. Adult somatic cell nuclear transfer is used to preserve the last surviving cow of the Enderby Island cattle breed. *Reprod. Fertil. Dev.* 1998; 10:369-378.
[p]Zakhartchenko et al. Adult cloning in cattle: Potential of nuclei from a permanent cell line and from primary cultures. *Mol. Reprod. Fertil.* 1999; 54:264-272.
[q]Zakhartchenko et al. Potential of fetal germ cells for nuclear transfer in cattle. *Mol. Reprod. Dev.* 1999; 52:421-426.
[r]Zakhartchenko et al. Effects of serum starvation and re-cloning on the efficiency of nuclear transfer using bovine fetal fibroblasts. *J. Reprod. Fertil.* 1999; 115:325-331.
[s]Lanza et al. Extension of cell life-span and telomere length in animals cloned from senescent somatic cells. *Science* 2000 Apr 28; 288:665-669.
[t]Hill et al. Evidence for placental abnormality as the major cause of mortality in first-trimester somatic cell cloned bovine fetuses. *Biol. Reprod.* 2000; 63:1787-1794.
[u]Hill et al. Development rates of male bovine nuclear transfer embryos derived from adult and fetal cells. *Biol. Reprod.* 2000; 62:1135-1140.
[v]Ogura et al. Production of male cloned mice from fresh, cultured, and cryopreserved immature Sertoli cells. *Biol. Reprod.* 2000; 62:1579-1584.
[w]Ogura et al. Birth of mice after nuclear transfer by electrofusion using tail tip cells. *Mol. Reprod. Dev.* 2000; 57:55-59.

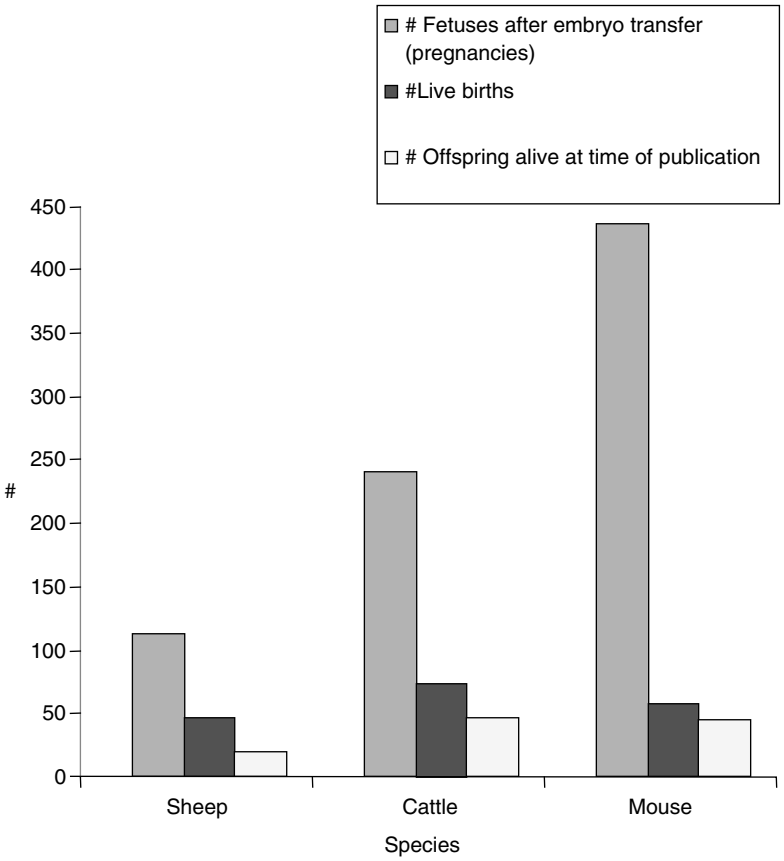

FIGURE 1 Survival Rates of Sheep, Cattle and Mouse Embryos Cloned from Adult, Fetal and Embryonic Cells.

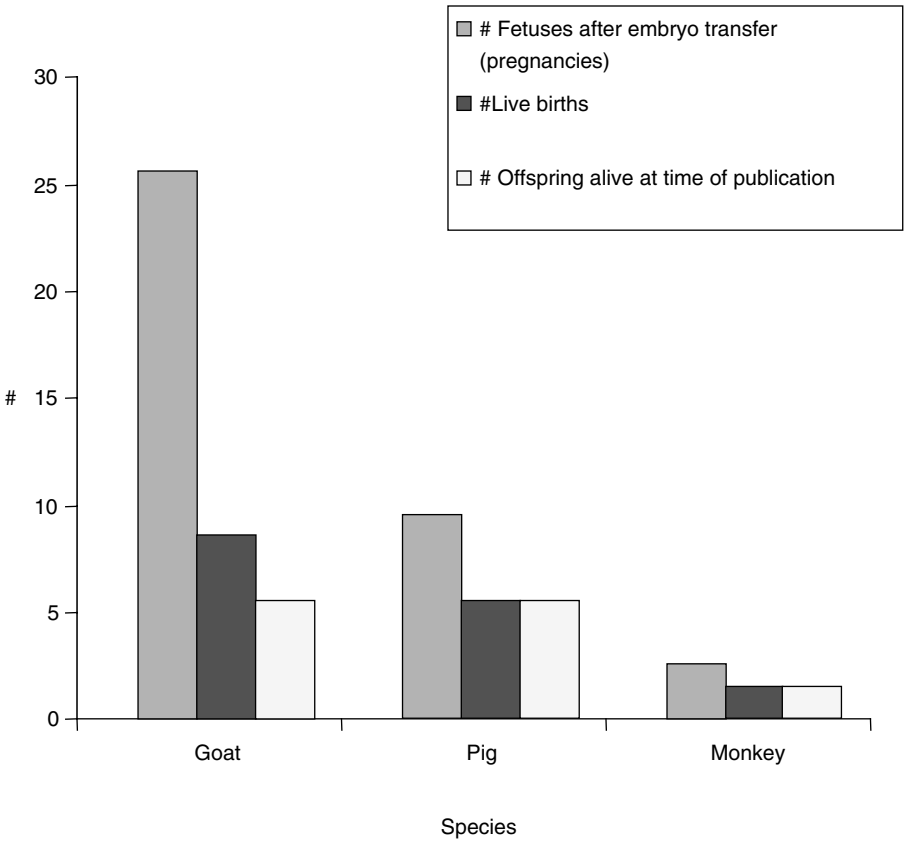

FIGURE 2 Survival Rates of Goat, Pig and Monkey Embryos Cloned from Adult, Fetal and Embryonic Cells.

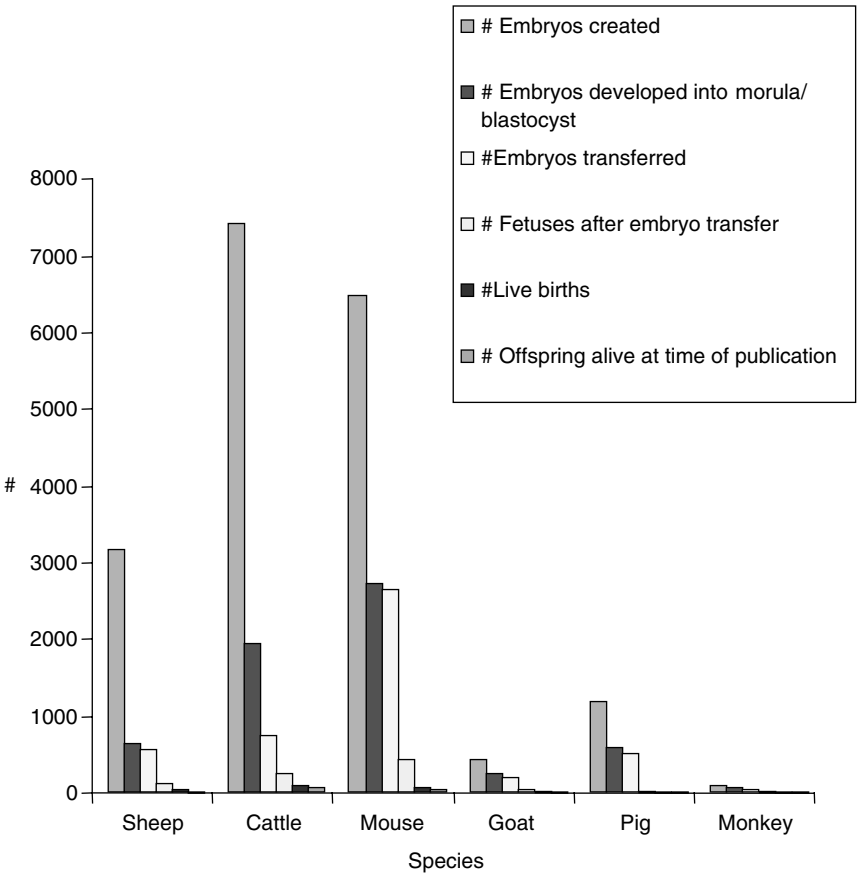

FIGURE 3 Efficiency of Cloning from Adult, Fetal and Embryonic Cells in Six Species.

