
GROWTH

Genetics & Hormones

Vol. 18 No. 4 www.gghjournal.com December 2002

THE CURRENT FRONTIERS OF IN VITRO FERTILIZATION

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INTRODUCTION

In the early 1980s when *in vitro* fertilization (IVF) became a clinical reality it was considered therapy for diseased fallopian tubes. However, its effectiveness soon made it applicable to other causes of infertility, such as endometriosis unresponsive to other therapy, oligospermia with at least a million sperm identified in the ejaculate, and in other possible indications such as infertility of unidentified etiology, and infertility thought to be due to immunological factors.

Improvements in both clinical and laboratory technology at the turn of the millennium made IVF the treatment of choice for all forms of tubal disease (except perhaps iatrogenic sterilization), for endometriosis if infertility was the principal complaint, and for oligospermia regardless of the sperm count, and even for cases of azoospermia in which sperm could be obtained directly from the testis and intracytoplasmic sperm injection (ICSI) used for a single sperm to cause fertilization and pregnancy. It should be said up front, that it appears as if the majority of cases of oligozoospermia are due to genetic causes with the gene primarily carried on the Y chromosome. Therefore, with the use of ICSI, there is a greater transmission of genetic disorders to the next generation since the Y sperm fertilizes the egg. In spite of this, few patients reject this therapy. Occasionally, IVF therapy is used in infertility of undetermined origin and in less frequent conditions, such as the female whose mucous destroys sperm before they can ascend into the uterus.

While the above are the best possible therapeutic options, in current practice, many patients do not receive contemporary therapy. There are numerous reasons for this, but primary among them is that when IVF came into use, the health insurance industry declined coverage on the basis that it was "experimental therapy".

Although IVF is the best possible therapy for several causes of infertility, the insurers continue to deny coverage, resulting in the application of obsolescent therapy for countless patients. For example, diseased fallopian tubes which prevent pregnancy are often surgically repaired because it is covered by insurance. There is reason to believe that contemporary therapy, i.e. IVF, used when medically indicated would be less costly and less risky than the obsolescent therapy supported by the insurance carriers. While some states now have mandated insurance coverage, this is suboptimal because of the restrictions and fixed prices which are often built into the legislation. On a population basis, the United States is now far behind other countries in utilizing IVF. In a study by Collins,¹ it was shown that many other nations are far more frequent users of IVF than the US (Figure 1).

EXPECTATION OF PREGNANCY

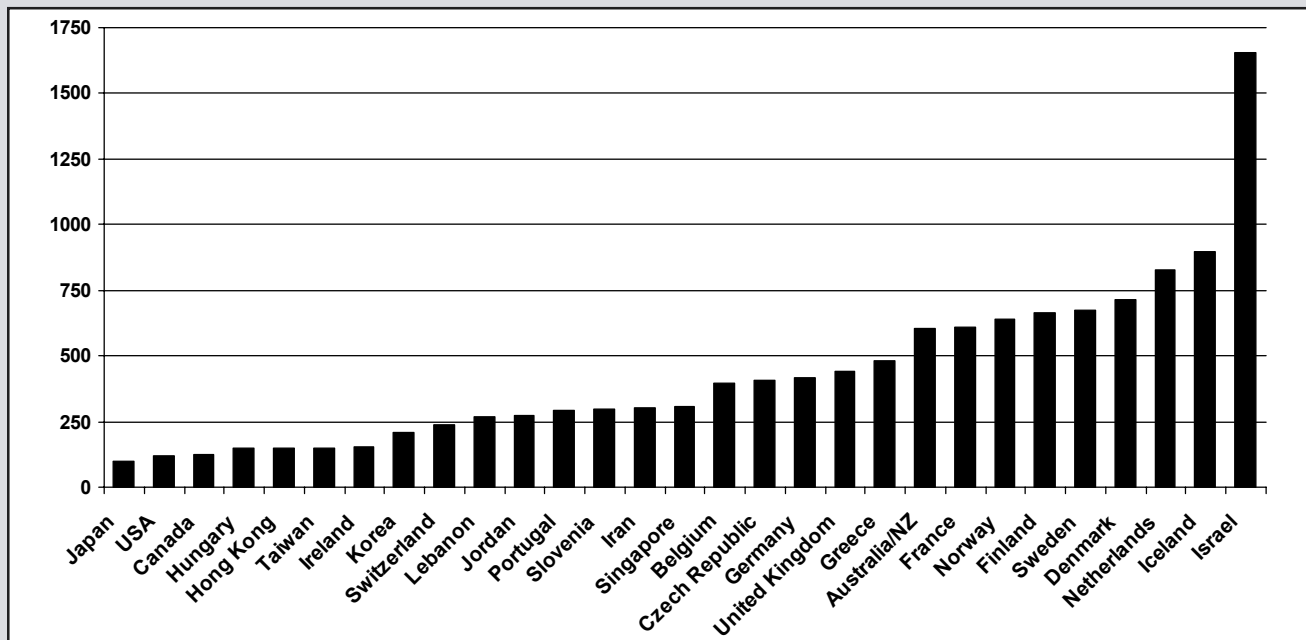
The 1998 official IVF Registry Report published in January 2002² showed that in the US there were 58,937 cycles involving IVF with a delivery rate per retrieval of 29.1% or 17,150 deliveries. There were 5,273 fresh donor oocyte cycles with a delivery rate for transfer of 41.2% (2,179 deliveries) and 11,228 frozen embryo transfer procedures with a delivery rate per transfer of 19.3% (2,167 deliveries). These percentages are as expected, as fresh donor procedures unequivocally are more successful than frozen embryo procedures. The Registry data are more than three years out-of-date and

