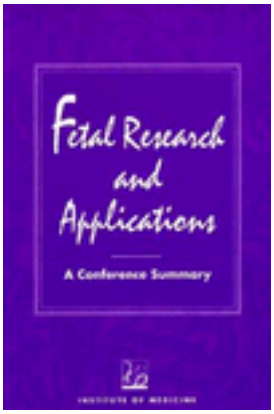

Fetal Research and Applications: A Conference Summary

Conference Committee on Fetal Research and Applications, Institute of Medicine

ISBN: 0-309-57341-6, 116 pages, 6 x 9, (1994)

Fetal Research and Applications

A Conference Summary

Conference Committee on Fetal Research and Applications
Division of Health Promotion and Disease Prevention
INSTITUTE OF MEDICINE

NATIONAL ACADEMY PRESS
Washington, D.C. 1994

NATIONAL ACADEMY PRESS 2101 Constitution Avenue, N.W. Washington, DC 20418

NOTICE: The project that is the subject of this report was approved by the Governing Board of the National Research Council, whose members are drawn from the councils of the National Academy of Sciences, the National Academy of Engineering, and the Institute of Medicine. The members of the committee responsible for the report were chosen for their special competencies and with regard for appropriate balance.

This report has been reviewed by a group other than the authors according to procedures approved by a Report Review Committee consisting of members of the National Academy of Sciences, the National Academy of Engineering, and the Institute of Medicine.

The Institute of Medicine was chartered in 1970 by the National Academy of Sciences to enlist distinguished members of the appropriate professions in the examination of policy matters pertaining to the health of the public. In this, the Institute acts under the Academy's 1863 congressional charter responsibility to be an adviser to the federal government and its own initiative in identifying issues of medical care, research, and education. Dr. Kenneth I. Shine is president of the Institute of Medicine.

Support for this study was provided by National Research Council funds.
Library of Congress Catalog Card Number 94-67254

International Standard Book Number 0-309-05176-2
Additional copies of this report are available from: National Academy Press 2101 Constitution Avenue, N.W. Box 285 Washington, DC 20055 Call 800-624-6242 or 202-334-3313 (in the Washington Metropolitan Area)

B474
Printed in the United States of America

The serpent has been a symbol of long life, healing, and knowledge among almost all cultures and religions since the beginning of recorded history. The image adopted as a logotype by the Institute of Medicine is based on a relief carving from ancient Greece, now held by the Staatlichemusseen in Berlin.

CONFERENCE COMMITTEE ON FETAL RESEARCH AND APPLICATIONS

UTA FRANCKE,* *Chair*, Professor of Genetics and Pediatrics, Stanford University Medical Center, Stanford, California

FREDERICK C. BATTAGLIA,* Professor, Department of Pediatrics, University of Colorado Health Sciences Center, Denver, Colorado

MARTHA CONSTANTINE-PATON, Professor of Biology, Yale University, New Haven, Connecticut

EZRA C. DAVIDSON,* Jr., Professor and Chairman, Department of Obstetrics and Gynecology, Charles R. Drew University of Medicine and Science, King-Drew Medical Center, Los Angeles, California

JOSEPH M. DAVIE,* Vice President of Research, Biogen, Inc., Cambridge, Massachusetts

NEAL L. FIRST,** Professor of Reproductive Biology and Biotechnology, Department of Meat and Animal Sciences, University of Wisconsin, Madison, Wisconsin

HAIG H. KAZAZIAN,* Jr., Sutland Professor of Pediatric Genetics, The Johns Hopkins University, Director, Center for Molecular Genetics, The Johns Hopkins Hospital, Baltimore, Maryland

Institute of Medicine Staff

CONSTANCE M. PECHURA, Study Director, Associate Director, Division of Biobehavioral Sciences and Mental Disorders

GARY B. ELLIS, Director, Division of Health Promotion and Disease Prevention (to January 1993)

MICHAEL STOTO, Director, Division of Health Promotion and Disease Prevention

TERRI BARBA, Project Assistant

Consultant

JUDITH M. SCHWARTZ, Contract Writer

* Member, Institute of Medicine
** Member, National Academy of Sciences

Acknowledgments

The committee and staff are grateful for the time and effort of the conference speakers. These individuals shared their considerable expertise through presentations that brought clarity and focus to difficult issues, ranging from legal and ethical dilemmas to scientific challenges and opportunities. Further, their work encouraged a remarkably free exchange of ideas. We hope that this report adequately reflects these important contributions.

We also wish to thank the conference participants, many of whom traveled long distances and took time out from busy schedules to attend. The discussions benefited greatly from these participants' questions and observations, and the report is richer for them.

Finally, we wish to thank the Institute of Medicine and the National Academy of Sciences for supporting this conference and the individuals who provided insightful review of the report draft.

Uta Francke

Chair

Contents

Fetal Research and Applications

Introduction

Research involving human fetuses and fetal tissues has been a subject of national debate and rancor for more than two decades. Despite the many demonstrated medical benefits of such research and the issuance of guidelines by various governmental and private ethics advisory bodies, federal support of this research has been severely curtailed. The only research so funded has been that which involved potential therapeutic benefit to the fetus, or involved no more than minimal risk to the fetus even if potential benefit to the mother could be demonstrated. Thus, scientific investigation in this area has depended almost exclusively on funds provided by pharmaceutical companies, private foundations, university budgets, and patient fees. The effects of federal restrictions, in addition to restricting the amount of research done, include the lack of a national, consensus-derived research agenda, absence of organized peer review of proposed research protocols, and advance of the clinical practice of medically assisted conception without the benefit of a robust scientific knowledge base.

In 1988 and 1989, about the time a three-year federal moratorium banning research on a fetus that involved more than minimal risk was scheduled to expire, reports were issued by the U.S. Congress Office of Technology Assessment and the Association of American Medical Colleges on biotechnology, infertility, and fetal research and fetal tissue research.[1] The Institute of Medicine (IOM) also issued a report in 1989 that contained policy recommendations and a re

[1] U.S. Congress, Office of Technology Assessment, *New Developments in Biotechnology: Ownership of Human Tissues and Cells*, OTA-BA-337, U.S. Government Printing Office, Washington, D.C., March 1987. U.S. Congress, Office of Technology Assessment, *Infertility: Medical and Social Choices*, OTA-BA-358, U.S. Government Printing Office, Washington, D.C., May 1988. Association of American Medical Colleges, Summary, *Fetal Research and Fetal Tissue Research*, Washington, D.C., 1988.

search agenda for increasing the science base underlying medically assisted conception.[2] This report dealt exclusively with issues related to in vitro fertilization (IVF), but its recommendations regarding the development of research policy and practice oversight, the establishment of research programs coordinated by the National Institutes of Health, multicentered data collection, and improvements in communication among researchers and clinical practioners could apply to other aspects of fetal research and fetal tissue research as well. Yet, until President Clinton lifted the federal moratorium (which had been extended following controversy about fetal tissue transplantation research) in January 1993 (58 FR 7468), nothing changed.

In 1992, following the recommendation of the IOM Program Committee, the National Academy of Sciences and the Institute of Medicine decided to support with their own funds a project, proposed by the IOM Board on Health Promotion and Disease Prevention, to examine the current state of fetal research and fetal tissue research. The IOM convened a committee to plan a conference on the topic. This report is the result of the committee's efforts.

The initial aim was to help inform private funders, who often lack the formalized peer review systems used by government funding agencies, about new and promising areas of research in the field and, thus, to serve as an outside assessment of research priorities. The committee, formed in 1992, was comprised of senior scientists, broadly knowledgeable in the field, but not themselves directly involved in human fetal studies. Although time and funds did not permit full elaboration of a detailed research agenda, the conference did provide an overview of current research and a forum for discussion of key questions for the future: What are the important problems in fetal research that can be approached with current or developing technology? What are the ethical boundaries?

The conference, organized into four sessions and involving 24 speakers, was held June 20–22 in Irvine, California. The first session introduced some of the ethical and legal issues of research with fetuses and fetal tissues; the following sessions on preembryo research, fetal research, and fetal tissue transplantation formed the main scientific portion of the conference (Appendix A). Discussion periods encouraged interaction among the speakers and conference participants, including other scientists, physicians, and bioethicists, as well as governmental advisers and public health and health industry representatives (Appendix B).

This report begins with an overview of fetal research and research with fetal tissue, followed by a short history and background regarding the regulatory and legislative actions governing this research over the past 30 years. These sections are provided to add breadth for the reader unfamiliar with the field and its complicated legislative history. The main body of the report summarizes the conference presentations and discussions. A glossary of terms is included and all

[2] Institute of Medicine, *Medically Assisted Conception: An Agenda for Research*, National Academy Press, Washington, D.C., 1989.

sections are written with a wide audience in mind, including lay persons and policymakers, as well as scientists and science administrators. Although there are no policy recommendations offered in this report, conclusions given by individual speakers and discussed by conference participants are emphasized. Further, Appendix C contains the summary of the recommendations and research agenda provided in the 1989 Institute of Medicine report on medically assisted conception to allow readers to put these recommendations in context with suggestions and topics discussed at the 1993 conference.

The prospect of federal funding for this area of research now seems brighter. Although some areas will remain controversial, even divisive, there are ample opportunities for progress where ethical consensus is possible. Greater attention is being brought to this field by changes in the political climate, the advance of technology, and the increasing concern among often polarized public constituencies. Dramatic evidence of these concurrent forces blazed recently across the nation's front pages with the news that human embryos could now be cloned.[3] The committee hopes that the background given and the research summarized here will provide insights for future progress and contribute to a fuller understanding of the social and ethical issues involved.

[3] Gina Kolata, "Cloning Human Embryos," *New York Times*, October 26, 1993.

Setting the Stage: Fetal Research, Fetal Tissue Research, and Historical Timeline of Regulation and Legislation[1]

FETAL RESEARCH

Fetal research is research done with living fetuses either inside the uterus (in utero) or outside the uterus (ex utero). Fetal research, in many contexts including this report, also refers to research with embryos. Some confusion about these terms is understandable, because they are used differently in various contexts. For example, although in legal writing the term "fetus" refers to all prenatal stages (see Figure 1 for various developmental stages), the term "embryo" is often used to denote the earliest stages following fertilization of an ovum by a sperm. Terms relating to different stages of embryonic development, however, are common and include *zygote* (the fertilized egg); early cleavage embryos produced by cell division up to the 50-to 60-cell stage, each cell of which is called a *blastomere*; and *blastocyst* (from the 60-cell stage to the point of implantation). Also commonly used are the terms "preimplantation" and "postimplantation" embryo, relatively self-evident in meaning, and "preembryo" referring to very early stages of development up to about an 8-or 16-cell mass.

Fetal research involves both invasive and noninvasive techniques (some of which are no longer used) and has led to improved techniques of in vitro fertilization and embryo transfer, and to major advances in the diagnosis and treatment of conditions that threaten the survival of fetuses and pregnant women. Some of these include

[1] Some of the material in this section is presented in greater detail in the 1988 report on fetal research and fetal tissue research from the Association of American Medical Colleges, cited in footnote 1 of the Introduction (p. 1). Although six years has passed since that report, little has changed. Additional material was drawn from a recent broad overview of the state of biomedical ethics in the United States; U.S. Congress, Office of Technology Assessment, *Biomedical Ethics in U.S. Public Policy*, OTA-BP-BBS-105, U.S. Government Printing Office, Washington, D.C., June 1993.

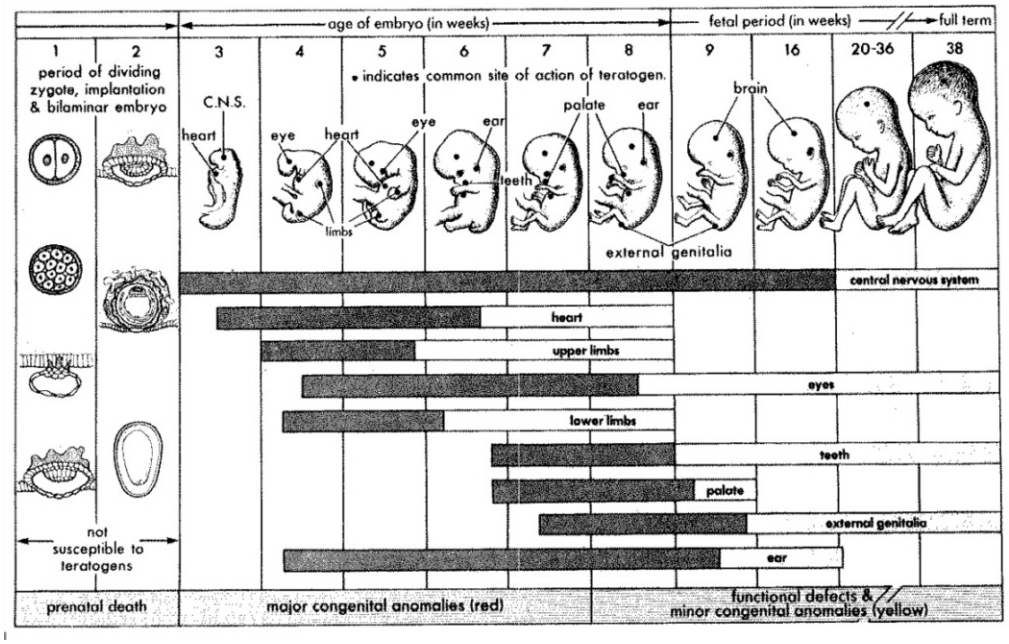

Figure 1
Schematic illustration of the critical periods in human development. During the first two weeks of development, the embryo is usually not susceptible to teratogens. During these predifferentiation stages, either a substance damages all or most of the cells of the embryo, resulting in its death, or it damages only a few cells, allowing the embryo to recover without developing defects. SOURCE: Moore, K.L., The Developing Human: Clinically Oriented Embryology, Third Edition, Philadelphia, W. B. Saunders Company, 1982. Reprinted with permission.

- tests of efficacy of rubella (German measles) vaccine;
- detection and treatment of Rh incompatibility (an immune system mismatch between the mother and fetus);
- development of diagnostic techniques including amniocentesis, ultrasonography, and chorionic villi sampling;
- detection of genetic and metabolic diseases in the fetus and assessment of other developmental problems, including fetal lung immaturity; and
- development of better techniques for obstetrical anesthesia and treatment of maternal hypertension, heart disease, and diabetes.

Much of the regulatory and legislative history concerns fetal research, rather than fetal tissue research. Fetal research has attracted greater scrutiny largely because it emerged earlier than the very controversial use of fetal tissue for transplantation. Fetal research also employed technology that emerged earlier (although tissue culture techniques were used for fetal tissue research about the same time) and had wide applications ranging from improving the survival of fetuses to improving the fertility of couples that have difficulty conceiving. Although current controversy is more closely focused on fetal tissue research, especially fetal tissue transplantation, fetal research by its nature involves the complete spectrum of ethical, legal, and social issues that attend to experiments on living fetuses in utero, embryos produced by in vitro methods, and even the very ownership of those embryos.

FETAL TISSUE RESEARCH

Fetal tissue research involves cells from dead fetuses that are harvested for the purpose of establishing cell lines or for use as transplantation material and other purposes. There are two sources of such fetal tissue—elective (or induced) abortions and spontaneous (or natural) abortions. Cell lines are established by culturing fetal cells in such a way that they continue growing and multiplying in laboratory dishes. Such cells can be used to test a drug's ability to damage genetic material or to test the effects of specific viral (or other types) of infection. Because the cells multiply, a small number of cells harvested from a dead fetus can be greatly expanded and used either as a source of more cell lines or for transplants.

Fetal tissue has been used for transplantation for two reasons. First, certain fetal tissues lack cell-surface markers found in mature tissue that induce immune system reactions in transplant recipients and lead to tissue rejection and transplant failure. Thus, fetal tissue eludes these body defenses. In addition, groups of different kinds of fetal cells can be separated from one another in the laboratory to remove those cells that may trigger a recipient's immune system. Second, certain areas of the body do not regenerate after birth or after a few years of life, so the use of mature tissue for transplantation is not possible. Adult brain cells,

for example, regenerate slowly if at all, but when fetal brain cells are transplanted they will grow readily.

It is interesting that fetal tissue research has produced one of the major medical breakthroughs of our time, the development of polio vaccine through the use of fetal cell lines in the 1950s, but also some of greatest current controversy about the use of such cells for transplantation. The first use of fetal cells for transplantation occurred in 1982, when Swedish physicians transplanted fetal brain cells into a patient suffering from Parkinson's disease, a progressive degenerative disease in which cells containing the neurotransmitter dopamine begin to die. Since that time, animal and human experiments (many of the human experiments have been done outside the United States or with private funds) have examined the usefulness of transplanting fetal cells to cure or lessen the effects of diabetes, certain blood disorders, radiation poisoning, and a variety of neurological disorders.

HISTORICAL TIMELINE OF REGULATION AND LEGISLATION

Specific reference to fetal research in federal regulations or legislation began in the 1970s. Public debate about fetal research, however, has its roots in the development of policies governing human subjects research, a process that took center stage in 1972 when the abuses of the Tuskegee Syphilis Study were exposed and a panel was convened to report on this abuse.[2] This panel also recommended that a permanent body be established to regulate human subjects research. Also, prior to the landmark Supreme Court decision in *Roe v. Wade* in 1973, fetal research was banned or regulated indirectly by many state abortion laws. Finally, for fetal tissue research in particular, regulation was applied under the Uniform Anatomical Gift Act that was ratified by the states in 1973 and regulated the use of human organs and tissues after death, prohibiting their sale for profit and their use for any but research or therapeutic reasons. Most likely, it was *Roe v. Wade* that opened the floodgates to federal regulation, however, because the legalization of abortion, although possibly increasing the potential supply of fetal tissue and cells, brought the debate into full view. The summary that follows provides an overview of that debate but, unless otherwise noted, pertains to fetal research only.

1973

Amendments were put forward in the U.S. Congress to ban research on a fetus outside the uterus, if that fetus had a beating heart. In addition, hearings were held by Senator Edward Kennedy of Massachusetts regarding the protec

[2] *Final Report of the Tuskegee Syphilis Study Ad Hoc Advisory Panel*, Department of Health, Education, and Welfare, April 28, 1973.

tion of human subjects in research. These hearings were in part fueled by controversy about reports of fetal research being done in Sweden.

1974

The National Research Act (Public Law 93-348) established a National Commission for the Protection of Human Subjects in Biomedical and Behavioral Research. This law further prohibited research on a fetus from an elective abortion until the commission had reported back to Congress. Just after passage of this law, a panel that had been convened in 1971 by the (then) Department of Health, Education, and Welfare (DHEW) issued its report. This report recommended that research resulting in no harm to the fetus be permitted, so long as that research might benefit other fetuses. The panel further recommended, as a protection to pregnant women, that no research be requested or initiated until after the abortion had been initiated. Despite the panel's report, the National Research Act took precedence and all such research was banned pending the National Commission report.

1975

After the National Commission issued its report (*Report and Recommendations: Research on the Fetus*), fetal research following abortion was permitted under subsequent DHEW regulations for therapeutic reasons, but otherwise held to the standard of "minimal risk." Minimal risk means that no more potential harm is tolerated than would be encountered in daily life. In the case of a fetus, almost all interventions exceed minimal risk, and the regulations did not distinguish between fetuses that were carried to term and those intended for abortion. The DHEW regulations, however, contained the possibility of waiver of the minimal risk standard on a project-by-project basis by a complicated procedure to be decided ultimately by an Ethics Advisory Board.

1978–1980

The first Ethics Advisory Board (EAB) was convened in 1978. The sole waiver issued by this body was to test the efficacy of using fetal blood samples for prenatal diagnosis of sickle cell anemia. The charter for the EAB expired in 1980, and despite publication of a draft charter in 1988, it has not been reactivated.

1985

The Health Research Extension Act was passed, reauthorizing the National Institutes of Health. This legislation contained two important additions. First, a three-year moratorium was imposed on issuing waivers for fetal research, so that

only research involving minimal risk or for therapeutic purposes would be allowed. Second, it called for the establishment of a Congressional Biomedical Ethics Board, comprised of members of Congress, to appoint and oversee a Biomedical Ethics Advisory Committee (BEAC).

1987

The Congressional Biomedical Ethics Board was established. Concurrently, the Uniform Anatomical Gift Act, governing fetal tissue research, was revised and submitted for ratification by the states.

1988–1989

In March 1988, Assistant Secretary for Health Robert Windom of the Department of Health and Human Services (formerly DHEW) imposed a moratorium on transplantation research with fetal tissue from induced abortions until an advisory committee could examine the ethical issues involved. The National Institutes of Health (NIH) convened an ad hoc committee, the Human Fetal Tissue Transplantation Research Panel, which issued a report in December 1988. The majority of panel members concluded that fetal tissue transplantation was acceptable and that the moratorium on use of the waiver provision should be lifted. There were a few panel members who, because of their views concerning abortion, disagreed strongly with the majority. This minority view prevailed when, in 1989, the Secretary of the Department of Health and Human Services extended the moratorium.

In 1988, legislation reauthorizing the NIH extended the fetal research moratorium, pending a report by the BEAC. Although scheduled to expire within a week, the BEAC met for the first time in September 1988 and addressed three topics, one of which was fetal research. In 1989, the BEAC became the victim of political disagreement within its governing Congressional Board and expired having issued no reports.

1993

President Clinton signed an executive order (58 FR 7468) lifting the moratorium and charging the National Institutes of Health to develop guidelines for fetal tissue transplantation research and for fetal research.

SESSION I

Ethical and Legal Issues of Fetal Research: History and Background[1]

ETHICS AND FETAL RESEARCH: PAST, PRESENT, AND FUTURE

JOHN C. FLETCHER

Center for Biomedical Ethics, University of Virginia Charlottesville, Virginia

Fetal research has been the dominant conflict in research ethics since the early 1970s. Fetal research can be categorized as therapeutic or investigative, involving a possibly viable or a nonviable fetus. The most controversial area, which has influenced all aspects of the debate, involves research affecting the fetus in elective abortion.

Beginning with the deliberations of the Peel Commission in the United Kingdom in 1972, and the United States National Commission for the Protection of Human Subjects from 1973 to 1974, three distinct ethical positions arose:

- Selectively higher research risks are permitted when abortion has been elected than when the fetus is wanted, based on the moral view of the pregnant woman as the primary decision maker in elective abortion, whose consent is required for research related to scientific and medical goals of benefit to society.
- Opposition to all investigative research in the context of abortion, based on the premise of the fetus as child: if the research will not benefit the child directly, the child is being exploited and the research is not morally justified.

[1] The first session on ethical and legal issues of fetal research was intended to give the audience a sense of the historical background of the area. In addition, the session was intended to make explicit the atmosphere in which important ethical/legal issues continue to be debated. It was not the purpose of this conference to provide in-depth treatment of these issues, however, and readers should consult referenced materials for a broader view of the controversies that exist.

- A "Golden Rule" position, adopted in the United States, based on the concept of equal protection of wanted and unwanted fetuses, with research limited to "minimal risks" and exceptions permitted only after review by an Ethics Advisory Board (EAB) and a waiver of restrictions by the Secretary of Health and Human Services.

Up to 1980, the role of the U.S. government in relation to research ethics was primarily that of patron of public discourse. During the 1980s, in a political climate influenced by antiabortion sentiment, the government dismantled most of the public process for debate and resolution, including the EAB. Federal support of fetal tissue transplant research was placed under an "indefinite moratorium" (lifted by President Clinton in 1993). As a consequence, there has been essentially no federally funded embryo or in vitro fertilization research.

Every major question related to fetal research in the United States (and many aspects of research with other vulnerable populations such as children, prisoners, and the mentally ill or impaired) needs reexamination and new policy parameters. A critical question for the future of research is whether selectively greater investigative risks are justified in instances of elective abortion, especially in the first trimester of pregnancy. Up to the present time, National Institutes of Health researchers have not been permitted to investigate the causes of cancer in human embryos obtained after in vitro fertilization (IVF). Yet, experiments with living children involving more than minimal risk, without prospect of direct benefit to the subjects but with the possibility of deriving greater knowledge about their disorders, have been allowed. The ability to transfer genes into fetal cells for therapeutic trials calls for new studies of the natural history of genetic diseases, including genetic causes of cancer. An Ethics Advisory Board to the Secretary of Health and Human Services should be restored to serve as an ongoing resource in an overall reevaluation of research ethics, beginning with fetal research.

