found that mouse embryos could be frozen at temperatures as low as −269°C for as long as 8 days—and survive. Some investigators think that the ability to store frozen embryos might eliminate or reduce the need for maintaining colonies of animals not in use. Since it is possible to transplant mouse embryos into foster-mothers in which they will develop into newborn mice, the frozen embryos could be thawed when needed and grown to term in foster mothers.

Whittingham, Mazur, and Leibo used two criteria of survival for their frozen embryos—development to the late blastocyst in culture and development to living mice in the uteri of foster-mothers. Up to 70 percent of the embryos frozen in the one-, two-, or eight-cell or blastocyst stage fulfilled the first criterion. Almost 1000 of the thawed embryos were subsequently transplanted into foster-mothers. Sixty-five percent of the animals became pregnant. Forty-three percent of the transplanted embryos developed into living fetuses (killed after 18 days of gestation) or newborn, apparently normal mice. The fetuses or pups carried genetic markers—dark eyes and coats—not possessed by their albino foster-mothers. Mazur says that they have now frozen mouse embryos for periods up to 1 year with survival of 80 percent of the thawed embryos.

According to Mazur and Leibo, who are specialists in cryobiology rather than embryology, formation of ice

Speaking of Science

In vitro Fertilization of Human Eggs:

Is in vitro fertilization of human eggs a valid means for alleviating the suffering of infertile couples? Or is it a dehumanizing and illicit intrusion of technology into one of the most profound aspects of human life? These are among the bioethical questions raised by current research in human embryology.

One rationale for much of this research is that it may permit a married couple, infertile because the woman's oviducts are defective, to have their own biological child. The woman could bear the child if an embryo, obtained from in vitro fertilization of her eggs by her husband's sperm, could be implanted in her uterus to develop.

The stumbling block for many who object to this procedure is the unknown element of risk to the embryo—destined to become a human being if pregnancy results. Paul Ramsey of the Department of Religion, Princeton University, Princeton, New Jersey, thinks that in vitro fertilization constitutes unethical medical experimentation on potential human beings. He argues that it is impossible to exclude the possibility that manipulations performed on the embryo outside the womb will damage it and result in production of a deformed or handicapped human. Experiments on animals might prove that the techniques of in vitro fertilization, embryo culture, and implantation were safe for animals. But only human experimentation could prove them safe for the human—and, according to Ramsey, that experimentation is unethical because of the risk involved.

Ramsey thinks that detection of embryo damage is not the answer to this dilemma. Before implantation, some abnormalities may escape detection or the detection methods themselves may be harmful; after implantation, amniocentesis (sampling of uterine fluids plus fetal cells) could identify some defects so that an abortion could be performed, but amniocentesis itself entails an added element of risk.

At present, no one knows whether in vitro manipulations of the embryo involve greater or less risk than does ordinary conception in vivo. But it is known that all conceptions are fraught with risk. Marc Lappé of the Institute of Society, Ethics, and the Life Sciences, Hastings-on-Hudson, New York, pointed out that up to 20 percent of human pregnancies, usually those in which the fetus is abnormal, may abort spontaneously. He also believes that research on the early mammalian embryo during the preimplantation stages indicates that it is quite resistant to damage. Most known teratogenic effects, for example, appear to occur after implantation during organ development.

Lappé thinks that in vitro fertilization can be a legitimate means of fulfilling a couple's desire for a child, if the risks prove to be acceptable and if the parents understand and consent to them. Nevertheless, he has suggested a moratorium on human research until animal experimentation, especially on subhuman primates, demonstrates that the risks of in vitro fertilization are at least no greater than those of normal conception.

Leon Kass, a scientist who frequently writes on bioethical topics, questions whether, in an age when overpopulation is a major concern, there are compelling reasons to proceed rapidly with the development of new means of producing babies—especially since, in his view, these techniques would introduce elements of depersonalization and dehumanization into the act of human procreation. Kass points out that there is an alternate solution to this problem of infertility, an operation for reconstruction of the oviducts. He suggests that additional effort be expended to improve the operation, now frequently unsuccessful, because it can cure the defect that causes the infertility without raising complex ethical issues.

Risk to a potential human being is not the only basis for objections to in vitro fertilization. To some individuals, the termination of fetal life—by whom, at what stage of development, by what method—is one of the central bioethical problems. For embryos would undoubtedly be killed, even in cases where implantation is the goal. More than one egg is fertilized, but only one is implanted.