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Figure 1

IVF/ICSI Cycles per Million Population



Adapted from Collins J. Cost-effectiveness of in vitro fertilization. *Seminars in Reprod Med* 2001;279-289.

for a variety of reasons can indeed be misleading to the unwary reader as different assisted reproductive technology (ART) programs have different performance guidelines and different methods of pooling the data.

It has long been known that fecundity, i.e. the probability of pregnancy per month of exposure, declines with the age of the female partner. This age factor cannot be overcome by the use of IVF; thus, therapeutic results reported in the ASRM/SART Registry² show a marked age related effect (Table 1). The therapeutic significance is that patients must be further educated about the eroding effect of age on the reproductive process and pregnancy should be undertaken as early as possible.

Multiple pregnancies have been a troublesome problem with IVF. Since the initiation of IVF and of ovulation induction (which also started around 1980) the multiple pregnancy rate in the US as reported by the Bureau of Vital Statistics (Figure 2) has increased each year through 2000, the last date for which data are available. Although the triplet and higher rate decreased slightly in 1999 and 2000, the increase in the rate for twins more than made up for this decrease so that the overall multiple pregnancy rate has increased each year. Examination of the 1998 ASRM/SART Registry reveals that of all deliveries 61.8% were single births, 31.7% of the deliveries were twins, 6.2% were triplets, and 0.3% were quadruplets or more. This is unacceptable and is caused by pressure from both patients and programs alike. They wish to have a high pregnancy rate which

can be accomplished with multiple transfers, but at the expense of multiple pregnancies which are undesirable. The goal should be to have a reasonable pregnancy rate with no more than 1% triplets.

Taking all these considerations into account, in 2002 a female who is a good responder, i.e. one who produces at least 5-6 mature oocytes to the required gonadic stimulation, is not over 38 years old, has both ovaries, and has a sperm producing partner, should expect to have a pregnancy 50% of the time with fresh transfer with a risk of less than 1% of having triplets and less than 4% of having twins.