FEDERAL AND STATE REGULATIONS OF FETAL RESEARCH: A CURRENT PERSPECTIVE[2]

LORI B. ANDREWS
American Bar Foundation, Chicago, Illinois

In 1971, medical investigators in Massachusetts undertook an experiment to learn how pregnant women metabolize certain antibiotics and whether the antibiotics cross the placenta. The research involved women planning to abort, who had consented to administration of the drugs. Specific consent had not been requested, however, for subsequent analyses of the aborted fetuses to determine

[2] See also Lori B. Andrews, "Regulation of Experimentation on the Unborn," *Journal of Legal Medicine* 14:25–56, 1993.

the effects of the antibiotics. After the study was published, an indictment was brought against the scientists under an 1814 grave-robbing statute. Although the criminal prosecution was later dismissed, the fact that it was brought at all indicates how politically charged the area of fetal research has become in the wake of *Roe v. Wade*, with significant consequences at the state level.

In addition to federal laws governing research on the unborn, 24 states have widely varying laws concerned with one or more of the following considerations:

- *Purpose*: Is the research intended to benefit the fetus, the mother, an infertile progenitor, or a tissue recipient, or is its purpose to acquire general scientific or medical knowledge?
- *Effect*: Does the research impose a risk to the life or health of the fetus? If so, what are the likelihood and the seriousness of risk?
- *Relation to abortion*: Does the research involve a fetus before, during, or after an induced abortion?
- *Fetal location*: Is the fetus in the womb or outside it?
- *Stage of development*: Has the fetus reached the stage at which it has acquired characteristics that warrant specific protection, such as the capacity for experiencing pain?

Who in fact is the beneficiary of research is a significant question often ignored by federal regulations and state statutes. Possible beneficiaries range from the pregnant woman or the fetus, to a couple, a fetal tissue transplant recipient, or the public at large. Particularly difficult issues arise when pregnant women are treated for illnesses with treatments that, although not experimental for the women, have unknown effects on the fetus and may, thus, be considered experimental for the fetus. Nine states define in utero research so broadly as to include just such a circumstance. Ironically, the research necessary to determine the fetal effects of many "standard" treatments has simply not been permitted. *Roe v. Wade* upheld the primacy of a woman's concern for her life and health, including her psychological health. Nonetheless, federal regulations regarding research (as opposed to therapeutic interventions) say that, even if an intervention may benefit the woman, it is permissible only if risk to the fetus is minimal.

With regard to the fetus itself, federal regulations distinguish between therapeutic and nontherapeutic activities, but six states do not, thereby potentially prohibiting innovative therapies even to save the fetus's life.

Research beneficial to the couple includes techniques for initiating pregnancy or for gathering information about the fetus. Such research has led to prenatal diagnostic techniques to detect the presence of genetic disorders (e.g., chorionic villi sampling, developed through research on patients planning first trimester abortion), now widely used to help a couple to decide whether or not to continue a pregnancy. Yet, seven states have laws that prohibit experimental prenatal diagnostic procedures on the fetus. Although in vitro fertilization is no longer considered experimental, some of its adjuncts are, including cryopreservation of

embryos for subsequent implantation or embryo donation to an infertile recipient. State laws that interfere with a couple's reproductive decisions by banning prenatal screening or infertility treatments, or that constrict the treatment options of a pregnant woman, have been struck down as unconstitutional by federal courts in Louisiana and Illinois.

The abortion controversy has had tremendous impact on the debate over fetal tissue transplantation research. Specific concerns include the fear that women will conceive and abort in order to donate tissue for treatment of an ailing loved one. To address this concern, an advisory panel of the National Institutes of Health (NIH) has recommended prohibition of the sale of fetal tissue or designation of a specific tissue recipient.

During the moratorium, one proposal was to establish a fetal tissue bank with tissue obtained from spontaneous abortions. Even in this circumstance, however, federal regulations defer to state law with regard to research on dead fetuses. Of 14 states that have dealt with this issue, nine allow such research with certain provisions for parental consent; four states permit only pathological examination or autopsy. Other states have regulations that echo the NIH recommendations. In states without specific regulations, decisions are based on the state's interpretation of the Uniform Anatomical Gifts Act.

Some of the greatest controversy occurs when the potential beneficiary of fetal research is less identifiable, especially the public at large. Although much information of medical relevance can be obtained through animal research, human research is ultimately necessary. Humans differ from animals. In rubella vaccination trials in monkeys, the vaccine did not cross the placenta; in human trials it did, and was therefore deemed unsafe for use with pregnant women. Many of the data that confirmed the efficacy and safety of amniocentesis were obtained from research involving fetuses prior to abortion. Of the 24 states with fetal research statutes, 12 either prohibit or seriously restrict general scientific research in utero and 18 prohibit it ex utero.

In the focus on federal research policies and funding mechanisms it is often overlooked that the legality of research involving embryos and fetuses has fallen mainly under the domain of state laws. These laws are not finely tuned and generally do not take into consideration the varied policy concerns that might be raised, based on the nature of the research and its intended beneficiary. The distinction, however, cannot be ignored by those directly involved in the research enterprise, regardless of their sources of support. For some researchers, the state in which they work will ultimately determine what experiments they may and may not undertake.

OPEN DISCUSSION PERIOD

The discussion began with an overview of the present state of regulation of fetal research in the United Kingdom. The Governmental Human Fertilization

Embryology Act allows British researchers a large degree of freedom in line with the first ethical position, based on the moral view of the pregnant woman as the primary decision maker. To aid research, the Medical Research Council maintains a tissue bank to collect and prepare fetal tissue for research.[3]

It is unclear where initiative for change in the United States will come from, but discussants agreed that the public is generally receptive to the goals of fetal research to lessen or alleviate serious health problems. It was further argued that scientists have a responsibility to interact with legislators and the public to help create the conditions for meaningful change. The difficulty of achieving such change, however, can be seen in the current state of organ transplantation. Repeated surveys have shown overwhelming public approval of organ transplantation. Yet, there are still no effective procedures for obtaining an adequate supply of organs, a problem that would be even more difficult for fetal tissue transplantation. Another special characteristic of fetal tissue banks is quality. In recently established tissue banks that are limited to spontaneously aborted fetuses, the material is frequently found to be abnormal or infected and, consequently, of little value for therapy or research. A necessary, but fundamental, change would be to establish tissue banks for the storage of normal, therapeutically useful tissue from elective abortions.

It was clear from the discussion that many ethical questions will reemerge. For example, does a woman seeking an abortion have the right to know everything that will be done to her fetus in a research project, based on the possibility that particular kinds of research might be objectionable to her? With the federal funding mechanisms for fetal research being reconstituted, it appears that such questions will again become the grist for public debate.

[3] A comparison of other countries' approaches to biomedical ethics questions, including fetal research and fetal tissue transplantation, is included in the Office of Technology Assessment report cited in footnote 1 of the Setting the Stage chapter (p. 4).

SESSION II

Preembryo Research

OVERVIEW: MODERN IDEAS IN EMBRYO RESEARCH

ROBERT EDWARDS[1]
Bourn Hall Clinic, Cambridge, United Kingdom

Much of the preembryo research conducted over the past 20 years has been related to the development of in vitro fertilization (IVF), now an accepted procedure worldwide. In thinking about IVF, we must always think first of the gametes: the sperm and egg. Some recent findings about gametes may have major bearing on IVF both scientifically and ethically. Two involve new mechanisms for sperm selection.

In the process of meiosis, through which gametes form, precursor cells called spermatocytes give rise to spermatozoa. Spermatocytes are diploid: they contain two sets of chromosomes. They divide into haploid cells, each containing one set of chromosomes. In experiments with a strain of mice, it was observed that the two classes of spermatozoa arising from meiosis were not equally successful in fertilizing eggs; one class fertilized 80 percent of the eggs. It was subsequently determined that this unexpected disparity results from differences in the way in which genetic information encoded in the DNA of one specific locus is transcribed in the two classes of spermatids. The controlling gene sequence—the string of "letters" that spell our the genetic message—has been identified in mouse spermatozoa. Similar sequences have been found in human spermatozoa, but their function in humans is as yet unknown.

This discovery provided a long-sought answer to the question of whether sperm are governed genetically by the haploid or diploid genome, at least with

[1] One of the original developers of in vitro fertilization.

regard to their ability to fuse with eggs. This research avenue may lead to the possibility of sperm selection to avoid genetic transmission of disease genes as an alternative to the current methods of prenatal testing (e.g., amniocentesis), which is done after embryo implantation in the uterus.

In other experiments, researchers have been developing refined methods for separating X and Y spermatozoa. X and Y are the chromosomes that determine an offspring's sex. Because may inherited diseases are X-linked, this new methodology may present another opportunity for preselecting spermatozoa that contain the Y chromosome. It could also intensify ethical quandaries concerning parental choice of a child's sex for other than medical reasons.

Research on female gametes (oocytes) is limited by lack of supply. Even superovulation, induced by fertility drugs in IVF, yields, at best, a small number of oocytes. The problem is now compounded by the availability of cryopreservation of embryos. Many researchers feel strongly that all eggs obtained from IVF patients should be fertilized, and the healthy embryos that result kept solely for the patient, in case of initial implantation failure or for future pregnancies. Aside from other considerations, IVF is a costly procedure. Consequently, the research that can be done with normal IVF embryos has been almost completely confined to what is directly relevant to the patient.

A potentially abundant source of oocytes exists in aborted female fetuses. Experiments with egg follicles extracted from infantile mice show that the follicles can be grown in culture to form artificial ovaries that ovulate, and the mature eggs can be fertilized to produce offspring. Governmental permission to conduct experiments with human fetal egg follicles has recently been granted in the United Kingdom, and experiments have been initiated to study ovarian growth and oocyte development. Permission has not yet been sought for fertilization of human fetal oocytes.

Fetal oocytes could conceivably be used for IVF, but such use raises serious ethical concerns. One concern is the sheer number of embryos that could be generated from oocytes from a single fetus. Another problem is compliance with consent requirements from an egg donor when that donor is an aborted fetus.

There has been considerable progress in preembryo science in animals and in techniques for making IVF safer and easier. For example, key information found regarding the mechanism by which the sperm attaches to and penetrates the zona pellucida (the egg's outer coat) led to the more successful practice of implanting those oocytes that exhibited zona-bound sperm. Understanding the role of calcium in activation of sperm has been important to micromanipulation of gametes. In addition, fascinating reproductive biological principles have been elucidated, such as the role of sperm-produced proteins in egg activation. Yet, the rate of successful implantations in patients remains low. The reasons are still not understood, and many studies are being directed toward solving the problem.

Science and medical practice need to know more about the maturation of male gametes and the way in which cells are allocated within embryos. Better

culture methods need to be developed and improved standards need to be defined by consensus of experts in the field. Much remains to be known about the fundamental biology of embryos, especially preimplantation embryos, with a view toward identifying vulnerable periods and states, both in vitro and in vivo.

Unexpected events may help to shed some light. It has lately been discovered that acyclic women, including women with no ovaries and postmenopausal women, have a much higher rate of IVF success than normally cyclic IVF patients. In 50-year-old women, the pregnancy rate was almost as high as in normal women of 25, and there have been successful pregnancies in women in their 60s. Why this is so, whether it has to do with the condition of the donated eggs or the uterus or some other factors, is unclear. What is clear is that the possibility of babies from fetal eggs and postmenopausal pregnancies ushers in a new set of ethical issue. Examples of these include the potential births of scores of genetic half-siblings and births of children to mothers who may not live long enough to raise the children to adulthood.

MICROMANIPULATION RESEARCH IN CLINICAL EMBRYOLOGY

JACQUES COHEN
Cornell University Medical Center, New York, New York

Research on important areas of human embryology, such as gamete interaction, embryo implantation, and abnormal development, has focused largely on efforts aimed at improving success rates of assisted reproduction. These studies have been advanced by the adaptation of powerful new laboratory tools. Micromanipulation is a technology that employs very fine glass microtools attached to robot arms hooked to a microscope and moved by remote control (Figure 2).

In embryo studies in experimental animals, micromanipulation is used to extract cells or cell components for genetic analysis and diagnosis, to inject sperm into eggs, to transplant cell nuclei for genetic study, or to remove damaged tissue. In human embryology it is used mainly to facilitate fertilization in cases where sperm cannot penetrate the zona pellucida and to help embryos hatch out of the zona, although its use for genetic analysis of preimplantation embryos is close at hand.

Male infertility is a widespread problem for which conventional IVF works in only a small percentage of cases. Assisted fertilization is a new and developing technology in which a number of approaches are being tried. In the most dramatic approach, a single sperm is placed directly into an oocyte. Although success rates are still relatively low and unpredictable, what is significant is that a man with virtually no sperm, or none that are motile, has a chance of becoming a father.

At present, IVF culture systems provide less than optimal environments, especially for older women, for the embryo to hatch out of the zona pellucida

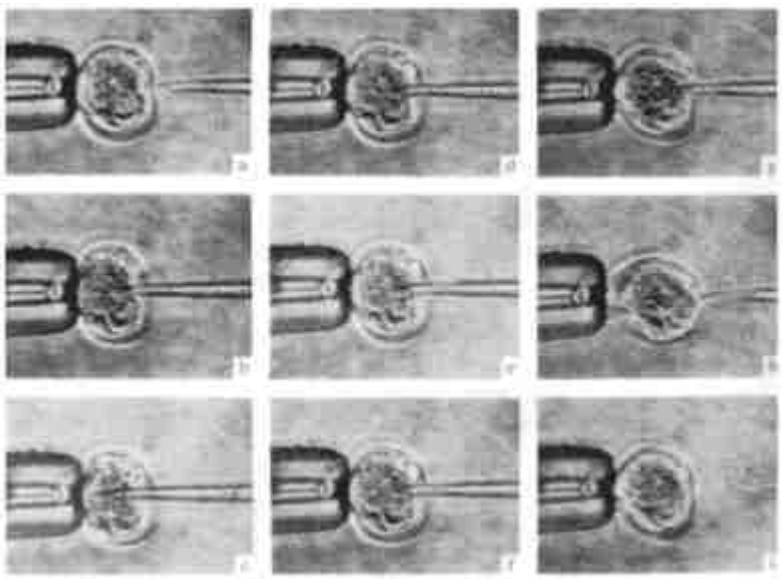

Figure 2
Aspiration of the blastocoelic cavity of a mouse blastocyst. (a–c) The blastocyst is secured to the holding pipette close to the inner cell mass and held by gentle suction. The sharply pointed biopsy pipette is introduced to the cavity via the mural trophectoderm. The outline of the cavity is not visible clearly in this figure, which is always focused on the point of the biopsy pipette. (d) The pipette is withdrawn slightly away from the inner cell mass. (e–f) Suction is applied to the biopsy pipette. The cavity collapses. Collapse has occurred in the vertical plane in this specimen, and no major change in volume is evident in the figure. (g–h) The biopsy pipette containing aspirated cells is withdrawn. The biopsy can then be expelled into a drop of medium for observation at high magnification. (i) The collapsed blastocyst seen in this figure reexpanded within three hours of the biopsy taken. SOURCE: Edwards, R.G., Preconception and Preimplantation Diagnosis of Human Genetic Disease, 1993. Reprinted, with permission, from Cambridge University Press.

and implant itself in the uterus. In human embryos the zona comes in different shapes and sizes, some thick, some thin. Thicker zonas yield low pregnancy rates. This problem can often be corrected by micromanipulating an opening in the zona shortly before the embryo is returned to the woman. In a series of assisted hatchings involving 790 patients, 339 became pregnant—much more than 50 percent of the younger patients, and more than 20 percent of patients over 39.

Research examining the chromosome abnormalities in human blastomeres suggests that such abnormalities account for a high percentage of IVF embryos that fail.[2] This represents one of the most pressing research questions in the field. Thus, methods of molecular biology, adapted for studying genetic anomalies in single embryonic cells removed by micromanipulation, offer great promise. One of these methods is called fluorescence in situ hybridization (FISH) in which complementary pieces of single-strand DNA with identified sequences are used to bind to single-strand DNA in the blastomere nucleus to identify specific sequences. The bound pieces are visualized with fluorescence microscopy. In this context, FISH can be used to identify specific chromosomes, including sex chromosomes in interphase nuclei and, thus, to determine the frequency of aneuploid blastomeres. Implantation would be limited to those embryos that are chromosomally normal.

PROGRESS IN REPRODUCTIVE BIOLOGY AND IMPLICATIONS FOR THE NEW REPRODUCTIVE TECHNOLOGIES IN HUMAN IVF

ALAN TROUNSON
Monash University Medical Center, Victoria, Australia

As with micromanipulation, a number of other areas of reproductive research are progressing toward innovations that should prove beneficial in IVF. A procedure now used with animals, which looks promising for adaptation to human patients, would lessen or eliminate the need for fertility drugs.

In human IVF as currently practiced, fertility drugs are used to induce superovulation. In a method that has proved very productive in artificial cattle breeding, developing follicles are directly removed from the ovaries of slaughterhouse animals and cultured and matured in vitro. Then, oocytes are fertilized and implanted. As modified for human patients, the technique would involve recovering immature eggs at any time during the natural ovulatory cycle, without any drug treatment or after very brief treatment with follicle-stimulating hormone.

There are several advantages to this technique. Oocyte recovery is markedly simpler than superovulation, which requires that the patient be closely monitored. It is significantly cheaper, which would make IVF available to more people. It garners many more eggs from a donor. It avoids or reduces adverse side effects of fertility drugs, such as premature births, deleterious effects of superovulation on uterine receptivity to implantation, and the potential for hormone-stimulated pathologies.

[2] Conference presenters differed on expected success rates of IVF, but generally about 10 percent of the attempts result in pregnancy. Success rates of 25 percent, however, are possible under ideal conditions.

In another area, progress in cryopreservation techniques has simplified and improved the success of gamete as well as embryo preservation. Freezing gametes rather than embryos avoids the technical difficulties associated with keeping embryos and the ethical dilemma created by increased numbers of unwanted embryos.

It is important to consider that not all techniques successful in animal work are likely to be transferred to human IVF. Embryo bisection is one of these, as are nuclear transfer and insertion of DNA.

The overriding concern in IVF is achieving healthy embryos. Methods are being developed for identifying and reducing the 30 to 40 percent of chromosomally abnormal embryos that occur in human IVF. (The rate of abnormalities is also high in natural conception.) Culturing methods for embryos need to be optimized by studying their nutritional requirements in detail. Results of studies in mice and sheep have yielded very different results, so that experiments on the growth conditions for human embryos are essential. As a consequence of improved embryo viability, the number of embryos implanted in IVF could be lowered, thus decreasing the frequent multiple births that arise from implantations and the attendant physical and familial problems.

Much of what has been learned about the antigens present on the zona pellucida or on sperm could be used to design reversible contraceptive agents, which would be free of the side effects of conventional drug therapies. From the perspective of a researcher working outside the United States, the present state of embryo research in the United States certainly reduces the keen competition from talented American scientists, but also limits the discoveries possible. This occurs at a time when, with techniques now available or being developed, answers to the major questions in reproductive biology are at our fingertips.

OPEN DISCUSSION PERIOD

All three presentations stimulated discussion concerning chromosomal abnormalities in fetuses and the dilemma of how research is to be pursued in this area if IVF embryos are kept solely for patients and oocyte fertilization is held in abeyance because of potential controversy. Numerous contrasting views were expressed on these topics and, most striking, the research questions and ethical dilemmas were intimately interwoven throughout the discussion.

Prior to the ban on federal funding, an Ethics Advisory Board (EAB) existed in the United States that considered the ethical issues related to IVF research and served as the forum for national debate. It is important to note that although this body discussed the use of extra embryos from IVF in research, no explicit approval was given. The Ethics Advisory Board, in fact, did not even address the issue of harvesting male and female gametes for the purpose of producing IVF embryos—for research or any other purpose. Research advances in other countries have now made such questions more immediate. Thus, the conference

discussion highlighted the new ethical questions that have emerged since the EAB and other national advisory bodies were dissolved.

Many of the conferees thought that couples undergoing IVF should be permitted to donate extra embryos for research purposes, and it was pointed out by some that the National Institutes of Health regulations on the use of embryos for research were being reconsidered.

Nowhere was the need for national debate through the reestablishment of some kind of ethics advisory body more apparent than in the discussion concerning the harvesting and fertilization of immature fetal oocytes. The many discordant views expressed could provide only a hint of the complexity of issues raised by this technical advance. For example, some thought it reasonable to fertilize fetal oocytes for embryo research, but not for implantation. Others considered implantation of such embryos as fundamentally similar to the presently accepted use of donated male and female gametes for IVF. A cautionary note was raised that fertilized fetal oocytes should first be studied to determine if they are normal. This concept was challenged by the suggestion that determining the normalcy of such embryos would be severely complicated by the lack of phenotypic information on the fetal donor. Assessment, then, could include only genetic analysis, which is not complete and may not be sufficient to ensure the health of the resulting child. As discussion deepened, other broad issues emerged such as the potential for wide dissemination of a single genotype, because many thousands of oocytes can be harvested from a single fetal donor.

Despite the compelling nature of the discussion outlined above, other technical and medical questions were raised concerning embryo implantation in postmenopausal women, the continued implantation of multiple embryos in IVF, and the selection of subjects for micromanipulation techniques. Although postmenopausal women up to 60 years of age have carried IVF pregnancies to term, concern was expressed regarding the greater medical risks and the social implications for the children of mothers of advanced age.

Regarding implantations of multiple embryos, a participant asked why, with the availability of cryopreservation for IVF embryos, this practice was still routine when it is well known that multiple births and their ensuing dangers (e.g., premature births, growth retardation, infant mortality, and personal burdens of triplets and quadruplets) are the common result. The reason is largely a matter of economics—multiple implantations increase the chance that one procedure will result in a viable pregnancy. Each implantation procedure is expensive, and whether or not this cost is covered by insurance is an important factor. In Australia, where IVF costs are covered, the number of embryos implanted in a single procedure has been significantly reduced, resulting in many fewer multiple births.

Experiences with microsurgical fertilization techniques for treatment of male infertility were shared in an attempt to arrive at selection criteria. There is growing evidence that severity of sperm deficiency does not correlate with poor

results. Rather, sperm with the most severe profiles appear to do best when injected into eggs through these techniques. A participant involved in data collection in this area reported that given the differences in technical competence at IVF centers, microsurgical fertilization does not result in fetal malformation any more significantly than normal IVF.

PREIMPLANTATION GENETIC ANALYSIS OF SINGLE HUMAN BLASTOMERES

MARK HUGHES
Baylor College of Medicine, Houston, Texas

Until recently, a couple who carries the gene for a genetic disease had three recourses: elect not to have children, adopt or use artificial insemination for recessive disorders, or terminate pregnancy if prenatal testing revealed the presence of disease in the unborn child. Preimplantation genetic analysis now provides a fourth option for some diseases.

Cystic fibrosis (CF), characterized by obstruction of the lungs leading to early death, is among the most devastating inherited disorders. One out of 25 Americans of northern European ancestry is a carrier of the CF gene. The disease is caused by a variety of mutations in a single gene that encodes a protein involved in chloride flux across cell membranes. Preimplantation analysis for CF and other genetic diseases has become possible through newly emerging techniques of molecular biology.

Developing a test that would definitively diagnose the presence of CF mutations requires being able to take the DNA in a single blastomere (one of the cells resulting from division of the fertilized egg) and generate huge quantities of it. A technique of molecular biology called polymerase chain reaction (PCR) can multiply a piece of DNA millions of times. This permits repeated comparative analyses of genes. The CF test that was developed, which yields results in a few hours, appears to be virtually 100 percent accurate for those cases in which the mutations in both parents have been identified, and it has been used successfully in a number of pregnancies.

Preimplantation analysis has also been developed for Lesch-Nyhan Disease, a severe neuromuscular disorder whose sufferers display bizarre self-mutilation behavior. To create this test it was necessary to amplify whole chromosomes, a feat achieved by a variation of PCR called primer extension preamplification, or PEP. PEP has also been used to develop a test for Duchenne's muscular dystrophy, which can arise from deletions in different parts of a single, enormous gene.

