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☐ Review Article ☐

Current Trends in Human IVF and Other Assisted Reproductive Technologies

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When the first "test tube baby", Louise Brown, was born following successful extracorporeal (in vitro) fertilization (IVF) and embryo transfer (ET) in 1978 (Steptoe and Edwards 1978), it offered new hope to women who were unable to conceive because of damaged fallopian tubes. It also opened the door to better understanding of human reproductive physiology and human fertilization and embryo growth, which led us to apply IVF to the treatment of couples with other types of infertility problems such as unexplained infertility (Lessing *et al.* 1988), male infertility (Awadalla *et al.* 1987) and immunological infertility (Thatcher and Decherney 1989). Not only have the indications of IVF expanded, but other assisted reproductive technologies have also been developed in the past five years.

Many of the new technologies are based on the basic principles of IVF-ET, and were developed to overcome several potentially faulty steps in human conception including failure of sperm to reach the distal fallopian tube, failure of oocyte pick-up by the fallopian tube, and non-fertilization in the presence of sperm and egg. These technologies include gamete intrafallopian transfer (GIFT), zygote intrafallopian transfer (ZIFT), and tubal embryo transfer (TET). Cryopreservation of human embryos, donor embryo transfer and gestational carrier as a surrogate are other new developments. Some of these new technologies have raised legal and ethical controversies for the infertile couples, the physicians, and society.

This review focuses on the current status of human IVF-ET and other new reproductive technologies involving extracorporeal manipulation of the gametes and addresses the issues of legal and ethical considerations.

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IN VITRO FERTILIZATION AND EMBRYO TRANSFER (IVF-ET)

Historical Review

The first experiments on mammalian in vitro fertilization date back to the Austrian scientist Schenk in 1878 (Hafez and Semm 1988). Following the first successful IVF in a rabbit in 1959, IVF was successfully achieved in other animal species in the 1960s (Knobil and Neill 1988). The ability to produce human life by IVF was the subject of fantasy and intense interest to many reproductive scientists and gynecologists long before it became a reality in 1978 (Steptoe and Edwards 1978). During the last decade, more than 5000 live births occurred worldwide from human IVF. The IVF procedure is primarily performed as a therapeutic method, but does offer a unique diagnostic value of assessing sperm-oocyte interaction in the management of infertility.

Essential Elements for a Successful IVF Program

A successful human IVF program requires an intimate relationship between clinical care and IVF laboratory procedures. The guidelines for IVF programs established by the American Fertility Society include the following: highly trained reproductive endocrinologists or physicians with a skill of oocytes retrieval and understanding of laboratory management of gametes, an embryologist or reproductive scientist with laboratory expertise in gametes, and facilities for clinical and laboratory procedures (Am fertil. Soc. 1984). Also, another important element is a team of supoort personnel such as a nursing coordinator, counselor and laboratory technicians. One of the most important steps in human IVF is the selection of pa-

tients. A complete infertility workup to evaluate the cause(s) must be done, and a thorough review of medical records and appropriate counseling are essential (Am Fertil. Soc. 1984).

Procedural Steps of Human IVF-ET

Human IVF-ET consists of four major steps: 1) induction of multiple follicular matruation, 2) retrieval of oocytes, 3) gametes and embryo culture in vitro, and 4) embryo transfer. Each step is critical in achieving successful conception.

Induction of multiple follicular stimuation: Although earlier human IVF babies were born from unstimulated, natural ovulatory cycles, the success rates with unstimulated ovulatory cycles remained low. In the early 1980s multiple follicular stimulation for oocyte maturation with various pharmacologic agents was introduced, and resulted in more oocytes available for IVF and a higher pregnancy rate (Trounson *et al.* 1981; Garcia *et al.* 1983). The goal of using various stimulating agents is to hyperstimulate the ovaries so as to obtain multiple mature oocytes. Commonly used agents are clomiphene citrate, human menopausal gonadotropin (hMG), pure follice stimulating hormone (FSH), or various combinations of these drugs (Yee and Vargyas 1986; Rosenwak *et al.* 1986). In all regimens human chorionic gonadotropin (hCG) is administered to induce the final maturation of preovulatory follicles approximately 34 to 36 hours before retrieving eggs. Each IVF program has its own method of multiple follicle development. One of the unsettling challenges is yet to identify those protocols that yield multiple mature follicles and in turn produce normal embryos and clinical pregnancies.