CRYOPRESERVATION

No program in IVF can be considered “full service” unless it offers cryopreservation which can hold frozen excess preembryos for future use. Indeed, in expressing the pregnancy rate for a particular IVF program, a misleading figure is given, unless the pregnancy potential from the frozen material is included. We have published³ a theoretical model in which a true expression of pregnancy rate resulting from stimulated cycles can be calculated. The interested reader is referred to this publication for full details. Briefly, it is quite impossible to properly evaluate the pregnancy outcome of a particular stimulation cycle unless supplementary pregnancies, if any, from cryopreservation are considered as part of the pregnancy rate of that particular stimulation cycle. This can be done by adding

Table 1

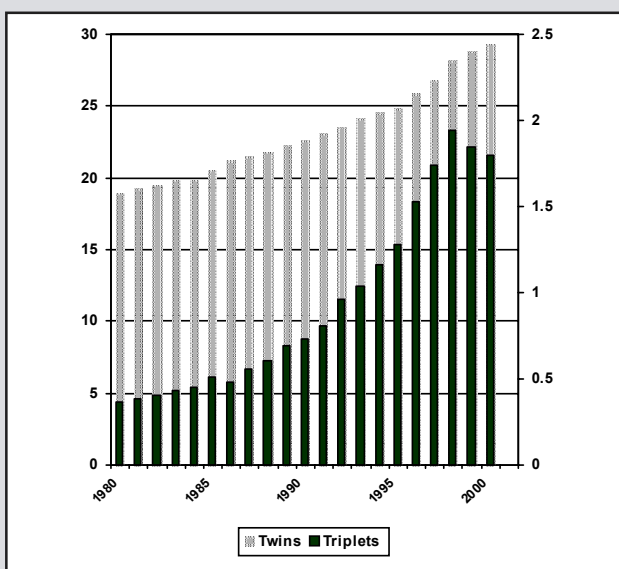
IVF procedures (with and without ICSI) by age group and cause of infertility.

1998 IVF procedures	No. of retrievals	Canceled cycles (%)	Transfers Per retrieval (%)	No. of pregnancies	No. of deliveries	Deliveries Per retrieval (%)	Multiple Births per Delivery (%)
No male factor infertility							
Women <35 years of age	16,648	10.0	93.4	6,878	5,948	35.7	43.4
Women 35-37 years of age	8,524	14.7	94.2	3,109	2,543	29.8	37.9
Women 38-40 years of age	7,063	19.5	92.7	2,006	1,498	21.2	29.0
Women >40 years of age	4,348	24.6	89.9	721	446	10.3	20.2
Male factor infertility							
Women <35 years of age	7,546	7.7	94.7	3,042	2,647	35.1	40.3
Women 35-37 years of age	3,147	11.6	94.8	1,206	1,000	31.8	35.5
Women 38-40 years of age	2,366	14.6	92.9	750	563	23.8	31.8
Women >40 years of age	1,129	19.1	91.9	231	144	12.8	13.9
1998 totals	50,771	13.9	93.6	17,943	14,789	29.1	38.2
1997 totals	44,170	14.0	93.4	15,047	12,302	27.9	39.0

SART/ASRM. ASRM/SART registry: 1998 results. Fertil Steril 2002.

Figure 2

Multiple Pregnancy Rate with IVF and Ovulation Induction



The rating of twins and triplets and more from the Bureau of Vital Statistics, U.S. Public Health Service.

all cryopregnancies to fresh pregnancies, or can be patient specific (i.e., considering cryopreservation as augmentation only among patients without a pregnancy from pre-embryos transferred fresh, or from previously transferred frozen material from the same harvest). For the patient-specific concept, cryopregnancies occurring among patients with a previous fresh or frozen pregnancy from the same harvest would be considered additive to the multiple pregnancy rate, i.e. twins, etc., but would be considered as 'delayed' multiple

pregnancies. Published results have not reflected the real purpose of cryopreservation; this is shown by the methods of presentation of cryopreservation in the publications of collecting agencies, such as the US Society for Assisted Reproductive Technology, the Great Britain Human Fertilization and Embryology Authority, the Australia-New Zealand Agency, and others. In general these publications report cryopreservation results as unrelated to a particular oocyte harvest or treat a cryopreservation as an additional transfer from the same cohort of prezygotes/pre-embryos, thus diluting the fresh pregnancy rate, as cryoresults are often not as good as fresh results.³

Generally speaking, expectation of a pregnancy from cryopreserved material is not as great as from fresh. Although the data are not exactly comparable, the ASRM/SART Registry for 1998 gave an overall pregnancy rate per transfer for fresh oocytes in IVF of 37.8% and 24.3% for cryopreserved material. With careful selection of fertilized eggs prior to cryopreservation, the pregnancy expectation from cryopreserved material approaches that of fresh material.

