Chapter 15

Frontiers of Reproductive Technology

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The field of reproductive endocrinology began to blossom in the 1930s with the description of reciprocal hormonal control of the ovaries and testes by the pituitary gland. Soon after, at the close of World War II, the modern era of biomedical research began. Inspired by Federal funding through the National Institutes of Health (NIH), rapid advances were recorded in the United States in broad areas of biology and medicine, including reproduction.

In the 1960s, the technique of radioimmunoassay enabled measurement of minute amounts of reproductive hormones and permitted characterization of both normal reproductive health and pathology. In the 1960s and 1970s, synergism between contraceptive research and fertility research led to identification and purification of numerous natural and synthetic reproductive hormones. This era also saw an increased research effort on mammalian eggs and early embryos that was facilitated by advances in nonhuman in vitro fertilization (IVF) and preimplantation development. Beginning in the 1970s, advances in microsurgery, fiber optics, and ultrasound–allowing for the first time routine visualization and retrieval of eggs—propelled novel reproductive technologies into clinical practice.

Today, advances in infertility prevention and treatment depend heavily on reproductive research in both humans and animals. Large domestic animal species such as cattle and pigs, because of their economic importance, play a particularly prominent role in reproductive research. Several methods of assisted reproduction are, in fact, better developed in animals than in humans. The frequency and success of embryo freezing, for example, have soared in the 1980s as the basis for a large commercial industry developed for improved breeding of cattle. The use of IVF, on the other hand, is most well developed in humans.

This chapter reviews the state of the art in selected areas of reproductive technology and, where possible, projects potential developments over the next decade. Humans have made great strides in understanding reproduction (for a historical perspective, see box 15-A), but a great deal of human reproduction remains a profound mystery.

The developments in reproductive technology discussed in this chapter have been accompanied by an emerging literature in the social sciences, Through the next decade, researchers are likely to report with increasing frequency on the positive and negative impacts of reproductive and allied technologies on the behavior of individuals and society as a whole.

IN VITRO FERTILIZATION

Ten years have passed since the birth of baby Louise Brown, the first human to be conceived by IVF. Some 5,000 IVF babies worldwide have been born since. If the prevailing rate of successful pregnancies (i.e., pregnancies resulting in live births) at today's most expert clinics–about 15 to 20 percent per case in which embryos are transferred—is ultimately achieved at other clinics, **some 6,000 successful IVF pregnancies per year may take place by the turn of the century. (One recent estimate assumes that 220 active IVF programs worldwide will undertake 30,000 IVF treatment cycles annually** by **then, Each treatment cy** - cle involves the attempted insemination of five oocytes, for a total of 150,000 oocytes per year. Twenty percent of the 30,000 cycles are assumed to result in successful term pregnancies, including some multiple births (22).) This means that IVF would account for fewer than 1 percent of the babies born each year in the United States.

Since the first report of pregnancy following IVF (12), the methods of human IVF and embryo culture have to a large extent been simplified and standardized, although there is no universally accepted set of techniques. One index of the research

Box 15-A.—A Look Back

From ancient times until the early 1600s, the view persisted that babies were conceived by mixing menstrual and seminal fluids. Aristotle maintained 2,300 years ago that development begins when the male provides the active form to the passive female substance. In 1651, William Harvey raised the fundamentally different claim that all animals are derived from an ovum, with the innate capacity to develop being influenced by the male semen. The discovery by Reinier de Graaf that follicles within the "female testes" were released prior to the appearance of embryos within the uterus gave credence to this belief, although the actual origin and nature of an egg remained obscure,

Despite the discovery of sperm by Anton van Leeuwenhoek in the 1670s, the view prevailed that all organisms arise from eggs that require the stimulating effects of male semen to develop. Most naturalists of the 1700s believed sperm to be parasites of the testis with no function in fertilization. With the introduction of cell theory in the rnid-1800s, sperm changed from being seen as parasitic organisms to cells necessary for fertilization. Only some 135 years ago—in the 1850s —was it resolved whether sperm played no role in fertilization, kept the seminal fluid in circulation, activated the egg by mere contact, or actually penetrated the egg. By then, fertilization was believed to involve the penetration and dissolution of the sperm within the egg, thus providing a basis for belief in inheritance,

The manufacture of microscope lenses free from chromatic and spherical aberration in the late 1800s and the refinement of fixing, staining, and sectioning techniques led to extensive investigations into cell and nuclear division. The discovery at that time of cell nuclei and chromosomes generated a further controversy, not resolved until the early 1900s, between those who argued that fertilization involved a complete fusion of male and female nuclear material and those who denied this. To the former, fertilization was a conservative blending process, while to the latter it led to variations in offspring. Recognition of meiosis and chromosomal recombination in the formation of sperm and egg cells resolved this debate.

SOURCE: Adapted from W F. Bynum, E.J Browne, and R. Porter, *Dictionary of the History of Science* (Princeton, NJ: Princeton University Press, 1981)

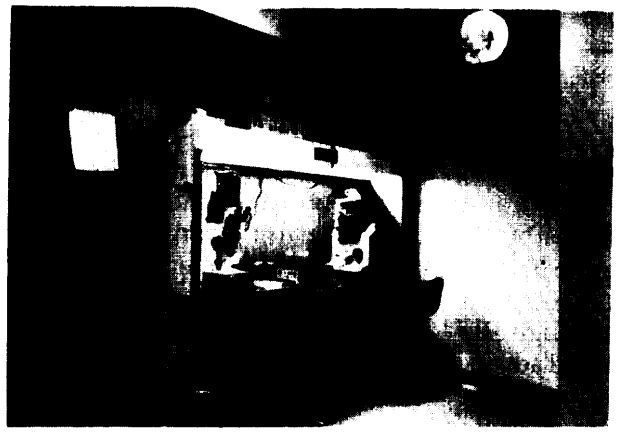


Photo credit: **Martin Quigley**

In vitro fertilization laboratory setup

activity in this area is the rapid growth of the biomedical literature on IVF and embryo transfer—from only 8 papers in 1980 to over 300 in 1986 (11). The laboratory techniques of IVF and embryo culture no longer represent the major weak point of noncoital reproduction, as repeatable techniques move from program to program (29). The principal determinant of the success of IVF today may well lie in the physiological state and developmental competence of sperm and eggs used in the procedure (38). An additional determinant is synchronization of the cultured, cleaving embryos with receptivity of the uterine endometrium, and transfer at the optimum time. Important scientific information concerning this uterine "window of receptivity" is now becoming available (33).

Indications for IVF have broadened and will likely continue to broaden beyond couples with untreatable, tubal-factor infertility to include couples with endometriosis or with cervical factor, male factor, or unexplained infertility-and essentially all infertile couples with whom conventional infertility therapy (see ch. 7) has been used unsuccessfully or for whom there is no other therapy available. As IVF is used in an increasing number of circumstances that do not positively preclude natural conception, conceptions that are actually independent of treatment can be expected in these programs (20,31). Such treatment-independent pregnancies will overstate the apparent success rate of IVF, although to what degree is uncertain.