Therefore, it is critically important to monitor the growth of follicles in order to better time the hCG administration and the egg retrieval. Serum levels of estradiol reflect the functional state of granulosa cells of the ovarian follicle, and correlate well with the growth of follicles. (Vargyas *et al.* 1982). Ultrasonographic imaging of the follicles has allowed physicians to monitor the growth of follicles accurately (Vargyas *et al.* 1982; Hackeloer *et al.* 1979; DeCherney and Lanfer 1984; Ritchie 1985). With the advent of transvaginal ultrasound technique, it is easier to scan the ovaries with more comfort for the patient. Thus, transvaginal scanning has entirely replaced the transabdominal approach in recent years. Ultrasound assessment of follicular development allows determination of the number and size of growing follicles and allows cancelling of IVF cycles when there is inappropriate follicular stimulation. It also detects any preexisting ovarian follicles or cysts prior to the initiation of treatment for multiple follicle development.

The ovarian follicular stimulation is best monitored by frequent determination of estradiol levels and ultrasound imaging of the follicles. When a follicle reaches a size greater than 16mm in diameter with hMG stimulation or greater than 18mm in diameter with clomiphene citrate stimulation, it is considered to be a mature preovulatory follicle. There is an excellent correlation between ultrasound findings and estradiol levels. In general, an estradiol level of 250-400 pg/ml per mature follicle is indicative of a preovulatory mature oocyte (Kerin *et al.* 1981). However, follicular growth patterns and hormonal behavior have been demonstrated to be quite different between various regimens. Generally clomiphene citrate treated cycles show larger follicles than hMG treated cycles.

Despite careful monitoring and individualized regimens of ovarian stimulation agents, some 25-35% of the treatment cycles have to be cancelled for the egg retrieval due to premature ovulation, inappropriate LH or estradiol increases, inappropriate follicular growth, or a single dominant follicle. Suboptimal treatment cycles lead to poor fertilization and lower pregnancy rates. Suboptimal stimulation may be caused by a premature luteinizing hormone (LH) surge before optimal maturity of the leading follicles or an androgen and LH-mediated atresia of secondary follicles. These problems can be prevented by pituitary suppression using gonadotropin releasing hormone agonist (GnRHa). Indeed, in the past several years numerous reports have shown the advantages of GnRHa use in conjunction with hMG-hCG stimulation (Awadalla *et al.* 1987; Neveu *et al.* 1987; Serafini *et al.* 1988). The principle benefit of GnRHa use is the reduction of cycle cancellation as it eliminates any endogenous LH effect due to pituitary suppression by the down-regulation mechanism.

Oocyte retrieval: Several of the follicle aspiration techniques for oocyte retrieval have been used to obtain the largest number of mature preovulatory oocytes with minimal damage to the oocytes and the least risk to the patient. These include laparoscopic retrieval (Steptoe and Edwards 1978), oocyte retrieval at the laparotomy for infertility-related conditions (Roh *et al.* 1988) and ultrasound-guided transabdominal or transvaginal aspiration (DeCherney and Lavy 1986). At present, ultrasound-guided transvaginal approach is most widely employed. It does not require general anesthesia and is relatively simple and safe. However, it can not be used for oocyte retrieval when a gamete intrafallopian transfer (GIFT) procedure is planned.