TABLE 2 Phenotypes Observed in Cloned Animals

1	2	3	4	5
Species[b]	Cell type[c]	Defects seen in miscarried fetuses[d]	# Live births[e]	Phenotype
Sheep	Embryo-derived epithelial-like	N/A	5	2/5 healtl 1/5 die unstate
	Adult mammary gland	N/A	1	1/1 healtl overwe
	Fetal fibroblast	2 fetuses from one of the cell types showed abnormal liver development	3	2/3 healtl unknow
	Embryo-derived epithelial-like	N/A	4	4/4 healtl
	Fetal fibroblast	1 died after delayed delivery, 2 died after sibling (2) died in utero, 2 stillborn	7	5/7 alive; defect; meconi
	ES cell line-derived epithelial-like injected into in vivo-matured oocytes	3 late aborted fetuses underdeveloped for age; edema, hydronephrosis, testicular hypoplasia; also fetuses had variety of other defects, including cleft palate and interventricular septal defect	2	2/2 healtl
	ES cell line-derived epithelial-like injected into in vitro-matured oocytes	1 late aborted fetus underdeveloped for age; edema, hydronephrosis, testicular hypoplasia	1	1/1 died failure, abnorm hypothe inadequ same ar modera althoug present by rese
	ES cell line-derived epithelial like	N/A	3	2/3 healtl perinata
	ES cell line-derived fibroblast-like	N/A	3	1/3 healtl respirat trample
	ES cell line-derived fibroblast-like	N/A	7	2/7 healtl failure, problen
	Fetal fibroblast	N/A	14	3/14 alive 30 hrs; those th unspeci defects

	5	6	7
ve hs[e]	Phenotypes of live born clones[f]	Placental defects, phenotypes[g]	Reference[a]
	2/5 healthy; 2/5 died perinatally and 1/5 died at 10 days with unknown or unstated pathology	N/A	Campbell 1996
	1/1 healthy - (Dolly) - later became overweight	N/A	Wilmut 1997
	2/3 healthy; 1/3 died perinatally with unknown pathology	N/A	Wilmut 1997
	4/4 healthy	N/A	Wilmut 1997
	5/7 alive; 1/7 euthanized with heart defect; 1/7 died perinatally with meconium lodged in lung	N/A	Schnieke 1997
	2/2 healthy	N/A	Wells 1997
	1/1 died perinatally with respiratory failure, was underweight and had abnormal placenta that researchers hypothesize may have provided inadequate nutrition to support growth; same animal also found to have moderate bilateral hydronephrosis, although enough kidney tissue was present for normal function (as stated by researchers)	necrosing placenta	Wells 1997
	2/3 healthy and fertile; 1/3 died perinatally with respiratory failure	N/A	Wells 1998[h] experiment 1
	1/3 healthy; 1 died perinatally with respiratory failure, 1 died after being trampled by mother	N/A	Wells 1998[h] experiment 2
	2/7 healthy; 5/7 died with respiratory failure, 4 of those also had kidney problem (hydronephrosis)	N/A	Wells 1998[h] experiment 3
	3/14 alive, healthy; 7/14 died within 30 hrs; 4/7 died within next 12 weeks: those that died had variety of unspecified kidney, liver and brain defects	N/A	McCreath 2000

continues

TABLE 2 Continued

1	2	3	4	5
Species[b]	Cell type[c]	Defects seen in miscarried fetuses[d]	# Live births[e]	Phenotype
Cattle	Blastomere (embryonic)	N/A	9	no mentio postnat but pho calves
	Embryonic stem cell	N/A	4	phenotyp
	Fetal fibroblast	1 fetus aborted early, one after 249 days gestation; the late aborted fetus had abnormal placenta (hydroallantois, enlarged placentomes, edematous chorioallantois and amnion); on necropsy, fetus was oversized and had abnormal lungs and heart	4	3/4 norm with pu to insuf and exh
	Adult mural granulosa from a 13 yr old cow	1 case late miscarriage attributed by researchers to hydrallantois	2	2/2 calves 1/2 was arrhyth 1/2 (the sucklin 2 days l rumenit
	Adult cumulus	N/A	5	2/5 healt no abn factors caused
	Adult oviduct epithelial	N/A	3	2/3 healt no abn factors caused
	Adult mural granulosa	7 miscarriages attributed by researchers to hydrallantois	10	10/10 birt all calv 1/10 re doxapra cardiop

ve hs[e]	5 Phenotypes of live born clones[f]	6 Placental defects, phenotypes[g]	7 Reference[a]
	no mention of birth condition or postnatal development of 9/9 calves, but photo shows 5/9 healthy-looking calves	N/A	Chesne 1993
	phenotypes not described	N/A	Sims 1994
	3/4 normal, healthy; 1/4 died perinatally with pulmonary hypertension leading to insufficient pulmonary perfusion, and exhibited heart and vessel defects	1/4 calves born with abnormal placenta	Cibelli 1998[i]
	2/2 calves had normal birth weight; 1/2 was initially treated for cardiac arrhythmia and is now healthy; 1/2 (the other) initially had poor suckling response and was euthanized 2 days later due to acute hemorrhagic rumenitis and abomastitis	N/A	Wells 1998[j]
	2/5 healthy; 3/5 died soon after birth; no abnormalities noted; environmental factors thought by researchers to have caused death	N/A	Kato 1998
	2/3 healthy; 1/3 died soon after birth; no abnormalities noted; environmental factors thought by researchers to have caused death	N/A	Kato 1998
	10/10 birth weights within normal range; all calves had strong suckling reflex; 1/10 required epinephrine and doxapram treatment to stimulate cardiopulmonary function at birth	abnormalities noted in the placentas (edematous membranes, high allantoic fluid volume, enlarged umbilical vessels), although these abnormalities did not compromise fetal development according to assessment of researchers	Wells DN 1999

continues

TABLE 2 Continued

1	2	3	4	5
Species[b]	Cell type[c]	Defects seen in miscarried fetuses[d]	# Live births[e]	Phenotype
	Adult mammary gland epithelium	1 induced abortion at late gestation due to hydrallantois: fetus oversized, cysts in kidney and liver, enlarged umbilical vessels	1	1/1 health
	Adult ear skin fibroblast	1 induced abortion at late gestation due to hydrallantois: fetus oversized, cysts in kidney and liver, enlarged umbilical vessels	1	1/1 slight had to l severe j noted t surface
	Fetal germ cell	N/A	1	1/1 died, thought pre-ter mother
	Fetal fibroblast	N/A	2	1/2 norm was ov with in
	Adult skin cell from ES cell clone	N/A	1	1/1 had e respon at 7 we necrops spleen a
	Adult muscle	N/A	4	1/4 health due to i 1/4 (th 18 days (1 that euthani with ar develop high bi
	Fetal fibroblast	1/5 from miscarriage at 8 weeks; 4/5 from mothers that died late in pregnancy: 2/5 had chronic pulmonary hypertension and placental edema	8	5/8 were these di dilated neonata in 1 dea hydralla 1/2 of t of all ca
	Senescent adult fibroblast	N/A	6	6/6 had i born wi polydyp hyperte at birth vaccina

	5	6	7
ve hs[e]	Phenotypes of live born clones[f]	Placental defects, phenotypes[g]	Reference[a]
	1/1 healthy and normal	N/A	Zakhartchenko 1999[k]
	1/1 slightly oversized at birth (57 kg) and had to be euthanized at 2 days due to severe joint abnormalities, and was also noted to have liver with abnormal surface and slightly indurated	N/A	Zakhartchenko 1999[k]
	1/1 died, no abnormalities found; death thought by researchers to be related to pre-term delivery due to health of the mother	N/A	Zakhartchenko 1999[L]
	1/2 normal and healthy; 1/2 (the other) was oversized and died 3 days after birth with insufficient pulmonary function	N/A	Zakhartchenko 1999[m]
	1/1 had enlarged right ventricle but responded well to drug treatment, died at 7 weeks due to severe anemia - at necropsy was found to have thymus, spleen and lymph node hypoplasia	N/A	Renard 1999
	1/4 healthy; 2/4 died w/in first 30 hrs due to inadequately inflated lungs, 1/4 (the other) could not stand after 18 days and was euthanized, 2 calves (1 that died 3 days later and the euthanized one) had astasia associated with arthrogryposis (abnormally developed joints); all cloned calves had high birth weight	N/A	Shiga 1999
	5/8 were normal at birth, but 1/5 of these died at 6 weeks with suspected dilated cardiomyopathy; 3/8 had neonatal respiratory problems resulting in 1 death at 4 days; 2/8 were hydrallantoic pregnancies and only 1/2 of these survived; birth weights of all calves normal	both calves that died had edematous placentas	Hill 1999
	6/6 had increased birth weight and some born with moderate polyuria/ polydypsia, several had pulmonary hypertension and respiratory distress at birth, some had fever following vaccination	N/A	Lanza 2000[n]

continues

TABLE 2 Continued

1	2	3	4	5
Species[b]	Cell type[c]	Defects seen in miscarried fetuses[d]	# Live births[e]	Phenotype
	Adult fibroblast from 17 yr old bull	N/A	6	4/6 healt perinat after la abnorm 2 calves weight
	Many adult and fetal types	N/A	24	high birth 2/24 di with E. malform researc Akaban observe abnorm of unkn showed variabil
	Adult and fetal fibroblast	placental problems	4	1/4 healt cardiop those 2 1/4 die systemi
	Adult fibroblast from 21 yr old bull	N/A	1	1/1 calf w pulmon diabete (discon antigen activati
Mouse	Blastomere (embryonic)	N/A	19	phenotyp
	Blastomere (embryonic)	N/A	25	6/25 (ide tested f fertile
	Adult cumulus	N/A	31	22/31 (71 9/31 (2
	ES cells and mural trophectoderm	N/A	4	phenotyp
	Adult fibroblast	N/A	3	3/3 pups with re

	5	6	7
ve hs[e]	Phenotypes of live born clones[f]	Placental defects, phenotypes[g]	Reference[a]
	4/6 healthy and normal; 2/6 died perinatally: 1 with Akabane virus, 1 after labor difficulty: but no abnormalities found upon necropsy; 2 calves had above average birth weight	N/A	Kubota 2000
	high birth weight observed in calves; 2/24 died after difficult labor; 1 died with E. coli septicemia; 8/24 died with malformations of joints thought by researchers to be caused by the Akabane virus; some calves also were observed to have kidney or gut abnormalities; 1/24 died at 3 months of unknown causes; some male clones showed aged characteristics and tissue variability in telomere length	N/A	Kato 2000
	1/4 healthy; 2/4 died within 5 days with cardiopulmonary problems, and one of those 2 calves had a gut infection; 1/4 died at 1 month with a chronic systemic bacterial infection	2/6 placentas examined from cloned pregnancies were normal; 4/6 were abnormal: 2/6 had flat cuboidal chronic epithelium and decreased vascularity; 2/6 had diminished cotyledonary structure	Hill 2000[o]
	1/1 calf with lung dysmaturity and pulmonary hypertension, juvenile diabetes that responded to treatment (discontinued at 2 months), low CD45 antigen expression (required for T cell activation)	N/A	Hill 2000[p]
	phenotypes not described	N/A	Cheong 1993
	6/25 (identical septuplet males) were tested for fertility and found to be fertile	NA	Kwon 1996
	22/31 (71%) were healthy and normal; 9/31 (29%) died in first week	N/A	Wakayama 1998
	phenotypes not described	N/A	Tsunoda 1998
	3/3 pups grossly normal, but 2/3 died with respiratory failure	unusually large placentas	Wakayama 1999

continues

TABLE 2 Continued

1	2	3	4	5
Species[b]	Cell type[c]	Defects seen in miscarried fetuses[d]	# Live births[e]	Phenotype
	Immature adult Sertoli cells	N/A	16	15/16 pup hernia,
	Tail tip fibroblast	all miscarriages were early in pregnancy	7	7/7 pups
	Adult cumulus	N/A	35	telomeres shorten suggest all mice (learnin strengt
	Adult cumulus	N/A	5 tested (does not say how many were born)	5 of the h for beha of prew delayed long-ter normal and mo postnat birth as but rese been ca backgro
	Fetal fibroblast	N/A	5	3/5 norm umbilic deficien
	Embryonic stem cell	N/A	28	28/28 hac did not of survi problem
Goat	Blastomere (embryonic)	N/A	45	45/45 hea
	Fetal fibroblast	all miscarriages were early in pregnancy	3	3/3 norm
	Fetal fibroblast infections	N/A	6	3/6 healt infectio