The most comprehensive data on IVF success rates in the United States come from 41 clinics that treated 3,055 different patients in 1986 (28). The clinics performed 4,867 stimulation cycles, or 1.6 **cycles per patient; the outcomes of these cycles are listed in table 15-1. Some 59 percent (2,864) of the stimulation cycles were followed** by **embryo transfer. The median number of embryos transferred was three.**

Embryo transfer led to clinical pregnancies—i.e., a positive fetal heart documented by ultrasound—in 485 cases, or 17 percent of the time. The 485 clinical pregnancies led to 311 live births, an unreported fraction of which were multiple births. Thus, embryo transfer led to a live birth less than 11 percent of the time. Put another way, about 6 percent of the initial stimulation cycles resulted in a live birth (28).

At the most expert IVF programs, success rates exceed the average. One program that treated 650 different patients from 1983 through 1987 is profiled in table 15-2. The program performed 723 oocyte recovery procedures that led to 662 embryo transfer cycles. The average number of embryos transferred was four (35).

Embryo transfer led to confirmed pregnancies—i.e., either a gestational sac confirmed by ultrasound or the products of conception identified by pathologic specimen—in 208 cases, or 31 percent of the time. The 208 clinical pregnancies led to 103 live births, an unreported fraction of which were multiple births. Thus, embryo transfer led to a live birth about 15 percent of the time. Put another way, about 14 percent of the oocyte recovery procedures (and a smaller percentage of the initial stimulation cycles) resulted in a live birth (35).

Table 15=1.—In Vitro Fertilization in the United States, 1986°

Outcome	Number
Patients seen	3,055
Stimulation cycles	4,867
Embryo transfer cycles	2,864
Embryos transferred per cycle (median)	3
Clinical pregnancies	485
Ectopic pregnancies	22
Miscarriages or stillbirths	156
Live births[c]	311

aRetrospective data reported voluntarily by 41 U.S. clinics.
bPositive fetal heart documented by ultrasound.
cIncludes an unreported number of multiple births.

SOURCE: Medical Research International and the American Fertility Society Special Interest Group, "In Vitro Fertilization/Embryo Transfer in the United States: 1965 and 1966 Results From the National IVF/ET Registry," *Fertility and Sterility* 49:212-215, 1966,

Table 15-2.-Statistical Profile **of an Expert In Vitro Fertilization Program, 1983.87°**

Outcome	Number
Patients seen[b]	650
Stimulation cycles	(not reported)
Oocyte recovery procedures	723
Oocytes recovered	3,759
Oocytes recovered per procedure (mean)	5
Embryo transfer cycles	662
Embryos transferred per cycle (mean)	4
Confirmed pregnancies[c]	208
Live births	103

aData reported by the three facilities of National Fertility Institute, Inc. (Northern Nevada Fertility Center, Reno, NV; Pacific Fertility Center, San Francisco, CA; Pacific IVF Institute, Honolulu, HI).
bAll women were under age 40; primary diagnoses were tubal disease (65 Percent), maie factor infertility (19 percent), and unexplained infertility (16 percent).
cGestational sac confirmed by ultrasound or products Of conception confirmed by pathologic specimen.
dIncludes an unreported number of multiple births.

SOURCE: G. Sher, Director, Pacific Fertility Center, San Francisco, CA, personal communication, Jan. 25, 1966.

It maybe difficult for the most expert IVF programs to sustain their success rates as their good reputations attract patients with the most difficult cases of infertility (e.g., unexplained infertility). Similarly, an increase in the average age of patients would likely trim an IVF program's success rates. Information about a clinic's patient mix is crucial to interpreting its success rates (see ch. 9).

IVF programs can serve as a source of biological materials, providing an opportunity for experimentation that adheres to legal and ethical principles and that yields valuable information about human fertilization. Table 15-3 gives an overview of components of the IVF procedure whose examination, correlated retrospectively with the outcome of a given case of IVF, could yield relevant information for human fertility research (38).

An offshoot of research surrounding IVF is likely to be, paradoxicaIIy, progress in contraceptive development. Contraceptive methods that precisely block the interaction between sperm and egg–thus preventing fertilization without systemic ef - fects on the body as a whole—have long been sought by reproductive scientists (42). By bringing sperm and egg together under laboratory scrutiny, IVF provides this research opportunity (22).

Table 15-3.—Sources of IVF Byproducts and Some Possibie Uses in Research

Source of IVF byproduct	Research use
Sperm sampling	Examination of sperm (membranes, enzymes, bound antibodies, effects of illness, studies of normal development)
	Analyses of seminal plasma (chemical composition, proteins, sperm antibodies, function of seminal vesicles, prostate function, screening for prostate cancer)
Recovery of oocyte/cumulus cells	Analyses of follicular fluid (hormones, proteins, sperm antibodies)
Sperm washing and preincubation	Examination of preincubated sperm (membranes, enzymes, bound antibodies, character of motility, fertilizing capacity)
Change of media after in vitro insemination	Analyses of spent insemination media (secretions of cells that surround the oocyte: steroids, peptides, proteins, biological effects)
	Examination of cultured cumulus cells (ultrastructure, steroid-producing enzymes, other proteins)
	Examination of supernumerary sperm
Embryo transfer	Examination of eggs that failed to cleave (ultrastructure, chromosomes, zona antibodies, interaction of sperm with zona pellucida)
	Analyses of spent growth media (steroids, proteins of embryonic origin)
Monitoring of pregnancy	Examination of spontaneously aborted conceptuses (chromosomes)

SOURCE: AdaDtad from J. Tesarik. "From the Cellular to the Molecular Dimension: The Actual Challenge for Human Fertilization Research," Gamete *Research* 13:47-89, *1988*

Despite the widespread practice of IVF in the United States, there is today a de facto moratorium on Federal funding of any research involving in vitro fertilization of human sperm and egg, fertilized ova, or early embryos. Research that involves in vitro fertilization of human sperm and eggs is in effect excluded from Federal support because of the absence since 1980 of an Ethics Advisory Board within the Department of Health and Human Services (DHHS); such a board is required to advise the Secretary as to the ethical acceptability of such research (45 CFR 46.204(d)).

Within DHHS, research funding for human IVF is under the jurisdiction of the Center for Population Research of NIH. Although the Center's Reproductive Sciences Branch (with a fiscal year 1987 budget of $83.1 million) supports research on, for example, sperm maturation and follicular hormone production, it does not support research that involves fertilizing human eggs with human sperm unless that research is directly related to IVF carried out as a part of an infertile couple's routine clinical care.