PREIMPLANTATION GENETIC DIAGNOSIS (PGD)

PGD has been available since about 1990.⁴ By this technique, one or two blastomeres are removed from the preembryos of the 6-10 cell stage and examined for single gene defects by the polymer chain reaction (PCR) or by fluorescent in situ hybridization (FISH) for gross chromosomal defects. Preembryos with defects are discarded and those found to be normal are transferred or frozen for future transfer.

Table 2
PGD referrals (n) according to indication

Chromosomal	647
X-linked	294
Autosomal recessive	290
Autosomal dominant	254
Mitochondrial	6
Two indications	9
Y-chromosome deletion	2
Social sexing	30
Unknown	29

ESHRE PGD Consortium Steering Committee (May 2001) Hum Reprod 17:235, 2002.

Diagnostic ability with PGD is precisely that of amniocentesis which is done at 15-18 weeks of pregnancy or chorionic villus sampling which is done at 10-14 weeks of pregnancy. PGD appeals to those who cannot morally terminate an affected fetus but who do not feel morally bound to implanting an in vitro affected preembryo. It also appeals to those who are prepared to undergo the requirements and expense of PGD and IVF simply to avoid the possibility of an elected termination, even though they may have no moral conflict in aborting an affected fetus.

The opportunity to use PGD is not offered by all centers, but its use is gradually increasing. According to data collected by the ESHRE,⁵ in 2001 there were 1,561 PGD procedures reported. The most common cause for referral was concern about chromosomal abnormalities. Specific gene disorders accounted for slightly over one-third of the cases (Table 2). Cystic fibrosis was the most common monogenic disorder.

PGD is not without an occasional error, and its efficiency in relation to fertility factors is somewhat less than IVF because of the limited number of preembryos that can be selected for transfer resulting from the screening out of affected fertilized eggs.

DONOR GAMETES

Donor *sperm* have long been used when infertility was due to sperm deficiencies. Currently, the use of donor *sperm* and *oocytes* can be considered standard practice for those who are prepared to accept nonfamilial genetic material. In some circumstances, donor gametes are used to replace gametes which are likely to or are known to harbor a mutant disease-causing gene. This is particularly valuable when the affected gene is not amenable to preimplantation genetic diagnosis.

When donor *sperm* are used either with or without IVF, the donors are vigorously screened. Requirements differ from center to center. At the Jones Institute the donors

must be 18 to 39 years of age, have a semen volume of 2 mL with a sperm count of at least 60 million, with sperm motility greater than 60%, and at least 7% of the sperm must be of normal form by strict criteria. There can be no excess of WBCs. More than 50% of the sperm must survive the cryo-survival test. The family history of the donor must be free of genetic disease. A physical examination must reveal no urethral discharge or genital warts or ulcers. Laboratory screening includes a serological test for syphilis, cytomegalovirus, hepatitis B and C, HIV-1 and HIV-2, and T-cell lymphotropic virus I and II. Serum tests must be negative for herpes, chlamydia and gonorrhea, and donors must pass a urine test for drug screening. In addition, donors must be free of cystic fibrosis and, if Jewish, tested for Hexosaminidase-A which causes Tay-Sachs disease. Black donors must be free of the sickle-cell trait. Potential Asian or Mediterranean donors with a positive hemoglobin electrophoresis for thalassemia are eliminated.

Semen quarantine is usually carried out for 6 months at which time the donor is checked for HIV and other possible potential problems before semen is released for use. All this is in accordance with the recommendations of the American Society for Reproductive Medicine (ASRM). Clinical pregnancy rates with donor *sperm*, with or without IVF, are consistent with a normal fecundity rate if there is no impediment to pregnancy on the part of the female.