Although there are analogies to abortion, Kass suggests that the issues involved in the two cases differ in part. Embryos produced by in vitro fertilization are wanted, used, and then deliberately killed; embryos that are aborted are usually the result of an "accidental" conception. Furthermore, André Hellegers, Director of the Kennedy Institute for the Study of Human Reproduction and Bioethics, Washington, D.C., points out that the basis for allowing abortion is conflict between the woman

crystals within cells during freezing usually produces irreversible damage. In their experiments with Whittingham, however, they avoided ice crystal formation by employing extremely slow rates of cooling—0.3° to 2°C per minute—to allow enough time for freezable water to flow out of the cells. They also found that slow warming—4° to 25°C per minute—was necessary for survival. A third requirement was the addition of a protective agent that helps prevent freezing damage, possibly through action on cell membranes.

In addition to using frozen embryos to preserve mutant strains of laboratory animals, or even of endangered species, they may also be useful for transportation of animals. Whitten, for example, recently shipped frozen mouse embryos to Whittingham in England. Whitten said that 57 embryos were implanted into foster-mothers; 21 developed into fetuses (killed before parturition) and 11 into newborn mice. Live animals require special handling during shipment and may also be subjected to quarantine against infectious disease if transported across national borders. Frozen embryos are much less likely to carry diseases, such as hoof-and-mouth disease or rabies, than are adults.

Large animals are particularly difficult to transport. Cattle breeders have been importing from Europe "exotic" breeds of beef cattle for improving their herds. Embryos frozen for transport or storage, or even for preserva-

Bioethical and Legal Considerations

and fetus; the fetus invades her privacy or is a threat to her physical or mental health. For embryos in vitro there can be no such conflict.

Use of in vitro fertilization techniques need not be restricted to treating infertility. They could also be applied to eugenics. This could either be positive eugenics, breeding "superior" human beings by mating eggs and sperm from donors with the desired qualities; or negative eugenics, discarding embryos carrying genetic defects (if these can be detected). Most investigators agree that the consequences of such tampering with human evolution—itself poorly understood—are unknown. The same could be said of the possibility of using these techniques to allow parents to predetermine the sex of their children. All of these applications require destruction of some embryos.

Experiments in human genetics and development could be performed on embryos obtained from in vitro fertilization. For example, controlled mating experiments in the human are not possible. The scientific difficulties (such as long generation time and small number of progeny) are almost as insurmountable as the ethical ones. However, such mating could be achieved in the test tube and the resulting embryos studied in the initial stages of development for expression of a genetic trait such as synthesis of an enzyme. One scientist suggested that these embryos not be maintained beyond the blastocyst stage. Some do not believe that these early embryos—which are barely visible to the naked eye and have not yet differentiated—are human life worthy of protection; others do, however.

Underlying any discussion of bioethics and the legality or morality of scientific research is still another thorny problem—one about which members of the scientific community are highly sensitive. That is the question of regulation of research. Who decides what is permissible and how is the decision enforced? In the case of research funded by the federal government, enforcement, at least, is relatively simple: deny funds for research not meeting the required guidelines.

When Robert Marston was director of the National Institutes of Health (NIH), Bethesda, Maryland, he commissioned an NIH task force, unofficially known as the Human Investigations Committee, to study the ethical and legal issues of human experimentation and to recommend guidelines for NIH-supported research. The committee report, titled "Draft: Special policy statement on the protection of human subjects involved in research, development, and demonstration activities" was published in the *Federal Register* on 16 November.

A subcommittee of the task force probed (among other things) questions relating to the use of human fetal material, whether derived from fertilization in vivo or in vitro, in research. A scientist who served on the subcommittee outlined some of their considerations on in vitro fertilization. (He requested that his name not be used to prevent a deluge of what he characterized as hate mail.) The scientist pointed out that there were legal issues in addition to moral or ethical issues.

Unlike natural conception, in vitro fertilization requires participation by a third party—the investigator or physician who fertilizes and implants the egg in the recipient. If a defective child develops from that egg, would the third party be legally liable for damages? And would the agency that funded the research be liable? At present NIH prohibits investigators with NIH funding from requiring participants in their research projects to sign waivers that release the institution from liability for damages. Even if a participant did sign such a waiver, it would not prevent him from suing for damages.

Furthermore, the question of who is legally responsible for caring for the child must be considered. The simplest case is that in which the couple is married; the husband donates the sperm, and the wife donates the egg and carries their child. But the use of in vitro fertilization need not be restricated to the simplest case. The transplant recipient and egg donor may not be the same individual. A man other than the husband may donate the sperm. A number of variations are possible. And finally there is the possibility, however remote, that the embryo can be brought to term completely in vitro. Will the child be without a parent?

The issues raised about in vitro fertilization of human eggs are profound and the views on these issues disparate. Nevertheless, virtually everyone expressed the opinion that only a continuing dialogue between scientists and public would permit a thorough exploration of the issues and an ultimate consensus.—J.L.M.