	5	6	7
ive hs[e]	Phenotypes of live born clones[f]	Placental defects, phenotypes[g]	Reference[a]
	15/16 pups normal; 1/16 had umbilical hernia, but was viable at birth	unusually large but structurally normal placentas	Ogura 2000[q]
	7/7 pups normal, healthy	N/A	Ogura 2000[r]
	telomeres lengthened rather than shortened in successive generations suggesting no inherited aging problem, all mice tested normal for behaviors (learning, memory, activity, agility, strength)	N/A	Wakayama 2000
ested does not ay how many were born)	5 of the healthy cloned mice were tested for behavioral defects - 3/10 measures of preweaning development were delayed but did appear and had no long-term effects; cloned mice were normal for learning, memory, activity and motor skills - these mice had high postnatal weight gain (not heavy at birth as in LOS) compared to controls but researchers suggest this may have been caused by the agouti gene in their background	N/A	Tamashiro 2000
	3/5 normal and healthy; 2/5 died with umbilical hernia and respiratory deficiency	placental hypertrophy and also placental structural abnormalities	Ono 2001
	28/28 had high birth weights, but this did not adversely affect clones in terms of survival; no respiratory or other problems	high placental weights	Eggan 2001
	45/45 healthy	N/A	Yong 1998
	3/3 normal, healthy	N/A	Baguisi 1999
	3/6 healthy; 3/6 died with respiratory infections	placentas within normal range for # of cotyledons	Keefer 2001

continues

TABLE 2 Continued

1	2	3	4	5
Species[b]	Cell type[c]	Defects seen in miscarried fetuses[d]	# Live births[e]	Phenotype
Pig	Adult granulosa	N/A	5	5/5 pigs v
	Fetal fibroblast	N/A	1	1/1, Xena
	Body cell and genital ridge cell	N/A	4	no pheno
Monkey	Blastomere (embryonic)	N/A	2	2/2 healtl

NOTE: N/A indicates that no data were available in the cited publication
NOTE: ES cell = embryonic stem cell.
NOTE: LOS = large offspring syndrome.

[a]The peer reviewed scientific article in which data for any given experiment were published. Full references can be found in the bibliography.
[b]The species of animal used in the experiment.
[c]The cell type used as the source of the donor nucleus for the nuclear transfer.
[d]Description of abnormalities seen in aborted cloned fetuses; in some cases, these abnormalities may be the cause of miscarriage.
[e]The number of live-born cloned animals.
[f]Description of observations of physical, physiological or genetic characteristics of live born cloned animals at time of publication of cited refernces, unless stated otherwise.
[g]Description of any characteristics, normal or abnormal, noted in the placentas of live born or miscarried cloned animals.
[h]Wells et al. Cloning sheep from cultured embryonic cells. *Reprod. Fertil. Dev.* 1998; 10:615-626.
[I]Cibelli et al. Cloned transgenic calves produced from nonquiescent fetal fibroblasts. *Science* 1998; 280:1256-8.
[j]Wells et al. Adult somatic cell nuclear transfer is used to preserve the last surviving cow of the Enderby Island cattle breed. *Reprod. Fertil. Dev.* 1998; 10:369-378.
[k]Zakhartchenko et al. Adult cloning in cattle: Potential of nuclei from a permanent cell line and from primary cultures. *Mol. Reprod. Fertil.* 1999; 54:264-272.
[L]Zakhartchenko et al. Potential of fetal germ cells for nuclear transfer in cattle. *Mol. Reprod. Dev.* 1999; 52:421-426.
[m]Zakhartchenko et al. Effects of serum starvation and re-cloning on the efficiency of nuclear transfer using bovine fetal fibroblasts. *J. Reprod. Fertil.* 1999; 115:325-331.
[n]Lanza et al. Extension of cell life-span and telomere length in animals cloned from senescent somatic cells. *Science* 2000 Apr 28; 288:665-669.
[o]Hill et al. Evidence for placental abnormality as the major cause of mortality in first-trimester somatic cell cloned bovine fetuses. *Biol. Reprod.* 2000; 63:1787-1794.
[p]Hill et al. Development rates of male bovine nuclear transfer embryos derived from adult and fetal cells. *Biol. Reprod.* 2000; 62:1135-1140.
[q]Ogura et al. Production of male cloned mice from fresh, cultured, and cryopreserved immature Sertoli cells. *Biol. Reprod.* 2000; 62:1579-1584.
[r]Ogura et al. Birth of mice after nuclear transfer by electrofusion using tail tip cells. *Mol. Reprod. Dev.* 2000; 57:55-59.

	5	6	7
ive hs[e]	Phenotypes of live born clones[f]	Placental defects, phenotypes[g]	Reference[a]
	5/5 pigs very healthy	N/A	Polejaeva 2000
	1/1, Xena, is healthy	normal placenta	Onishi 2000
	no phenotypes described	N/A	Betthauser 2000
	2/2 healthy	N/A	Meng 1997

TABLE 3 Developmental Capacity of Cytoplasts Reconstituted with Nuclei from Embryonic Cells

1	2	3	4	5	6
Species	Recipient cytoplast	Donor cell type	% Early Development: % Blastocyst (# Blastocysts/ # Cultured)	% Term Development: % Offspring (# Live births/ # Transferred)	References
Mouse	Zygote	Inner cell mass	16% (23/142)	19% (3/16)	Illmensee
		Trophectoderm	1% (1/68)	0	
	Zygote	Pronuclear	95% (20/21)	no transfer	McGrath
		2-cell	13% (19/151)	no transfer	
		4-cell	0 (0/81)	no transfer	
		8-cell	0 (0/111)	no transfer	
		Inner cell mass	0 (0/84)	no transfer	
	Zygote	8-cell	0(0/32)	no transfer	Robl 1986
		Inner cell mass	0 (0/84)	no transfer	
	Zygote	8-cell	0(0/32)	no transfer	Robl 1986
	2-cell blastomere	2-cell	93% (40/43)	24%[a] (10/41)	
	Zygote	8-cell	51% (45/89)	0[a] (0/11)	
		Cumulus cell	0 (0/91)	no transfer	Wakayam 2000[b]
	2-cell blastomere	4-cell	72% (49/68)	22% (10/46)	Robl 1987
		8-cell	35% (49/139)	8% (4/48)	
		Inner cell mass	0 (0/91)	no transfer	
	Mll oocyte	2-cell	23% (20/88)	15% (3/20)	Kono 199
		8-cell	4% (1/26)	0 (0/1)	
		Inner cell mass	13% (11/87)	0 (0/11)	
		2-cell	78% (36/46)	29% (10/34)	Cheong 1
		4-cell	71% (30/42)	22% (6/27)	
		8-cell	46% (18/39)	18% (3/17)	
		4-cell[c]	83% (58/70)	43% (2/58)	Kwon 199
		Inner cell mass[c]	64% (23/36)	11% (2/18)	Tsunoda
		Trophectoderm[c]	62% (16/26)	8% (2/25)	
		ES cell	5% (47/931)	0 (0/56)	Tsunoda
		ES cell	29% (312/1087)	6% (8/132)	Wakayam 1999[f]
Sheep	Mll oocyte	8-cell	33% (8/24)	75% (3/4)	Willadsen 1986
		16-cell	27% (13/49)	21% (3/14)	Smith 198

	6	7
m opment: spring e births/ nsferred)	References	Significant findings
3/16)	Illmensee 1981	First demonstration of developmental potential in mammals. Reproducibility of results questioned.
nsfer	McGrath 1984	Biologically impossible to achieve development with transcriptionally active nucleus.
nsfer		
nsfer		
nsfer		
nsfer		
nsfer	Robl 1986	Development more advanced with cytoplast prepared from 2-cell than zygote.
nsfer		
nsfer	Robl 1986	Development more advanced with cytoplast prepared from 2-cell than zygote.
(10/41)		No development beyond 12 days gestation.
11)		
nsfer	Wakayama 2000[b]	No development when zygotic cytoplasts were used.
10/46)	Robl 1987	Term development when 4- and 8-cell nuclei used but not more advanced.
/48)		Importance of cytoplast environment.
nsfer		
3/20)	Kono 1991[g]	Development to term from embryonic nuclei transferred to enucleated oocyte.
1)		
11)		
10/34)	Cheong 1993	Embryonic nuclei in G1 phase of the cell cycle can direct term development when transferred to Mll cytoplasts.
6/27)		
3/17)		
2/58)	Kwon 1996	Serial nuclear transfer of metaphase-arrested embryonic nuclei results in term development.
2/18)	Tsunoda 1998	Term development following serial nuclear transfer of inner cell mass and trophectoderm nuclei.
/25)		
56)	Tsunoda 1993	Implantation sites but no term development.
/132)	Wakayama 1999[f]	Late-passage actively dividing ES cell nuclei are able to direct development to term.
3/4)	Willadsen 1986	Term development from cleavage stage blastocysts.
3/14)	Smith 1989	Transcriptionally active nuclei are able to direct development to term.