The Center reports receipt of 10 grant applications related to human IVF between 1980 and 1987. One proposal, for example, involved injection of human sperm into human ova in an attempt to overcome infertility that was thought to be due to sperm antibodies in the female. Three others proposed to correlate sperm characteristics (e.g., motility) with successful pregnancies. Seven of the ten grant applications were approved on scientific merit, but did not rank high enough to be funded. In failing to achieve a fundable ranking, these applications were not candidates for the next step, ethical review. Thus, from 1980 to 1987, no grant application involving human IVF actually made it to the point where review by the Ethics Advisory Board was required (21).

This blanket statement is misleading, however. Investigators indicate that they ***do not submit***

proposals involving in vitro fertilization of human egg and sperm because of a widespread awareness of the de facto ban on such research. The dimensions of this chilling effect of the moratorium on IVF research are such that NIH estimates it might receive more than 100 grant applications related to human IVF if the Ethics Advisory Board were extant (21). At the moment, funding for research on human IVF comes from the private sector, including pharmaceutical companies and IVF patients, through their fees, and from university and medical center operating budgets.

GAMETE INTRAFALLOPIAN TRANSFER

Since the first description of gamete intrafallopian transfer (GIFT) in 1984 (6), numerous reports have appeared confirming its utility in treating some types of infertility. One report combining data from clinics in nine countries ranked the chief diagnoses among GIFT patients as unexplained infertility, endometriosis, and male factor infertility (see table 15-4). Overall, 29 percent of the stimulation cycles resulted in clinical pregnancies established by GIFT, making it biologically competitive with, if not superior to, IVF.

Table 15-4 indicates a broad range of effectiveness of GIFT, depending on the factors contributing to a couple's infertility. Success in achieving a clinical pregnancy by means of GIFT ranged from only 10 percent among couples with immunologically based infertility to a peak of 56 percent success among women with premature ovarian failure. It is important to note that as many as one in three clinical pregnancies fails to go to term.

It is unlikely, however, that gamete intrafallopian transfer will replace IVF. In most cases of damage to the oviducts, for example, GIFT is not an option (because the gametes need to be placed into the oviduct), whereas IVF is possible (because fertilized ova are placed in the uterus, bypassing damaged oviducts). Yet in the years ahead gamete intrafallopian transfer will likely become an increasingly popular option for cases of chronic unexplained infertility, for some cases of endometriosis, for cases where artificial insemination by donor has failed, for infertility due to cervical factors, for men with various seminal deficits, and for women with premature ovarian failure.

Proficiency with gamete intrafallopian transfer is rapidly spreading among clinicians, and there is no apparent technical barrier to it being offered By most units that deal with reproductive medicine and treatment for infertility. Unlike IVF, no requirement exists for expertise in, or a facility for handling, embryo culture. On the other hand, a clinical drawback to GIFT is that—if no pregnancy ensues—the procedure provides no diagnostic information about the fertilizability of the female's oocytes by the male's sperm. Defects in

Table 15-4.—Clinical Pregnancies Following GIFT[a]

Etiology	Number of stimulation cycles	Number of clinical pregnancies	Success rate[b] (percent)
Unexplained infertility	796	247	31
Endometriosis	413	132	32
Male factor	397	61	15
Tubo-peritoneal	210	61	29
Failed artificial insemination by donor	160	66	41
Cervical	68	19	28
Immunological	30	5	10
Premature ovarian failure	18	10	56
Total	2,092	601	29

[a]Results of a multinational cooperative study.
[b]Percent of stimulation cycles leading to a clinical pregnancy. As many as one in three such clinical pregnancies fails to go to term.

SOURCE: R.H. Asch, UCI/AMI Center for Reproductive Health, Garden Grove, CA, personal communication, December 1987.

the fertilizing ability of sperm or oocytes that might have been identified during IVF can go unnoticed with gamete intrafallopian transfer, when gametes are placed in the oviduct. An additional drawback is that, unlike IVF, gamete intrafallopian transfer usually requires the woman to undergo general anesthesia.

UTERINE LAVAGE TO RETRIEVE A FERTILIZED OVUM

Since 1983, about a dozen viable pregnancies have been reported as a result of flushing a preimplantation embryo (a fertilized ovum) from the uterus of a fertile donor and transferring it to an infertile recipient (8,9,16). This procedure initially seemed promising, particularly for infertile recipients without ovaries or with premature ovarian failure.

The future of this technique is uncertain (37). First, the success of IVF and gamete intrafallopian transfer, using multiple donor eggs, in treating women with premature ovarian failure exceeds that of fertilized ovum transfer where, to date, only one fertilized ovum at a time is transferred. Also, it remains to be shown that safe supranormal stimulation of the ovaries of ovum donors is possible in the ovum transfer technique.

Second, active IVF or gamete intrafallopian transfer programs, with sufficient patient populations providing an abundance of extra donor eggs and with a ready number of hormonally receptive recipients, can more easily arrange for donation of unused eggs. With increasing success in freezing eggs in years down the road, however, IVF and gamete intrafallopian transfer patients who now donate may become reluctant to do so when they themselves could receive the same eggs at a later date. At the same time, efficient freezing would eliminate the need to synchronize donor with recipient in the lavage procedure.

Third, certain risks to the fertile donor, such as ectopic pregnancy, multiple pregnancy as a consequence of supranormal stimulation of ovulation, and transmission of disease (e.g., acquired immunodeficiency syndrome) via semen, may render this technique impractical if not reduced to negligible levels. Such risks may be unacceptable for some in light of the suitability and success rates of IVF and GIFT.

FREEZING EMBRYOS

Embryo freezing is an attractive adjunct to IVF to conserve embryos, obviate the need for repeat egg retrieval procedures, and reduce the risk of multiple pregnancy when several embryos (i.e., more than three or four) are available for transfer. In Australia and Europe, about 60 children have been born from the transfer of thawed embryos; the first such U.S. birth occurred in 1986. Two dozen or more IVF programs in the United States have stored frozen embryos, but the technique is still experimental and requires additional research to improve success.

Initial research in France suggests that three factors influence human embryo survival after thawing: the developmental stage of the frozen embryos, the appearance of the embryo at the time of freezing, and the mode of ovarian stimulation in the IVF cycle **(39). In the** French study, optimal success was obtained by using programmed hormonal stimulation and selecting for l-cell embryos or 2- and 4-cell embryos with a favorable appearance. There was also a tendency for better pregnancy rates if embryo storage did not exceed 1 to 2 months.

Research with cryopreserved animal embryos suggests that embryos frozen and stored in liquid nitrogen at – 1960 C remain viable for 10 years or more, similar to cryopreserved sperm. Embryo freezing technology is especially well developed for laboratory mice and cattle; in farm animals (cattle, sheep, goats, and horses) as a group, the expected pregnancy rates from frozen-thawed embryos range from 35 to 55 percent (18). The farm animal embryos that exhibit the best viability fol-

lowing freezing are those frozen at the morula or blastocyst stage of development, well beyond the stage (i.e., 1 to 8 cells) at which most human embryos have been frozen to date.