Other tests can now detect mutations of two forms of Tay-Sachs disease and sickle cell anemia. Blastomere diagnosis will be applicable to an inherited disorder for which the gene mutation has been identified. This technology can even be employed in certain diseases for which the defective genes are not yet identi

fied. For example, in X-linked recessive disease, only male children will be affected. Thus, if one can identify the sex of the IVF embryos, one could implant only those that were female. Although identifying sex from multiple cells is relatively easy, the chances of misdiagnosis in the analysis of single cells from embryos with conventional methods are still high. In an elegant test that amplifies regions from two related genes, one on the X and the other on the Y chromosome, unequivocal sex diagnoses can be made.

It is unclear how many couples who can conceive normally will choose preimplantation analysis, which involves going through IVF, rather than conventional prenatal testing. Certainly many couples have serious difficulties with the idea of terminating a pregnancy. The decision for other couples may involve more than religious or moral positions on termination of their pregnancies. Many of the couples already have a child affected with the same disease, and the cycle of pregnancy, prenatal testing, and possible pregnancy termination carries with it an undesirable message that although the parents love their child, they do not want another child like that one. The spur to developing the test for Lesch-Nyhan disease was a woman who already had an afflicted son, four subsequent pregnancies that were aborted because of the presence of the disease, and five spontaneous abortions. To date, hundreds of patients have inquired about preimplantation analysis at IVF centers all over the world.

The molecular biological techniques involved in preimplantation diagnosis require those doing the testing to have extensive training and experience. As the demand for this approach widens, a serious concern is that IVF centers may attempt preimplantation analysis without adequate competence in medical genetics and molecular biology. What is needed is guidance by the National Institutes of Health and the establishment of a preimplantation working group to provide a mechanism for interaction among molecular biologists, medical geneticists, and obstetricians.

STUDIES ON DNA SEQUENCES IN SINGLE HUMAN GAMETES

NORMAN ARNHEIM
University of Southern California, Los Angeles, California

As with preimplantation embryo studies, PCR and PEP are being applied to genetic analysis of human gametes. Because of the difficulty in obtaining oocytes, most of this research has concentrated on sperm. Among the diseases being studied are the muscle-wasting diseases spinobulbar muscular atrophy and myotonic dystrophy. Huntington's disease, a severe and ultimately fatal neurological disorder, is also being studied with this technique.

A common characteristic of all these diseases is abnormal repetition of DNA sequences within specific genes. The number of repeats marks the difference between being an unaffected carrier for the disease and being afflicted with it.

Current research, looking at genes in sperm from men who start out with different numbers of repeats, is aimed at learning about mutation rates for the gene expansion, and possible contraction, events in these conditions.

In the double set of chromosomes within diploid cells, one inherited from the mother and the other from the father, paired genes may be identical or different in their DNA sequences. The different forms of the gene are called alleles. Studies of sperm by using PCR and PEP are being applied to the investigation of recombination or crossing-over events during meiosis, in which chromosomes intertwine and exchange sections that are detected as new allele combinations. Aberrant types of crossing-over events can sometimes lead to altered genes that can cause disease. A critical problem concerns the fact that crossing-over events occur at different frequencies in male and female gametes. Because of limited access to human female gametes, studies are conducted mostly with laboratory animals, but human oocyte research will ultimately be needed to resolve these questions.

Access to early human embryos is also critical for understanding fragile X syndrome, and possibly other diseases involving DNA repeats. Recent evidence suggests that the repeat expansion that leads to full-blown disease may occur not during meiosis, but shortly after fertilization. Therefore, it is questionable whether a full expansion could be detected by blastomere diagnosis.

In strict Mendelian genetics there is no functional difference between a gene inherited from the male or female parent. In mammals, however, some genes are expressed differently in an offspring—depending on which parent contributed that gene—through a process called genetic imprinting. A number of human diseases have been found to be associated with genetic imprinting, particularly certain types of juvenile-onset cancer. An experimental system is needed to investigate the mechanisms that control the differential marking of genes in sperm and oocyte meiosis that affects their later expression in the embryo.

OPEN DISCUSSION PERIOD

Discussion opened with a debate regarding the percentage of the genome that is actually amplified by PEP and means by which to optimize the technique. The usefulness of early embryos for studies of imprinting was also questioned because imprinting may occur only postfertilization.

Technical questions were focused on the relative merits of PCR techniques versus FISH. Whereas FISH can clearly identify certain chromosomal abnormalities, PCR techniques are more suited for identification of mutations and abnormalities in discrete regions of chromosomes. Further investigation of both these, and other, methods to analyze genes and chromosomes in single cells from embryos is needed.

A participant asked the cost of preimplantation genetic analysis. Because it is still a non-billed experimental protocol, its ultimate cost is difficult to esti

mate, but should not be much higher than prenatal testing on chorionic villi sampling or amniotic material with the same DNA technology. The cost of IVF was a recurrent topic of concern. In the United States it can range from $1,500 to $12,000 a cycle. On average, three or four cycles are performed in the course of treatment. An interesting comparison may emerge between IVF pregnancy rates of parents utilizing preimplantation genetic analysis, who have normal reproductive systems, and rates among the more common IVF parents who have reproductive difficulties.

A persistent question concerning genetic analysis is confidentiality, for example, when an embryo is implanted that is known to be an unaffected carrier of genetic disease. Such concerns exist against a broader background involving a growing number of genes being sequenced and the way in which decisions will, or should, be made—and by whom—regarding access by government or private insurance companies to a patient's genetic profile.[3] As one participant pointed out, everyone carries genetic traits potentially detrimental to one's self or one's offspring. As information accrues, the way in which insurance risks are calculated may have to be reconsidered.

[3] For more thorough discussion of these issues, see U.S. Congress, Office of Technology Assessment, *Cystic Fibrosis and DNA Tests: Implications of Carrier Screening*, OTA-BA-533, U.S. Government Printing Office, Washington D.C., August 1992; Institute of Medicine, *Assessing Genetic Risks: Implications for Health and Social Policy*, National Academy Press, Washington D.C., 1993; and Institute of Medicine, *Social and Ethical Impacts of Advances in Biomedicine*, National Academy Press, Washington D.C., 1994 (in press).

SESSION III

Fetal Research

OVERVIEW: THE FETUS AS A PATIENT: THE ORIGIN OF THE SPECIALTY

JOHN QUEENAN
Georgetown University Medical Center, Washington, D.C.

Before the last half-century, the fetus in the womb was essentially inaccessible to medical diagnosis or treatment. The beginnings of fetal medicine came in the late 1940s with the use of penicillin to treat syphilis in pregnant women, which prevented the congenital effects of the disease in their offspring. Over the last 40 years, revolutionary new technologies have opened the way to direct intervention to correct fetal disorders, initiated in large part through efforts to combat Rh disease.

The Rh factor is a substance on the surface of red blood cells. In a small percentage of the population the factor is missing. Rh incompatibility between a mother and fetus can incite the mother's immune system to attack the fetal blood cells. Normally, an Rh-negative woman's first such pregnancy is not adversely affected, but with the immune reaction established, subsequent pregnancies become jeopardized. Before effective therapy was developed, the mortality rate was 50 percent.

The first leaps forward in the treatment of Rh disease came in the 1950s with exchange blood transfusion of the baby immediately after birth, or preterm delivery, when the mother's Rh history was known. These measures cut the death rate in half, but there remained the problem of undiagnosed disease. The application of amniocentesis, in which a sample of amniotic fluid is drawn by needle from the uterus and analyzed for various genetic and chemical characteristics, provided the means for detecting and evaluating a fetus at risk. In the

1960s, methods were developed to perform intrauterine transfusions. As a result of these advances, the Rh disease death rate dropped to 5 percent.

With intrauterine transfusion a fetus could, for the first time, be treated directly to correct the anemia and heart failure that can occur in Rh disease. However, because the blood was transfused into the peritoneal cavity (the fluid-filled space between the abdominal wall and abdominal organs), it entered the fetal bloodstream too slowly to be very effective against hydrops, a severe, potentially fatal edema, or fluid accumulation. In the past few years, it has become possible to transfuse the fetus intravascularly, directly through the umbilical vein, and reverse hydrops.

When amniocentesis and intrauterine transfusion began, there were virtually no methods for accurately guiding a needle into the uterus. Ultrasound, in which sound waves bouncing off the fetus create a moving picture in real time, has made access to the womb substantially safer. Ultrasound and intrauterine intravascular transfusion have now lowered fetal mortality from Rh disease to 2 percent.

Ultrasound and intravascular transfusion have revolutionized fetal medicine. In addition to Rh disease, ultrasound detects life-threatening obstructions in the urinary tract, chest, and skull; diaphragmatic hernias; and cardiac dysrhythmias. Intrauterine intravascular intervention can reverse nonimmune hydrops, a fetal edema unrelated to Rh hydrops. The causative agents of this condition, including viral infections, number 100 or more. Untreated, the mortality rate can range from 50 to 90 percent.

Many fetal disorders cannot yet be treated, or treated adequately. Surgical interventions to repair severe developmental abnormalities have yielded uneven results and require further exploration. Innovative approaches are needed, which may solve such problems as inborn errors of metabolism, blood diseases, and structural abnormalities. Among technologies on the horizon, fetal gene therapy and new applications of intravenous intervention hold promise for eliminating a variety of fetal ills.

PERCUTANEOUS UMBILICAL BLOOD SAMPLING

KENNETH MOISE, JR.
Baylor College of Medicine, Houston, Texas

The procedure for intravenous entry into the fetal bloodstream is called by different names. The term PUBS, for percutaneous umbilical blood sampling, is commonly used in the United States; equivalent terms include cordocentesis and funipuncture. In PUBS, a needle guided by ultrasound is introduced into a blood vessel (usually the vein) of the umbilical cord to assess fetal status and treat fetal disease, particularly diseases of the blood.

PUBS was developed primarily in France, in the 1980s, to diagnose fetal toxoplasmosis, an infectious disease that can be transmitted by a pregnant wom

an to her fetus and can damage the fetal nervous system. Although relatively rare in the United States, toxoplasmosis is a common problem in France and elsewhere.

Between 1987 and 1991, an international registry monitored the applications of PUBS. Data collected from 7,000 procedures conducted in 11 centers showed PUBS being used most frequently to obtain chromosome diagnoses faster (within 48 hours) than is possible with amniocentesis (which requires weeks for results) in situations where delay might have serious clinical implications. In addition to toxoplasmosis, Rh disease, and nonimmune hydrops, PUBS has been used to diagnose fetal thrombocytopenia, a hemorrhaging condition caused by maternal autoimmune antibodies to platelets that cross the placenta, and to monitor drug levels and hyperthyroidism.

Therapeutically, PUBS has been indispensable in reversing hydrops in Rh disease. Data on 400 Rh fetuses transfused by PUBS, as reported at a 1989 PUBS conference, showed an 82 percent survival rate for hydropic fetuses. Survival rates vary from center to center, probably reflecting the severity of the cases handled. As PUBS is tried for reversing other conditions, varying degrees of success are being reported. Eight fetuses have been treated by means of PUBS for nonimmune hydrops caused by one of the recently identified agents, B19 parvovirus. All eight procedures resulted in the birth of healthy babies. In five attempts to correct chronic loss of blood between the fetus and its mother, only one transfusion was successful. Efforts to transfuse blood platelets into the fetus in cases of maternal-fetal platelet incompatibility proved impractical because platelets have a short half-life and multiple transfusions are required. Platelet transfusions performed near term, however, have averted the necessity for cesarean delivery.

Stem-cell transfusion is a new promising application of PUBS in which the cells that give rise to different classes of blood cells are transfused into fetuses with some type of blood cell disease. Of five stem-cell transfusions reported, three of the fetuses survived. One survivor had bare lymphocyte syndrome, a condition in which white blood cells fail to produce cell-surface molecules essential to immune response. Another had severe combined immunodeficiency (SCID). The third had beta thalassemia, an anemia widespread among people of Mediterranean origin, in which hemoglobin production is defective.

PUBS is not without risk. The PUBS registry's overall fetal fatality rate was around 1 percent. Other surveys have shown somewhat higher or lower rates. Survival is critically related to the particular disease and how far it has advanced; in fetal infection, mortality can be 11 percent, and in nonimmune hydrops as high as 31 percent. There is a risk of slowed heart rate, usually transient but more common if the umbilical artery is used, and of hemorrhage. In PUBS transfusions, there is the danger of the fetus moving and bumping into the needle, causing lacerations of the blood vessels. To prevent this, the fetus is often given drugs that induce temporary paralysis. When such risks are clearly

explained to the mother, PUBS can be a very effective and lifesaving procedure. The risks, however, mitigate against the use of PUBS for prenatal screening when there are no indications of fetal illness.

EMBRYOSCOPY AND NEW ADVANCES IN FETAL DIAGNOSIS AND TREATMENT

E. ALBERT REECE
Temple University, Philadelphia, Pennsylvania

Although great strides have been made in fetal medicine, current diagnostic and therapeutic techniques have, for the most part, been limited to the second and third trimesters of pregnancy. In the early stages of pregnancy, ultrasound produces only a generalized, blurry picture of the embryo. Conventional fetoscopy, in which a viewing scope inside a needle is inserted into the womb, requires a needle too large to be used safely for early embryonic examination. Embryoscopy, a newly developed refinement of fetoscopy using a much finer needle, provides both direct visualization of an early embryo in exquisite detail and access into the embryonic circulation.

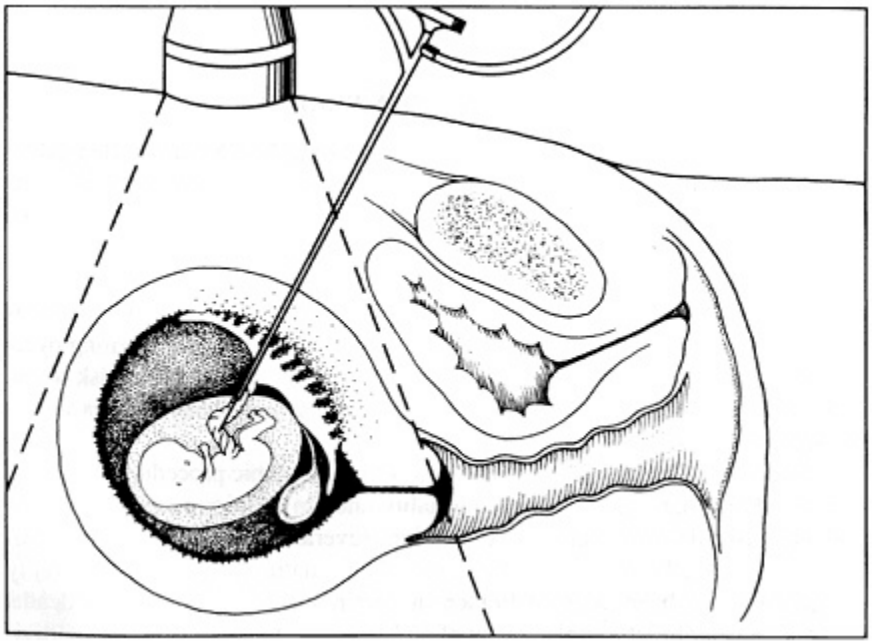

Figure 3
Schematic of embryoscopy method using transabdominal approach. SOURCE: Reece, E.A. "Embryoscopy: New Developments in Prenatal Medicine," Current Opinion in Obstetrics and Gynecology, 4:450, 1992. Reprinted with permission.

Embryoscopy can be performed through the vagina or the abdomen. In the transvaginal approach the scope is guided through the cervical canal, the chorion (the outer embryonic membrane), and into the exocoelomic space. The limitation is around 12 weeks, when the chorion and amnion fuse and there is risk of rupturing the amnionic membrane. To reduce complications, a new transabdominal approach has been developed, similar to amniocentesis, in which the needle, guided by ultrasound, goes directly through the uterine wall and into the amniotic cavity (see Figure 3 on p. 29). Recent trials of transabdominal embryoscopies were performed on more than 12 patients undergoing pregnancy terminations. They yielded complete anatomical surveys of the fetus. Blood sampling and infusion of dye were conducted and did not harm the fetus or the mother.

Transabdominal embryoscopy should significantly enhance capabilities for early diagnosis and therapy. It could pave the way for treatment of genetic and stem-cell diseases during a period when the fetus is immunologically naive, thus avoiding the problem of immune rejection of transplanted or injected donor cells. Many disease genes have been identified, and model systems for human gene delivery have been applied successfully in experiments in both culture and laboratory animals. Although the optimal times for intervention would have to be worked out, it is altogether conceivable that stem-cell and gene therapy could be initiated early enough in gestation to reduce or prevent irreversible damage to a developing embryo.

OPEN DISCUSSION PERIOD

The advisability of using paralyzing drugs during PUBS was challenged by a participant who also employs the procedure. Their use was argued to be necessary for PUBS transfusion, which takes longer than blood sampling and so runs a greater risk of fetal movement. In an early case in which the fetus was not immobilized, it moved and dislodged the needle, which caused fatal lacerations to the vessels. Another discussant brought up the possibility of a woman contemplating PUBS being misled by the PUBS registry statistics concerning overall safety. In all cases, counseling should be based on the particular risk to the individual fetus, and the higher risks associated with specific fetal diseases should be outlined clearly.

A questioner wanted to know how many embryoscopic procedures had been performed, in what context, and the mortality rate. In addition to the 12 transabdominal cases described in the presentation, several hundred transvaginal embryoscopies have been conducted in cases of terminating pregnancies. Seventy procedures have been done in France in continuing pregnancies; two deaths resulted. Regarding the applicability of embryoscopy to gene therapy, a participant wondered whether sufficient blood could be drawn to retrieve enough cells into which altered genes could be inserted. Blood sampling in early embryos is difficult, and the maximum amount that can be taken has not been determined.

MATERNAL NUTRITION AND METABOLISM: ROLE IN INTRAUTERINE AND LONG-TERM DEVELOPMENT OF THE OFFSPRING

BOYD METZGER

Northwestern University, Chicago, Illinois

That good maternal and infant nutrition is essential for optimal pre-and postnatal development is universally accepted. Exactly what constitutes good nutrition, and how malnutrition impedes development, have not been clearly defined. Recent advances in epidemiology and clinical medicine have uncovered important clues linking nutrition and metabolism during prenatal development to long-term health. Pregnancy complicated by maternal diabetes is a situation in which these issues have been explored in depth.

The environment in which a fetus grows is conditioned by the maternal metabolism and the function of the placenta, through which all nutrients must pass. The placenta has the capacity to modify nutrients and contribute its own metabolic and hormonal input. Insulin plays an essential role in determining the composition of the maternal metabolic environment and the fetus's nutrient intake.

In the traditional model that has been used to explain the effects of maternal diabetes on fetal development, insulin deficiency in the mother disturbs insulin-sensitive nutrients, including glucose and many amino acids, the subunits of proteins. These nutrients, modified by the placenta, produce an altered nutrient mix that impinges on the beta cells of the fetal pancreas and leads to fetal hyperinsulinism. (Insulin is made by clusters of beta cells, called the islets of Langerhans, dispersed through the pancreas.) Excessive insulin production alters fetal development and the maturation of insulin-sensitive cells, including lung, heart, liver, and especially, fat cells.

A study was set up 15 years ago to test the model in a group of diabetic mothers and their fetuses, and to follow long-term effects on the offspring. As is typical of babies of diabetic mothers, the babies in the study were fat at birth and returned to a normal weight level shortly after. The situation later reversed, so that by about age six the incidence of obesity was very high, and by early adolescence so was the incidence of impaired glucose metabolism.

The most important insight that has come out of these studies to date is that the tendency toward obesity and metabolic abnormality in the children correlated directly with their state of hyperinsulinism during gestation. Furthermore, the effects were the same whether the mother was diabetic before pregnancy or developed gestational diabetes. In addition, impaired fetal islet-cell development has been linked to pre- or postnatal growth deficiency, in particular, secondary to protein deficiency in infancy.

The propensity to obesity and impaired glucose tolerance in the offspring of diabetic mothers establishes a setting in which these conditions may be perpetuated congenitally from generation to generation. Confirming data from animal

studies show that altered fetal islet function is sustained through subsequent generations. Among the Pima Indians of Arizona, who tend to marry within their own group, a large percentage of the population suffers from severe obesity and diabetes.

The next step is to learn how to intercede in this process. If intrauterine malnutrition and growth retardation in the first year of life could be prevented, and maternal diabetes more effectively treated so as to prevent fetal hyperinsulinism, the cycle of congenital diabetes could be broken and the ever-increasing incidence of the disease arrested.

HORMONAL REGULATION OF HUMAN FETAL GROWTH

STUART HANDWERGER
Children's Hospital Medical Center, Cincinnati, Ohio

Although there is considerable information about the hormonal factors that regulate postnatal growth, these factors appear to have scant effect, if any, on the fetus. Relatively little is known about hormonal regulation of fetal growth. Gaining such knowledge is of critical importance clinically since aberrations in fetal growth are frequently involved in diabetes and in many of the other common pathological conditions of pregnancy. Intrauterine growth retardation may have a lifelong effect on the incidence of illness and early death.

A number of findings have demonstrated that the pituitary growth hormone so important to growth after birth (deficiency leading to short stature and excess to gigantism) is not at work prenatally—infants born without a pituitary gland are normal in size. Although pituitary growth hormones are present in a normal fetus, fetal tissues lack receptors for them. (Receptors are the molecules on cells that initiate cellular response to hormones.) Insulin-like growth factor (IGF-1), whose production is increased postnatally by the action of pituitary growth hormone, does appear to be at work in fetal growth. Recent experiments have shown that the absence of IGF-1 leads to significant fetal growth failure. This suggests that in the fetus, something other than pituitary growth hormone is driving IGF production.

Fetal growth seems to be regulated, to a large extent, by a hormone in the placenta called human placental lactogen (hPL). Its role was first suggested by the striking similarities in its structure and function to pituitary growth hormone and to prolactin, another pituitary hormone, and the genes that encode the three hormones are closely related. Placental lactogen is made in the placenta and secreted into the maternal blood beginning at about six weeks into gestation. Production continues to increase, reaching a concentration at week 34 greater than that of any other hormone in the mother. Decreased concentrations in the mother have also been correlated with fetal growth failure.

Placental lactogen is active in both maternal metabolism and fetal tissue. In

the mother, hPL contributes, along with growth hormone and prolactin, to preparing the breast for lactation, and it stimulates maternal IGF production, which may be involved in increasing uterine size. More importantly, it increases the availability of glucose and amino acids for transport across the placenta to the fetus. In fetal tissues, placental lactogen directly stimulates IGF production, uptake of amino acids, and the activity of an enzyme important in protein and carbohydrate synthesis.

Contrary to expectation, the release of hPL is not regulated by the factors that regulate growth hormone or prolactin, but by high-density lipoproteins (HDLs). Lipoproteins are complex compounds of lipids and protein. Almost all of the effects of HDL that have been observed postnatally (in cholesterol metabolism) involve the lipid part of the compound. In contrast, the release of hPL in the fetus is effected by the protein part.

Aberrations in hPL secretion have been detected in many complications of pregnancy, including diabetes and preeclampsia, a condition associated with intrauterine growth retardation. It may be possible to prevent these conditions by administering placental lactogen or drugs that increase hPL concentrations. Before that can be ascertained, however, considerably more human research is required to understand the mechanisms involved.

CLINICAL EVALUATIONS OF FETAL LOSS: RELEVANCE AND NEW DIRECTIONS

CYNTHIA CURRY
Valley Children's Hospital, Fresno, California

Approximately 15 percent of all clinically recognized pregnancies end in spontaneous abortion. The increasing tendency of women to postpone childbearing has made such loss all the more distressing, because in some cases a spontaneous abortion is the first clue to problems that may compromise every succeeding pregnancy. In these and most other cases of spontaneous abortion, knowing the underlying causes is important to the parents and critical to a physician's ability to counsel patients concerning future pregnancies. Yet, with some notable exceptions, clinical evaluation of fetal loss, despite its diagnostic and psychological benefit, has been largely neglected. Numerous factors are responsible for this neglect. For example, complete evaluations require the expertise of geneticists, pathologists, and radiologists, among others. Thus, the evaluations require careful planning and coordination, and they must be financially supported in some way. In addition, it is often unclear which physician should be in charge.