continues

TABLE 3 Continued

1	2	3	4	5	6
Species	Recipient cytoplast	Donor cell type	% Early Development: % Blastocyst (# Blastocysts/ # Cultured)	% Term Development: % Offspring (# Live births/ # Transferred)	References
		Inner cell mass	38% (6/16)	11% (1/9)[d]	
		Cultured cell line	14% (34/244)	14% (5/34)	Campbell
Cattle	Pronuclear	Pronuclear	13% (5/38)	100% (2/2)	Robl 1987
		2- to 8-cell	0 (0/10)	no transfer	
	Mll oocyte	2- to 8-cell	12% (13/111)	0 (0/12)	Prather 19
		9- to 16-cell	16% (8/50)	28 (2/7)	
		17- to 32-cell	8% (2/24)	no transfer	
		Morula (64-cell)	23-35%	22%[e] (104/463)	Bondioli 1
		Morula (31-cell)	24% (152/641)	15% (9/59)	Chesne 19
		Inner cell mass	7% (20/304)	13% (2/15)	Collas 199
		Inner cell mass	5% (30/629)	8% (2/26)	Keefer 19
		Cultured inner cell mass	27% (109/406)	12% (4/34)	Sims 1984
		Fetal germ cell (PGC)	20-38% (30/149-53/140)	5% (1/20)	Zakhartch 1999
Rabbit	Mll oocyte	8-cell	not assessed	4% (6/164)	Stice 1988
		8- to 16-cell	49% (34/69)	21% (23/110)	Prather 19
		32-cell	33% (14/43)	15% (10/67)	Collas 199 Callas 1
		Inner cell mass	20% (17/83)	no transfer	
		Trophectoderm	0 (0/52)	no transfer	
Pig	Mll oocyte	2-cell	9% (1/11)	0 (0/33)	Prather 19
		4-cell	8% (7/83)	3% (1/34)	
		8-cell	19% (11/57)	0 (0/21)	
Goat	Mll oocyte	Morula[c]	31% (18/57)	31% (45/141)	Yong 1998
Monkey	Mll oocyte	8-cell	52%[e] (53/101)	4% (2/53)	Meng 199

[a]Development assessed at 8.5 days post coitum.
[b]Wakayama et al. Nuclear transfer into mouse zygotes. *Nat Genet* 2000; 24:108-9.
[c]Achieved using serial nuclear transfer.
[d]Development assessed at 42 days of pregnancy.
[e]Embryonic development assessed at the 2-cell stage prior to transfer.
[f]Wakayama et al. Mice cloned from embryonic stem cells. *Proceedings of the National Academy of Sciences, USA* 1999; 96:14984-89.

	6	7
m opment: spring e births/ nsferred)	References	Significant findings
1/9)[d]		
5/34)	Campbell 1996	Nuclei from cell lines from embryonic discs are able to support development to term.
(2/2)	Robl 1987	Cleavage stage embryonic nuclei are unable to direct embryonic or term development when transferred to enucleated zygotes.
nsfer 12)	Prather 1987	Term development from transcriptionally active donor embryonic nuclei.
/7) nsfer (104/463)	Bondioli 1990	Nuclei from morula stage embryos can direct midgestation development.
9/59)	Chesne 1993	Nuclei from morula stage embryos can direct development to term.
2/15)	Collas 1994	Direct injection of inner cell mass nuclei into Mll cytoplasts can direct development to term.
/26)	Keefer 1994	Totipotency of inner cell mass nuclei confirmed.
4/34)	Sims 1984	Nuclei from inner cell mass cultured for up to 28 days are able to direct development to term.
/20)	Zakhartchenko 1999	Fetal germ cells can direct development to term.
/164)	Stice 1988	First production of genetically verified nuclear transfer rabbits from embryonic donor nuclei.
23/110)	Prather 1989	High rates of development from transcriptionally active embryonic nuclei.
10/67)	Collas 1990; Callas 1991	Normal embryonic development from inner cell mass donor nuclei.
nsfer nsfer 33)	Prather 1989	Cleavage stage embryonic nuclei can direct term development in pigs.
/34) 21) 45/141)	Yong 1998	Serial nuclear transfer of transcriptionally nuclei results in high rates of development.
/53)	Meng 1997	Embryonic nuclei can support term development in the monkey.

[g]Kono T et al. Development of enucleated mouse oocytes reconstituted with embryonic nuclei. *J. Reprod. Fertil.* 1991; 93:165-72.

SOURCE: Lewis, IM, MJ Munsie, AJ French, R Daniels and AO Trounson, 2001. The Cloning Cycle: From Amphibia to Mammals and Back. *Reproductive Medicine Reviews* 9:1 pp. 3-33.

TABLE 4 Developmental Capacity of Cytoplasts Reconstituted by Nuclei from Fetal and Adult Somatic Cells

1	2	3	4	5
Species	Recipient cytoplast	Donor Cell Type	% Early Development: % Blastocyst (# Blastocysts/ # Cultured)	% Term Developm % Offspri (# Live bir # Transfer
Mouse	Zygote Mll oocyte	Cumulus Cell Thymocyte	0 (0/91) 7% (6/88)	No Trans 0
		Cumulus Cell	67% (101/151)	2% (31/13
		Neuronal Cell	22% (50/223)	2%[a] (1/46
		Sertoli cell (mature) Cumulus Cell	40% (63/159) 20% (19/93)	2%[a] (1/59
		Cultured follicular cell; Adult Male fibroblast; Cumulus cell.	34% (51/151) 50% (207/414) 52% (206/393)	3% (1/30) 1% (2/177 1%[c] (2/20
		Fibroblast cell Sertoli Cell (immature)	23% (38/162) 33% (94/284)	0[c] (0/38) 4% (6/134
Sheep	Mll oocyte	Fetal fibroblast	27% (34/124)	8% (3/40)
		Adult Mammary (epithelial)	12% (29/247)	3% (1/29)
		Transgenic Fetal fibroblast	5-21% (5/82-19/89)	5/21% (1/
		Transgenic Fetal fibroblast	6-28% (14/109, 43/154, 4/71, 19/83)	0-28% (4/ 8/43, 0/
Cattle	Mll oocyte	Cumulus cell	13% (5/38)	0 (0-19)
		Fetal fibroblast	12% (33/276)	14% (3/28

	5	6	7
	% Term Development: % Offspring (# Live births/ # Transferred)	References	Significant Findings
6/88)	No Transfer 0	1. Wakayama 2000[a] - same footnote information as earlier Wakayama 2000 in Table 3 2. Callas, 1992	1. No development when zygotic cytoplasts were used. 2. Somatic nuclei are able to direct embryonic development through no term development.
)	2% (31/1315)	Wakayama 1998[b]	Direct-injected cumulus cell nuclei direct term development; however, Sertoli and neuronal nuclei do not.
	2%[a] (1/46)		Findings do not support the requirement of G0/G1 nuclei for term development.
	2%[a] (1/59) 0 (0/3)	Kato 1999	Serial nuclear transfer of cultured follicular cells but not cumulus cell nuclei results in term development.
)	3% (1/30); 1% (2/177);	Wakayama 1999[c]; Ogura 2000[d]	Male-derived adult somatic cell nuclei can direct term development;
)	1%[c] (2/206)		Immature, actively dividing Sertoli cell nuclei can direct term development.
	0[c] (0/38)		
	4% (6/134)		
	8% (3/40)	Wilmut 1997	Inducing cell to enter quiescence by serum starvation may assist in nuclear reprogramming.
	3% (1/29)		First demonstration that nuclei from differentiated somatic fetal or adult origin can direct development to term.
9/89)	5/21% (1/21-1/5)	Schnieke 1997	Term development of transfected somatic cell nuclei suggests an alternative method for the production of transgenic animals. One male lamb was born.
9, 71, 19/83)	0-28% (4/14, 8/43, 0/4, 2/19)	McCreath 2000	Production of gene-targeted sheep by nuclear transfer from cultured somatic cells.
	0 (0-19)	Callas 1994	Nuclei from adult somatic cells can direct embryonic development.
	14% (3/28)	Cibelli 1998[e]	Cultured activity dividing fetal fibroblast nuclei can direct development to term.

continues

TABLE 4 Continued

1	2	3	4	5
Species	Recipient cytoplast	Donor Cell Type	% Early Development: % Blastocyst (# Blastocysts/ # Cultured)	% Term Developm % Offspri (# Live bir # Transfer
		Adult Male Fibroblast	21-37% (24/114 - 43/115)	7% (2/7)
		Cumulus Cell	49% (18/37)	33% (2/6)
		Cultured Oviductal cell	23-34% (196/842 - 29/84)	50% (2/4)
		Cultured Granulosa	28% (152/552)	10% (10/1
Rabbit	MII oocyte	Adult granulosa	Number not specified	0
Pig	MII oocyte	Granulosa cell line	Not assessed	1.3% (5/4
		Fetal fibroblast	1-31% (total 88/615)	No Trans
			93% (2-, 4- and 8-cell, 110/118)	0.9% (1/1
Goat	MII oocyte	Transgenic Fetal fibroblast	Not assessed	3% (3/112
Monkey	MII oocyte	Fetal fibroblast	57% (57/100)	0
		Adult fibroblast	44% (4/9)	0

SOURCE: Lewis, IM, MJ Munsie, AJ French, R Daniels and AO Trounson, 2001. The Cloning Cycle: From Amphibia to Mammals and Back. *Reproductive Medicine Reviews* 9:1 pp. 3-33.
NOTE: Cytoplast = Enucleated Egg.

[a]Wakayama et al. Nuclear Transfer into mouse zygotes. *Nat Genet* 2000; 24:108-9.
[b]Wakayama et al. Full-term development of mice from enucleated oocytes injected with cumulus cell nuclei. *Nature* 1998; 394:369-74.
[c]Wakayama T. and Yanagimachi R. Cloning of male mice from adult tail-tip cells. *Nat Genet* 1999; 22:127-8
[d]Ogura A, et al. Production of male cloned mice from fresh, cultured and cryopreserved immature Sertoli cells. *Biol. Reprod.* 2000; 62:1579-84.
[e]Cibelli J., et al. Cloned transgenic calves produced from nonquiescent fetal fibroblasts. *Science* 1998; 280:1256-8.