The timing of oocyte retrieval is critical as the ob-

ject is to retrieve mature oocytes. It must be performed as close as possible to the expected time of ovulation based on careful monitoring of the follicular growth in order to avoid spontaneous ovulation and immature oocytes. In most IVF programs, the oocyte retrieval is carried out 34 to 36 hours after hCG injection. Although the oocyte recovery rates and fertilization rates vary somewhat between different techniques, they range between 70 and 90% for oocytes recovery and 60 and 80% for fertilization (DeCherney and Lavy, 1986; Seifer *et al.* 1988).

IVF (gametes and embryo culture): A successful IVF depends largely on the efficient IVF laboratory staffed by experienced hands in gamete-embryo culture. The IVF laboratory steps include the following: a) recognition of oocytes from the aspirated follicular fluid, b) accurate grading of the maturity of oocytes, c) oocyte culture, d) preparation of sperm for insemination, e) insemination of oocytes with sperm, and f) monitoring of fertilization and embryo growth (i.e. pronuclear formation and cleavage) (Marrs 1986). The quality of IVF laboratory systems such as the culture incubator and culture medium must be checked frequently to assure their efficiency. Numerous types of culture media have been successfully used by different IVF laboratories.

Embryo transfer: The embryo transfer (ET) is carried out by transcervically replacing the embryo(s) using a teflon catheter into the fundal portion of the uterine cavity. Embryo(s) are suspended in a culture-transfer medium in the teflon catheter tip, which is passed through the cervix into the uterus. The embryos for ET are usually between 2-cell and 16-cell stages. While 70-80% of oocytes retrieved will be fertilized, only 15-25% of embryos transferred will implant. The successful implantation of an embryo is dependent on the quality of the embryo, the receptivity of the endometrium to implantation, and other unknown factors.

How many embryos should be transferred remains controversial. However, there seems no advantage of transferring more than three or four embryos. Also, there is no definite scientific evidence of the necessity of luteal phase support following ET. However, the majority of IVF programs utilize some form of luteal support (i.e. progesterone or hCG administration) since implanation failure constitutes the major impediment to successful IVF outcome (Yovich *et al.* 1984).

Clinical Results of IVF

Due to a lack of standardized definitions of "fertilization" and "conception", reported IVF success rates vary significantly from one program to another causing confusion. The pregnancy rates generally run below 20%. The live birth rate in IVF is significantly lower than in general pregnancy (Meldrum *et al.* 1987; Lin *et al.* 1988; National IVF-ET Registry 1990).

In the United States the American Fertility Society has established a registry under the Society of Assisted Reproductive Technologies, which collate the data for all registered U.S. programs. In 1988, the overall clinical pregnancy rate was 16% of the embryo-transfer cycles and the rates of abortion and tubal pregnancy were 25% and 4% respectively (National IVF-ET Registry 1990). The greatest risk of spontaneous abortion in IVF is associated with women older than 40 years of age; about 50% of pregnancies will miscarry (Romen *et al.* 1987; Gudzick *et al.* 1986).

OTHER ASSISTED REPRODUCTIVE TECHNOLOGIES

While IVF-ET has improved from its original form, clinical pregnancy rates remain relatively stable at approximately 15%. Numerous outgrowths from IVF-ET principles have brought a broader application to clinical management of the infertile couple.

Gamete Intrafallopian Transfer (GIFT)

Asch and his co-workers (1984) reported the first successful pregnancy from GIFT, where sperm and oocytes were directly placed into a woman's fallopian tubes. In contrast with IVF, GIFT facilitates physiologic processes (in vivo) to achieve fertilization and pregnancy. The presence of at least one normal tube is a prerequisite for GIFT. Steps in the GIFT procedure include induction of multiple follicular development (as in IVF), and laparoscopic oocyte retrieval with intrafallopian transfer of the gametes (oocytes and sperm).