To be complete, evaluation of fetal loss should include a history of the pregnancy, examination of the fetus, chromosomal studies, photographs, x rays, autopsy, and examination of the placenta and cord. The mother's history should be taken, including results of fetal monitoring tests. It is important to know

about the mother's glucose control as well as any maternal illnesses. Congenital myotonic dystrophy is an inherited condition that can be difficult to diagnose in a mother who is asymptomatic; yet a significant percentage of future pregnancies carry a 50 percent risk of being affected by it.

Causes of death differ in fetuses that have become macerated (e.g., not delivered for sometime after fetal death has occurred) and those that are freshly expelled. Macerated fetuses have a significantly higher chance of having been aborted because of chromosomal abnormality, a placental problem, or multiple malformations. Fresh fetuses are more likely to have died of nonrecurrent causes, such as cord accidents or infection. Chromosome studies need to be performed in fetuses when the mother has had recurrent losses. Such studies should also be done in any fetus with ultrasound-detected or obvious external abnormalities and in fetuses with hydrops, growth retardation, or maceration.

In a study of 500 cases of pregnancy loss, a cause could be established in 90 percent of intact and 30 percent of nonintact fetuses. Chromosomal abnormalities accounted for 19 percent; placental abnormalities, 11 percent; infections, 7 percent; cord abnormalities, 6 percent; neural tube defects, 8 percent; renal, central nervous system, or cardiac abnormalities, 6 percent; twins, 5 percent; recognizable syndromes, 4 percent; skeletal deformities, 2 percent; and other causes, 11 percent. Of particular significance was the finding of maternal immune rejection (possibly preventable) in about 10 percent of the losses. Certain placental pathologies were typical of this condition and underscore the importance of examining the placenta in cases of fetal loss. The fetal findings in maternal "immune rejection" included growth retardation and hydrops. Most of the women also had a history of recurrent miscarriages.

Many fetal conditions are yielding to advanced diagnostic and therapeutic modalities. The role of the placenta in fetal loss offers a major new direction for exploration. Families want and deserve answers as to why a miscarriage happened and what can be done to prevent its happening again. The best way to provide such answers is to know more about the precise mechanisms of fetal loss.

OPEN DISCUSSION PERIOD

A participant asked whether altered islet-cell function in the children in the diabetes study involved permanent down-regulation of insulin receptors. The question cannot be answered at present. Insulin sensitivity and beta-cell function continue to be measured in the children who show impaired glucose tolerance, and comparative information is being sought through animal studies.

Another participant asked what is known about the receptor for placental lactogen and where it is distributed in the fetus. Studies of sheep fetuses show the presence of hPL as early as six weeks into gestation, binding to cells in the liver, ovary, testis, and adrenal gland. In mice there is also a high degree of

binding in the brain, which raises the question of whether hPL could affect maternal behavior. Isolating and purifying the fetal hPL receptor, which has been pursued for a number of years, has proved extraordinarily difficult. Most of the studies of human placental lactogen have been done on tissues at term, well past the period of most rapid fetal growth. Second trimester studies have not been done because of the difficulties of obtaining tissue.

As to the evaluation of aborted fetuses, a medical school dean wondered who pays for the tests. Patient insurance covers examination of a fetus less than 20 weeks as a surgical procedure. After 20 weeks, placental examination is covered. The study reported in the presentation was supported by grants. Nevertheless, such tests are often supported by academic medical institutions as part of overall research and training and, as such, are threatened by increasing financial constraints on these institutions.

THERAPEUTIC INTERVENTIONS IN UTERO

RICHARD BERKOWITZ
Mount Sinai School of Medicine, New York, New York

Current methods for intrauterine fetal therapy encompass noninvasive measures, principally bed rest and transplacental drug delivery, and invasive procedures, such as preterm delivery, blood sampling and transfusion, percutaneous shunts, and open surgery.

Bed rest, the most common form of noninvasive therapy, is generally thought of as cheap and without significant side effects. Bed rest in the hospital, however, is not cheap, and being confined to bed for weeks can have major side effects. More important, it rarely works. Transplacental therapy, in which drugs given to the mother pass through the placenta to the fetus, effectively treats a variety of conditions. Drugs or vitamins that cross the placenta have been used to accelerate fetal lung maturation, reduce the risk of neural tube defects, and manage cardiac arrhythmias.

Among invasive therapies, percutaneous umbilical blood sampling, which provides direct entry into the fetal blood circulation, enables diagnosis, intravenous transfusions, and drug delivery. PUBS has been used recently to diagnose and track the effectiveness of therapy for alloimmune thrombocytopenia.

Like Rh disease, alloimmune thrombocytopenia is caused by maternal-fetal incompatibility. In this case, maternal antibodies attack the platelets in fetal blood, which causes a marked reduction in their number (i.e., thrombocytopenia) and may lead to devastating intracranial hemorrhaging. The treatment that has been used is gamma globulin administered intravenously to the mother, sometimes with steroid hormones.

To evaluate and refine this therapy, a trial was conducted with women who

had previously delivered babies with the disease and had been shown, by PUBS testing, to be carrying severely thrombocytopenic fetuses. Half of the group was given gamma globulin alone, and half, gamma globulin with low doses of a steroid (dexamethasone). Fetal platelet counts were reassessed with PUBS three weeks after the beginning of the regimen and again six weeks later. If at three weeks the fetal platelet count was less than 20,000/ml^3, the mothers were additionally given high doses of a steroid, prednisone, for the remainder of the pregnancy. Among the women who did not require the additional therapy and have since delivered, there was no essential difference in outcome with or without hormones.

A therapeutic success was defined as a birth platelet count greater than 30,000/ml^3 since there have been no reports of intracranial hemorrhage with higher platelet concentrations. This was achieved in 82 percent of cases. Fifty percent of those fetuses whose initial platelet count was less that 20,000/ml^3 showed a therapeutic response at the time of the second PUBS. Of greatest importance, however, is the fact that regardless of the platelet count at birth, no infant in this study had an intracranial hemorrhage. These data strongly suggest that medical therapy for this disorder will be effective in the vast majority of cases.

An additional finding from this multicenter study was the critical role of fetal platelet concentration as a risk factor for PUBS-induced fetal loss. Eight untreated fetuses, all with a platelet concentration of less than 20,000/ml^3 (six actually had counts of less than 10,000/ml^3), died following the initial diagnostic PUBS procedure. As a result, the investigators recommended obtaining measurement of the platelet concentration before removing the needle from the umbilical blood vessel and, if the concentration was less than 50,000/ml^3, transfusing previously prepared antigen-negative packed platelets into the fetus prior to removal of the needle. No fetal losses have occurred after adoption of this regimen.

Percutaneous shunting is a form of intervention designed to drain abnormal accumulations of fluid within the fetus that can severely compromise the growth and development of adjacent organs. The shunts are put into the obstructed area by a needle guided by ultrasound. Shunts have been used to alleviate obstructions of the bladder outlet, pleural space, and ventricles of the brain. A registry of bladder shunt procedures showed an overall survival of about 40 percent, but with a high risk of renal disease later in life. Long-term results have been more encouraging for hydrothorax, which hampers development of the heart and lungs. Shunting in utero has been much less successful for hydrocephalus, however, where fluid builds up within the ventricular system of the brain. Although 83 percent of the shunted hydrocephalic fetuses survived, more than half had severe brain impairment, and as a result, there is now a moratorium on the procedure for this indication.

The most radical form of invasive intervention is now being attempted out

side the uterus. In a small number of selected cases where closed procedures cannot be performed, the uterus has been opened and surgery performed directly on the fetus. The results of this approach, have thus far been mixed, and its ultimate role remains to be determined.

FETAL THERAPY

MITCHELL GOLBUS
University of California Medical Center, San Francisco, California

A number of advances, derived through animal research, made it possible to think of attempting open surgical interventions (outside the uterus) on fetuses whose prospects for survival in the absence of intervention were dismal, but for whom interventions inside the uterus were not possible. The first fetal surgeries were performed on fetal bladders to correct urinary tract obstructions in instances in which shunting was not advisable. A shunt tends to clog after a number of weeks and, so, will only buy enough time for a fetus close to the point at which it can be safely delivered. In earlier stages, it becomes necessary to replace the shunt repeatedly, thereby putting the fetus in extreme danger.

Despite technical advances, though, such surgeries present major challenges. Specific anesthetics must be used that provide uterine relaxation as well as anesthesia. Opening the uterus causes preterm labor, which must be prevented by continuous intravenous administration of relaxing (tocolytic) agents through indwelling catheters for the remainder of the pregnancy. Fetal status during the surgery requires monitoring.

Of the eight original bladder surgeries, only three were successful. Two of the failures resulted from gastrointestinal problems that could not be recognized prenatally. Two involved kidney abnormalities at a time when it was not known how to evaluate fetal renal status. The fifth was caused by the failure of the mother to stay on tocolytic medications, which resulted in premature labor and delivery. Of the surviving children one required a kidney transplant and the other two are doing well.

The failures from inadequate diagnosis of other compromising conditions can now be alleviated by improved evaluation and testing. Although such tests can predict which fetuses are likely to survive the surgery and be delivered, they cannot predict future renal function. Nevertheless, many couples appear willing to accept the idea of a child who may need a kidney transplant, because they give consent to the surgery after being fully informed about the risk of later renal failure. Even so, the investigators wonder whether such couples can really appreciate the enormity of the burdens and concomitant risks of kidney transplants in the absence of experiential knowledge and in the face of trying to save their fetus.

Diaphragmatic hernia surgery was attempted next. These hernias cause the

stomach and other organs to lie in the thorax, crowding the heart and lungs and preventing their normal development. Although postnatal surgery can be done, mortality is high. In situations where the bowel is in the chest, there is not enough room for the lungs to develop. The bowel can be surgically moved back into the abdomen in the prenatal surgical procedure, but because the mass is too large for the abdomen that has developed, the gap must be covered with a patch and closed postnatally. This prenatal surgery could not be tolerated before 22 weeks. Unlike bladder surgery, which takes 10 minutes from the time the uterus is opened until it is closed, hernia repair requires an hour. In addition, surgery to bring the liver down from the chest proved impossible. Of the first 30 prenatal diaphragmatic hernia surgeries, only four fetuses survived. Among 100 fetuses with diaphragmatic hernia who did not have in utero surgery, the survival rate was 40 percent. Thus, despite the enormous knowledge gained, the majority of investigators have instituted a moratorium on prenatal procedures for diaphragmatic hernia.

Two other types of fetal surgery have shown more promise. Fetuses with cystic adenomatoid malformation of the chest, which impedes development of the lungs, can survive without intervention, but not if the condition is compounded by nonimmune hydrops. Surgery on six such fetuses resulted in four that did well, although intervention early in the hydrops was critical to their survival. Of five surgeries performed to remove an anomalous twin that was threatening the life of the living twin, four survived—a rate considerably better than nature's 50 percent.

Although a good deal has been learned about fetal conditions that are more or less amenable to open surgery and there have been some successful outcomes, it does not seem likely that the existing technology offers an approach that will save large numbers of endangered fetuses.

TRANSPLANTATION OF FETAL LIVER HEMATOPOIETIC STEM CELLS IN UTERO

ESMAIL ZANJANI
Ioannis A. Lougaris Medical Center, Reno, Nevada

Hematopoietic stem cells (HSCs) give rise to all the elements of the blood. In addition, they have the ability to self-replicate, so that their numbers do not decrease as mature cells form. In the adult, HSCs are found primarily in bone marrow. It is possible to transplant these stem cells to replace defective marrow cells, but in an immunologically competent adult, all of the patient's own marrow cells must be destroyed and the marrow repopulated with donor cells. Because the number of donors with compatible cell types is limited, there is the danger of graft rejection and the resulting need for sustained immunosuppressant therapy. In addition, there is a danger of graft-versus-host disease (GVHD), which results from the immunologically mature donor cells attacking the host's

cells. As is well known, fetal cells are not mature immunologically and, thus, make excellent donor cells for transplant.

Many of the congenital abnormalities that can be treated with HSC transplantation after birth can be diagnosed early in gestation. The question arises then of whether one can transplant stem cells into a compromised fetus to take advantage of an immature immune system and therapy avoid the problems of rejection and immunosuppressant therapy. The additional use of fetal donor cells, produced abundantly by the fetal liver, could circumvent the possibility of GVHD.

In early experiments in sheep, stem cells from adult animals were transplanted into fetuses. The sheep that were born showed acceptance (engraftment) of the transplanted cells, but they all developed graft-versus-host disease and died. In subsequent experiments, stem cells from fetal donors were used and none of the recipients developed GVHD. In fact, the donor cells were still active in the sheep several years after birth.

The next question was whether fetal HSC transplantation could actually correct a disease. Batten's disease causes blindness in sheep. Animals diagnosed with the disease in utero and treated with donor fetal HSCs were born normal. They were sacrificed at 15 months of age for pathological examination, and the data, although not yet complete, indicate that at the time of death the sheep were still free of disease.

Human victims of Hurler's disease, which is due to an enzymatic deficiency that causes accumulation of mucopolysaccharides in many organs, usually die before the age of 10 from respiratory infection and heart failure. In 1990 a fetus diagnosed with Hurler's was given fetal HSC transplants, injected into the peritoneal cavity. The child was born prematurely and showed signs of pulmonary disorder typical of early birth, but exhibited no indication of GVHD. However, the child was enzyme deficient at birth and, by six months, clearly exhibited the disease. Starting at about one year, tests began to show low levels of the missing enzyme, a trend that continued over the next year. The child is now two years old and still shows evidence of disease. Whether failure to cure him resulted from problems inherent in the particular condition or an insufficient level of cell transplantation is not known.

Research to intervene early in fetal life to alleviate specific diseases is hampered in humans by the difficulty of obtaining human fetal tissue for experimentation, the condition of the tissue that can be obtained (most of which comes from spontaneous abortions and is infected or genetically abnormal), and the social and political issues surrounding fetal tissue use.

Studies are now under way to improve methods of purification and preservation of stem cells and to determine persistence of engraftment. For example, incubation of stem cells in medium with certain growth factors seems to improve the engraftment of the donor cells in sheep. Fetal sheep stem cells have been grown in culture and their numbers expanded, with the idea that transplanting

more cells will improve success. Human fetal stem cells have also been successfully transplanted in sheep. Among the questions being explored is whether the pool of human HSCs could be expanded by using animals as a kind of "living" culture system. Will a small population of human fetal cells transplanted in fetal sheep or pigs expand in the adult animal's bone marrow? Will the cells be accepted by and beneficial to human recipients? These and other questions await further research.

OPEN DISCUSSION PERIOD

The data reported from the alloimmune thrombocytopenia trial led one conferee to suggest that the time had come for gamma globulin treatment to be considered cost-effective for coverage by third-party payers. It costs more than $1,000 a week per patient. Even with extended treatment, however, it is less than the cost of caring for a hemorrhaging infant or the cost of repeated transfusions.

In a discussion of success rates for treating obstructions that affect renal function, the point was made that the condition is often not diagnosed until the kidney damage is too extensive to save the fetus. Another participant wondered whether the collective incidence of prenatal problems that could be diagnosed and treated if caught early warranted the use of ultrasound in every pregnancy. Ultrasound scanning at 18 weeks would allow appropriate dating important for the evaluation of intrauterine growth retardation, including AIDS pregnancy, and is done for every pregnancy in Britain, Scandinavia, Germany, and France. Although not contesting its medical value, another participant replied that a recent literature search did not support the statistical cost-effectiveness of routine ultrasonography in pregnancy.

The feasibility of fetal cardiac surgery was raised. Some cardiac conditions might be candidates for prenatal surgery in the future, and animal studies have begun to explore this.

The discussions kept returning to the issue of the ultimate value and usefulness of fetal surgery. One point made was that given the difficulties encountered in surgical trials for diaphragmatic hernia, it was best that the trials were limited to one institution rather than being conducted by different centers using different protocols. In the view of one of the speakers, as more is learned about molecular and cellular factors controlling fetal growth and development, new approaches, such as stem-cell transplantation and gene therapy, stand to help many more patients than fetal surgery.

A participant questioned the use of intraperitoneal rather than intravenous transfusion for stem-cell transplantation. Technical difficulties have made intravenous cell transplantation less successful than intraperitoneal transplantation in sheep, but there are likely species differences. The resulting questions about how many cells were transplanted by the two routes led to an interesting scheme for "preparing" a fetus to receive a stem-cell transplant after birth, which has

been tried in mice. This approach would be to transfuse or intraperitoneally inject stem cells into a fetus in low numbers, with the assumption that the fetal immune system as it matured would recognize the transplanted cells as "self." After birth, a full stem-cell transplantation with cells from the original donor pool could take place with a much reduced chance of graft rejection.

APPLICATIONS OF MOLECULAR BIOLOGY AND GENETICS TO DEVELOPMENTAL TOXICOLOGY

DANIEL NEBERT
University of Cincinnati Medical Center, Cincinnati, Ohio

The role of environmental substances in causing infertility, in utero toxicity, and birth defects is an important public health issue. Studies of the genetic mechanisms that control metabolic pathways have begun to clarify the ways in which toxic substances induce abnormalities in the developing fetus.

Enzymes of a group collectively called cytochrome P450 (P450) are involved in metabolizing the foods, drugs, and environmental chemicals that enter the body. Cytochrome P450 enzymes belong to a group called Phase I enzymes that add oxygen molecules to incoming substances. The oxygen functions as a sort of handle that Phase II enzymes, another group, use to hook or conjugate the substance to other chemical compounds in the body as a preparation for excretion. Many substances, such as foods or certain chemicals in cigarette smoke, enter the body already oxygenated. It has long been known that such oxygenated "intermediate" compounds cause cancer and birth defects in mice—for example, benzopyrene metabolites cause epithelial tumors, cleft palate, and kidney lesions, among other problems. In some cases, Phase II enzymes may actually potentiate the toxicity of an oxygenated compound by blocking its breakdown, thereby allowing the concentration of the toxic compound to increase.

In the 1960s, research was initiated to describe the functional relationships between various genes that encode drug-metabolizing enzymes (DMEs), including P450, that process a specific class of incoming compounds known as aromatic hydrocarbons. An early study that examined the P450 concentrations in human placenta found greater concentrations of the enzyme in placentas from women who had smoked more cigarettes during pregnancy. Other research focused on the receptors, proteins that "recognize" and bind certain molecules, for classes of incoming compounds. These and other lines of investigation provided a rich understanding of the mechanisms of chemical toxicity and laid a useful foundation for more intensive work. For example, it became possible in the 1970s to identify the genes for two types of receptors that bind aromatic hydrocarbons, one of which binds such compounds with vigor (high affinity) and one of which binds only weakly (low affinity).

Knowledge of aromatic hydrocarbon (Ah) receptor differences allowed investigators to give benzopyrene to pregnant mice and demonstrate that the offspring of mice with the low-affinity receptor had more birth defects and in utero toxicity. This finding is supported by work in humans showing that pregnant women with the low-affinity Ah receptor had babies with more birth defects than mothers with the high-affinity receptor, despite smoking the same number of cigarettes.

Another series of experiments was set up to examine the interrelationship of two Phase I and four Phase II DME genes in mice. In normal function, Phase II genes turn on and off to maintain a steady-state balance of metabolic activity. In the study, all six of the DME genes were activated by benzopyrene and by dioxin. It was assumed in the early 1970s that these DMEs existed only after birth and were not present in the fetus. Thus, the role of DMEs in birth defects could be attributed to the genetic susceptibility of the mother.

Doubt was cast on that assumption by findings from studies of the mechanisms of birth defects in mice, which suggested that the response in the embryo to certain environmental chemicals given to the mother occurred as early as day 5 and varied with the genetic makeup of the fetus. With the advent of more sensitive molecular techniques, it has been possible to confirm and expand this finding. By using methods of in situ hybridization in which genetic probes search out and combine with complementary genetic material, P450 expression was localized in mouse embryos. It occurred first in endothelial cells lining the uterus, then in the developing yolk sac, and by about day 12 or 14, in the developing liver and lung.

Recent experiments have been aimed at pinpointing the specific function of P_1450, an extensively studied specific P450, in normal fetal development. By using a variant of the very sensitive technique of polymerase chain reaction, which provides a large amount of a particular gene for study, a striking increase in the expression of a gene encoding P_1450 was discovered in the fertilized egg before the two-cell stage—expression that decreases in later stages of embryonic development. This finding supports the idea that the gene and its product are important at critical times in the preimplantation embryo.

Extrapolation from animal models provides an incomplete picture of human biology. There are drug-metabolizing enzymes in mice that are not present in humans and vice versa. One way to attack this problem is to make transgenic mice and insert human genes into these mice, thus inducing the production of proteins and enzymes that react as they would in humans to incoming chemicals. The use of transgenic mice can help to further our understanding of the complex interplay among enzymes that process drugs and toxic substances from food and the environment. In addition to the P450 genes, other DME genes are now implicated in developmental toxicity. Armed with sensitive molecular techniques and assays, we can now begin to manipulate this complex system experimentally in developing embryos. A complete understanding of the mechanisms

through which toxic substances cause birth defects clinically, however, can come only from application of the knowledge gained thus far to the study of human fetal tissue.

OPEN DISCUSSION PERIOD

A participant asked how many human genes would have to be expressed in transgenic mice to provide an adequate model of drug and environmental responses in humans. The number of genes involved in the important responses is estimated to be between 10 and 20. With so-called knockout technology, mouse genes can be made nonfunctional and replaced by comparable human genes. Transgenic mice can be bred to be simultaneously deficient for more than one gene. As the pharmacogenetic variations at P450 genes in humans become well-characterized, test tube assays can be set up to determine which women should or should not receive certain prescribed drugs on the basis of individual Phase I and Phase II cytochrome P450 alleles.

SESSION IV

Fetal Tissue Transplantation

PLASTICITY AND COMMITMENT IN THE DEVELOPING MAMMALIAN BRAIN

SUSAN MCCONNELL
Stanford University, Stanford, California

The mammalian brain is composed of a huge variety of nerve cells, or neurons, which arise from common progenitor cells of an embryonic structure called the neural tube. The hollow space inside the neural tube eventually becomes the ventricles of the brain; progenitor cells line the inner, ventricular, surface of the tube. In the fully formed brain, a multilayered sheet of neurons, the cerebral cortex, covers the cerebral hemispheres. Some regions of the cortex are involved with movement and sensation, but other are involved with higher-order analysis and reasoning. Thus, the cortex is responsible for our highest cognitive and perceptual processes. Studies of the development of the cerebral cortex are helping to explain how neurons achieve their individual identities and may shed light on the mechanisms of fetal brain injury.

The cortex is particularly amenable to this kind of investigation because its neurons are organized into six distinct layers, each containing specific types of neurons that make highly specialized axonal connections (Figure 4). The upper layers of cortical neurons typically send their axons to other cortical cells, and neurons in the lower levels communicate with neurons located beneath the cortex in deeper regions of the brain.

Progenitor cells give birth to daughter cells through the process of mitosis, in which they double their chromosomal package and then split in half. In the forming of the cerebral cortex, the first cells to be born and migrate out from the ventricular surface are destined for the deepest layers of the cortex, layers five and six. They travel along special, elongated cells, called radial glial fibers,

which are transiently present during development and serve as a guide wire for the early migrations of cortical neurons. Neurons born later migrate past the early settlers to the top of the cortical plate. Because the different layers are generated in a precise sequence, a cortical neuron's "birthday" provides a remarkably accurate tool for predicting its ultimate position and connections.

Two explanations have been put forth to account for the correlation between cell birthday and ultimate destiny. One possibility is that a neuron's fate is determined in advance according to a kind of internal clock in its progenitor cell. An alternative view is that neurons within a given layer adopt their identities in response to environmental influences, which are likely to be chemical in nature and could occur before or during the cells' migration.