FREEZING EGGS

Three births have been recorded in Australia and West Germany from eggs that were frozen and thawed. The routine capability to freeze and store eggs, in much the same manner that sperm are frozen for later use, would obviate much of the need to freeze embryos, thereby reducing the ethical and legal dilemmas inherent in the cryopreservation of human embryos (see chs. 11 and 13). An egg's chromosomes, however, are less hardy than a sperm's (which are highly condensed), and their fragility may make them intolerant of the rigors of freezing and thawing. The possibility of developmental anomalies arising in offspring conceived from frozen eggs—a major unanswered question—is cited as justification for chromosomal analysis of such embryos before attempting transfer of other such embryos for pregnancy. As with several of the technologies discussed in this chapter, this raises the issue of embryo use for research rather than pregnancy (22).

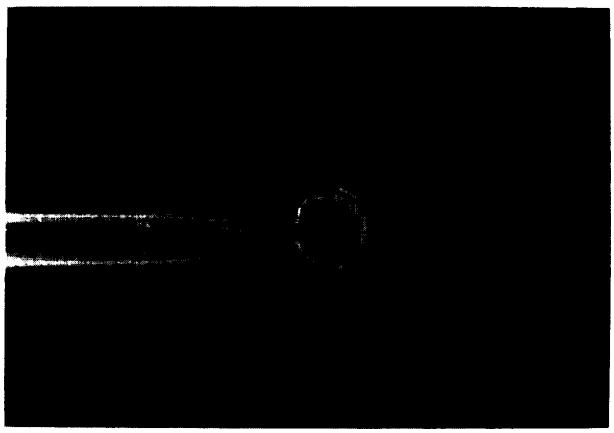
The most pressing clinical applications for freezing eggs arise in situations where women face the loss of fertility due to pelvic disease, surgery, or imminent radiation or chemotherapy for ovarian cancer or other malignancies. Patients could have one or more cycles of ovarian hyperstimulation so that oocytes could be collected and stored prior to the fertility-threatening treatment (26).

Oocyte freezing and thawing is technically more difficult and costly than embryo freezing and thawing. First, unlike a multicellular embryo, an egg consists of a single cell surrounded by a single membrane, damage to which kills the egg. Second, eggs must be frozen soon after retrieval, on a daily basis in a laboratory, whereas embryos can be maintained in culture and frozen in batches every other day or so. Third, in order to ultimately obtain a sufficient number of fertilized embryos, extra eggs must be frozen and thawed to account for the failure of some eggs to fertilize, For the latter reason, cryopreservation of eggs would have to be more successful than cryopreservation of embryos in order to be competitive, Therefore, in the near term, egg freezing is unlikely to surpass embryo freezing (40,41). With improved technology (2), however, and if chromosomal damage is not a factor, egg freezing could take its place alongside cryopreservation of sperm in the mainstream of reproductive technology.

MICROMANIPULATION OF SPERM INTO OVA

In animals, the microscopic surgical placement of a single sperm into an egg can achieve fertilization and trigger cleavage, but there has been little success in triggering embryonic development and producing offspring. Establishing such a treatment for humans would permit infertile men who either produce a reduced number of sperm (severe oligospermia), produce ejaculates with a large percentage of abnormal sperm, or produce sperm that are unable to fertilize their wives' ova to attempt fertilization by means of IVF by sperm micromanipulation (22)34). The ability to inject sperm cells could also be useful in conjunction with a reliable technique for separating X- and Y-bearing sperm (discussed in next section), if that technique had a low yield of viable sperm (15).

One type of micromanipulation of sperm involves insertion of a sperm under the egg's outer membrane, the zona pellucida, by using a fine glass needle that mechanically breaches the membrane. Successful fertilization of a human oocyte (but not embryonic development) was reported with this technique in Australia in 1987 (24). Another type of micromanipulation, called "zona drilling, " involves chemically etching the egg's outer membrane to create an opening for sperm penetration. The egg is anchored into place on a dish and



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cessful human and domestic animal pregnancies may be recorded before the next decade is out. This technique is of potentially great value for treating male factor infertility, most of which is due to unexplained oligospermia. The technique carries the risk, at least in theory, of fertilizing an egg with a sperm that otherwise would have been unable to fertilize the egg, with unknown developmental consequences.

Micromanipulation of a different sort also lies on the visible horizon—i.e., the multiplication of genetically identical individuals through cloning. Initial research in sheep and cattle indicates that nuclei from multicellular embryos can be injected into unfertilized, enucleated eggs and produce offspring of the same genotype (18)47). The research necessary to develop such a technique in humans is unlikely to soon be deemed ethically acceptable in the United States for several reasons, not the least of which is concern about deliberately creating genetically identical humans.

SEXING SPERM CELLS

The only established difference between female (X-bearing) sperm and male (Y-bearing) sperm is DNA mass, with Y-bearing sperm being about 3 percent lighter in weight than X-bearing sperm. Many articles have appeared in the scientific literature on attempts to separate X- and Y-bearing sperm. Most studies have been conducted on the semen of laboratory animals, most often rabbits. Methods evaluated over the years (1) include separation by mass, electric charge, or staining, and sperm migration through cervical fluids. other methods focus on manipulation of vaginal chemistry by diet or douching in order to select sperm. Although individual experiments using some of these approaches have been encouraging, usually the results could not be replicated.

The most recent approach to sexing human sperm cells involves the use of a protein solution, bovine serum albumin, to separate X- and Y-bearing sperm (13). According to the theory behind this procedure, Y-bearing sperm migrate preferentially into the protein solution and can then be washed and used for artificial insemination in an attempt to conceive a male. This method is protected by patents in the United States and abroad and has been used by some 50 clinics worldwide to produce about 400 babies. Although it is reported to tip the sex balance of offspring from the norm of just over 50 percent males to as high as 75 percent males, some members of the scientific community remain skeptical of even this degree of success attributed to semen sexing technology. In 1986, the American Fertility Society stated that current techniques for separating X- and Y-bearing sperm are not adequate to provide reasonable assurance of success (3).

Selection for X-bearing sperm has been attempted with at least two methods. In one, X-bearing sperm are separated from Y-bearing sperm using solutions with different densities (23). In another, sperm are flushed through columns that preferentially retain Y-bearing sperm because of their smaller size. The X-bearing sperm flow through the column and can be used for insemination. Too few births have been reported to date to adequately evaluate this technique for producing females,

It is noteworthy that the ability to sex semen of farm animals and influence the sex ratio of animal progeny would be of great economic benefit to commercial livestock producers. A male calf of a dairy cow may be worth only $5o, for example, while a female calf may be worth 10 times as much. The strong economic incentive in the livestock industry for sex selection–as well as human interest in selecting the sex of children, in some cases to avoid sex-linked genetic diseases—will continue to drive research in sexing of sperm for the foreseeable future.