When donor *eggs* are supplied, the donor has a similar historical review for genetic problems, as well as laboratory studies. However, it is impractical to quarantine an *egg* for six months, as the *eggs* do not freeze nearly as well as the *sperm*. Therefore *egg* quarantine is essentially never done. HIV testing in the *egg* donor is done by the antigen test rather than the antibody test, as a prompt answer can be obtained, although there is some uncertainty as to the time required for the appearance of the antigen. Clinical pregnancy rates for donor *eggs* in IVF are a cut above that obtained by IVF in general - due to the younger age of the donor. The pregnancy rate with donor *eggs* is consistent with the age of the donor and unrelated to the age of the recipient. There is great uncertainty about an upper age limit for the use of donor *eggs*.

ASRM has issued a guideline indicating that donor *eggs* should not be used in a recipient at an age above a woman's normal reproductive life. This guideline probably has been left purposely vague. The guidelines must have been violated as there are accounts of recipient mothers 60 years of age and over. Each program must adopt its own standard in regard to age limit. Some variations in the standard donor egg scenario have occurred. For example, there have been

menopausal grandmothers who were prepared to receive an anonymous donor egg for their daughter - such an egg, of course, fertilized by the daughter's husband. There are no guidelines for these offbeat situations, thus each program must handle them on an individual basis. Calling for assistance might be appropriate, such as the utilization of sociologists, and/or an ethics committee, or other outside resources to establish guidelines and share responsibility for these decisions.

Suffice it to say, when donor eggs are used, and especially if the recipient's age is 40 or above, a preconception medical evaluation is in order. Such an evaluation would look for those conditions which might cause complications during pregnancy or those which might be aggravated by pregnancy, such as obesity, hypertension, and diabetes. Only those women who are totally medically fit should be considered as recipients.

An upper age limit for a prospective father is sometimes an issue *with or without* donor sperm. This seems to arise when a prospective father is 60 or above and marries a much younger wife. One must ask, "Does the program have a responsibility in this circumstance to consider the welfare of the child; specifically, is there any reason to be concerned about how a man of 60, 70 or 80 years of age can function responsibly, mentally and physically, with teenage children?" A program probably has no responsibility here, but the issue is thought provoking.

CONCLUSION AND A FINAL WORD

Prior to IVF it was common for physicians who treated infertility patients to tell them that everything had been tried, and it was now time to consider adoption or a childless future. Basic IVF technology changed much of that, as did the addition of donor gametes for those prepared to accept alien genetic material; the physician is now able to offer an option to essentially all couples. The era of IVF also has made it possible to go beyond

the mere solution of the problem of infertility. Preimplantation genetic diagnosis now makes it possible to eliminate disease-causing mutant genes. Thus, we are beginning to diminish the number of children born with handicaps. Such children previously were thought to represent an intrinsic risk of bearing children.

If the era of IVF has written a new chapter in the treatment of infertility, are there additional chapters to be written? To be sure! The aging oocytes represent a challenge. Can they be rejuvenated? I think it will be possible. IVF is inefficient, but changing this represents a problem. With eight fertilized two-cell zygotes in the dish, experience tells us that on average only two or three of these have the potential to progress to a term fetus. We are far from perfect in identifying which ones are the two or three. Can our selection potential be improved? I think it will be possible. Cryopreservation is very efficient for *sperm* but very inefficient for the *egg* due to its size. Can cryopreservation of the egg be achieved? I think it will be possible.

These are only examples. There are several other possibilities - some of which may be considered by some in the realm of science fiction, but all aimed at improving the human condition. Reproductive medicine and its developing technology have placed us in the midst of a reproductive revolution.

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Abstracts from the Literature

Genetic Screening for Maternal Uniparental Disomy of Chromosome 7 in Prenatal and Postnatal Growth Retardation of Unknown Cause

This very enlightening paper from Finland is worth reading by all pediatric subspecialists for its wealth of information. The authors first relate that uniparental disomy (UPD) associated with growth retardation has been found in at least 9 chromosomes (2,6,7,9,14,16,17,20 & 22) and concluded that UPD thus may provide explanations for some cases of growth retardation of unknown cause. Inheritance of *both*

parental genomes is essential for normal growth and development.

In their study, these authors focused on UPD of chromosome 7 and particularly on maternal or matUPD7. The study was prompted as matUPD7 has been reported in approximately 10% of patients with Russell Silver syndrome (RSS) and in a few patients with intrauterine growth retardation (IUGR) without RSS.