In animal experiments designed to determine the developmental potential of cortical neurons, cell groups containing progenitor cells were removed from developing rat brains at specific stages and transplanted into brains at different stages of development. For example, cells normally destined to become layer-six neurons were transplanted, before migration, into an environment in which the deep layers had already been generated and upper-layer neurons were in the process of migrating. What resulted was that the progenitor cells transplanted at

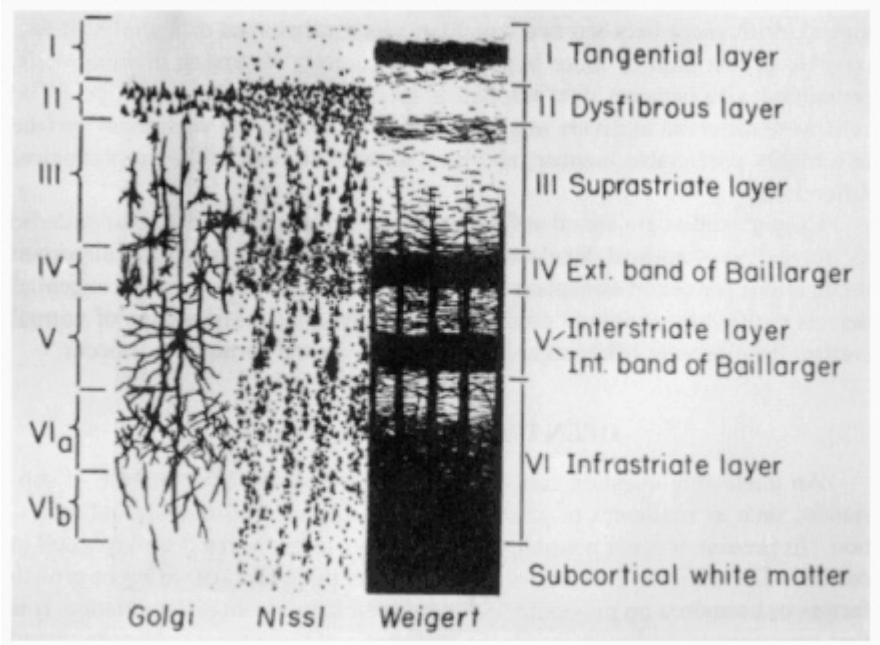

Figure 4
Cell layers and fiber arrangement of the human cerebral cortex. SOURCE: Carpenter, M.B., Core Text of Neuroanatomy, Third Edition, Williams and Wilkins, 1985. Reprinted with permission.

the point in the cell cycle just before mitosis migrated to their normal destination, as dictated by their birthday. Cells transplanted while still in the early phase of the cell cycle changed their normal fate and migrated to the cortical layer dictated by the brain into which they had been transplanted. Thus, the influence of the environment was a potent force in cell differentiation, but only if the cells are present just before mitosis. Further, these influences affect only those cells that have not divided yet, but they have no effect on migrating cells.

This scheme is complicated by the results of experiments suggesting that there is an asymmetric division in mitosis in the generation of cortical neurons. That is, instead of the two daughter cells migrating to their appropriate levels, it seems that sometimes only one daughter cell migrates and the other returns to an early stage in the cell cycle and is capable of dividing again. Further, this cell could later give rise to neurons in other cortical layers. The implication of these findings is that the message from the environment, acting on the mother cell in the early stages of the cell cycle, can be erased later by the daughter cell that does not migrate and returns to the cell cycle.

One proposed mechanism for asymmetric division was that the mother cell splits in a plane parallel to the ventricular surface, thus leaving one cell still adherent, or moored, to the ventricular surface while the other cell migrates away. Another idea was that there might be observable structural, or morphological, differences between two daughter cells that behaved differently. It was possible to test both of these hypotheses by directly observing dividing cells, containing a fluorescent dye, through a specially equipped microscope. The cells were observed to divide in a plane perpendicular to the ventricular surface in a highly predictable manner, and they showed no noticeable morphological differences.

Current studies are aimed at finding the molecular mechanisms that underlie the regulation of cortical development. These mechanisms may have important implications for neural transplant studies. There are, however, many congenital defects typified by abnormal cortical development, and a knowledge of normal cortical development is key to understanding how such perturbations occur.

OPEN DISCUSSION PERIOD

An interesting question concerned what was known about the role of substances such as hormones or growth factors in cortical cell mitosis and migration. At present, it is not possible to tell an upper-layer from a deep-layer cell in culture. Therefore, in order to be able to assess the effects of different growth factors or hormones on progenitor cells and their subsequent differentiation, it is first necessary to find markers for the different cell layer types. Also discussed was whether plasticity is possible in every cell cycle. It is suspected that late progenitors lose the ability to produce deeper-layer cells, and experiments to test that are under way.

Noting that the effects of lead in retarding cognitive development are still unexplained, a participant suggested that the culture system described in which a slice of developing cortex is maintained in vitro might provide an excellent assay for observing the influence of toxic substances on early stages of cortical neurogenesis and migration.

STEM-CELL TRANSPLANTATION IN HUMAN BRAIN DISEASE

RONALD MCKAY
National Institute of Neurological Disorder and Stroke, Bethesda, Maryland

The central problem of modern neurobiology is the molecular complexity of the nervous system. For all the proteins active in the nervous system there is generally a large family of genes, as well as a whole series of steps in the expression of the genes, that increases the functional diversity of the proteins. One path toward developing a strategy for understanding neuronal complexity is to focus initial attention on stem cells, the multipotential cells from which neurons form during fetal development.

Neuroepithelial stem cells, which line the walls of the embryonic neural tube (the neuroepithelium), generate all the neurons and glial cells of the central nervous system. Many proteins thought to contribute to driving differentiation of these and other body cells are expressed only in highly specific subsets of cells: for example, certain classes of neurons or specific types of endocrine, skin, or muscle cells. A protein called nestin is expressed in all precursor cells throughout the embryonic nervous system (and in early muscle cells) that can give rise to neurons or to the supporting cells of the brain called glia. Nestin expression is turned off abruptly in the nervous system when the cells become postmitotic, functionally committed neurons (or glia). The portion of the nestin gene that controls its expression in nervous system precursor cells differs from the part that controls muscle expression. That fact, along with nestin's ubiquity in the nervous system, makes it an ideal protein for experimental manipulations of neuroepithelial stem cells.

Neural stem cells in culture continue to express nestin for long periods. To preserve them for study, the cells are immortalized through a technique using viruses that carry oncogenes, genes that give rise to cancer. One of the qualities of cancer cells is that they can function indefinitely. Cells into which these viruses are introduced can be manipulated in culture so that the oncogenes lose their cancer-inducing capability while still bestowing immortality.

Further manipulation of the culture conditions could induce the stem cells to differentiate into neurons and glia in vitro, but only very inefficiently. By contrast, when they were implanted in the well-studied hippocampus region of brains of newly born rats, they differentiated with great efficiency. The key question,

however, was, Would they display the characteristics of hippocampal neurons? To answer this question, it was necessary to demonstrate that the transplanted cells took on the structure and position of some type of hippocampal neuron. By labeling the stem cells with a radioactive marker prior to transplantation, it was found that the transplanted cells differentiated into the type of neuron called granule cells in the hippocampus. Much is known about these granule cells including their precise connections with other areas of the brain and the fact that they contain specific, identifiable proteins. In addition, they respond in a highly predictable manner to the neurotransmitter glutamate or to compounds that behave like glutamate (called agonists). These responses include up-regulation of a distinctive oncogene and changes in the sensitivity of the cells' electrophysiological responses under specific types of stimulation (called long-term potentiation). The transplanted, differentiated cells were found over a long series of experiments to exhibit all these normal characteristics of hippocampal granule cells.

These findings in animals demonstrate that embryonic neural precursor cells can be preserved in culture for long periods of time yet still be capable of differentiating when placed into a recipient brain. Transplantation of embryonic cells into adult brains raises the possibility of providing a useful therapy for neurode-generative diseases, many of which are intractable and impose terrible personal, social, and economic costs. Experimentally, not only can cultured stem cells integrate with a recipient brain, they can be genetically manipulated before transplantation to allow a more precise analysis of gene function in the normal and diseased state. Moreover, there are data to support the idea that this approach need not be confined to the central nervous system.

Applications of these techniques, however, must await extensive further research including extending the investigations to primates. Peter Medawar, a pioneering contributor to understanding of the immune system, noted that the idea of transplantation always elicits a kind of social taboo, but that the important question is, Does it work? With regard to stem cells the answer appears to be yes.

OPEN DISCUSSION PERIOD

One participant wondered whether the strategy described for genetic manipulation of stem cells could be used to produce disease mutations for investigational purposes. Although there is considerable debate about how to do this, and different approaches are being tried, there seems no reason to presume that overwhelming technical difficulties would prevent such an application.

Another question concerned genetic changes in immortalized cell lines that might influence the neuronal functions studied. The differences among primary cells, immortalized cells, and transformed (cancerous) cells constitute an important issue. Immortalized cells may not turn out to be the appropriate ones for treating disease.

A final question was raised about age limits for the recipients of transplanted stem cells. Although the accepted dogma is that conditions do not exist in the adult brain for neuronal differentiation, ongoing research by a variety of laboratories suggests that the efficiency of differentiation of transplanted cells decreases with age, eventually reaching a very low level. It is an area, nevertheless, that is under active investigation.

FETAL TISSUE TRANSPLANTATION FOR PATIENTS WITH PARKINSON'S DISEASE

RICHARD ROBBINS
Yale University School of Medicine, New Haven, Connecticut

Upwards of a million people in the United States have Parkinson's disease, a progressively degenerative condition affecting movement. The tremor and motor-control loss that characterize it result from the death of a group of brain cells that produce the neurotransmitter dopamine. What causes the cells to die—whether a virus, an environmental toxin, or another agent—is not known.

Therapy using L-dopa, the precursor of dopamine, alleviates the symptoms of parkinsonism for five to ten years, after which patients frequently develop resistance to it. Other approaches have been tried, including slow-release dopamine implants and transplants of the patient's own adrenal cells, which had to be stopped because of adverse side effects. Experimental work in this disease progressed slowly for decades, because there was no good animal model of the disease. That situation was changed almost 10 years ago by a tragic accident. An illegally produced "designer" drug containing a contaminant, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), was taken by a number of young healthy individuals, who then developed very severe parkinsonism. Once the cause was established and MPTP was shown to produce the same effect in animals, a badly needed animal model was suddenly available. The work of the Yale-Rochester private implantation group at St. Kitt's clearly demonstrated that fetal dopaminergic tissue could innervate MPTP-damaged brain regions and reverse the neurological defects. The idea of transplanting fetal dopaminergic cells into Parkinson's patients grew out of this work.

Thirteen Parkinson's patients have received fetal tissue transplants in Yale University's Neural Transplant Program. Because of the long-standing ban on federal funding of fetal tissue transplantation, the program has been privately funded. The tissue used in the program is collected from elective abortions. The women who consent to its donation are in no way influenced in their decision to terminate pregnancy; the request for donation is made only after the women have already signed consent forms for the abortion. The normal abortion procedure is also not changed in any way. Donors cannot specify who will receive the tissue, no money is exchanged, and no one involved profits financially.

The neurons in the donor tissue, usually from a seven- or eight-week fetus, are still immature, captured soon after they have committed to becoming dopaminergic cells, but before they have fully differentiated. The tissue is microdissected by a neuroanatomist into small blocks that contain supporting glial cells. These cells are believed to secrete growth factors important to the development and differentiation of dopamine neurons. Cryopreservation is used to store the tissue, because about six weeks of testing are needed to ensure that the tissue is free of infectious agents. The dopamine content of the cells is also evaluated. Storage also permits surgery to be scheduled at a time of optimal health of the patients.

When a patient is ready for surgery, an atlas of his or her brain is created by means of magnetic resonance imaging to ensure safety and accuracy in the placement of the implant. The procedure, which takes about six hours, is done under local anesthesia and the patient usually goes home the following day. All of the patients undergo intensive preoperative and follow-up neurological and psychological evaluation. Their status is tracked, along with that of a nonoperated control group, in a "blind" manner so that the examiners do not know which patient has received a transplant and which has not. Improvement in motor function generally begins several months after surgery and continues for up to two years.

Although not cured of their disease, these patients perform better than they did under optimal medication before implantation, and they perform still better if they have medication, in reduced dosages, after surgery.

Continuing research is directed toward questions such as the following that are critical to the progress of implantation therapy for Parkinson's disease:

- What is the optimal age of fetal tissue for implantation?
- How much of the tissue should be placed—and where?
- What are the most sensitive noninvasive measurements of graft viability?
- Which cells in the graft are responsible for improvement?
- Should growth factors be added to the grafts?
- How would graft rejection be detected?
- How much immunosuppression should be used? For how long?
- Are outcomes among transplant centers comparable?
- Will there be enough fetal tissue for sufficient trials?
- Should procedures to collect tissue be modified?
- Should dopamine cells be genetically engineered for controlled proliferation?

Dopamine neurons arise in a part of the midbrain called the substantia nigra and communicate with the striatum, further forward in the brain, specifically with the caudate and putamen areas. The events in cell differentiation are being studied in cultured substantia nigra neurons and glial cells for insights into why cells die in Parkinson's patients. The effects of growth factors are being examined to see if they can be used to increase survival of dopamine cells in culture.

Tests have been set up to look for dopaminergic toxins or other possible toxic factors in patients' caudate tissue.

A major problem for research and treatment is the difficulty of obtaining healthy fetal tissue. Treating a few patients per year will not make a significant dent in the large population of Parkinson's sufferers. One possible solution is to create an immortalized dopaminergic cell line. Because this may involve viral vectors or oncogenes, methods for preventing the cells from turning cancerous must be ascertained. The cells must also be able to release dopamine in a regulated manner, because too much dopamine is as bad as too little.

Despite its present limitations, fetal tissue transplantation has been shown to restore neurologic function in patients with severe parkinsonism. Research to verify the clinical improvements and to define the factors that are directly responsible for the improvements must proceed. The lifting of the ban on fetal tissue research will hopefully enable research on this disease to go forward more quickly.

IMPROVING FETAL NEURONAL GRAFT TECHNOLOGY THROUGH THE USE OF NONHUMAN PRIMATES TO MEET THE NEEDS OF HUMAN THERAPEUTIC APPLICATIONS FOR PARKINSON'S DISEASE

JOHN R. SLADEK, JR.
Neuroscience Institute, Chicago Medical School, Chicago, Illinois

Twenty years of study using rodent models and, more recently, nonhuman primates have demonstrated that grafts of embryonic nerve tissue implanted in the brain alleviate the symptoms of experimentally induced parkinsonism. These studies were based on the elegant work of a number of researchers who had investigated the viability and physiology of transplanted tissues. The development of the primate model of parkinsonism and successful neural grafting, reported in 1985, opened the way to clinical trials with patients with Parkinson's disease. Results to date, although very promising, have pointed up the need for more refined methods of graft tissue selection and preparation to achieve greater graft-cell viability and growth. Continuing animal studies are providing new data that, when used in comparative studies with human cells, should lead to better understanding and treatment.

One of the questions that has been explored recently in studies with rhesus monkeys is the optimal age of fetal donor cells for transplantation. The use of ultrasound in a primate breeding colony has allowed access to embryos at progressive stages of development. Tissues from different stages have been grafted into recipient animals, and samples of the graft retrieved later for examination. Computer programs have been adapted to extrapolate information from a sample to create a three-dimensional reconstruction of the graft and measure the percentage of transplanted cells that survive.

These experiments have yielded a number of valuable results. Very impor

tantly, the grafts contained all of the cell types normally found in the region of the brain in which dopamine cells arise, and they were filled with growing axons, the cell fibers from which dopamine is released (Figure 5). Most significantly, the studies revealed that there is a very brief window of time during neurogenesis when developing cells are best suited for survival in a graft. Translating that information from the monkey to the human gestational period has not yet been done, in large part because of the prohibition on fetal tissue research.

Dopamine neurons, arising in the substantia nigra region of the midbrain, normally send out their axons across the brain to target cells in the striatum. In both laboratory and clinical trials, grafts have been implanted directly into the striatum. Because the normal system is thus short-circuited, there was uncertainty as to whether factors in the natural environment that are missing in the transplant environment are important to dopamine cell control. To look into this, tissue from both the substantia nigra and the striatum of the same donor embryo were grafted, about 2 mm apart, in a host brain. The nigral cells sent their axons preferentially to the donor striatum. Moreover, some of the damaged brains appeared to regenerate in a pattern similar to normal development in response to the graft.

Other co-graft experiments showed for the first time that long-distance targeting, from one side of the brain to the other, could be effected. This has suggested that growth-factor signals in the embryonic striatum are so strong that they might be used as stepping stones by growing axons. By grafting dopamine cells into the host substantia nigra, their natural location, and placing growth-factor packets along the route to the striatum, it may be possible to reconstruct the natural pathway.

Data from animal and human cells need to be compared. Experiments grafting human cells into nonhuman primates are necessary to compare long-distance cell targeting and circuit reconstruction. The optimal human donor-cell age needs to be determined, as does the optimal age for a graft recipient. Experiments in aged monkeys are necessary to learn when best to intervene in the course of disease, a critical question. For example, work from our laboratory suggests that L-dopa, especially long-term therapy, may itself reduce the effectiveness of dopamine released from the graft. The comparative survival in grafts of cryopreserved tissue, freshly dissected tissue, and short-term cultured cells also needs to be assessed. Resolving these issues is essential to providing graft-cell yield that can best compensate for the progressive loss of dopamine neurons in Parkinson's disease.

OPEN DISCUSSION PERIOD

The comments and questions that followed these presentations underscored how much remains to be learned about fetal-cell therapy for Parkinson's disease. One participant asked if there was any evidence in patients in the Yale trials as to whether the effects of transplants are due to the formation of axonal connections

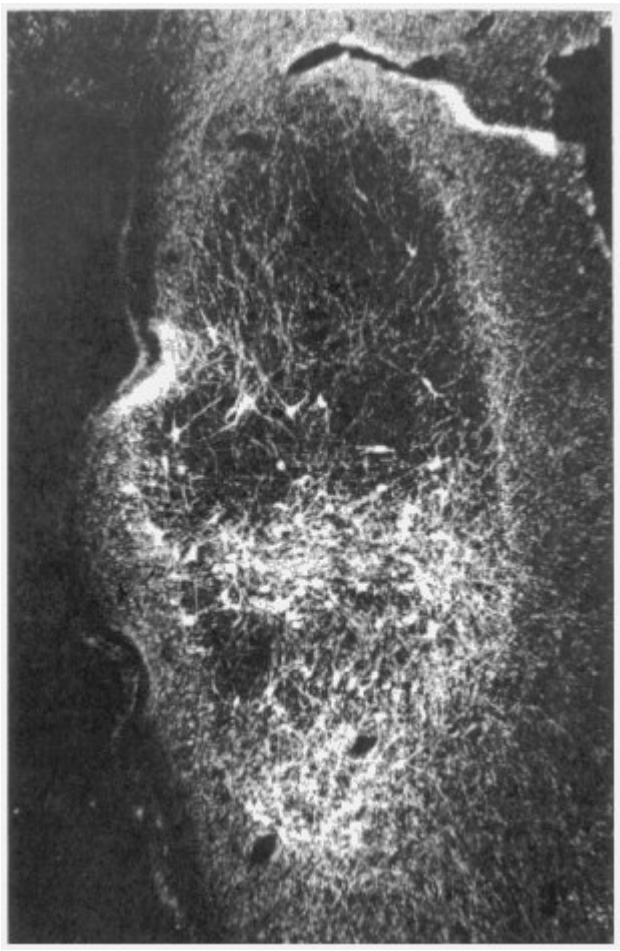

Figure 5
Transplantation of embryonic neural tissue can result in the survival of thousands of neurons of a specific transmitter type as illustrated in this photomicrograph. Selection of tissue samples from the optimal stage of neural development has yielded over 22,000 surviving dopamine neurons following grafting into a nonhuman primate model of Parkinson's disease. This represents about one-third the normal adult complement of dopamine neurons derived from the mesencephalon, a critical brain region in Parkinson's disease. These neurons also were characterized by the presence of extensive outgrowth of axons and dendrites into the host brain, which suggests the opportunity for functional integration between grafted and host tissue. SOURCE: Sladek, J.R., Redmond, D.E., Roth, R.H., et al. "Fetal Dopamine Cell Survival After Transplantation Is Dramatically Improved at a Critical Donor Gestational Age in Non-Human Primates," Experimental Neurology, 122:22, 1993. Reprinted, with permission, from Academic Press, Inc.

or to diffuse release of dopamine. The answer is not known. It may be that the glial cells implanted with dopamine neurons make growth factors, which in turn encourage other dopamine neuron systems to grow. The implant may also increase sensitivity to L-dopa, among other possibilities. Another question concerned how much fetal material is needed for a human transplant. The amount currently used is about equivalent to two fetal midbrains, but fetuses are rarely retrieved intact, so only a fragment of midbrain is usually available.

Among questions generated by the presentation of animal research data was whether adult host cells would form reciprocal connections with fetal cells grafted into the midbrain. The question of neuron-target interactions will require detailed experimental reconstruction and analysis at both ends of the system. A participant asked if there was any evidence that co-grafting provides better recovery. Although the monkey studies are still in the process of analysis, rodent models appear to improve more rapidly after co-grafting.

Information was requested about human transplantation therapy programs and results worldwide. In addition to the Yale program and one at the University of Colorado in this country, there are programs in Sweden, Cuba, England, and Poland. During the fetal tissue research ban in the United States, an American neurosurgeon took patients to China for implantation. Specific data on results are beginning to be collected. Patients in a joint American and Swedish program have been described as showing the most marked improvement. Interestingly in terms of recipient age, however, these patients are all young people who developed drug-induced parkinsonism from use of MPTP. It is important to remember the differing etiologies and the fact that MPTP-induced parkinsonism is not progressive.

FORCES THAT MOLD THE ANTIBODY REPERTOIRE: LESSONS FROM FETAL LYMPHOCYTES

ROGER PERLMUTTER
University of Washington, Seattle, Washington

The fundamental property of the immune system is its ability to recognize the entire universe of potentially pathogenic foreign invaders, such as viruses and bacteria. Recognition is achieved by a virtually unlimited repertoire of receptor molecules on white blood cells, or lymphocytes. Lymphocytes express specific receptors in response to specific molecules on invaders (antigens) that mark them as foreign. This process also accounts for rejection of grafts of nonself tissue. Remarkably, immune receptors are encoded by a relatively small number of genes. They achieve their diversity through a complex feat of genetic recombination.

Lymphocyte receptors are formed from pairs of protein chains. Each chain contains a constant region and a variable region. The variable regions are encoded in gene segments capable of vast numbers of recombinatorial rearrangements.

Superimposed on this system are other processes that enhance receptor flexibility. The system's extreme heterogeneity has made it difficult to study the way in which the immune repertoire is generated. Studies of fetal lymphocytes, conducted over several decades, have provided a useful entrée into the system.

It would be logical to assume that, because their gene rearrangements are random, lymphocyte types arise randomly, but in fact, they develop at specific times during an animal's life. This process has been studied in greatest detail in the mouse, where a whole hierarchy exists for the acquisition of lymphocyte receptors that bind particular antigens. To investigate this process in human development, the fetal liver, which contains arising B lymphocytes, has been examined at different stages of growth.

Antibodies are released by B lymphocytes and function as immune receptors dispatched by the cells into the body's fluids. Research has revealed that of the 200 variable-region gene segments that function in the adult, only about 12 or 13 are active in the fetus. Studies of developing T lymphocytes have shown that the organization of their receptor genes looks very much like the pattern of limited diversity and characteristic developmental order seen in antibodies.

Interestingly, fetal antibody gene segments are very similar in humans and mice. At the level of the nucleotide (the building block of DNA) sequences, the segments are 92 percent similar. This close relationship has facilitated comparative research to begin to determine what is so special about the variable gene segments that are expressed early in life. One thing that has been discovered about them is that they will bind self-components, something ordinarily prohibited in immune response. Immune tolerance to self-antigens is essential to survival. Fetal antibodies include many that appear identical to antibodies responsible for autoimmune disease, such as rheumatoid arthritis, in which immune cells attack other cells of their own body.