In humans, there is likely an upper limit to the popularity of sex selection by separation of X- and Y-bearing sperm, should a reliable method become available. The process requires artificial insemination of the woman once the sperm are separated, and the overwhelming majority of couples are likely to prefer intercourse. Thus, the intrusive nature of artificial insemination will probably limit the broad use of sex selection, regardless of how reliable the selection of X- and Y-bearing sperm becomes in the future.

SEXING EMBRYOS

Although sexing the human fetus by evaluating chromosomal spreads in embryonic cells (collected by chorionic villi sampling or amniocentesis) is a well-established clinical procedure, sexing human embryos by such karyotyping has only recently been considered. Applying this procedure involves doing a biopsy of the embryo to examine the sex chromosomes of one cell. Such karyotyping has been reported in mice and cattle, but this method is unlikely to be developed for use in humans unless there is a general acceptance of human embryo biopsy. In such a case, embryonic cells would be evaluated from the biopsied part of the embryo, and the embryo would be made ready for transfer (18).

Researchers in the United Kingdom have demonstrated the identification of human male embryos using a commercially available DNA probe for Y-chromosomal DNA (46). The embryos, created solely for research purposes, were at the 2-cell through blastocyst stages. The DNA probe was applied to whole embryos, not samples of cells; it left the embryos unviable and the process took 4 to 8 days. Sexing embryos by this method will ultimately require embryo splitting and, because of the time required for the process, embryo freezing with later transfer. This is likely to become technically feasible, but will remain a laboratory technique without popular application for the foreseeable future. Basic studies of nonhuman preimplantation embryos may provide new approaches to embryo sexing.

GENETIC SCREENING OF GAMETE DONORS

It is impossible to exclude all sperm or egg donors capable of transmitting genetic disorders. Indeed, most couples conceiving a genetically abnormal child through intercourse show no characteristic that distinguishes them from couples having genetically normal children. In fact, the more severe an autosomal dominant trait, the greater the likelihood that the trait will have arisen in an affected individual as a result of a new mutation—i.e., one arising in the egg or sperm responsible for fertilization. Likewise, there is usually no recorded history of exposure to deleterious agents, nor are there consistently identifiable socioeconomic factors. The same can be expected for IVF, gamete intrafallopian transfer, and artificial insemination (37). In practice, relatively few prospective donors are excluded for genetic reasons (45).

Despite these limitations, some genetic screening is possible. A goal of excluding donors likely to place a pregnancy at greater risk than the rate of seriously anomalous offspring expected for the general population–about 3 percent–has been called realistic (36). No uniform criteria for such screening exist, but various guidelines have been

suggested. Some methods include lengthy and often unwieldly donor screening forms for physicians to use while eliciting a complete history and conducting a physical examination.

Guidelines proposed in 1986 by the American Fertility Society (4) recommend that sperm donors: have no malformations, have no nontrivial Mendelian disorder, have no adult-onset disease with a genetic component (e.g., hypertension or epilepsy), not be a heterozygote for an autosomal recessive gene known to be prevalent in the donor's ethnic group for which heterozygosity can be determined, have no chromosomal rearrangement, be young, and have an Rh type compatible with that of the prospective mother. Similar exclusions pertain to egg donors.

In addition, first degree relatives (i.e., parents and offspring) of male and female gamete donors should not have any nontrivial anomalies, autosomal dominant disorders of reduced penetrance or late age of onset, or autosomal recessive disorders of a high frequency in the population. For prospective female donors, those with heterozygosity for an X-linked recessive disease are also excluded.

The practical impact and uniform application of these guidelines are today unknown. OTA surveyed practitioners of artificial insemination in the United States, asking-among other questions —if the practitioner uses professional society guidelines and, if so, which ones (43). The guidelines are certain to assume increasing importance with the continued practice of noncoital reproduction and as the capability to test for human genetic disorders grows.

In recent years, the powerful techniques of molecular biology have been used to locate genes or chromosomal loci responsible for several inherited diseases, such as Huntington's disease, Duchenne's muscular dystrophy, familial Alzheimer's disease, autosomal dominant manicdepressive disease, myotonic dystrophy, and familial amyloidotic polyneuropathy. Likely to be located in the near future are genetic loci for neurofibromatosis, familial spastic paraparesis, and torsion dystonia. Further in the future lies the possibility of locating the gene for virtually any dominantly inherited disorder, provided that sufficiently large families with the disorder are available for analysis (27). With tests for a growing number of human genetic disorders likely to become available through the next decade, diagnostic testing of gamete donors for a handful of genetic diseases may become routine.

HEALTH OF INFANTS CONCEIVED BY IVF OR GIFT

Initial reports indicate that babies conceived in the laboratory through IVF face the same low risk of birth defects as babies conceived through intercourse. This finding comes from several studies, including one of 164 babies conceived between 1983 and 1985, half by IVF and half by normal means (48). A French study of 2,342 IVF pregnancies around the world found no significant increase in the rate of birth defects, once the data were corrected for the risks of increased maternal age and multiple pregnancies (10). Among 574 live births following IVF at 41 U.S. clinics in 1985 and 1986, 18 chromosomal abnormalities or congenital anomalies were recorded (3 percent) (28). In contrast, a study from North Carolina of 70 IVF pregnancies reports an excessively high 6 anomalies (30).

As with IVF, early indications are that gamete intrafallopian transfer confers no risk of excessive congenital abnormalities. The first 800 GIFT cases worldwide resulted in one chromosomal abnormality, a trisomy 21 (7).

Rigorous proof that neither IVF nor gamete intrafallopian transfer contributes to an increased prevalence of anomalies in offspring is today limited primarily by small numbers of potential subjects (37). For example, a sample size of 1,151 IVF pregnancies and 1,151 controls would be required to exclude (with 95 percent certainty) a threefold increase in chromosomal abnormalities that have an incidence of 0.5 percent. To detect a twofold increase, the required sample size would be 4,668 in each group. A sample size of 244 IVF

pregnancies would be required to exclude a threefold increase in total congenital anomalies (3 percent incidence), whereas 748 would be necessary to detect a doubled increase.

The IVF or gamete intrafallopian transfer population is also less than ideal to study because of inherent limitations in sample characteristics. Pooling tabulated outcomes of IVF or gamete intrafallopian transfer pregnancies—although fashionable—is hazardous because groups of women achieving such pregnancies come from diverse geographic areas and countries, with possible exposure to a host of potentially deleterious agents. An even more important confounder is the varying history of infertility among pooled patients: idiopathic infertility, for example, may be related to (as yet undetectable) genetic abnormalities in sperm or eggs. Finally, criteria for anomaly assessment are not standardized.