	5	6	7
/	% Term Development: % Offspring (# Live births/ # Transferred)	References	Significant Findings
	7% (2/7)	Kubota 2000	Nuclei from male adult fibroblast can direct development to term.
3/115)			
	33% (2/6)	Kato 1995	High rates of term development following transfer of cumulus and oviduct nuclei.
	50% (2/4)		
29/84)			
)	10% (10/100)	Wells 1999	Production of calves from cultured granulosa cells.
specified	0	Collas and Rob, unpublished	First production of genetically verified nuclear transfer rabbits.
	1.3% (5/401)	Polejaeva 2000	Term development following serial nuclei transfer of cumulus cells.
8/615)	No Transfer	Onishi 2000	Term development following direct injection of nuclei from fetal fibroblast cells.
d 8-cell,	0.9% (1/110)		
	3% (3/112)	Baguisi 1999	Production of transgenic goats from transfected fetal fibroblast nuclei.
	0	Wolf 1999	Donor nuclei from cell lines are capable of limited embryonic development.
	0		

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Workshop Agenda and Speaker Biographical Information

AGENDA

Committee on Science, Engineering and Public Policy
Board on Life Sciences

Panel on Scientific and Medical Aspects of Human Cloning
August 7, 2001

The National Academies
Auditorium
2101 Constitution Avenue; 2100 C Street, NW
Washington, DC

8:30 a.m.	**Welcome** ***Bruce Alberts***, President, The National Academy of Sciences, Chair, The National Research Council ***Irving Weissman***, Chair, National Academies Panel on Scientific and Medical Aspects of Human Cloning and Karel and Avice Beekhuis Professor of Cancer Biology, Stanford University
8:35 a.m.	**Overview of Embryology** Moderator: ***Irving Weissman*** Speaker: ***Virginia Papaioannou***, Professor of Genetics and Development, Columbia University
8:50 a.m.	Discussion
9:00 am	**Scientific Issues Underlying Cloning** Moderator: ***David Galas***, Vice President, Keck Graduate Institute of Applied Life Sciences and Panel Member Speakers: ***Rudolf Jaenisch***, Professor of Biology, MIT Whitehead Institute ***Eric Schon***, Professor of Genetics and Development, Columbia University
9:45 a.m.	Discussion
10:00 am	Break
10:15 am	**Reproductive Cloning in Animals** Moderator: ***Brigid Hogan***, Hortense B. Ingram Professor, Department of Cell Biology, Vanderbilt University School of Medicine and Panel Member

Speakers:
Alan Colman, Research Director, PPL-Therapeutics
Jonathan Hill, Assistant Professor of Theriogenology, Cornell
Peter Farin, Assistant Professor, Dept. of Farm Animal Health and Resource Management, North Carolina State University
Ryuzo Yanagimachi, Professor of Anatomy and Reproductive Biology, University of Hawaii

11:15 a.m. Discussion

11:30 am **Cloning for Stem Cells**
Moderator: ***Anne McLaren***, Principal Research Associate, The Wellcome Trust and Research Campaign, Institute of Cancer and Developmental Biology, University of Cambridge and Panel Member
Speakers:
Jose Cibelli, Vice-President of Research, Advanced Cell Technologies
Peter Mombaerts, Head of Laboratory of Developmental Biology and Neurogenetics, Rockefeller University
Alan Trounson, Deputy Director, Institute of Reproduction and Development, Monash Institute, Australia

12:00 p.m. Discussion

12:15 p.m. Lunch

1:00 p.m. **Reproductive Cloning in Humans**
Moderator: ***Irving Weissman***
Speakers:
Severino Antinori, Director, International Associated Research Institute
Brigitte Boisselier, Director, Clonaid
Panayiotis Michael Zavos, Director and Chief Andrologist, The Andrology Institute

1:45 p.m. Discussion

2:15 p.m. Break

2:30 p.m. **Applicability of Animal Cloning Data to Human Cloning**
Moderator: ***Irving Weissman***
Speaker: ***Ian Wilmut***, Director, Roslin Institute

2:45 p.m. Discussion

3:00 p.m. **Assisted Reproductive Technologies**
Moderator: ***Arthur Beaudet***, Chair, Department of Molecular and Human Genetics, Baylor College of Medicine and Panel Member
Speakers:
André Van Steirteghem, Professor of Embryology and Reproductive Biology, Brussels Free University, Brussels, Belgium
Alan Trounson, Deputy Director, Institute of Reproduction and Development, Monash Institute, Australia
Jay Cross, Associate Professor, Dept. of Biochemistry & Molecular Biology and Obstetrics & Gynecology, University of Calgary
Eugene Pergament, Professor of Obstetrics and Gynecology, Northwestern University Medical School

4:00 p.m. Discussion

4:30 p.m. Break

4:45 p.m. **Human Cloning: Some Public Policy Issues**
Moderator: ***Mark Siegler***, Lindy Bergman Professor and Director of the MacLean Center for Clinical Medical Ethics, University of Chicago and Panel Member
Speakers:
John Robertson, Vinson and Wilkins Chair, University of Texas School of Law, Austin
R. Alta Charo, Professor of Law and Bioethics, University of Wisconsin-Madison

5:30 p.m. Discussion

5:45 p.m. Final Thoughts

6:00 p.m. Adjourn

SPEAKER BIOGRAPHICAL INFORMATION

Severino Antinori is professor of reproductive physiopathology at the Medical Faculty of the Tor Vergata University in Rome. He is also scientific director of the International Research Association for Human Reproduction. He was formerly professor of physiopathology of reproduction at the University of Pisa. He is president of the Italian Society for Reproductive Medicine and vice-president of the International Association of Assisted Reproductive Medicine Centers and Laboratories. He has published more than 180 papers, mainly on male sterility, menopausal pregnancies, and human reproduction. He has published in *The Lancet* and *The Journal of Assisted Genetics*.

Brigitte Boisselier is the director of Clonaid, the first human-cloning company. She received a PhD in physical chemistry from the University of Dijon, France, in 1982 and another in analytic chemistry from the University of Houston in 1985. She has published extensively in *Inorganic Chemistry* and *Analytic Chemistry*, and she holds three patents for chemical processes. Dr. Boisselier's primary focus has been on the analysis of porphyrins with various metal-carbon and metal-metal bonds. She continues to carry on research stemming from her dissertation, which focused on porphyrins and the influence of axial and equatorial ligands on reduction-oxidation characteristics. A strong advocate of undergraduate research and scholarship, Dr. Boisselier wrote *Science et Conscience*, a book for the general public on advances in science.

R. Alta Charo is professor of law and bioethics at the University of Wisconsin (UW) Law and Medical Schools, where she teaches bioethics and biotechnology law, food and drug law, reproductive rights, torts, and legislative drafting. In addition, she has served on the UW Hospital clinical ethics committee, the UW Institutional Review Board for the protection of human subjects in medical research, and the UW Bioethics Advisory Committee. Before her arrival at UW in 1989, Professor Charo served as associate director of the Legislative Drafting Research Fund of Columbia University, Fulbright Junior Lecturer in American Law at the Sorbonne in Paris, legal analyst for the Biological Applications Program of the congressional Office of Technology Assessment, American Association for the Advancement of Science, and Diplomacy Fellow for the Policy Development Division of the Office of Population at the US Agency for International Development. She was a member of the 1993 National Institutes of Health Human Embryo Research Panel and since 1996 has been a member of the presidential National Bioethics Advisory Commission.

Jose B. Cibelli is vice president of research at Advanced Cell Technology, Inc. He received a DVM from at the University of La Plata, Argentina, in 1989 and a PhD from the University of Massachusetts in Amherst in 1998. From 1989 to 1993, he was a veterinarian at the Cooperative of Artificial Insemination of Venado Tureto, Argentina, and has several years of research experience at the Department of Veterinary and Animal Science at the University of Massachusetts in Amherst, where he did his doctoral dissertation (in the laboratory of James Robl) on the production of transgenic cattle. Dr. Cibelli is one of the pioneers in cloning with transgenic somatic cells in bovine cows for the production of animals and embryonic stem cell-like cells. His work focused on the production of transgenic cattle. In January 1998, Dr. Cibelli's efforts led to the announcement of the generation of the world's first transgenic calves by cloning. That was followed by publications in *Science*, *Nature Biotechnology*, and *Nature Medicine*.

Alan Colman is research director of PPL Therapeutics, a biotechnology firm based in Edinburgh, Scotland (PPL Ltd.), Blacksburg, Virginia (PPL Inc.), and New Zealand (PPL NZ). He obtained a BA in biochemistry from Oxford University (1971) and a PhD under John Gurdon, a pioneer in nuclear transfer, from the Laboratory of Molecular Biology in Cambridge, England (1974). After a series of academic appointments in Oxford and Warwick Universities, he became professor of biochemistry in the University of Birmingham. With Ron James (managing director of PPL), he has been involved with PPL since its inception in 1987, first as part-time research director, becoming full-time (and leaving Birmingham) in 1993. PPL has recently attracted considerable media attention because of its participation in the technique of somatic cell nuclear transfer. That work led to Dolly, the world's first sheep cloned from an adult somatic cell; Polly and Molly, the first cloned transgenic livestock; Diana and Cupid, the first livestock with targeted genetic changes; and Millie and others, the first cloned pigs.

Jay Cross is an associate professor of biochemistry and molecular biology at the University of Calgary, an investigator of the Canadian Institutes of Health Research, and a senior scholar of the Alberta Heritage Foundation of Medical Research. He received a PhD from the University of Missouri and a DVM from the University of Saskatchewan, Canada. He is an expert in the molecular genetics of early embryonic development, focusing on the placenta and cardiovascular system and using transgenic and gene knockout mice. He has written extensively about the development and biology of the placenta in different mammalian species.

Peter Farin is an assistant professor in the Department of Farm Animal Health and Resource Management at North Carolina State University. He received his MS in animal science in 1980 from the Colorado State University, where he also got his DVM. He received his PhD in veterinary medical sciences in 1995 from North Carolina State University. He has published over 30 journal articles and 25 abstracts. He received a specialty-board certification from the American College of Theriogenologists in 1991. Dr. Farin has been a clinical instructor in the Department of Veterinary Medicine and Surgery at the University of Missouri.

Jonathan Hill is an assistant professor in the Department of Veterinary Clinical Sciences at Cornell University. He is a board-certified veterinary animal reproduction specialist and received his PhD in reproductive physiology from Texas A&M University in 1999. His studies at Texas A&M included observations on the clinical and pathological features of the world's first somatic cell-cloned calves, production of a calf cloned from a 21-year-old Brahma bull, and observations on the causes of failure in first-trimester cloned pregnancies. He has extensive clinical and research experience with the in vitro production of embryos via cloning and in vitro fertilization, in vivo embryo collection, embryo transfer, pregnancy monitoring, and neonatal care.