What is emerging from this research is confirmation that a tremendous amount can be learned by starting with a relatively limited repertoire as a foundation on which to build understanding of the complex progression that leads to the immensely larger immune receptor repertoire. It is important, however, to examine these issues in human fetal lymphocytes.

ISOLATION OF CANDIDATE HUMAN STEM CELLS USING SCID-HU MICE IMPLANTED WITH HUMAN FETAL TISSUE

IRVING WEISSMAN[1]
Stanford University School of Medicine, Stanford, California

Hematopoiesis is the process through which all the different types of blood cells are formed, from red blood cells, to T and B lymphocytes, to a number of different types of so-called white blood cells. Given the multitude of human

[1] Dr. Weissman informed conference participants that some of the work he would be presenting was done at Systemix, a company in which Dr. Weissman has financial interests.

diseases that affect these cells, it is understandable that this process is the subject of intense study. All the blood cells formed in hematopoiesis derive from primitive stem cells, and by definition, a stem cell must have two properties: the ability to give rise to every kind of blood cell and to more stem cells.

A search of several years' duration was conducted to isolate true stem cells —cells not yet committed to a particular destiny—in the bone marrow cell populations of experimental mice. Sorting methods involved the use of monoclonal antibodies that, when fully catalogued and mapped, can home in on specific types of cells in a mix by recognition of distinct cell-surface antigens (markers). The search showed only 1 in every 2,000 marrow cells to be free of markers that would indicate they were type-committed.

To make sure that the marker-free cells had full stem-cell potential, they were isolated and injected into mice that had been irradiated to kill their existing marrow cells. When such lethally irradiated mice are injected with unsorted donor marrow, it takes about 200,000 cells to save them; it required only 85 to 100 of the isolated cells to do the same job.

Further experiments showed that a single stem cell could give rise to 100 million blood cells of all types, including many hundreds or thousands of new stem cells. When progeny stem cells from the original cell were collected from the first recipient mouse and subsequently injected into irradiated animals, all of the animals' marrow became fully reconstituted. This finding also meant that one could now study the full developmental repertoire of a single stem cell.

Growth factors have important effects on both primitive and mature blood cells. One very useful effect is to cause stem cells to migrate from the bone marrow into the peripheral blood. By applying these factors, stem cells can then be harvested from the peripheral blood for clinical use—a procedure vastly more simple than harvesting from bone marrow. Thus, this procedure will undoubtedly provide the most enriched and utilized source for marrow or hematopoietic transplants in the future.

T lymphocytes arise in the thymus and move to target organs such as the skin and other epithelial areas of the body where they serve specific functions. The order of appearance of these targeted T cells in the fetus follows a developmental timetable. Experiments have shown that the ability to make skin-homing cells, for example, switches on and off only during a specific and limited time window after which the production of skin-homing cells stops. It has also been shown that if an adult stem cell is inserted in the fetus, it will give rise only to adult-programmed types of T cells. These findings suggest the significance of the age of the donor stem cells for any future fetal therapy and imply that donor cells should be the same age as the recipient.

The study of human hematopoietic stem cells began by harvesting cells from human fetal livers (like bone marrow, a rich source of such cells in the fetus) and growing them in culture to identify the different types. Stem cells were found that could generate all the necessary blood cell types in culture, but it

was unknown what would happen in vivo. Although the needed experiments could not be done in humans, an invaluable alternative was found in a mutant mouse strain that exhibits severe combined immunodeficiency (SCID). SCID mice cannot make T cells or B cells and are, therefore, tolerant of foreign tissue. Human fetal thymic grafts implanted in SCID mice produce human T cells tolerant of both the mouse and their own human tissue, but fully reactive to other tissue. These grafts, as well as transplanted human fetal bone marrow, will reconstitute all the human blood cell types.

From a clinical standpoint, the development of the SCID-human (SCID-hu) model and the study of human stem cells make a number of strategies possible. For example, it has been found that stem cells from a human being with a certain type of leukemia, which lack the genetic change associated with leukemia, give rise to normal, healthy blood cells. Purified stem cells could then be harvested from a patient with leukemia and used to repopulate the bone marrow after the diseased cells were destroyed by irradiation. Other cancers that may be responsive to such treatment include myeloma and lymphoma. Some diseases that affect blood cells are inherited, such as severe combined immunodeficiency disease and thalassemia. If stem cells from the siblings of affected patients could be isolated, they could then be transplanted successfully because such cells do not give rise to graft-versus-host disease. In cases where suitable sibling cells are not available, it may be possible in the future to put corrective genes directly into the patient's own stem cells. A potential that has aroused great excitement is stem-cell treatment for AIDS.

Human immunodeficiency virus (HIV) attacks and cripples the immune system. Its port of entry is a subset of T cells designated CD4. A number of studies indicate that HIV does not penetrate stem cells. If that is true, it may be possible to insert into the stem cells of AIDS patients genes that encode mechanisms to stop viral replication or the pathogenic effects of HIV on CD4 cells. Attempts to make such gene constructs have begun and the SCID-hu model is valuable in these efforts also.

In the process of mapping thymic development in the SCID-hu mouse, it was discovered that the very first progeny cell in the human thymus (unlike the mouse) is the CD4 cell. HIV injected into the SCID-hu mouse infects these human CD4 cells, which means that SCID-hu can be used as a model to study viral infection. SCID-hu can also be used to study the development of many different tissue types. For example, fetal lungs or bone can be implanted successfully in SCID mice and provide cells for study. In addition, to study genetic diseases it may be possible to implant fetal tissue that is known through early diagnostic testing to be affected by some type of genetic disease.

Another stem-cell potential derives from a phenomenon that has been known for a long time: namely, that in an irradiated animal in which bone marrow has been replaced with donor marrow, any organ can be grafted from that same donor and not be rejected by the host. In recent experiments studying how this

might affect autoimmune diseases, stem cells from a strain of mice that do not get diabetes were implanted into mice that invariably develop diabetes. Stem-cell implants were done, after lethal or sublethal irradiation, at a time when the recipients' lymphocytes were collecting in the pancreas, indicating the onset of autoimmune attack that would eventually destroy the insulin-producing pancreatic cells. The transplanted mice never developed diabetes.

XENOGENEIC TRANSPLANTATION: THE USE OF ANIMALS FOR ORGAN DONORS

DENISE FAUSTMAN
Massachusetts General Hospital, Charlestown, Massachusetts

There is a long and growing list of human disorders for which cell or organ transplantation promises more effective therapy. The problem is that the human tissue available for transplant, now or in the future, cannot begin to meet the need. Removing the barriers to xenogeneic (cross-species) transplantation could open the way to organ banks with an unlimited supply of replacement cells and organs.

Xenogeneic tissue has advantages and disadvantages compared to human tissue—some obvious, some less obvious, some potential. Among the advantages, in addition to being plentiful, animal donors often lack, or could be genetically modified to lose, susceptibility to human disabilities or pathogens, such as HIV. The disadvantages include differences in size and structure, as well as critical metabolic incompatibilities that must be avoided or compensated for. Animal studies have shown, for example, that although the ability to secrete insulin is intrinsic to all pancreatic islet cells, the cells regulate blood sugar differently across species. Thus, a diabetic human given goat islet cells could possibly go into hypoglycemic coma, because the goat cells respond to glucose levels appropriate for that species and secrete more insulin than is appropriate for humans.

For liver transplants, there are also hemodynamic problems. In the recent experiment in which a baboon liver was implanted in a human subject, the baboon was chosen because its cardiac output is similar to that of humans and it is resistant to AIDS (from which the patient was suffering), but the clotting factors produced by the baboon liver differed significantly and resulted in severe clotting abnormalities in the recipient.

The primary barrier to the clinical reality of xenografts is that the recipient mounts a much more pronounced rejection response to nonhuman donor tissues. The strategy currently used in all clinical transplant protocols to circumvent immunogenic graft rejection is immunosuppression. Although the graft may take, the patient, stripped of immune defenses, succumbs easily to massive infection. A solution to this problem may lie in research currently under way, based on new information about the molecular mechanisms that regulate the T lymphocytes, especially those known as killer cells that attack tissue transplants.

A killer T cell attacks a foreign graft in a series of steps that begin when the T cell recognizes and sticks to a binding site on the target cell. The binding triggers a cascade of events, including activation of the T cell's receptor, that culminate in the T cells' drilling a hole in the target cell and killing it. In an experimental approach that works in reverse of immunosuppression, instead of inactivating T cells the donor tissue is altered in such a way that its surface antigens are hidden from the recipient's T cells. This has been done by taking antibodies to the key antigen of the recipient cells and separating the binding part of the antibody from its "handle," or the part to which T killer cells stick in the initiation step of donor cell destruction. These separated antibody fragments were shown in tissue culture experiments, and eventually in transplantation experiments, to mask the antigenic sites of the donor tissue and protect it from the host's immune system.

In the initial experiments, monoclonal antibodies were used to engage the donor cell binding sites, thereby making them unavailable for binding by T cells. Human islet cells, so disguised, were implanted in mice and survived up to 200 days without T cell attack. In subsequent experiments, another approach was tried, in which the genes for the donor cell binding sites were inactivated or knocked out. With both masking and knockout strategies, researchers have now created masked donor heart, liver, kidney, and insulin-secreting cells. There are no clear answers yet as to whether surface masking or genetic engineering protects donor tissue in the long-term. There is renewal with time of the antigenic cell-surface proteins that are not masked and are therefore vulnerable, although this renewal seems slower than normal. However, there are also hints in some tissue types, possibly due to slow renewal, that the recipient's immune system may become tolerant to these cell-surface markers.

Many barriers to xenogeneic grafts must still be overcome, but these experiments have shown that it is possible to design new methods to manipulate cells. Xenogeneic transplant therapy offers the chance to enhance and broaden treatment options for many diseases and, in some cases, to provide permanent cures. We may one day be treating problems of cholesterol metabolism and hemophilias with xenogeneic liver cells or bone marrow stem cells, diabetes with xenogeneic insulin-secreting cells, and cardiac disease with pig heart transplantation.

Highlights and Themes

The final part of the conference included summaries of the highlights of each session and discussion of common themes. The field of fetal research carries great promise to alleviate suffering from certain human diseases and to contribute fundamental new knowledge to our understanding of human biology. The political, ethical, and legal controversies elaborated in the first session, however, continue to entangle this field. In the United States, without an Ethics Advisory Board, such controversies resulted in an outright prohibition of most fetal research funded by the federal government. During this period, researchers have obtained support from private sources in the United States, have collaborated with groups outside the United States, and together with these groups and other researchers around the world, have made significant progress. Yet, critical gaps still remain.

The lifting of the U.S. funding moratorium will certainly provide greater resources to investigations of human reproduction, embryogenesis, genetic abnormalities, fetal failure, fetal therapy, and fetal tissue transplantation. Debate continues about whether or not these benefits can be realized only after the reconstitution of an ethics advisory body that can serve as a platform for national discussion and consensus building. While some scientists believe an ethics advisory body should be reconstituted, others do not. Further, it is not yet clear how Congress and the Executive Branch will structure any new ethics advisory body. Thus, although prospects for future federal support are brighter now, additional barriers will continue to exist as a consequence of widely varying state laws. How an ethics advisory body will function and whether or not national guidelines or laws will be established are just two of the many complex questions that await resolution.

All of the speakers in the second session on preembryo research echoed the

theme that progress in this field has been hampered by lack of federal funding in the United States. As a result, much of the U.S. work on technologies related to in vitro fertilization (IVF) has been done in animal models. For example, good predictors of embryologic development have been found in work with oocytes from cows, but this knowledge has not been validated completely with human oocyte research. While the demand has increased for assisted conception technologies, clinics have adapted techniques developed in animals (see Appendix C).

Beyond these difficulties there is a pressing need for greater understanding of developmental failures and abnormalities, particularly genetic abnormalities. It is not known how many of these are normal and occur with equal frequency in natural conception, or how many may be a product of the process of IVF. A related issue is how to predict developmental competency in order to increase the success rates of IVF. Both areas would be served best by research with human oocytes.

Current procedures in IVF carry risks that might be lessened by more research focused on oocyte maturation. The typical procedure now is to give a patient superovulatory or fertility drugs that are implicated in uterine changes and hormone-induced pathologies. Better knowledge of oocyte harvesting, in vitro maturation, and preservation could eliminate the need for fertility drugs in women who require IVF to become pregnant.

A baseline understanding of all the factors involved in human embryo development is still lacking, including cell cycles, secretory products, environmental tolerances, growth factors, differentiation events, and their controls. In vitro embryos produced or donated for research and oocytes derived from aborted fetuses would provide opportunities to shed light on both normal development and the mechanisms of genetic diseases and human cancers. The ability to perform micromanipulations on oocytes and early embryos has allowed the fertilization of oocytes by sperm that could not normally penetrate the zona pellucida. It has also made possible the elegant single-cell assays that are successful in identifying diseases such as cystic fibrosis and Tay-Sachs disease. Thus, preimplantation genetic screening is possible; its use also raises important ethical considerations regarding sex selection, use of screening information and confidentiality, and many other issues.

The third session on fetal research underscored the historic strides that have been made in fetal medicine. A near revolution has occurred in the ability to gain relatively safe access to the fetus from early pregnancy to delivery, opening the way to many avenues of diagnosis and treatment. Techniques we now take for granted, such as amniocentesis and ultrasonography, would not have been possible without the kinds of research that have been restricted in the recent past.

Newer techniques under investigation offer great promise. Embryoscopy is likely to permit earlier diagnosis than other methods. For some fetal conditions, early treatment will be critical. Access to the fetal blood supply through percutaneous umbilical sampling not only can aid in fetal diagnosis, but also can permit

effective interventions for a variety of conditions that threaten the survival of a fetus. Another area needing further attention, however, is methodology for the prenatal detection of certain central nervous system problems, such as cerebral palsy, that arise in seemingly normal babies born of normal pregnancies. New information about nutritional, placental, and environmental factors that impinge on fetal development points to the value of both prenatal and long-term studies in solving birth defects and later health problems. One example from nutritional research is delayed onset of congenital diabetes resulting not from genetic transmission, but rather from unstable glucose levels during fetal life as a consequence of maternal diabetes. Another example comes from studies of the molecular biology of enzymes, which suggest that genetic factors regulating enzyme function may produce important differences from one fetus to another in susceptibility to environmental toxins such as components of cigarette smoke. Postmortem studies are also required in cases of spontaneous abortion to identify the contributions of placental and other pathologies and to diagnose undetected genetic abnormalities that will inevitably compromise a woman's future pregnancies.

In the arena of fetal therapy there have been successes and disappointments. Among the successes is the replacement of fetal blood in Rh disease; work that additionally identified specific risk factors for percutaneous umbilical blood sampling (PUBS) will aid physicians in giving better information to patients considering this procedure. In contrast, attempts at surgical interventions and open fetal surgery have been only partly successful. For example, although placement of shunts to alleviate bladder obstructions in fetuses is frequently successful, later-developing renal disease complicates the usefulness of the procedure in the long-term. Certain other interventions, including the placement of shunts for hydrocephalus, cannot be justified because they alleviate the fluid buildup but do not reverse the severe brain damage. Critical to the evaluation of potential invasive fetal therapies is the degree to which the therapy will increase survival over what would be observed normally and whether or not the therapy results in improved function in the long-term. Nevertheless, research in such areas can lead to better understanding of underlying disease processes and more effective methods for delivering donor cells.

Through both the third and the fourth sessions, the importance of research on stem cells was emphasized. Fetal stem cells, unlike their mature counterparts, lack the cell-surface markers that trigger immune system responses and, especially in the case of neural cells, are multipotent or capable of regeneration and differentiation. Thus, the use of human fetal stem-cell transplants to correct enzymatic deficiencies and neural damage is an active area of research. The research described in the fourth session on fetal tissue transplantation is beginning to provide a foundation for understanding two complex biological systems, the nervous system and the immune system. The major issue for neurobiologists is getting fetal neurons transplanted into patients to perform at sufficient levels to modify disease. The clinical potential of neural transplants is exemplified by

what has already been accomplished with Parkinson's disease. Although optimal results are still in the future, the work to date supports the assertion that we will be able to reduce the immense human costs of certain central nervous system diseases.

Transplant work continues to rely, however, on basic research aimed at defining the precise patterns of neural development, such as the complex patterning of the cerebral cortex that arises from a set of precursor or stem cells controlled by both genetic and environmental mechanisms. Animal experiments have also defined critical time periods during which fetal neurons are most useful for transplant. Such experiments have demonstrated the amazing plasticity of fetal neurons and have shown the potential for manipulation of the local environments of transplant sites to encourage maximal growth of transplanted neurons.

Transplantation research and practice are limited also by adequate sources of stem cells, and for this challenge, basic research may offer great promise. If fetal stem cells are scarce, it makes sense to try to find ways to preserve them in culture until they are needed. For neural stem cells, this is not easily accomplished. One approach has been to "immortalize" the stem cells with genetic manipulations, and then to transplant these cells and examine their function. In animal experiments, this technique appears to have produced stable stem-cell lines that, once transplanted, function normally in the host brains.

The primary problem for transplantation in organ systems other than the brain is immune rejection. Current research is seeking the solution to problems of both immune rejection and transplant function largely through studies of hematopoietic stem-cell differentiation in the fetus. After markers were found that identify true hematopoietic stem cells, great strides have been made in experimental bone marrow transplantation because transplantation of a very few fetal stem cells was found to be as successful as the implantation of hundreds of thousands of unsorted cells. The development of the SCID-hu (severe combined immunodeficiency-human model) mouse further expanded this work and it is now possible to study the complete developmental process of a single human stem cell. In addition, because the SCID mouse cannot make lymphocytes, all of the lymphocytes (and many of the other hematopoietic cells including stem cells) harvested from a SCID mouse that has been implanted with human stem cells are human lymphocytes. Thus, the SCID mouse can be used as a "culture environment" to expand the available pool of human stem cells. The SCID-hu mouse also provides an excellent model for studying viral infections. This fact raises the potential for exploring the use of stem cells, which are genetically engineered to block specific pathogenic mechanisms of viral infection, to treat AIDS.

One type of transplantation that is particularly vulnerable to immune rejection is the transplantation of tissue from one species to another, a so-called xenograft. However, if xenografts were more successful, they could offer one solution to the problem of too few organs and tissues being available to meet

existing needs. Research to make xenografts more viable has contributed to the development of techniques to mask antigenic sites on transplanted cells, or to knock them out genetically, thereby disguising the transplanted cells from the host's immune system.

The value of a new technique such as embryoscopy is that it provides access to the fetus before the fetus is immunologically competent. This has the potential of opening up a large array of genetic diseases to fetal therapy.

In summary, then, the conference provided a broad overview of the diversity and direction of current fetal research and a starting point for creating a future research agenda. Examination of the impressive progress that has been made in the face of severe obstacles underscores the need for national guidelines and resolution of the problem of contradictory state regulations. The importance of consensus in the scientific community with regard to ethical considerations surrounding fetal research was a continuing theme throughout the conference. Many participants felt that the scientific community must take a leadership role in partnership with ethicists, policymakers, and others, in helping to frame the debate, develop guidelines, and establish common ground.

Appendixes

Appendix A

Conference Agenda

CONFERENCE ON FETAL RESEARCH AND APPLICATIONS
The Arnold and Mabel Beckman Center
Irvine, California
June 20–22, 1993

Sunday, June 20, Auditorium

5:00 p.m.	OPENING SESSION
	Welcome and Overview of Conference
	Uta Francke, M.D. Stanford University Medical Center Stanford, Calif.
5:15	SESSION I ETHICAL AND LEGAL ISSUES OF FETAL RESEARCH: HISTORY AND BACKGROUND
	John Fletcher, Ph.D. University of Virginia Charlottesville, Va. "Ethics and Fetal Research: Past, Present, and Future"
	Lori Andrews, J.D. American Bar Foundation Chicago, Ill. "Federal and State Regulations of Fetal Research: A Current Perspective"
6:45–7:45	RECEPTION IN ATRIUM

Monday, June 21, Auditorium

8:00 a.m.	BREAKFAST
8:30	SESSION II PREEMBRYO RESEARCH
	Moderator: Neal First, Ph.D. University of Wisconsin Madison, Wisc.
	Overview: Professor Robert Edwards Bourn Hall Cambridge, England "Modern Ideas in Embryo Research"
9:30–10:20	Jacques Cohen, Ph.D. Cornell University Medical Center New York, N.Y "Micromanipulation Research in Clinical Embryology"
	Professor Alan Trounson Monash University Victoria, Australia "Progress in Reproductive Biology and Implications for the New Reproductive Technologies in Human IVF"
10:20–10:35	BREAK
10:35–11:20	Mark Hughes, M.D., Ph.D. Baylor College of Medicine Houston, Tex. "Preimplantation Genetic Analysis of Single Human Blastomeres"
	Norman Arnheim, Ph.D. University of Southern California Los Angeles, Calif. "Studies on DNA Sequences in Single Human Gametes"
11:30–1:00	LUNCH IN REFECTORY

1:00 p.m.	SESSION III FETAL RESEARCH
	Moderators: Frederick Battaglia, M.D. University of Colorado Denver, Colo.
	Haig Kazazian, Jr., M.D. Johns Hopkins University Baltimore, Md.
	Overview: John Queenan, M.D. Georgetown University Medical Center Washington, D.C. "The Fetus as a Patient: The Origin of the Speciality"
2:00–3:15	Kenneth Moise, Jr., M.D. Baylor College of Medicine Houston, Tex. "Percutaneous Umbilical Blood Sampling"
	E. Albert Reece, M.D. Temple University Philadelphia, Pa. "Embryoscopy and New Advances in Fetal Diagnosis and Treatment"
	Boyd Metzger, M.D. Northwestern University Chicago, Ill. "Maternal Nutrition and Metabolism: Role in the Intrauterine and Long-Term Development of the Offspring"
3:15–3:30	BREAK
3:30–4:45	Stuart Handwerger, M.D. Children's Hospital Medical Center Cincinnati, Oh. "The Role of Placental Hormones in the Regulation of Human Fetal Growth"

	Cynthia Curry, M.D. Valley Children's Hospital Fresno, Calif. "Clinical Evaluations of Fetal Loss: Relevance and New Directions"
	Richard Berkowitz, M.D. Mount Sinai School of Medicine New York, N.Y. "Therapeutic Interventions in Utero"
4:45	CLOSING COMMENTS AND ADJOURN
Tuesday, June 22	
8:00 a.m.	BREAKFAST IN REFECTORY
8:30–10:10	SESSION III (CONTINUED)
	Mitchell Golbus, M.D. University of California San Francisco, Calif. "Fetal Therapy"
	Speaker originally scheduled for this time slot was unable to attend conference
	Esmail Zanjani, Ph.D. Ioannis A. Lougaris Medical Center Department of Veterans Affairs Reno, Nev. "Transplantation of Fetal Liver Hematopoietic Stem Cells in Utero"
	Daniel Nebert, M.D. University of Cincinnati Medical Center Cincinnati, Oh. "Applications of Molecular Biology and Genetics to Developmental Toxicology: Update and Future"
10:10–10:30	BREAK

10:30	SESSION IV FETAL TISSUE TRANSPLANTATION
	Moderator: Martha Constantine-Paton, Ph.D. Yale University New Haven, Conn.
10:40–12:20	Susan McConnell, Ph.D. Stanford University Stanford, Calif. "Plasticity and Commitment in the Developing Mammalian Brain"
	Ronald McKay, Ph.D. National Institutes of Health Bethesda, Md. "Stem-Cell Transplantation in Human Brain Disease"
	Richard Robbins, M.D. Yale University New Haven, Conn. "Fetal Tissue Transplantation for Patients with Parkinson's Disease"
	John Sladek, Jr., Ph.D. Neuroscience Institute Chicago, Ill. "Improving Fetal Neuronal Graft Technology Through the Use of Nonhuman Primates to Meet the Needs of Human Therapeutic Applications for Parkinson's Disease"
12:30–1:30	LUNCH IN REFECTORY
1:30–2:45	Irving Weissman, M.D. Stanford University School of Medicine Stanford, Calif. "Isolation of Candidate Human Stem Cells Using SCID Mice Implanted with Human Fetal Tissue"
	Roger Perlmutter, M.D., Ph.D. University of Washington Seattle, Wash. "Forces That Mold the Antibody Repertoire: Lessons from Fetal Lymphocytes"

	Denise Faustman, M.D., Ph.D. Massachusetts General Hospital Charlestown, Mass. "Xenogeneic Transplantation: The Use of Animals for Organ Donors"
2:50–3:05	BREAK
3:05–3:35	CLOSING COMMENTS
	Moderator: Uta Francke
	Frederick Battaglia Martha Constantine-Paton Ezra Davidson Joseph Davie Neal First Haig Kazazian

Appendix B

Conference Participants

CONFERENCE ON FETAL RESEARCH AND APPLICATIONS
The Arnold and Mabel Beckman Center
Irvine, California
June 20–22, 1993

Dr. Simone Ballandras
Franco-American Association for Fetal Medicine
Paris, France

Gillian Beatty
Whittier Institute for Diabetes and Endocrinology
La Jolla, Calif.