MATERNAL HEALTH CONSEQUENCES OF IVF AND GIFT

Women undergoing IVF or other forms of noncoital reproduction are not comparable to the general population. Their pregnancy outcomes, therefore, are not likely to be comparable. Inability to have achieved pregnancy readily dictates that such couples are older than the childbearing population at large. As a result, they are expected to be at increased risk for a variety of age-related adverse perinatal outcomes (25). older women naturally have had a longer time to manifest certain illnesses (e.g., chronic high blood pressure) that might not have been evident had they been able to achieve pregnancies earlier in life. Research will be needed to verify the present thinking that there is no apparent reason to suspect maternal complications in excess of those found in a comparable age group.

Adequate studies of maternal health consequences are today lacking, and there are at least two practical problems in conducting such research. First, the worldwide experience with IVF totals about 5,000 births; that of gamete intrafallopian transfer is about half that number, and fertilized ovum transfer far less. These numbers are too small for statistically rigorous studies. Second, IVF couples generally come from diverse geographic venues, even when treated at a given center. Successful pregnancies are usually delivered in a couple's local community, where outcomes are not monitored in a consistent fashion.

In the United States, one report of maternal consequences of IVF consists of 125 pregnancies conceived between 1981 and 1984 (5). These resulted in 100 deliveries, producing 115 babies. Only 12 deliveries actually happened at the IVF clinic (Norfolk, VA); 88 deliveries occurred elsewhere in the United States and in three foreign countries. The spontaneous clinical abortion rate (18.4 percent) was slightly higher than that of the general population, but similar to that of women undergoing ovulation induction. In 1986,485 clinical pregnancies among 41 U.S. IVF clinics resulted in 151 miscarriages (31 percent) (28). Another study reports a spontaneous clinical abortion rate of 28 percent among women 40 years and older undergoing IVF (32).

A higher than normal rate of delivery by cesarean section—despite no indication of increased likelihood of fetal distress-has been noted among IVF pregnancies (5). The increased rate of cesarean section may be a consequence of high levels of anxiety generated in physician and patient alike by an IVF pregnancy. This leads to a tendency to take every medical precaution at delivery, a circumstance generally favoring cesarean rather than vaginal delivery.

When ovarian stimulation with human menopausal gonadotropin or clomiphene citrate is undertaken prior to IVF or gamete intrafallopian transfer, hyperstimulation can land the woman in the hospital. Among IVF clinics, this has been reported at the rate of 1.2 to 1.5 patients per 1,000 stimulation cycles (28).

PSYCHOLOGICAL EVALUATION OF PARTICIPANTS IN NONCOITAL REPRODUCTION

The circle of participants in reproduction today encompasses biological parents, legal parents, and immediate family members of participants (e.g., siblings of a child conceived through assisted reproduction technology). It also includes medical personnel who share responsibility for the successes and more numerous failures of the procedures, as well as those whose participation in assisted reproduction was rejected (e.g., unsuitable gamete donors or surrogate mothers). Each group of individuals is subjected to unique stimuli and can be expected to exhibit a range of psychological responses. Yet few participants have been systematically studied, and little is known about the psychology of participants in assisted reproduction.

The intended child is the principal participant in assisted reproduction and arguably the individual whose psychological status is of greatest concern. Three major psychological questions regarding the child are:

- What are the developmental sequelae, if any, of prenatal procedures such as in vitro embryo culture or embryo cryopreservation?
- In the case of surrogate motherhood, what is the child's relationship with his or her birth mother (even if the relationship occurs only in the child's fantasy life)?
- In the case of ovum donation or artificial insemination by donor, what is the child's relationship to his or her genetic parent(s) (again, even if this occurs only in the child's fantasy life)?

From the child's perspective, ovum donation or artificial insemination by donor may at times dif - fer from adoption–with unknown psychological consequences to the child. Adoption, for example, can involve parents giving up a child for the child's own good. In contrast, gamete donation can involve parents giving up gametes with no knowledge of the child to be born and at times for the parent's financial reward.

The major research question regarding participants in assisted reproduction is the descriptive

Photo credit: Martin ***Quigley***

Identification of egg under microscope in IVF laboratory

measurement of what happens to them psychologically from the first contact with infertility evaluation and treatment through the years that follow. This type of controlled, longitudinal research is essential. The National Institute of Child Health and Human Development is moving toward establishing a health surveillance system of women undergoing treatment for infertility with IVF (44). Similar systems would be useful for the children so conceived, the genetic (donor) parents and spouses, and spouses of those who underwent infertility treatment (who may themselves undergo treatment),

Along with controlled, longitudinal research, studies are needed of the baseline psychological status of each group of participants. Such studies are necessary both to quantify the subsequent psychological effects of assisted reproduction as well as to assist in the selection of participants. Research is needed, for example, to determine whether couples seeking assisted reproduction have special psychological problems that would render them unfit candidates for treatment.

No widely accepted psychological criteria currently exist that couples seeking a child must meet before they can be considered as participants in

an assisted reproduction program. Neither, of course, are there standard criteria for a couple seeking a child by means of intercourse. Establishing criteria for couples desiring to undertake assisted reproduction may permit caregivers to impose their values on the selection process—raising questions about who should be involved in and responsible for developing such criteria (17). Yet such criteria have been assessed as useful for helping to guide the behavior of the caregiver assisting a couple. Criteria could include the following (14):

- The presence of a stable psychosocial environment. An applicant couple on the brink of divorce, for example, would be poor candidates. Likewise, applicants troubled by addictive behaviors (i.e., drug or alcohol abuse) would be unsuitable.
- Evidence of authentic motivation. Each spouse should be participating because of the desire to raise a child, and not, for example, under the threat of divorce if he or she did not participate.

Research is needed on the predictive validity of these criteria, as well as on useful criteria for other participants in assisted reproduction, particularly the genetic parents (the donors).

A final critical question is how an infertile couple resolve their childlessness if infertility persists and adoption is rejected. What are the developmental factors, thought processes, or emotional involvement necessary to accept childlessness? What are the societal attitudes that affect a couple's ability to live contently without children? It is especially important to identify the treatment strategies that mental health professionals can bring to bear to assist a couple in the resolution of this final stage of infertility.

SUMMARY AND CONCLUSIONS

Two reproductive technologies first applied to humans within the past decade are today helping a small, but measurable, fraction of infertile couples form families and will continue to do so for the foreseeable future. The chances of achieving a successful pregnancy in the hands of the most expert practitioners are estimated to be about 15 to 20 percent for one completed IVF cycle and about the same for one completed attempt at gamete intrafallopian transfer. The number of infertile couples offered these techniques is likely to increase in the future as the techniques are applied to infertility of more varied causes than at present. The once-promising technique of uterine lavage to retrieve a fertilized ovum, followed by embryo transfer, is unlikely to continue to be offered to infertile couples.