All published reports showed superior results with the GIFT procedure compared to IVF-ET (National IVF-ET Registry 1990; Pouly *et al.* 1989; Asch 1987). The U.S. Registry in 1990 reported the clinical pregnancy rate as 27%, the abortion rate 20%, and the ectopic pregnancy rate 5% (National IVF-ET Registry 1990). While the pregnancy rates in patients with unexplained infertility and mild endometriosis were reported to be about 25%, the pregnancy rates in male factor infertility were 10-15% in most studies. The success of GIFT is dependent on the number and quality of oocytes transferred and upon the quality of sperm (Craft and Brinsden 1989). Although the GIFT procedure yields a higher pregnancy rate, it has several disavantages;

the necessity of laparoscopy or minilaparotomy, no diagnostic information about fertilization when conception does not occur, and the relatively restricted indications as GIFT requires at least one healthy fallopian tube. However, the GIFT procedure offers an opportunity to evaluate the pelvic condition at the time of laparoscopy for oocyte retrieval and gametes transfer (Barad *et al.* 1988). In order to make the GIFT procedure simpler, some investigators are attempting to transfer oocytes and sperm transcervically into the fallopian tubes under ultrasonographic guidance (Hughes *et al.* 1988). Such a technical advent will make the GIFT procedure much more practical and attractive.

Zygote Intrafallopian Transfer (ZIFT)

The ZIFT procedure employs a combination of the advantages of IVF (to have the diagnostic value of assessing the presence of fertilization) and GIFT (to yield a higher conception rate) (Devroey *et al.* 1989). There are several variations of this new technology; tubal embryo transfer (TET) (Balmaceda *et al.* 1988), pronuclear stage tubal transfer (PROST) (Yovich *et al.* 1987) and tubal pre-embryo transfer (TPET) (National IVF-ET Registry 1990). The development of a safe transvaginal aspiration of follicles under ultrasound guidance made ZIFT possible as patients are subjected to only one laparoscopy for the transfer of zygotes or embryos fertilized in vitro.

The obvious advantage of ZIFT over GIFT lies in its diagnostic value that there is proof of fertilization before transfer into the tubes. Its theoretical advantage over IVF is based on the assumption that the intratubal milieu is more favorable for development of embryos than the in vitro condition or in utero.

Indeed, the pregnancy rates of ZIFT appear to be better than those of GIFT or IVF. Clinical pregnancy rates for unexplained infertility and male factor infertility are reported to be 29% and 25% respectively (Yovich *et al.* 1988; Hamori *et al.* 1988). Despite its superior results, ZIFT requires two procedures, one for oocyte retrieval and another for intrafallopian transfer, and is more costly. Therefore, it is best indicated in patients with a failed GIFT or IVF attempt.

Cryopreservation of Embryos

The advent of cryobiological technology has brought a wide use of cryopreservation of human sperm. Recently cryopreservation of human embryos was added to new reproductive technology. Various methods with different cryoprotectants have been successfully used (Friedler *et al.* 1988). Cryopreservation of preimplantation embryos (pronuclear stage to blastocyst stage) is widely used in many IVF programs to preserve excess embryos for the patients. This technique allows one to limit the number of embryos transferred into the patients uterus at any one time. It is believed that no more than three or four embryos should be transferred at one time because of the increasing risks of multiple gestation. The ethical and safety questions of cryopreservation remain to be further answered. At present the clinical pregnancy rate in frozen embryo transfer is lower than that in IVF with an approximately 5 to 10% clinical pregnancy rate and a 25% spontaneous abortion rate (National IVF-ET Registry 1990; Fugger 1989).

Donor Oocyte or Embryo Programs

Oocyte donation or embryo donation is the only method of achieving pregnancy in patients with gonadal dysgenesis, premature ovarian failure, and surgical oophorectomy. Other indications include infertile women over 40 years of age and those with familial genetic disorders such as Huntington's chorea. The donated oocytes are fertilized by the husband's sperm in vitro, and are transferred into the woman's uterus which was synchronized by exogenous estrogen with the time of oocyte recovery from the donor (Serhal and Craft 1989; Navot *et al.* 1986; Rosenwak 1987). Embryos donated by another couple can be transferred to the recipient uterus synchronized with estrogen. The donated oocytes or embryos may be transferred into the tubes as in GIFT or ZIFT (Asch *et al.* 1988).