Rudolf Jaenisch is one of the founders of transgenic science (gene transfer to create mouse models of human disease). His laboratory has produced mouse models leading to new understanding of cancers and various neurological diseases. He also has made important contributions to cloning technology. Studies of cloned mice will help to decipher how the genome from an adult cell is reprogrammed to create a new organism. A founding member of the Whitehead Institute and professor of biology at Massachusetts Institute of Technology, he received his doctorate in medicine from the University of Munich in 1967. He came to the Whitehead from the University of Hamburg, Germany, where he was head of the Department of Tumor Virology at the Heinrich Pette Institute. In 1996, he was awarded the Boehringer Mannheim Molecular Bioanalytics Prize.

Peter Mombaerts is associate professor and head of the Laboratory of Developmental Biology and Neurogenetics at The Rockefeller University in New York. He received his MD in 1987 from the Catholic University of Leuven, Belgium, and his PhD in biology in 1992 from the Massachusetts Institute of Technology. His research interest is developmental neurobiology. The approach he takes is genetic manipulation of mice, including transgenesis, targeted mutagenesis, and cloning by nuclear transfer. He has a long-standing collaboration with Teruhiko Wakayama and Anthony

Perry, who developed mouse-cloning technology. Dr. Mombaerts has won numerous awards, including an Alfred P. Sloan Fellowship, a Guggenheim Fellowship, and the Presidential Early Career Award for Scientists and Engineers, which he received in 1997.

Virginia E. Papaioannou is professor of genetics and development at the College of Physicians and Surgeons of Columbia University. She received her BSc in biological sciences in 1967 from the University of California, Davis and her PhD in genetics in 1972 from the University of Cambridge, England. Dr. Papaioannou is the senior editor of *Differentiation* and associate editor of *Molecular Reproduction and Development*. Previously, she was the director of the course on molecular embryology of the mouse at Cold Spring Harbor, NY, and was a professor at Tufts University School of Medicine and Veterinary Medicine. She is the author or coauthor of more than 90 articles and reviews.

Eugene Pergament is a medical geneticist and professor of obstetrics and gynecology at Northwestern University Medical School. He received a BS from Yale University, a PhD in genetics from Purdue University, and an MD from the University of Chicago. Dr. Pergament is certified in clinical genetics and cytogenetics by the American Board of Medical Genetics and is a founding member of the American College of Medical Genetics. He serves on numerous local, regional, and national committees and was a member of the Executive Board of the Organization for Teratogen Information Services.

John A. Robertson holds the Vinson and Wilkins Chair at the University of Texas School of Law at Austin. He received his BA from Dartmouth in 1964 and his JD from Harvard University in 1968. He has written and lectured widely on law and bioethical issues. He is the author of two books in bioethics—*The Rights of the Critically Ill* (1983) and *Children of Choice: Freedom and the New Reproductive Technologies* (1994)—and numerous articles on reproductive rights, genetics, organ transplantation, and human experimentation. He has served on or been a consultant to many national bioethics advisory bodies, and he is cochair of the Ethics Committee of the American Society for Reproductive Medicine.

Eric A. Schon is professor of genetics and development in neurobiology at Columbia University. He received his BS in chemical engineering from Columbia University in 1968 and his PhD in biological chemistry from the University of Cincinnati in 1982. He did his postdoctoral research in biochemistry in 1982-1983 at Harvard University. Dr. Schon's research has included mitochondrial DNA rearrangements in neuromuscular disease

and cellular and animal models of mitochondrial disease. He has over 150 publications, many of them on mitochondrial genetics. He is a member of the New York Academy of Sciences and the American Association for the Advancement of Science, and he was a member of the Scientific Advisory Committee of the Muscular Dystrophy Association in 1995-1998.

Alan Trounson is deputy director of the Institute of Reproduction and Development at Monash Institute, in Australia. He pioneered in vitro fertilization technology with Karl Wood. He received his BSc from the School of Wool and Pastoral Sciences of the University of New South Wales, in Sydney, and his PhD in agriculture from Sydney University in 1974. He has received numerous awards, including the Ford Foundation Senior Research Fellowship. He is the author or coauthor of numerous reviews and book chapters and over 200 journal articles.

André Van Steirteghem is dean and professor of embryology and reproductive biology at the Medical School of the Dutch-speaking Brussels Free University (VUB). He is chairman of the Department of Radioimmunology and Reproductive Biology at the VUB Hospital and the laboratory and scientific director of the Centre for Reproductive Medicine. In the last 2 decades, the VUB Centre for Reproductive Medicine has developed into one of the world's largest centers of assisted reproductive technology. Major developments at the Centre included the first clinical application of intracytoplasmic sperm injection (ICSI), the largest prospective followup study of children born after assisted-reproduction technology, and one of the largest programs of preimplantation genetic diagnosis.

Ian Wilmut is the director of the Roslin Institute in Scotland. He was the first to clone a mammal (in 1996), a Finn Dorset lamb named Dolly, from fully differentiated adult mammary cells. Dr. Wilmut's work, published in 1997, pushed the concept of cloning into the news and public debate. Dr. Wilmut attended the University of Nottingham for his undergraduate work. In 1971, he received a PhD in animal genetic engineering from Darwin College of the University of Cambridge. In 1974, he joined the Animal Research Breeding Station in Scotland, which is now known as the Roslin Institute, and he has conducted research there ever since.

Ryuzo Yanagimachi is professor of anatomy and reproductive biology at the University of Hawaii Medical School. His research focuses on assisted reproduction in mammals. He cloned the first male mammal from adult cells (tail tip). He also developed the Honolulu technique of injecting donor nuclei from cumulus cells (a differentiated population of ovarian granulosa cells that undergo terminal differentiation and arrest in G0 in

response to the midcycle surge of luteinizing hormone thus introducing a delay that seems to make blastocyst formation more likely). He also made clones of a clone in mice.

Panayiotis Michael Zavos is a professor emeritus of reproductive physiology-andrology at the University of Kentucky; founder, director, and chief andrologist of the Andrology Institute of America; cofounder and codirector of the Kentucky Center for Reproductive Medicine and IVF; and president and CEO of ZDL, Inc., a private corporation that markets infertility products and technologies worldwide. He received a BS in biology and chemistry in 1970, an MS in biology and physiology in 1972, and an EdS in 1976 from Emporia State University, in Kansas. Dr. Zavos received his PhD in reproductive physiology and statistics in 1978 from the University of Minnesota in Twin Cities.

D

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Animal Cloning

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Glossary

This glossary was developed by the panel from several sources, including the National Institutes of Health report, *Stem Cells: Scientific Progress and Future Research Directions* [1] and the National Bioethics Advisory Committee report *Cloning Human Beings* [2]. Boldface words in glossary definitions refer to other terms defined in the glossary.

Adult stem cell – An **undifferentiated** cell found in a **differentiated** tissue in an adult organism that can renew itself and can (with certain limitations) differentiate to yield all the specialized cell types of the tissue from which it originated.

AI – See **Donor insemination**

Amniocentesis – A prenatal test performed by inserting a thin needle through the abdomen into the **uterus** and withdrawing a small amount of amniotic fluid (the fluid around the **fetus**) for laboratory testing. The fluid contains skin, kidney, and lung cells from the fetus that can be tested for chromosomal abnormalities, and the fluid itself can be tested for biochemical abnormalities. Amniocentesis is usually performed during the 15th week of pregnancy or later.

Andrology – The science dealing with the structures, functions, and disorders of the male reproductive system.

Antigen – Any substance or molecule that is recognized by the body as

"foreign" and that stimulates a specific immune response when it enters the tissues of an organism.

ARTs – See **Assisted reproductive technologies**

Artificial insemination – See **Donor insemination**

Assisted reproductive technologies (ARTs) – Fertility treatments or procedures that involve laboratory handling of **gametes** (**eggs** and **sperm**) or **embryos**. Examples of ARTs include **in vitro fertilization (IVF)** and **intracytoplasmic sperm injection (ICSI)**.

Autoimmune disease or **disorder** – A category of diseases and disorders in which one's own cells are mistakenly identified as "foreign" by the body and are therefore attacked by the immune system, causing tissue damage.

Blastocoel – The fluid-filled cavity within the **blastula**.

Blastocyst – A **preimplantation embryo** in placental mammals (about 3 days after fertilization in the mouse, about 5 days after fertilization in humans) of about 30–150 cells. The blastocyst stage follows the **morula** stage, and can be distinguished by its unique morphology. The blastocyst consists of a sphere made up of a layer of cells (the **trophectoderm**), a fluid-filled cavity (the **blastocoel** or **blastocyst cavity**), and a cluster of cells on the interior (the **inner cell mass**, or **ICM**). The ICM, consisting of **undifferentiated** cells, gives rise to what will become the **fetus** if the blastocyst is implanted in a uterus. These same ICM cells, if grown in **culture**, can give rise to **embryonic stem cell** lines. At the time of **implantation** the mouse blastocyst is made up of about 70 **trophoblast** cells and 30 ICM cells.

Blastocyst cavity – The fluid-filled cavity within the **blastocyst,** sometimes referred to as the **blastocoel**.

Blastomere – A cell from a **morula**-stage **embryo**.

Blastula – Term (often used in lower vertebrates) to describe an early stage in the development of an **embryo** consisting of a hollow sphere of cells enclosing a fluid-filled cavity called the **blastocoel**. The term *blastula* sometimes is used interchangeably with **blastocyst**.

Cell line – A general term applied to a defined population of cells that has been maintained in **culture** for an extended period and usually has undergone a spontaneous process, called **transformation**, that

allows the cells to continue dividing (replicating) in culture indefinitely.

CGH – See **Comparative genomic hybridization**

Chimera – An organism composed of cells derived from at least two genetically different individuals.

Chorion – The outermost of the two membranes surrounding the **embryo**/**fetus**, part of which forms the fetal portion of the **placenta**.

Chorionic villus sampling (CVS) – A prenatal test performed by removing a small sample of the **placenta** from the **uterus** with either a catheter (a thin flexible tube) or a needle. The sample can be tested for genetic abnormalities. Chorionic villus sampling is usually done between the 10th and 12th weeks of pregnancy.

Chromosomes – Structures composed of very long **DNA** molecules (and associated proteins) that carry most of the hereditary information of an organism. Chromosomes are divided into functional units called **genes**, each of which contains the genetic code (instructions) for making a specific **protein**. A normal human body cell (**somatic cell**) contains 46 chromosomes; a normal human reproductive cell (**gamete**) contains 23 chromosomes.

Cleavage – The process of cell division in the very early **embryo** before it becomes a **blastocyst**.