The next decade will likely see the proliferation of embryo freezing as an adjunct to IVF, although early success with freezing eggs would likely preclude embryo freezing. Freezing eggs, however, stands as a formidable technical task and may involve an insurmountable biological obstacle—damage to the fragile chromosomes of the oocyte.

Researchers seeking to examine fertilization of human sperm and eggs, fertilized ova, or early embryos face a de facto moratorium on funding of such investigations by the Department of Health and Human Services, unless the study is directly related to IVF carried out as part of an infertile couple's routine clinical care. Research to study, for example, why some sperm do not fertilize eggs, or why some eggs are not fertilizable, is not funded by the National Institutes of Health.

Successful pregnancies following microinjection of a single sperm into an egg—recorded in neither animals nor humans, to date—would mark dramatic progress in the treatment of male factor infertility, most of which is caused by too few or abnormal sperm.

Reliable separation of X- and Y-bearing sperm for sex selection remains elusive despite many such attempts. When sex selection of sperm cells becomes possible, its use will be limited by the willingness of couples to undergo artificial insemination. The development and use of techniques to sex human embryos is likely to be retarded because such techniques involve splitting embryos

into one part for sexing and another part for transfer. Research on nonhuman preimplantation embryos may lead to alternative approaches for the sexing of embryos.

Techniques for screening sperm and ova donors for a limited number of genetic anomalies lie in the foreseeable future. The practical application of genetic screening by practitioners of artificial insemination is uncertain, however, and no amount of screening will exclude all donors capable of transmitting genetic disorders.

The health of infants conceived by IVF or by gamete intrafallopian transfer and that of their mothers does not appear to deviate from norms for comparable populations. Because of the small numbers of individuals that have used these technologies to date, however, such estimates are necessarily preliminary, and ongoing surveillance would be prudent. Similarly, studies of the psychology of participants in assisted reproduction—particularly the children–are warranted.

This brief review of selected reproductive technologies and areas of reproductive research suggests that, while Aldous Huxley's vision of a Brave New ***World*** has not been realized, some frontiers of reproductive technology have been broached and others are being approached. As a result, infertile couples today have a wider range of options than before, and some of today's babies already have a qualitatively different pedigree. One noted science fiction writer recently offered his view of what the pedigree of tomorrow's babies might look like (see box 15-B).

Box IS-B.—A Look Ahead

Commonwealth of California, Department of Health's Vital Records
CERTIFICATE OF LIFE

subject:	**Baby Boy, Miller**
Date of Conception:	**Nov. 15, 2018, 12:15 p.m.**
Place:	**Comprehensive Fertility Institute, Beverly Hills, CA**
Number of Parents:	**Three, including surrogate mother—mother donated egg, father sperm**
Method of Conception:	**In vitro fertilization followed by embryo transfer. Mother's body had rejected her artificial fallopian tube. After 8 days on Pergonal, mother produced two eggs. Both were removed during routine laparoscopy and screened for possible defects. Eggs united with father's sperm. After 48 hours in incubator, embryos were removed from growth medium and placed in surrogate's womb. Only one embryo attached itself to uterine wall.**
Prenatal Care:	**Ultrasound at 3 months. Fetal surgery performed at 5 months.**
Date/Time of Birth:	**Jason Lawrence Miller born July 20, 2019, 4:15 a.m.**
Father:	**Jason L. Miller, Sr.**
Mothers:	**Amy Wong (natural); Maribeth Rivers (surrogate)**
Birth Method:	**Newly lifed in Morningstar Birthing Center, division of Humana Corporation. Natural delivery after 5-hour labor. Labor pains controlled though acupuncture. Therapeutic touch used for last hour of labor. Child's father, adopted sister, and natural mother attended the delivery.**
Weight/Length:	**10 lb.; 25 in.**
Eye Color:	**Green**
Projected Life Span:	**82 years**

SOURCE. Adapted from AC Clarke, *July 20, 2019: Life in the 21st Century* (New York, NY Macmillan, 1986)

CHAPTER 15 REFERENCES

1. Alexander, N.J., "Prenatal Sex Selection," *Current Therapy of Infertility, vol. 3,* R.D. Amelar (cd.) (Philadelphia, PA: B.C. Decker, 1987).
2. Al-Hasani, S., Diedrich, K., van der Ven, H., et al., "Cryopreservation of Human Oocytes," *Human Reproduction* 2:695-700, *1987.*
3. American Fertility Society Ethics Committee, "Ethical Considerations of the New Reproductive Technologies," *Fertility and Sterility* 46:1S-94S, *1986.*
4. American Fertility Society, "New Guidelines for the Use of Semen Donor Insemination: *1986, "Fertility and Sterility* 46:95S-104S, *1986.*
5. Andrews, M. C., Muasher, S.J., Levy, D. L., et al., "An Analysis of the obstetric Outcome of 125 Consecutive Pregnancies Conceived In Vitro and Resulting in 100 *Deliveries, "American Journal of Obstetrics and Gynecology* 154:848-854, *1986.*
6. Asch, R. H., Ellsworth, L. R., Balmaceda, J. P., et al., "Pregnancy After Translaparascopic Gamete Intrafallopian Transfer" (letter), *Lancet, Nov. 3, 1984,* pp. 1034-1035.
7. Asch, R. H., Balmaceda, J. P., Cittadini, E., et al., "GIFT: International Cooperative Study, The First 800 Cases," presentation at Fifth World Congress on In Vitro Fertilization and Embryo Transfer, Norfolk, VA, Apr. 5-10, 1987.
8. Buster, J. E., Bustillo, M., Thorneycroft, I. H., et al., "Nonsurgical] Transfer of In Vivo Fertilized Donated Ova to Five Infertile Women: Report of Two Pregnancies," *Lancet,* July 23, 1983, pp. 223-226.
9. Bustillo, M., Buster, J., Cohen, S., et al., "Nonsurgical Ovum Transfer as a Treatment in Infertile Women," *Journal of the American Medical Association 251: 1171-1173, 1984.*
10. Cohen, J. J., and Mayaux, M.J., "IVF Pregnancies: Results of an International Study," presentation at Fifth World Congress on In Vitro Fertilization and Embryo Transfer, Norfolk, VA, Apr. 5-10, 1987.
11. DeCherney, A. H., Book Review, *New England Journal of Medicine* 316:954, *1987.*
12. Edwards, R. G., and Steptoe, P. C., "Pregnancies Following Implantation of Human Embryos Grown in Culture," presented at Scientific Meeting, Royal College of obstetricians and Gynecologists, London, Jan. 26, 1979.
13. Ericsson, R.J., Langevin, C. N., and Nishino, M., "Isolation of Fractions Rich in Human Y Sperm," *Nature* 246:421-425, 1973.
14. Fagan, P. J., Schmidt, C. W., Jr., Rock, J. A., et al., "Sexual Functioning and Psychologic Evaluation of In Vitro Fertilization Couples," *Fertility and Sterility* 46:668-672, *1986.*
15. Foote, R. H., '(In Vitro Fertilization and Embryo Transfer in Domestic Animals: Applications in Animals and Implications for Humans," *Journal of In Vitro Fertilization and Embryo Transfer* 4:73-88, *1987.*
16. Formigli, L., Formigli, G., and Roccio, C., "Donation of Fertilized Uterine Ova to Infertile Women," *Fertility and Sterility* 47:162-165, *1987.*
17. Frankel, MS., Program Head, Committee on Scientific Freedom and Responsibility, American Association for the Advancement of Science, Washington, DC, personal communication, Aug. 28, 1987.
18. Godke, R. A., "Animal Reproductive Technologies and Their Potential Applications for Human Fertility Problems: A Review," prepared for the Office of Technology Assessment, U.S. Congress, Washington, DC, March 1987.
19. Gordon, J. W., and Talansky, B. E., "Assisted Fertilization by Zona Drilling: A Mouse Model for Correction of Oligospermia," *Journal of Experimental Zoology* 239:347-354, *1986.*
20. Haney, A. F., Hughes, C. L., Jr., Whitesides, D. B., et al., "Treatment-Independent, Treatment-Associated, and Pregnancies After Additional Therapy in a Program of In Vitro Fertilization and Embryo Transfer," *Fertility and Sterility* 47:634-638, *1987.*
21. Haseltine, F. P., Director, Center for Population Research, National Institute of Child Health and Human Development, National Institutes of Health, Bethesda, MD, personal communication, Sept. 25, 1987.
22. Hodgen, G. D., Scientific Director, Jones Institute for Reproductive Medicine, Norfolk, VA, testimony before the Select Committee on Children, Youth, and Families, U.S. House of Representatives, May 21, 1987.
23. Iizuka, R., Kaneko, S., Aoki, R., et al., "Sexing of Human Sperm by Discontinuous Percoll Density Gradient and Its Clinical Application," *Human Reproduction* 2:573-575, *1987.*
24. Laws-King, A., Trounson, A., Sathananthan, H., et al., "Fertilization of Human Oocytes by Microinjection of a Single Spermatozoon Under the Zona Pellucid," *Fertility and Sterility* 48:637-642, *1987.*
25. Lehmann, D. K., and Chism, J., "Pregnancy Outcome in Medically Complicated and Uncomplicated Patients Aged 40 Years or Older," *American Journal of Obstetrics and Gynecology* 157:738-742, *1987.*
26. Marrs, R. P., Stone, B. A., Serafini, P. C., et al., "Frontiers of Reproductive Research," prepared for the Office of Technology Assessment, U.S. Congress, Washington, DC, August 1987.
27. Martin, J. B., "Genetic Linkage in Necrologic Dis-