Richard E. Behrman, M.D., J.D.
Center for the Future of Children
David and Lucile Packard Foundation
Los Altos, Calif.

Luca Bernandini
Hitachi Research Center
University of California
Irvine, Calif.

Dr. John D. Biggers
Laboratory of Human Reproduction
Harvard Medical School
Boston, Mass.

Awie Botes
Pacific Fertility Center
University of California
Torrance, Calif.

Michael Brush
Hitachi Research Center
University of California
Irvine, Calif.

Monica J. Casper, C.Phil.
Department of Social and Behavioral Sciences
University of California
San Francisco, Calif.

Philip Chan, Ph.D.
Department of Obstetrics/Gynecology
Loma Linda University
Loma Linda, Calif.

Dr. Usha Chitkara
Perinatal Diagnostic Center
Department of Gynecology/Obstetrics
Stanford University School of Medicine
Stanford, Calif.

Italo Ciuffardi, M.D.
University of California
Irvine, Calif.

Cynthia Cohen, Ph.D., J.D.
National Advisory Board on Ethics in Reproduction
Washington, D.C.

Dr. Randy Cohen
University of California
San Diego, Calif.

Johannah Corselli, Ph.D.
Loma Linda University Medical Center
Loma Linda, Calif.

Loetania N. Dantas
Department of Obstetrics/Gynecology
University of California
Irvine, Calif.

Delbert H. Dayton, M.D.
National Institute of Child Health and Human Development
National Institutes of Health
Bethesda, Md.

Mark Denker, M.D.
University of California
Irvine, Calif.

Nelly Durr
Hitachi Research Center
University of California
Irvine, Calif.

Jamie Eberling, Ph.D.
Lawrence Berkeley Laboratory
Center for Functional Imaging
Berkeley, Calif.

Frank Falkner, M.D.
International Child Health
University of California
Berkeley, Calif.

Carlos Felix, M.D.
University of California Medical Center
Santa Ana, Calif.

Delbert A. Fisher, M.D.
Nichols Institute Reference Lab
San Juan Capistrano, Calif.

Russell Foulk, M.D.
Division of Medical Education
University of California, Irvine Medical Center
Irvine, Calif.

Monica Goldsman, M.D.
University of California
Santa Ana, Calif.

Julie Growcock
Hitachi Research Center
University of California
Irvine, Calif.

Dr. Minoru Hoshimaru
Department of Neurosciences
University of California
La Jolla, Calif.

Mark Johnson, M.D.
Department of Gynecology/Obstetrics
Stanford University School of Medicine
Stanford, Calif.

Ahmad Kamal
Beckman Laser Institute and Medical Clinic
University of California
Irvine, Calif.

Dr. Mary Kent
Promega Corporation
Madison, Wisc.

Hillary Klonoff-Cohen, Ph.D.
Department of Family and Preventive Medicine
University of California
La Jolla, Calif.

David Korn, M.D.
Stanford University, School of Medicine
Stanford, Calif.

Carl M. Leventhal, M.D.
National Institutes of Health
Bethesda, Md.

Fred Levine, M.D., Ph.D.
Center for Molecular Genetics
University of California
La Jolla, Calif.

Alejandro Manzur
University of California
Irvine, Calif.

Douglas L. Meinecke, Ph.D.
National Institutes of Health
Rockville, Md.

Fred Miller, M.S.
Pacific Fertility Center
University of California
Torrance, Calif.

Ines Moretti-Rojas
Hitachi Research Center
University of California
Irvine, Calif.

Ben A. Murray, Ph.D.
Department of Developmental and Cell Biology
University of California
Irvine, Calif.

Joseph Neev
University of California
Irvine, Calif.

Julie Neidich, M.D.
Perinatal Diagnostic Center
Stanford University School of Medicine
Stanford, Calif.

Teri Ord
University of California
Irvine, Calif.

Dr. Theo Palmer
Department of Neurosciences
University of California
La Jolla, Calif.

Pasquale Patrizio, M.D.
Division of Reproductive Endocrimology and Infertilization
University of California
Irvine, Calif.

Dr. Roger Pedersen
Laboratory of Radiobiology and Environmental Health
University of California
San Francisco, Calif.

Dr. Richard Pink
Department of Neurosciences
University of California
La Jolla, Calif.

Roy M. Pitkin, M.D.
Department of Obstetrics/Gynecology
University of California
Los Angeles, Calif.

Stirling M. Puck, M.D.
Vivigen/IG
Santa Fe, N.M.

Patrick Quinn
San Fernando Valley Fertility and Reproduction Center
Tarzana, Calif.

Catherine Racowsky
University of Arizona
Tuscon, Ariz.

Dr. Heather Raymon
Department of Neurosciences
University of California
La Jolla, Calif.

Richard Robertson
University of California
Irvine, Calif.

Francisco Rojas
Hitachi Research Center
University of California
Irvine, Calif.

R. Salem
Pacific Fertility Center
University of California
Torrance, Calif.

Dr. Andrew Scheinman
Department of Neurosciences
University of California
La Jolla, Calif.

Carol R. Scheman
Food and Drug Administration
Rockville, Md.

Mitchel C. Schiewe, Ph.D.
Department of Obstetrics/Gynecology
University of California
Irvine, Calif.

Angela L. Scioscia, M.D.
University of California
San Diego, Calif.

Anita Singh
Hitachi Research Center
University of California
Irvine, Calif.

Irene Stith-Coleman, Ph.D.
Head of Biomedical Policy Section
Congressional Research Service
Washington, D.C.

Chung-Ho Sun, Ph.D.
Beckman Laser Institute
University of California
Irvine, Calif.

Yona Tadir
Beckman Medical Clinic
University of California
Irvine, Calif.

Diane Thibodeau, M.D.
Loma Linda University Medical Center
Loma Linda, Calif.

Donald Tredway, M.D., Ph.D.
Dept of Obstetrics/Gynecology
Loma Linda University
Loma Linda, Calif.

Sijian Wang
Center for Molecular Genetics
University of California
La Jolla, Calif.

Marie Wilson
Beckman Laser Institute
University of California
Irvine, Calif.

Jen Yu, M.D., Ph.D.
Department of Physical Medicine and Rehabilitation
University of California Medical Center
Irvine, Calif.

Appendix C

Summary of Medically Assisted Conception: An Agenda for Research

The following is a reprinted summary of the Institute of Medicine report *Medically Assisted Conception: An Agenda for Research*, which was published in 1989. Although focused on research pertaining to in vitro fertilization, the recommendations regarding research policy and coordination are still useful and applicable to the topics discussed in the present conference report. A review of the research agenda offered in this 1989 report will reveal that progress has been made in some areas, but is still lacking in others. For example, the success rates of in vitro fertilization (IVF) have not changed appreciably since 1989. Likewise, little progress has been made in developing new contraceptives. In contrast, there has been progress in maturing oocytes in culture and in analyzing genetic abnormalities in early embryos. It is hoped that the inclusion of this summary will help to deepen the readers' appreciation of issues, from both the science and the public policy arenas, that still confront the fields of fetal research and fetal tissue transplantation.

MEDICALLY ASSISTED CONCEPTION

An Agenda for Research

Report of a Study by a Committee of the

INSTITUTE OF MEDICINE
Division of Health Sciences Policy

NATIONAL RESEARCH COUNCIL
Board on Agriculture

National Academy Press
Washington D.C. 1989

NOTICE: The project that is the subject of this report was approved by the Governing Board of the National Research Council, whose members are drawn from the councils of the National Academy of Sciences, the National Academy of Engineering, and the Institute of Medicine. The members of the committee responsible for the report were chosen for their special competencies and with regard for appropriate balance.

This report has been reviewed by a group other than the authors according to procedures approved by a Report Review Committee consisting of members of the National Academy of Sciences, the National Academy of Engineering, and the Institute of Medicine.

The Institute of Medicine was chartered in 1970 by the National Academy of Sciences to enlist distinguished members of the appropriate professions in the examination of policy matters pertaining to the health of the public. In this, the Institute acts under both the Academy's 1863 congressional charter responsibility to be an advisor to the federal government and its own initiative in identifying issues of medical care, research, and education.

The National Research Council was organized by the National Academy of Sciences in 1916 to associate the broad community of science and technology with the Academy's purposes of furthering knowledge and advising the federal government. Functioning in accordance with general policies determined by the Academy, the Council has become the principal operating agency of both the National Academy of Sciences and the National Academy of Engineering in providing services to the government, the public, and the scientific and engineering communities. The Council is administered jointly by both Academies and the Institute of Medicine.

This project received support from the National Academy of Sciences Basic Science Fund and the U.S. Department of Agriculture (Contract 59-32U4-8-63/R).

2101 Constitution Avenue, N.W.
Washington, D.C. 20418

(202) 334-3300

IOM 89-08

INSTITUTE OF MEDICINE

NATIONAL RESEARCH COUNCIL
Board on Agriculture

COMMITTEE ON THE BASIC SCIENCE FOUNDATIONS OF
MEDICALLY ASSISTED CONCEPTION

KENNETH J. RYAN,* (Chairman), Professor and Chairman, Department of Obstetrics and Gynecology, Harvard Medical School, Boston, Massachusetts

JOHN D. BIGGERS, Professor of Physiology, Harvard Medical School, Boston, Massachusetts

MARIAN D. DAMEWOOD, Assistant Professor, Department of Gynecology and Obstetrics, The Johns Hopkins Medical Institutions, Baltimore, Maryland

CLAUDE DESJARDINS, Professor of Physiology, Department of Physiology, University of Virginia School of Medicine, Charlottesville, Virginia

NEAL L. FIRST,** Professor of Reproductive Physiology, Meat and Animal Science Department, University of Wisconsin, Madison, Wisconsin

GARY D. HODGEN, Professor and Scientific Director, Eastern Virginia Medical School, Norfolk, Virginia

ROBERT B. JAFFE,* Professor and Chairman, Department of Obstetrics, Gynecology and Reproductive Science, School of Medicine, University of California, San Francisco

HOWARD W. JONES, Jr., Professor of Obstetrics and Gynecology, Eastern Virginia Medical School, Norfolk, Virginia

STANLEY P. LEIBO,*** Research Associate Professor, Departments of Obstetrics, Gynecology and of Urology, Baylor College of Medicine, Houston, Texas

---

*Member, Institute of Medicine
**Member, National Academy of Sciences
***During the study, Dr. Leibo was in the Research and Development Division of Rio Vista International, Inc.

JONATHAN VAN BLERKOM, Professor of Molecular, Cellular, and Developmental Biology, Department of Molecular, Cellular and Developmental Biology, University of Colorado at Boulder, Boulder, Colorado

EDWARD E. WALLACH, Professor and Chairman, Department of Gynecology, The Johns Hopkins Medical Institutions, Baltimore, Maryland

STUDY STAFF

Institute of Medicine

RUTH ELLEN BULGER, Director, Division of Health Sciences Policy

JESSICA TOWNSEND, Study Director

CONSTANCE M. PECHURA, Meeting Rapporteur

WALLACE K. WATERFALL, Editor, Institute of Medicine

Board on Agriculture
JAMES TAVARES, Associate Executive Director

TABLE OF CONTENTS

ACKNOWLEDGEMENTS

The committee appreciates the generous contribution of time and knowledge of those who presented papers at the committee's workshop at the Arnold and Mabel Beckman Center, Irvine, California, August 21-23, 1988. The thought-provoking papers contributed to the workshop, and the presence of exceptional scientists and clinicians stimulated outstanding discussions. The committee gained invaluable knowledge and insight into issues in research in reproductive and developmental biology that provided the basis for its deliberations. I want to extend the gratitude of the committee to all who attended the workshop. By offering those resources that we all value -- time and knowledge -- the committee's work was greatly facilitated.

Kenneth J. Ryan
Chairman
Committee on the Basic Science Foundations
of Medically Assisted Conception

PREFACE

This report is the result of a study by a committee of the Institute of Medicine (IOM) and the National Research Council's Board on Agriculture. The study results from a 1984 meeting of an ad hoc group convened by the National Academy of Sciences to discuss aspects of genetic engineering of the human germline. It was noted that because of a policy that, in effect, prohibited the use of federal funds for research involving human embryos, the clinical practice of in vitro fertilization and embryo transfer (IVFET) was in danger of outstripping its scientific foundations. Moreover, the United States had not systematically reviewed the current state of knowledge and practice of IVFET. In 1986, the IOM's Board on Health Sciences Policy convened a meeting of experts in the fields of human and animal research, clinical practice, law, ethics, and public policy to consider some of the issues raised at the earlier meeting. This group was asked to advise the IOM on whether it should pursue a study in any of the areas in which difficult issues had been raised by the practice of medically assisted conception—namely, professional, social, legal, and ethical issues, in addition to questions of science policy. The group identified several topics to which the IOM could make a substantial contribution. An important result of the meeting of this group was to highlight the scientific contribution of research relating to animal uses of medically assisted conception, and the lack of communication between those working to further human clinical IVFET and those working in the animal sciences. This study of the basic science foundations of medically assisted conception results from the recommendations made by this group of experts.

Scope of the Study

A committee was appointed whose membership included individuals with expertise in the clinical practice of in vitro fertilization, research in animal and human reproductive and developmental biology, and physiology. The committee was asked to examine the basic science foundations of medically assisted conception, and develop an agenda for basic research in reproductive and developmental biology that would contribute to advances in the clinical and agricultural practice of IVFET. This research would also be applicable to other concerns in human and animal reproduction such as male and female infertility and contraception. Because of limitations on research using human embryos, the committee was also asked to identify animal systems that provide useful models for specific aspects of medically assisted conception. In addition, the committee was asked to address ways of diminishing barriers to progress in reproductive research and consider ways of fostering communication between investigators oriented to human clinical questions and those interested in studies of animals.

Although the charge to the committee includes consideration of ways of lowering barriers to progress in research, some of which are caused by legal and ethical concerns, the committee was not constituted to resolve or make recommendations on such issues. Rather, it was to confine its deliberations to consideration of ways that scientists might contribute to the furtherance of the debate on legal and ethical issues related to medically assisted conception.

The committee held two meetings. At the first, a plan was developed for a workshop that would bring together clinicians and investigators to review the status of assisted conception in animals and humans, and suggest productive areas for future research. The committee carefully reviewed current research directions in reproductive science, and selected research areas and investigators to present them at the workshop. The committee was aware that it was neither feasible nor desirable to provide a comprehensive view of reproductive research. Rather, the committee selected areas of research that show particular promise or describe particularly problematical areas in relation to IVFET. This workshop was also conceived as a major effort in bringing together representatives from the human and animal research worlds to enable them to exchange ideas, enhance their understanding of ways in which they can contribute to each other's work, and together participate in a joint activity that might establish continuing ties.

The committee's second meeting, after the workshop, was spent in developing this report and its recommendations.

SUMMARY

This study was prompted by a concern that, because of a policy that in effect prohibits the use of federal funds for research involving human embryos, the clinical practice of in vitro fertilization and embryo transfer (IVFET) was pushing to the limit of its scientific foundations. In addition, there was a perception that animal science had made substantial progress in the development of IVFET but, because of a lack of communication among those working to further human clinical IVFET and those working in the animal science area, the knowledge developed in one sector was not being conveyed to the other. As a result progress in each sector was seen as being slowed.

An Institute of Medicine committee was appointed to examine the basic science foundations of medically assisted conception, to develop an agenda for basic science research that would contribute to advances in the clinical and agricultural practice of IVFET, to suggest animal systems that provide useful models for specific research areas, to identify ways of diminishing barriers to progress, and to recommend ways of bringing together the veterinary and human reproductive research workers.

The centerpiece of the study was a workshop at which clinicians and investigators reviewed the status of assisted conception and the related basic research in humans and animals, and suggested productive areas for future research. This workshop brought together representatives from the human and animal research worlds to enable them to exchange ideas, enhance their understanding of ways in which they can contribute to each other's work, and participate in a joint activity that could establish continuing ties.

Social Concerns That Can be Addressed By IVFET

Research directed at pushing forward the frontiers of medically assisted conception has the potential to provide benefits beyond the limited number of couples whose infertility may be solved by IVFET. There are expectations that such research would enable practitioners to identify genetic defects in embryos without damaging them, and to determine the sex of embryos without damaging them, so that those with sex-linked genetic diseases can be identified at a very early stage. Major areas to which advances in basic reproductive biology would make large contributions include:

o Infertility. The magnitude of the problem of human infertility is reflected by the number of women with "impaired fecundity" -- 4.4 million or 8.2 percent of women of childbearing age in 1982 (National Center for Health Statistics, 1985). By one estimate, more than half of the 4.2 million women who have been surgically sterilized for non-contraceptive reasons, and half of the 4.4 million subfecund women would like to become pregnant. Furthermore, one million women between the ages of 15 and 44 who were or had been married reported at least one medical visit for infertility in 1982 (Fuchs and Perrault, 1986). Although data cannot describe the emotional toll of infertility, the communications media are beginning to portray some of the distress. The lengths to which couples will go in attempting to conceive and the formation of nationwide support groups for childless people are indicators of the pain of childlessness. Although IVFET is a solution for only limited numbers of infertile couples, research that advances the practice of IVFET also has the potential of advancing other forms of infertility treatment.

Numerous infertility treatments exist, including education to give couples sufficient knowledge of reproductive biology, surgical repair, artificial insemination, and the use of drugs to induce ovulation. Two major new technologies are IVFET and gamete intrafallopian transfer (GIFT). These are complicated technologies. The simple description that follows will facilitate understanding of the research agenda developed by the committee.

For IVFET, eggs are removed from the woman either during a natural cycle or after growth and maturation of oocytes has been stimulated by such drugs as human menopausal gonadotrophin. This latter method has the advantage of allowing more than one oocyte to be harvested. The egg is placed in a petri dish together with washed sperm that have been treated to ensure capacitation. If fertilization is achieved, the process of cleavage starts, and somewhere between the 2- and 16-cell stage, the embryo is transferred to the uterus. Pregnancy is established when the developing embryo implants itself into the wall of the uterus. More than one embryo may be transferred to the uterus.

For GIFT, growth and retrieval of eggs are performed in a manner similar to that used for IVFET. Semen is collected and placed in a catheter with the eggs, and they are then transferred to the fallopian tube. Fertilization takes place in vivo.

Sometimes donated sperm, eggs, or fertilized zygotes are used in assisted conception. For example, excess zygotes collected from a patient undergoing IVFET can be fertilized and implanted in a recipient uterus that has been synchronized with the donor's cycle.

o Contraception. Advances in the basic science that would improve the clinical practice of assisted conception would, at the same time, help in the search for better contraceptive technologies. Despite widespread use of such contraceptive methods as sponges, surgical sterilization, intrauterine devices, and birth control pills, there remain unresolved

problems of safety and efficacy. The search for improved forms of contraception is spurred not only by the desire of individuals to gain control over their reproductive lives, but also by the social cost of unwanted pregnancies and the problems caused by fast-growing populations in countries unable to provide an adequate standard of living for the present population.

o Agriculture. The application of assisted conception techniques has made rapid inroads in the domestic cattle industry. Artificial insemination is the norm, with 70 percent of dairy cows conceiving in this manner in 1985. In less than two decades, a multimillion dollar IVFET bovine industry has developed. About 25 percent of embryo transfers in 1984 were of frozen embryos. Artificial insemination has resulted in genetic improvements in dairy cattle that have doubled milk production per cow in thirty years (First, Crister, and Robl, 1985). Embryo transfer technology increases the rate of production of valuable cows. The adoption of new reproductive technologies to enhance the production of food-producing animals has the potential for lowering the cost of food and quickening the process by which animals genetically suited to difficult climates can be created.

o Biodiversity. Advances in reproductive technologies may sustain biodiversity by improving the reproductive efficiency of endangered species.

o Primates for Research. A limited number of primates are in captivity and available for research, and there is a possibility that the capture of more may be halted because of concerns for the future of the species. It will be increasingly important to maximize the reproductive capabilities of the primates available to science.

Barriers to Progress in In Vitro Fertilization and Embryo Transfer

Since the birth of Louise Brown in England in 1978, in vitro fertilization with embryo replacement has become an established method of treatment for certain types of infertility that do not respond to alternative methods of treatment. However, the chances of success in IVF are relatively low. In 1985, 14.1 percent of stimulation cycles resulted in clinical pregnancies. In 1986 this figure rose to 16.9 percent (Fertility and Sterility, 1988). But, the proportion of women entering treatment who attain a live birth is far lower -- only 8.9 percent of oocyte retrievals ended in live birth (Journal of the American Medical Association, 1988). Why are the odds for successful IVFET so low? The state of clinical practice of IVFET today is limited by lack of knowledge of some of the basic reproductive biology involved. This is caused, in part, by the many ethical questions raised by research in pursuit of the needed information. Difficulties in resolving these issues have caused the research to be deprived of federal funding.

Ethical and Social Issues

Some of the ethical or social issues that arise from the various forms of assisted conception are unrelated to decisions about the progress of research. Examples of these are questions about the protection of the rights of gamete donors, gestational parents, and social parents; the ownership of cryopreserved embryos; and the sale of gametes and embryos.

Some ethical questions have a direct bearing on research, and have had important consequences for the funding of research. The major questions focus on the status of the embryo at each stage of its development. How the embryo is regarded dictates what is morally acceptable to do to it.

At one end of this spectrum of thought is the position taken by the Roman Catholic Church. The Vatican's Instruction on Respect for Human Life states that "from the first moment of its existence until birth . . . no moral distinction is considered between zygotes, pre-embryos, embryos or fetuses" (cited in Fertility and Sterility, 1988b). Therefore, the absolute sanctity that is accorded to post-natal human life begins with the zygote. This concept makes it impossible to discard spare embryos or use them for research purposes. At the other end of the spectrum is the position that an embryo is merely biological material like any other group of living cells. The special value that might be attached to that material results from the expectations or aspirations of others (Office of Technology Assessment, 1988).

Midway between these two positions is one that holds that "the human embryo is entitled to profound respect; but this respect does not necessarily encompass the full legal and moral rights attributed to a person" (Department of Health, Education, and Welfare, 1979). Holding this position, the Ethics Advisory Board (established by the Department of Health, Education and Welfare (DHEW) in 1979) concluded that research was acceptable on embryos up to 14 days after fertilization.

The Federal Government and Embryo Research

Policy concerning research on human subjects has been slowly evolving since the 1960s. A study group was convened at NIH to develop guidelines, and a National Advisory Commission on Health Science and Society was proposed by Senator Walter Mondale in 1968 to examine developments in medical research. Following reports of the infamous Tuskegee syphilis experiments, DHEW recommended that Congress establish a permanent body to regulate federally funded research using human subjects.

In the 1970s the abortion issue became linked to the issue of embryo research. After the Roe v. Wade decision legalized abortion under certain conditions, concern developed that women would be pressured into having abortions and the sale of aborted embryos might occur. In 1974, the federal government created the National Commission for the Protection of

Human Subjects (P.L. 93-348). Until this commission reported to Congress, research on the living fetus was prohibited unless it was used to help that fetus survive. In 1975, DHEW issued regulations based on the findings of the commission. These regulations did not cover embryo research. The commission also recommended establishing an Ethics Advisory Board (EAB) to review requests for research on embryos and in vitro fertilization. However, in 1980, the Secretary of DHHS allowed the EAB charter to expire. Thus, no research could be approved, and federal funding of embryo research was de facto prohibited. As a result, embryo research has relied on private funding from patient care revenues, pharmaceutical companies, and university budgets.