Cleavage pattern – The pattern in which cells in a very early **embryo** divide; each species of organism displays a characteristic cleavage pattern that can be observed under a microscope. Departure from the characteristic pattern usually indicates that an embryo is abnormal, so cleavage pattern is used as a criterion for **preimplantation screening** of embryos.

Clone – 1) An exact genetic replica of a **DNA** molecule, cell, tissue, organ, or entire plant or animal. 2) An organism that has the same nuclear **genome** as another organism.

Cloning – The production of a **clone**. (For the purpose of this report, generating an individual animal or person that derives its nuclear **genes** from a **diploid** cell taken from an **embryo**, **fetus**, or born individual of the same species.)

Comparative genomic hybridization (CGH) – A chromosomal screening technique that permits the detection of quantitative changes in chromosomal copy number without the need for cell **culturing**. It provides a global overview of chromosomal gains and losses

throughout the whole **genome** (including extra, missing, and broken **chromosomes**), but cannot detect small changes in DNA sequence or changes in the **imprinting** state of a **gene**.

Culture – Growth of cells, tissues or **embryos in vitro** on an artificial nutrient medium in the laboratory.

CVS – See **Chorionic villus sampling**

Cytoplasm – The contents of a cell other than the **nucleus**. Cytoplasm consists of a fluid containing numerous structures, known as organelles, that carry out essential cell functions.

DI – See **Donor insemination**

Differentiated – Having developed into a specialized cell or tissue type

Differentiation – The process whereby an unspecialized early embryonic cell or **stem cell** acquires the features of a specialized cell, such as a heart, liver, or muscle cell.

Diploid – Refers to a cell having two sets of chromosomes (in humans, 46 chromosomes). In contrast, a **haploid** cell, such as a **gamete**, has only one set of chromosomes (23 in humans).

DNA – A chemical, deoxyribonucleic acid, found primarily in the **nucleus** of cells (some is also found in the **mitochondria**). DNA is the genetic material that contains the instructions for making all the structures and materials the body needs to function. **Chromosomes** and their subunits, **genes**, are made (primarily) of DNA.

DNA methylation –See **Methylation**

Donor insemination (DI) or **Artificial insemination (AI)** – Deposition of **sperm** from a male donor inside a female reproductive tract for the purpose of achieving pregnancy.

EBs – See **Embryoid bodies**

EG cells – See **Embryonic germ cells**

ES cells – See **Embryonic stem cells**

Egg – The mature female reproductive cell.

Embryo – A group of cells arising from the **egg** that has the potential to develop into a complete organism. In medical terms, **embryo** usually refers to the developing human from **fertilization** (the **zygote** stage) until the end of the eighth week of gestation when the beginnings of the major organ systems have been established.

Embryo splitting – Separation of an early-stage **embryo** into two or more embryos with identical genetic makeup, essentially creating **identical twins** or higher multiples (triplets, quadruplets, etc.).

Embryoid bodies (EBs) – Irregularly shaped clumps of cellular structures that arise when **embryonic stem cells** or **embryonic germ cells** are cultured. Embryoid bodies usually contain tissue from all three of the germ layers: endoderm, mesoderm, and ectoderm. Embryoid bodies are not part of normal development and occur only **in vitro**.

Embryonic germ (EG) cells – **Pluripotent stem cell lines** that migrate, during early development, to the future **gonads** to form the **progenitors** of **egg** or **sperm** cells. The properties of EG cells are similar to those of **embryonic stem cells**, but may differ in the **DNA methylation** of some imprinted regions.

Embryonic stem (ES) cells – Primitive (**undifferentiated**) cultured cells from the **embryo** that have the potential to become a wide variety of specialized cell types (that is, are **pluripotent**). They are derived from the **inner cell mass** of the **blastocyst**. Embryonic stem cells are not embryos; by themselves, they cannot produce the necessary cell types, such as **trophectoderm** cells, in an organized fashion so as to give rise to a complete organism.

Embryonic stem (ES) cell lines – Populations of dividing cells established from **embryonic stem cells** and **cultured** in the laboratory. Within embryonic cell lines are cells that can produce more embryonic stem cells or, under conditions of **differentiation**, give rise to collections of cells that include most or all cell types that can be found in a postimplantation embryo, **fetus**, or developed organism.

Enucleation – A process whereby the nuclear material of a cell is removed, leaving only the **cytoplasm**. When applied to an **egg**, the removal of the maternal **chromosomes**, which are not surrounded by a nuclear membrane.

Epigenetic effects – Changes in **gene expression** that occur without changing the **DNA** sequence of a **gene**; for example, in the epigenetic effect called genomic **imprinting**, chemical molecules called methyl groups attach to DNA and "turn off" the gene's expression.

Extraembryonic tissues – Intrauterine tissues derived from the **zygote** that support the **embryo** (for example, the **placenta**, the umbilical cord, and membranes such as the amniotic sac).

Fertilization – The process whereby male and female **gametes** (**sperm** and **egg**) unite.

Fetus – 1) Legally, refers to the developing organism from the completion of **implantation** in the **uterus** to the time of birth. 2) In medical terms, refers to the developing human from the end of the eighth week to birth. At the end of the eighth week, the **embryo** is 2.0–3.0 cm (0.8–1.2 in.) long and weighs 1–4.5 g (0.04–0.16 oz). The major organ systems (for example, the nervous and cardiovascular systems) and rudiments of limbs, fingers, and toes have formed.

Fibroblast – Cells that give rise to part of the connective tissue.

Fluorescence in situ hybridization (FISH) – A technique that can be used for **prenatal diagnosis**, in which specifically designed fluorescent molecules are used to "light up" particular **genes** or sections of **chromosomes** to make them visible under a microscope. The fluorescence makes even small abnormalities in the chromosomes visible.

Gamete – A reproductive cell (**egg** or **sperm**). Gametes are **haploid** (having only half the number of chromosomes found in **somatic** cells – 23 in humans), so that when two gametes unite at **fertilization**, the resulting one-cell embryo (**zygote**) has the full number of chromosomes (46 in humans).

Gene – A functional unit of heredity that is a segment of **DNA** in a specific site on a **chromosome**. A gene directs the formation of a **protein** or **RNA** molecule.

Gene expression – The process by which **RNA** and **proteins** are made from the instructions encoded in **genes**. Alterations in gene expression change the function of the cell, tissue, organ, or whole organism and sometimes result in observable characteristics associated with a particular gene.

Genome – The complete genetic material of an organism.

Genomic imprinting – See **Imprinting**

Germ cell or **Germline cell** – A **sperm** or **egg**, or a cell that can develop into a sperm or egg; all other body cells are called **somatic cells**.

Germinal vesicle transfer – See **Oocyte nuclear transfer**

Germline cell – See **Germ cell**

Gestation – The period of development of an organism from **fertilization** of the **egg** until birth.

Gonad – The reproductive organ that contains the developing **sperm** or **eggs**. The mature male gonads are the testes, and the mature female gonads are the ovaries.

Graft-versus-host disease – A condition that occurs after tissue transplantation in which the donor-derived T cells attack the host's tissues.

Haploid – Refers to a cell (usually a **gamete**) having only one set of **chromosomes** (23 in humans). In contrast, body cells (**somatic cells**) are **diploid**, having two sets of chromosomes (46 in humans).

Hematopoietic stem cell – A **stem cell** from which all red blood cells, white blood cells, and platelets develop.

Heteroplasmy – See **Mitochondrial heteroplasmy**

Identical twins – See **Monozygotic twins**

Implantation – The process by which an **embryo** becomes attached to the inside of the **uterus** (7-14 days in humans).

Imprinting – A process whereby **DNA** obtains biochemical marks that instruct a cell how and when to express certain **genes**. Imprinting often results in **gene expression** from only one copy of a gene – either the maternal or paternal copy.

In utero – Latin: literally, "in the **uterus**."

In vitro – Latin: literally, "in glass"; in a laboratory dish or test tube; in an artificial environment.

***In vitro* fertilization (IVF)** – An assisted reproduction technique in which **fertilization** is accomplished outside the body.

In vivo – Latin: literally, "in the living" subject; in a natural environment.

Informed consent – A process in which a patient gives written consent (agreement) to undergo a medical procedure after having been provided with information about the nature of the procedure, risks, potential benefits, alternatives, and so on by his or her doctor.

Inner cell mass – The cluster of cells inside the **blastocyst**. Before **implantation**, these can give rise to **embryonic stem cell lines**. After

implantation, the inner cell mass gives rise to all the tissues of the **fetus**, as well as some of the membranes around it.

Institutional review board (IRB) – An administrative body in an institution (such as a hospital or university) established to protect the rights and welfare of human research subjects recruited to participate in research activities conducted under the auspices of that institution. The IRB has the authority to approve, require modifications in, or disapprove research activities in its jurisdiction, as specified by both federal regulations and local institutional policy.

Intracytoplasmic sperm injection – An assisted reproductive method in which a **sperm** is injected directly into an unfertilized **egg** with a microscopic needle; this procedure is used in cases of severe **male factor infertility**.

IVF – See **In vitro fertilization**

Karyotype – The full set of **chromosomes** of a cell arranged with respect to size, shape, and number. This arrangement allows visual comparison of the chromosomes and identification of gross abnormalities (e.g. extra, missing or broken chromosomes).

Major histocompatibility complex (MHC) – A group of **genes** that code for cell surface **proteins** that play a major role in histocompatibility (tissue compatibility; Latin: histo = tissue) in transplantation. Differences between the MHC proteins of a transplant donor and recipient are the major cause of transplant tissue rejection.

Male factor infertility – Condition in which a male patient is infertile for such reasons as very low **sperm** count, sperm that cannot swim properly, sperm that are unable to penetrate the **egg**, or blocked sperm ducts.

Meiosis – Cell division in the specialized tissues of ovaries and testes that results in the production of **sperm** or **eggs,** which contain half the number (23 in humans) of **chromosomes** found in somatic cells. During **fertilization**, the **nuclei** of the sperm and egg fuse to produce a **zygote** with the full number of chromosomes (46 in humans).

Methylation – A biochemical process involving the addition of chemical tags called methyl groups (-CH3) to **DNA**. Methylation can be a signal for a **gene** or a section of a **chromosome** to turn off **gene expression** and become inactive or "silent."