eases," New *England Journal of Medicine 316:1018-1020, 1987.*

28. Medical Research International and the American Fertility Society Special Interest Group, "In Vitro Fertilization/Embryo Transfer in the United States: 1985 and 1986 Results From the National IVF/ET Registry," *Fertility and Sterility* 49:212-215, *1988.*
29. Meldrum, D. R., Chetkowski, R., Steingold, K. A., et al., "Evolution of a Highly Successful In Vitro Fertilization-Embryo Transfer Program," *Fertility and Sterility* 48:86-93, *1987.*
30. Nebel, L., Mashiach, S., Rudak, E., et al., "Congenital Anomalies and Neonates Following IVF-ER; Teratological Analysis," presentation at Fifth World Congress on In Vitro Fertilization and Embryo Transfer, Norfolk, VA, Apr. 5-10, 1987.
31. Roh, S.1., Chin, N., Awadalla, S. G., et al., "In Vitro Fertilization and Embryo Transfer: Treatment-Dependent Versus -Independent Pregnancies," *Fertility and Sterility* 48:982-986, *1987.*
32. Romeu, A., Muasher, S. J., Acosta, A., et al., "Results of In Vitro Fertilization Attempts in Women 40 Years of Age and Older: The Norfolk Experience," *Fertility and Sterility* 47:130-136, *1987.*
33. Rosenwaks, Z., "Donor Eggs: Their Application in Modern Reproductive Technologies," *Fertility and Sterility* 47:895-909, *1987.*
34. Schrader, S. M., Andrologist/Toxicologist, Experimental Toxicology Branch, National Institute for Occupational Safety and Health, Cincinnati, OH, personal communication, September 1987.
35. Sher, G., Director, Pacific Fertility Center, San Francisco, CA, persona] communication, Jan, 25, 1988.
36. Simpson, J. L., "Genetic Screening for Donors in Artificial Insemination," *Fertility and Sterility 35: 395-396, 1981.*
37. Simpson, J. L., Professor and Chairman, Department of Obstetrics and Gynecology, The Health Science Center, The University of Tennessee, Memphis, TN, personal communications, June and August 1987.
38. Tesarik, J., "From the Cellular to the Molecular Dimension: The Actual Challenge for Human Fertilization Research," *Gamete Research* 13:47-89, *1986.*
39. Testart, J., Lassalle, B., Forman, R., et al., "Factors Influencing the Success Rate of Human Embryo Freezing in an In Vitro Fertilization and Embryo Transfer Program," *Fertility and Sterility 48:107-112, 1987.*
40. Trounson, A., "Preservation of Human Eggs and Embryos," presentation at 42d Annual Meeting of the American Fertility Society, Toronto, Ontario, Sept. 29, 1986.
41. Trounson, A., "Preservation of Human Eggs and Embryos," *Fertility and Sterility* 46:1-12, *1986.*
42. U.S. Congress, Office of Technology Assessment, *World Population and Fertility Planning Technologies: The Next 20 Years,* OTA-HR-157 (Springfield, VA: National Technical Information Service, 1982).
43. U.S. Congress, Office of Technology Assessment, *Artificial Insemination: Medical and Social Issues,* forthcoming.
44. U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, request for proposals No. NICHD-CE-87-6, July 1, 1987.
45. Verp, M. S., Cohen, M. R., and Simpson, J. L., "Necessity of Formal Genetic Screening in Artificial Insemination by Donor," *Obstetrics and Gynecology* 62:474-479, *1983,*
46. West, J. D., Angell, R. R., Thatcher, S. S., et al., '(Sexing the Human Pre-Embryo by DNA-DNA In-Situ Hybridisation, " *Lancet*, June 13, 1987, pp. 1345-1347.
47. Willadsen, S. M., "Nuclear Transplantation in Sheep Embryos, " *Nature* 320:63-65, *1986.*
48. Wirth, F.H., Morin, N., Johnson, D., et al., "Followup Study of Children Born as a Result of IVF," presentation at Fifth World Congress on In Vitro Fertilization and Embryo Transfer, Norfolk, VA, Apr. 5-10, 1987.