Since 1985, efforts have been made that, if successful, might establish some rules under which embryo research could proceed. However, the chances of such an outcome in the near future appears to be slim. A Congressional Biomedical Ethics Board, composed of six senators and six representatives, has been appointed. This group established a Biomedical Ethics Advisory Committee. In 1988 the Department of Health and Human Services announced its intention to revive the Ethics Advisory Board and publish a proposed charter. A final charter is awaited.

Domestic and Foreign Decisions Concerning Embryo Research

The two professional societies in the United States that represent the physicians most involved in human IVFET have considered ethical questions about the practice of IVFET and embryo research. In 1986 the Committee on Ethics of the American College of Obstetricians and Gynecologists (ACOG) (1986) issued a statement that acknowledged the ethical issues posed by the creation of embryos outside a uterus, the dilemma of surplus embryos, and the acceptability of research using early human embryos. The ACOG committee recommended that human embryos should be used only if nonhuman embryos could not provide the needed knowledge. It also recommended banning research on embryos that had reached the age of 14 days. The American Fertility Society (AFS) also issued a report in 1986, approving experiments on embryos up to 14 days (Fertility and Sterility, 1986). A year later, after consideration of the Vatican's Instruction for Human Life in its Origin and on the Dignity of Procreation, issued by the Congregation for the Doctrine of Faith, the AFS issued another report. This report stated that progressive degrees of respect are due with progressive development of embryos, and that experimentation can be justified and is necessary if the human condition is to be improved (Fertility and Sterility, 1988b).

The government of the United States, since 1979, has not followed the lead of nations that have systematically examined issues related to human IVFET. Since 1979, at least 85 statements have been prepared by

committees representing at least 25 countries. Four Australian committees found research on early (preimplantation) embryos to be ethically unacceptable. Eleven committees approved at least some kinds of early embryo research. Six of these accept such research only on embryos left over from clinical activities. Five committee statements (including the 1979 DHEW Ethics Advisory Board) would allow the creation of embryos for research purposes. Although the majority of committees favor limiting research on embryos to up to fourteen days, one committee allowed it only to seven days, and one only through the first cleavage (Walters, 1987).

In sum, numerous groups have wrestled with questions related to the ethical problems of embryo or fetal research. Some have based their conclusions on religious tenets, some on an interpretation of scientific knowledge, some on a mixture of both.

Other Barriers to Scientific Progress

Other factors besides ethical considerations are slowing the progress of research in areas of reproductive biology related to assisted conception.

Deficiencies in the Science Base Papers presented at the committee's workshop and the research agenda developed from that workshop indicate deficiencies in the scientific underpinnings of reproductive biology, and identify many areas in which further research efforts would make major contributions to improvements in medically assisted conception. The deficiencies are on three levels: basic science knowledge; knowledge needed to improve the technologies being used for medically assisted conception, such as cryobiology; and knowledge needed to improve both human and animal clinical practice of IVFET.

Research Funding Approximately $155 million annually is spend on research in reproductive processes. Federal agencies are the principal support for research. In 1986 they provided $109 million for research in reproductive processes (National Institutes of Health, undated). Federal funds for research relating to agricultural animal reproduction are available from the U.S. Department of Agriculture.

Funding for basic research in reproductive biology is undoubtedly constrained by the lack of vocal and focused advocacy groups. Lacking such a voice a major increase in federal support is unlikely.

Lack of Communication Among Researchers Discussion with the scientists and clinicians at the committee's workshop revealed an underuse of available mechanisms for communications among the individuals involved with various aspects of research in reproductive biology — basic, clinical, animal sciences, etc. Also revealed was a desire for greater

communication to allow cross-fertilization of ideas and development of ongoing relationships among investigators pursuing similar approaches to problems.

Sources of Research Material for Experiments with Humans and Other Primates The committee's workshop provided many excellent examples of instances in which information about reproductive physiology derived from animal models has been useful in understanding human physiology. However, animal models cannot suffice for investigating all central questions; progress is some areas requires the use of human tissue. An example of this is investigation of reasons for developmental failure of human embryos.

Although specific primates are good models for some aspects of human reproductive physiology, there are only a limited number of monkeys of desirable species in captivity and many of them are presently being used for AIDS research.

Research Agenda

A workshop was held August 21-23, 1988 at the Arnold and Mabel Beckman Center in Irvine, California. Overviews of the experience gained by the clinical practice of IVFET and of the practice of assisted conception in food-producing animals directed attention to unanswered questions that will require basic science research for their resolution. These questions reflect important gaps in our knowledge of the biology of all the stages of reproduction from the development of male and female gametes to the process of embryo implantation. The topics listed below are areas in which further research was recommended by workshop participants and committee members. Work in these areas is expected to increase understanding of the biology of reproduction with the hope that increased knowledge will eventually lead to improvements in the practice of IVFET in humans and other animals, or to advances in contraception. Research areas are listed here in summary form and apply both to lower animals and human beings unless specifically noted. The complete summary of the workshop is contained in Chapter Two of the full report.

Basic Science

Male Gametogenesis

- Definition of the role of cell adhesion molecules in interactions between Sertoli cells and developing sperm cells.

- Understanding the function of differential protein synthesis in different stages of sperm development.

o Determination of the role of paracrine factors including fibroblast growth factor, somatomedin C, epidermal growth factor, and interleukin-1 on the development and differentiation of male gametes.

o Structural analysis to identify normal and abnormal sperm and the development of markers for abnormal sperm.

o Understanding of the biochemistry of sperm capacitation.

Female Gametogenesis

o Analysis of the effects of superovulation or hormonal stimulation protocols on oocyte development and maturation. This work should also examine differences between species.

o Development of ways to mature oocytes in vitro.

o Investigation of ways to naturally stimulate oocyte and follicular development.

o Investigation into the biochemistry of meiotic arrest and the factors, such as cyclic AMP, purines, calcium, and maturation-promoting factor, that may mediate this process.

o Development of ways to produce or synthesize hormones from non-human primates to be used in ovarian stimulation.

o Definition of the role of ovarian estrogen in oocyte maturation and ovulation and the interactions between estrogen and paracrine factors including fibroblast and epidermal growth factors, insulin-like growth factor, transforming growth factor, and inhibin.

o Definition of the point at which oocytes become sensitive to factors that influence their development.

o Elucidation of the processes that underlie oocyte depletion, to determine why oocytes are lost at a predictable rate throughout life.

o Investigation into ways to augment natural hormone release.

o Investigation into the biochemistry of protein synthesis and modification in ovarian cells.

Fertilization

o Investigation into the biophysics of cell membranes as it relates to sperm and egg interactions at fertilization.

o Continued investigation to identify the genes for zona proteins in various species, especially humans.

o Further delineation of the role of zona proteins, especially ZP2 and ZP3, in sperm binding.

o Understanding of the biochemistry of the modification of zona proteins in preventing polyspermy.

o Elucidation of the molecular determinants of antibody formation to zona proteins and their possible role in contraceptive strategies.

o Definition of the biochemical mechanisms of the cortical reaction in the egg and the effects of this reaction on zona proteins.

o Determination of the physiological significance of germinal vesicle breakdown and the biochemistry of sperm chromatin decondensation.

o Definition of the molecular events associated with formation of the male and female pronuclei.

o Definition of the molecular events during zygote formation and the first cleavage.

Preimplantation Development

o Definition of the metabolic requirements of early embryos at different stages.

o Determination of embryonic gene expression.

o Assessing the potential of individual embryonic cells and defining the point at which embryonic cells are committed to particular fates.

o Identification of substances produced by early embryos that signal changes in the uterus prior to implantation.

o Improvements in embryo multiplication and embryo splitting, especially for food producing animals.

Implantation

o Definition of the biochemical events that make the uterus permissive to implantation.

o Definition of the factors released by embryos that cause endometrial changes at the site of implantation.

o Identification of the role of embryo-released factors in suppressing the immune responses of the mother.

o Isolation and analysis of substances released by endometrial cells and their effects on embryos.

o Continued work with in vitro models of human implantation to study the biochemistry and mechanisms of embryo-endometrial interactions, especially the role of extracellular matrix proteins and the biochemistry of trophoblast invasion of the endometrium.

Technological Advances

o Improved cryopreservation techniques, including freezing and thawing protocols for eggs and embryos.

o Improved resolution of ultrasonography for localization and noninvasive harvest of oocytes, eggs, embryos—would have particular usefulness for non-human primates and food producing animals.

o Development of new culture media and methods for in vitro maturation of oocytes.

o Development of safe methods of biopsy of early embryos for preimplantation diagnosis of genetic diseases.

Clinical Research Opportunities

The following areas are those in which a coordinated data collection effort across IVFET clinical centers would help improve the quality and success rates of IVFET nationally and, possibly, internationally.

o Evaluation of hormonal stimulation protocols in terms of number of oocytes harvested, quality of oocytes, and rate of fertilization success.

o Documentation on the incidence of abnormal implantation rates in IVFET practice and correlation of incidence with particular stimulation protocol used.

o Collection of information regarding the incidence of abnormal zygotes and embryos, failed fertilization, and developmental arrest of embryos.

o Analysis of data pertaining to synchronization of embryonic stage with endometrial stage and development of methods to improve synchronization.

o Collection of information on sharing of spare eggs and arrested embryos for research purposes.

Conclusions and Recommendations

Developing Research Policy

Lack of a mechanism for dealing with ethical disagreement over the use of embryos in research has slowed the rate of progress in research by, in effect, placing a moratorium on the use of federal funds for eight years. This has had undesirable results: the human clinical practice of IVFET is less effective than it might have been had research progressed at a faster pace; other socially desirable goals such as improved contraception, better techniques to preserve endangered species, and more cost-effective methods of producing food have developed at a pace slower than optimal.

The recent appointment of the Biomedical Advisory Committee by the Biomedical Ethics Board, to report to Congress by November 1990 on embryo research issues, could be a step toward a solution. The committee applauds the intention to revive the Ethics Advisory Board of the Department of Health and Human Services to rule on the ethical acceptability of research relating to human embryos, which is required before federal funding of such a research grant can be considered. However, until these groups become fully functional and show evidence of progress, their impact must remain in question.

If these groups can assume leadership roles in resolving the difficult issues of reproductive research, and develop guidelines for research that are based on information provided by science, and on concepts that are ethically acceptable to society, research in reproduction will be able to move forward. But if these groups become paralyzed because of political considerations or an inability to develop a framework for the resolution of differences of opinion, another organization should take over the role. The committee recommends that, if the groups currently being formed fail to come to conclusions concerning embryo and fetal research, a non-governmental organization should be established to develop guidelines for embryo and fetal research that are based on the most advanced knowledge that science can muster, and with serious consideration of the expressed values of society. The group should be composed of individuals with expertise in the relevant scientific disciplines, representatives of the lay public, and experts in the legal, ethical, and social issues. The organization should be housed in an institution that would allow it to conduct its deliberations free from any undue pressures from political and special interest groups. A model for such activities can be found in the Voluntary Licensing Authority of Great Britain.

Basic Science Foundations

The number and range of topics included in the research agenda indicate the exciting potential for productive scientific exploration. The committee believes that fundamental research to enhance the basic science foundations of reproductive biology should be stimulated and supported. This includes studies of human beings, laboratory animal models, and food-producing animals. The knowledge that would be generated is fundamental to an understanding of how to reverse infertility, to new approaches in the area of contraception, and to increasing the world's food supply.

It is important that male as well as female reproductive biology be studied and that investigators make use of some opportunities that are largely ignored today. These opportunities occur as a result of clinical activities as well as research activities.

The committee recommends that a vigorous program of funding for a basic science agenda in reproductive biology be maintained in a coordinated fashion by an appropriate office in the National Institutes of Health.

Applied Research

Research needs to be stimulated concerning technologies used in medically assisted conception in food producing animals and in human beings. Lack of support in these areas is leading to inadequate scientific underpinnings for safe and effective clinical practice. An example of a technique used, but not carefully evaluated for possible detrimental effects, is freezing eggs or zygotes. Further experiments should be conducted to assess the effects on safety and viability of this technology which is standard practice in many IVFET clinics. Other areas of technology that need to be developed include less invasive ways to retrieve oocytes, ways to mature oocytes in vitro, and ways to assess the quality of spermatozoa or eggs to be used for fertilization.

The committee recommends that applied research into technologies used in medically-assisted conception be undertaken to provide a firm foundation for the safe and effective practice of in vitro fertilization and embryo transfer. Such applied research should be coordinated by the appropriate office at the National Institutes of Health.

Clinical Research Opportunities

Perhaps the most obvious missed opportunity is the failure to learn from the diverse experiences of the approximately 160 clinical programs that provide IVFET. In addition to scientific questions, there are

questions to do with the organization of clinics and the outcomes of procedures, the answers to which would enable practitioners to work more effectively, and policy makers to make decisions on the basis of the best available information.

Clinical IVFET centers can provide unique opportunities for important studies. For example, human oocytes that fail to fertilize in vitro could be used to investigate the phenomenon of failed fertilization. Research that seeks to understand the basis of reproductive failure, and its relationship to hyperstimulation should be encouraged. Coordinated studies utilizing the mass of material and experience from IVFET centers could begin to answer these and other questions.

The committee applauds the activities of the various professional societies that have issued non-binding statements about the quality of practice of IVF. The American Fertility Society has also provided a voluntary registry for centers.

The committee believes that a mechanism is needed to monitor and evaluate clinical practice so that existing information that is relatively easy and inexpensive to collect can be disseminated. This would enable clinicians to build on the broadly based experience of the community and help ensure that patients have access to information about developments in IVFET and to well-informed physicians. The committee recommends that a mechanism for multi-centered data collection be established to monitor and evaluate human and veterinary practices of medically assisted conception in order to improve the safety, effectiveness, and quality of clinical practice. A cooperative group composed of the relevant professional societies should be established to fund and initiate data collection under the direction of an inter-society council composed of representatives of each participating organization.

Improving Communications

The IOM Workshop on Medically Assisted Conception brought together researchers from basic science, clinical practice, and animal sciences. The resulting interaction was viewed as extremely helpful by investigators from each of these communities. The committee recommends that a mechanism (or multiple mechanisms) be found for fostering continued communication between researchers in diverse areas of reproductive science. The initiative should come both from NIH research administrators who could sponsor additional workshop opportunities, as well as from the professional societies, either individually or through an intersociety council.

REFERENCES

American College of Obstetricians and Gynecologists. 1986. Ethical Issues in Human In Vitro Fertilization and Embryo Placement. Committee on Ethics ACOG Committee Opinion Number 47. Washington, D.C.

Department of Health, Education and Welfare. 1979. HEW Support of Research Involving Human In Vitro Fertilization and Embryo Transfer. Report and Conclusions. May 4. Washington, D.C., U.S. Government Printing Office.

Fertility and Sterility. 1988a. Invitro Fertilization/Embryo Transfer in the United States: 1985 and 1986 Results from the National IVF/ET Registry. 49(2):212-215.

Fertility and Sterility. 1988b. Ethical considerations of the new reproductive technologies. By the Ethics Committee (1986-7) of the American Fertility Society in light of Instruction on the Respect for Human Life in its Origin and on the Dignity of Procreation issued by the Congregation for the Doctrine of the Faith. Feb;49(2 Suppl 1):1-7S

Fertility and Sterility. 1986. Ethical Considerations of the New Reproductive Technologies. The Ethics Committee of The American Fertility Society. Sep;46(3 Suppl 1):1S-94S.

First, N.L., Critser, E.S., and Robl, J.M. 1985. Boving Embryo: Development, Cloning, Sexing and Transfer of Genes for Immunology of Reproduction and Contraception, P. Talwas, ed. Elsevier, 1985.

Fuchs, V.R. and Perreault, L. 1986. Expenditures for Reproduction-Related Health Care. Journal of the American Medical Association. Vol. 225, No.1. January 3:76-81.

Journal of the American Medical Association. 1988. IVF Registry Notes More Centers, More Births, slightly Improved Odds. 259(13):1920-1921.

National Institutes of Health. Undated. Inventory and Analysis of Federal Population Research. Fiscal Year 1986. U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health.

Office of Technology Assessment. 1988. Infertility: Medical and Social Choices. OTA BA BP 48. August. Washington, D.C.

Walters, LeRoy. 1987. Ethics and New Reproductive Technologies: An International Review of Committee Statements. Hastings Center Report. June:3-9.

Glossary

Allele.	Individual variation in a particular gene.
Alloimmune thrombocytopenia.	A fetal disorder in which the mother's immune system makes antibodies against the blood platelets of the fetus.
Amniocentesis.	Prenatal testing method in which fluid from the sac surrounding the fetus (amniotic sac) is analyzed for sex determination and for genetic and biochemical properties.
Aneuploidy.	Presence of abnormal numbers of chromosomes.
Antibody.	B-lymphocyte receptor dispatched by the cell into body fluids that recognizes and binds to cell-surface proteins on foreign cells.
Antigen.	Cell- surface protein on an infectious agent, cancer cell, or cell of another organism that marks it as foreign.
Axon.	Long, slender extensions of neurons down which neuronal electrical impulses travel, culminating in the release of neurotransmitter across the synapse to a target cell.
B lymphocytes, or B cells.	Specialized cells of the immune system, products of hematopoiesis, that release antibodies.
Blastomere.	One of the cells resulting from division of the fertilized ovum.
Cerebral cortex.	Multilayered sheet of neurons that covers the cerebral hemispheres of the brain and coordinates and controls many functions including sensation, movement, and cognition.
Chorionic villi sampling (CVS).	Prenatal testing method in which fetal cells are aspirated from early placenta (villi) and analyzed for genetic abnormalities.
Cryopreservation.	Preservation of biological tissue by freezing.

Cystic fibrosis. A genetic disease that affects a number of organ systems, particularly the respiratory, gastrointestinal, and reproductive systems, and leads to early death.

Cytochrome P450. Specific type of enzyme involved in the metabolism of foods, drugs, and environmental chemicals.

Diploid. Cell containing double pairs of chromosomes in the nucleus. In general, all cells except the gametes (ovum and sperm) are diploid.

L-Dopa. A precursor of dopamine used as a treatment for Parkinson's disease.

Dopamine. A neurotransmitter. Malfunction of dopamine cells in specific brain regions gives rise to Parkinson's disease.

Embryo. Unborn conceptus in early weeks of pregnancy, usually up to the ninth week.

Enzymes. Proteins that induce chemical reactions in other molecules.

Fetus. Unborn conceptus in later weeks of pregnancy, usually after ninth week.

Fluorescence in situ hybridization (FISH). Technique for finding specific regions of DNA in a large mix (or in cells) by using chemically labeled, single strands of complementary nucleotide sequences to serve as probes that are detected by fluorescent compounds.

Fragile X syndrome. The most common form of inherited mental retardation. (See X-linked diseases.)

Gamete. Reproductive germ cell; a sperm is the male gamete, an oocyte the female gamete.

Gamma globulin. A preparation of blood proteins, containing antibodies, that can be administered to boost immune responses.

Genome. The entire genetic repertoire of a cell.

Graft-versus-host disease. Rejection of the recipient's body cells or tissues by the immune responses of the donor, a particular problem in transplantation of hematopoietic tissue such as bone marrow.

Haploid. Cell with a single set of chromosomes, as in sperm and oocytes.

HDL. High-density lipoprotein. Lipoproteins are compounds of fats and proteins. In adults, HDL participates in cholesterol metabolism: in the uterus, HDL seems to regulate placental hormones involved in fetal growth.

Hematopoiesis. Process though which precursor cells give rise to all the varieties of blood cells.

Hematopoietic stem cells. Precursor cells that give rise to different blood cells and to other stem cells.

Hippocampus. Area of the brain involved in memory formation.

HIV. Human immunodeficiency virus, the causative agent of acquired immunodeficiency syndrome (AIDS).

Hormones. Blood-borne messenger molecules of the endocrine system that regulate cellular activity in distant organs.

Huntington's disease. An inherited, progressive neurological disorder that begins in early middle age and leads to eventual loss of motor and mental function.

Hurler's disease. An inherited mucopolysaccharide storage disease, caused by an inborn enzyme deficiency, that causes abnormalities in a variety of organ systems and leads to early death.

Hydrops. Severe fetal edema.

Immortalized cell. Cell that divides indefinitely because of the introduction of viral, cancer-inducing oncogenes. The cancer-inducing properties are removed.

Imprinting. Preferential or exclusive expression of a gene from a paternally or maternally derived chromosome.

In vitro. In the test tube or culture dish.

In vivo. In a living organism.

Islet cells. Also called beta cells. Specialized cells clustered in the pancreas that are responsible for insulin production.

Lesch-Nyhan disease. A disease inherited only by males (X-linked) associated with lack of function of a specific enzyme and marked by neurological dysfunction, mental retardation, and self-mutilation.

Lymphocyte. White blood cell.

Meiosis. Division of a precursor, diploid, germ cell to produce haploid gametes.

Mitosis. Division of cells other than germ cells to produce daughter cells.

Monoclonal antibody. Antibody engineered for exceptional specificity for targeting antigens.

MPTP. A compound that induces severe symptoms of Parkinson's disease. MPTP has been used to create animal models of parkinsonism for research.

Myotonic dystrophy. Inherited muscle-wasting disease.

Neural tube. Embryonic structure from which the brain develops, it contains the precursor cells that give rise to different classes of neurons.

Neuron. Nerve cell.

Neurotransmitter. Chemical messenger of communication between neurons and other neurons, or neurons and muscle cells.

Nucleotide.	Constituent molecule, or subunit, of DNA and RNA. The order of specific nucleotide sequences forms the genetic code for protein production.
Oncogene.	Gene that induces cancerous transformation in a cell.
Oocyte.	Female gamete.
Percutaneous umbilical blood sampling (PUBS).	Method of entering the bloodstream of the fetus through the umbilical vein, also called cordocentesis.
Peritoneal cavity.	Space between the lining of the abdominal cavity and the abdominal organs.
Polymerase chain reaction (PCR).	Technique whereby millions of copies of a DNA fragment can be generated from a single copy.
Primer extension preamplification (PEP).	Variation of PCR that allows amplification of whole chromosomes and genomes.
Receptors.	Cellular molecules that recognize and bind other external molecules including neurotransmitters, hormones, and antigens.
Rh disease.	Condition brought on by immune system incompatibilities between mother and fetus, based on the presence or absence of a specific protein on blood cells (Rh factor), in which the mother's immune system attacks fetal blood cells.
SCID-hu mouse.	Mouse strain with severe combined immunodeficiency, into which human cells have been implanted.
Sex-linked diseases.	See X-linked diseases.
Spermatocyte.	Precursor stage in development of sperm.
Spinobulbar muscular atrophy.	Inherited muscle-wasting disease, X-linked.
Stem cells.	Multipotent precursor cells from which differentiated cells arise.
Striatum.	Target area of the brain for dopamine-releasing neurons of the substantia nigra.
Substantia nigra.	Area of the brain containing dopamine-releasing cells that project to another brain area, the striatum. This connection is important in motor control and is damaged in the brains of patients with parkinsonism.
T lymphocytes, or T cells.	Cells involved in immune system response to antigens and graft rejection.
Tay-Sachs disease.	An inherited disorder, marked by inability to break down a fatty substance in the brain, resulting in mental and physical degeneration.
Tocolytics.	Drugs that prevent preterm labor.

Transcription. Process by which RNA is synthesized on a DNA template.

Transformed cell. Cancer cell.

Transgenic animals. Animals, often mice, to whose genome new, functional DNA fragments have been added by transection of fertilized eggs.

Ultrasound. Prenatal diagnostic tool that uses sound waves to create moving images of the fetus.

X and Y chromosomes. Chromosomes that differ in the two sexes. Human females have two X chromosomes (XX) and males have XY.

X-linked diseases. Diseases caused by a mutation in a gene on the X chromosome. Females are usually protected by the presence of a normal X, but may transmit the abnormal X to their offspring. In contrast, male children will be affected by the disease if their (single) X chromosome carries the mutation. An example of this type of inherited disorder is hemophilia.

Xenograft. Graft of tissue derived from one species implanted into different species.

Zona pellucida. Oocyte outer coat.