MHC – See **Major histocompatibility complex**

Minor H antigens – See **Minor histocompatibility antigens**

Minor histocompatibility antigens or **Minor H antigens** – A group of **proteins** (in addition to those encoded by the **major histocompatibility complex** (**MHC**) that can cause transplant tissue rejection. Minor H antigens can cause tissue rejection even when donor and recipient are matched for MHC. Immune response to minor H antigens is far less potent than response to MHC-encoded proteins, so the rejection is a slower process.

Mitochondria – See **Mitochondrion**

Mitochondrial heteroplasmy – An atypical condition characterized by the presence of more than one type of mitochondrial **DNA** in a single individual. Normally, each individual has only one type of mitochondrial DNA, inherited from his or her mother through the **egg** at **fertilization**. (**Mitochondria** from the **sperm** are systematically eliminated by the egg at fertilization.) Cloned organisms may exhibit mitochondrial heteroplasmy (having a mixture of mitochondria from both the donor cell and the recipient egg) because this elimination system may be bypassed during the cloning process.

Mitochondrion (plural, **Mitochondria**) – A cellular structure in the **cytoplasm** that provides energy to the cell. Each cell contains many mitochondria. In humans, a single mitochondrion contains 37 **genes** on a circular mitochondrial **DNA**, compared with about 35,000 genes contained in the nuclear **DNA**.

Monozygotic twins – Twins derived from one **egg** and one **sperm** (often called **"identical twins"**).

Morula – The **preimplantation embryo** 3–4 days after **fertilization**, when it is a solid mass composed of 12–32 cells (**blastomeres**). After the eight-cell stage, the cells of the preimplantation embryo begin to adhere to each other more tightly, becoming "compacted." The resulting embryo resembles a mulberry and is called a morula (Latin: morus = mulberry).

Multipotent stem cells – **Stem cells** from the **embryo**, **fetus**, or adult, whose progeny are of multiple **differentiated** cell types and usually, but not necessarily, all of a particular tissue, organ, or physiological system.

Mutation – A change in **DNA** that alters a **gene** and thus the gene's product, leading in some cases to deformity or disease. Mutations can occur spontaneously during cell division or can be triggered by environmental stresses, such as sunlight, radiation, and chemicals.

Nuclear transfer – A procedure in which a **nucleus** from a donor cell is transferred into an **enucleated egg** or **zygote** (an egg or zygote from which the **nucleus**/**pronuclei** have been removed). The donor nucleus can come from a **germ cell** or a **somatic cell**.

Nuclei – See **Nucleus**

Nucleus (plural, **Nuclei**) – The compartment of a cell that contains the **chromosomes**.

Oocytes – The developing female reproductive cells (the developing **eggs**) produced in the ovaries.

Oocyte nuclear transfer or **Germinal vesicle transfer** – An assisted reproductive technique involving transfer of an **egg** nucleus (usually from a woman with age-related infertility or mitochondrial disease) into a healthy donor egg whose nucleus has been removed. This reconstituted egg can then be fertilized by a **sperm in vitro**. This technique may restore fertility to older women or to prevent the passing of mitochondrial disease to offspring.

Ooplasmic transfer – An assisted reproduction technique that essentially enhances the defective (egg **cytoplasm**) from the patient's **egg** with healthy cytoplasm from a donor egg. This "enhanced" egg can then be fertilized by a **sperm in vitro**. This procedure may restore fertility to older women.

PCR – See **Polymerase chain reaction**

PGD – See **Preimpantation screening**

Placenta – A **vascular** organ-like structure that develops in the **uterus** during pregnancy, serving to anchor the **embryo** or **fetus** after **implantation**. The placenta enables oxygen and nutrients to pass from the maternal blood to the embryo or fetus. It also eliminates carbon dioxide and waste products from the embryo or fetus by passing them to the mother, who excretes them through her liver, kidneys, or lungs.

Pluripotent stem cells (PSCs) – **Stem cells** that include in their progeny all cell types that can be found in a postimplantation **embryo**, **fetus**, or developed organism.

Polymerase chain reaction (PCR) – A technique for making multiple copies of a specific stretch of **DNA** or **RNA**; can be used to test for **mutations** in DNA. For example, if a stretch of DNA is mutated, the copies of it made with the PCR can be longer or shorter than normal.

Precursor cells or **Progenitor cells** – In fetal or adult tissues, these are partially **differentiated** cells that divide and give rise to differentiated cells.

Preimplantation embryo – The very early, free-floating **embryo**, from the time the **egg** is fertilized (**zygote**) until the beginning of **implantation** (in humans, a period of about 6 days). Also includes embryos resulting from **nuclear transfer**, in all the developmental stages through the **blastocyst** stage.

Preimplantation screening or **Preimplantation genetic diagnosis (PGD)** – Before an **in vitro-fertilized embryo** is implanted in a woman's **uterus**, it can be screened for specific genetic **mutations** that are known to cause particular genetic diseases or for chromosomal abnormalities. One or more cells are removed from the **preimplantation embryo** for testing.

Prenatal diagnosis – Detection of abnormalities and disease conditions while a **fetus** is developing in the **uterus**. Many techniques for prenatal diagnosis, such as **chorionic villus sampling** and **amniocentesis**, require sampling **placental** tissue or fetal cells found in the amniotic fluid or fetomaternal circulation. Others, such as **ultrasonography**, can be performed without cell or tissue samples.

Progenitor cells – See **Precursor cells**

Pronuclei – See **Pronucleus**

Pronucleus (plural, **Pronuclei**) – Refers to the **haploid nucleus** of **egg** or **sperm** prior to **fertilization**, and immediately after fertilization, before the sperm and egg nuclei have fused into a single **diploid** nucleus.

Protein – A large complex molecule made up of one or more chains of amino acids. Proteins perform a wide variety of activities in the cell.

PSC – See **Pluripotent stem cells**

Recloning – See **Serial nuclear transfer**

Reprogramming – Resetting the developmental clock of a **nucleus**; for example, resetting the developmental state of an adult **differentiated** cell nucleus so that it can carry out the genetic program of an early embryonic cell nucleus, making all the **proteins** required for embryonic development. In **somatic cell nuclear transfer**, components of the recipient **egg cytoplasm** are thought to play an important role in reprogramming the somatic cell nucleus to carry out the functions of an embryonic nucleus.

RNA (Ribonucleic acid) – A chemical that is similar in structure to **DNA**. One of its main functions is to translate the genetic code of DNA into structural **proteins**.

Serial nuclear transfer or **Recloning** – The first step of this technique is a normal **nuclear transfer**, in which a **nucleus** is transferred into an enucleated **egg**, forming an **embryo**. In the second step, a nucleus from the resulting cloned embryo is transferred into another **enucleated** egg or an enucleated **zygote** (a fertilized egg with both male and female **pronuclei** removed). The second step can be repeated one or more times. This technique allows the nucleus to have two (or more) opportunities to be **reprogrammed** by egg **cytoplasm** (one during the original nuclear transfer, and more during subsequent nuclear transfers), thus potentially improving the chance of successful reprogramming of the nucleus.

Somatic cell nuclear transfer (SCNT) – Transfer of the **nucleus** from a donor **somatic cell** to an unfertilized **egg** cell from which the maternal **chromosomes** have been removed.

Somatic cell – Any cell of a plant or animal other than a reproductive cell or reproductive cell **precursor**. Latin: soma = body.

Sperm – Mature male reproductive cells.

Stem cells – Nonspecialized cells that have the capacity to divide indefinitely in **culture** and to differentiate into more mature cells with specialized functions.

Stochastic – Random or involving a random variable.

Telomerase – An enzyme composed of a catalytic **protein** component and an **RNA** template and that synthesizes the telomeric **DNA** at the ends of **chromosomes**. When active, telomerase can continually add to the length of the telomeres on the ends of chromosomes within a cell, thus conferring on that cell the ability to continue dividing past its normal lifespan.

Telomeres – "Caps" (made of repeated DNA sequences) found at the ends of **chromosomes** that protect the ends of the chromosomes from degradation. The telomeres on a chromosome shorten with each round of cell replication. Telomere shortening has been suggested to be a "clock" that regulates how many times an individual cell can divide (that is, when the telomeres of the chromosomes in a cell shorten past a particular point, the cell can no longer divide).

Tissue culture – See **Culture**

Totipotent cells – **Stem cells** that have unlimited developmental capability. The totipotent cells of the very early **embryo** (an embryo prior to the **blastocyst** stage) have the capacity to **differentiate** into **extraembryonic** tissues, membranes, the embryo, and all postembryonic tissues and organs.

Transcription – Making an **RNA** copy from a **gene** or other **DNA** sequence. Transcription is the first step in **gene expression**.

Transformation – A genetic process resulting in a heritable alteration of the properties of a cell. In the case of **cultured** cells, transformation often refers to the acquisition of new properties, such as unlimited culture lifespan.

Translation – The process of forming a **protein** molecule from information contained in messenger **RNA**.

Trophectoderm – The outer layer of the developing **blastocyst** that will ultimately form the embryonic side of the **placenta**.

Trophoblast – The **extraembryonic tissue** arising from the outer layer of the **blastocyst**, involved in **implantation** and later in development of the **placenta** and **chorion**.

Ultrasonography – Commonly called "ultrasound." An imaging technique that uses high-frequency sound waves to create an image. During pregnancy, ultrasonography can be used to provide an image of the developing **fetus**, including the entire body, organs and surrounding tissue.

Undifferentiated – Not having developed into a specialized cell or tissue type.

Unipotent stem cell – A **stem cell** that both divides and gives rise to a single mature cell type, such as a spermatogenic stem cell, which only gives rise to sperm.

Uterus – The muscular pear-shaped organ (in humans, located in the lower part of a woman's abdomen) in which the **fetus** develops.

Vascular – Composed of or having to do with blood vessels.

WGA – See **Whole genome amplification**

Whole-genome amplification (WGA) – A technique that allows production of enough **DNA** from a single cell to do multiple genetic analyses; involves nonspecific **polymerase chain reaction (PCR)** amplification of an entire **genome**, providing templates for later PCR to produce more copies of the genome.

X chromosome – One of the two sex **chromosomes**, the other being the **Y chromosome**. Females have two X chromosomes, and males have one X chromosome and one **Y chromosome**.

X inactivation – Normal inactivation of one of the two **X chromosomes** in females.

Y chromosome – The **chromosome** that determines male gender.

Zygote – The one-cell **embryo** formed by the union of **sperm** and egg at **fertilization**.

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