Gestational Carrier (Surrogate Uterus)

Use of a surrogate uterus as a gestational carrier became feasible with IVF and GIFT. The gametes or fertilized embryos of patients with functioning ovaries who are unable to carry their pregnancy due to hysterectomy or uterine anomalies are transferred to the surrogate uterus of a gestational carrier. The woman providing a surrogate uterus will carry the pregnancy and deliver a child, who will be adopted back by the child's biological parents. The gestational carrier woman would not contribute to the genetic composition of the child (The Ethics Committee of American Fertility Society 1986).

Micromanipulation of Gametes in IVF

The fertilization capacity of men with abnormal semen is significantly lower than that of men with normal semen (Awadalla *et al.* 1987; Mahadevan and

Trounson 1984). The fertilization rates in male factor infertility vary between 25 and 50% while 60 to 80% rates are expected in men with normal semen. Although IVF has increased the incidence of pregnancy in male factor infertility, the efficiency is significantly compromised by any dysfunction of the spermatozoa. In the fertilization process, the most important barrier for sperm to overcome is the zona pellucida. The spermatozoa with functional deficiency or inadequate numbers are less capable of traversing this barrier.

Various techniques of micromanipulation have been reported to circumvent the barrier of zona pellucida, thus promoting more effective sperm penetration into the ooplasm. These methods which are still under investigation, have resulted in many live births in recent years and include the following techniques: direct injection of sperm into the ooplasm (Markert 1983), "zona drilling" (Gorden *et al.* 1988), insemination of sperm under the zona pellucida (subzonal insemination) (Laws-king *et al.* 1987), and partial zona dissection of the oocyte (Malter and Cohen 1989). Amongst these methods, subzonal insemination (insertion of one or a few sperm under the zona pellucida or into the perivitelline space), zona drilling (to create small holes in the zona pellucida with the use of acidic Tyrode's solution) and partial zona dissection (a method to open the zona pellucida partially using a micro-pipette) are most promising and are being actively investigated. Common to all methods is the purpose of promoting sperm penetration through the zonal barrier.

ETHICAL AND LEGAL ASPECTS OF THE ASSISTED REPRODUCTIVE TECHNOLOGIES

The availability of extracorporeal fertilization and other new technologies has brought much excitement and hope to the medical world and patients suffering from infertility. From the scientific point of view, these procedures are viewed as an extension of traditional infertility treatment. However, society at large has shown appropriate concern as to the proper place, legally and ethically, of these new advanced technologies. In order to answer these important questions, numerous organizations, governmental and professional, have proposed various guidelines (Ethics Committee of American Fertility Society 1986; Andrews 1986; Dept. of Health and Social Security 1984).

The Ethics Committee of the American Fertility Society published its recommendations on various procedures in 1986. The usual practice is that following approval of its institutional ethics committee each institution develops guidelines on all new procedures to protect the rights and ethics of all participants. However, at present what is legally and/or ethically acceptable in this new world of highly technical reproductive procedures is far from clear, and will undoubtedly remain controversial. Generally, the guidelines should be based on the following ethical principles: 1) the human embryo should be treated with respect even if it may not be given full legal status as a person, 2) the reproductive technology should not create serious harm or risks, physical or psychological, to the participants, 3) participation in the new technology should be voluntary, and 4) the application of such new technology should not be harmful to society.

There are numerous legal barriers to these new reproductive technologies. The fetal research laws are an example. Without a clear legal definition of the terms "life", "embryo", and "fetus", any new technologies on gametes and fertilization are subject to legal controversies. In the case of cryopreservation of embryos, the ownership of embryos presents a difficult legal interpretation (Robertson 1987). Likewise, in the gestational carrier situation, the genetic parents may have difficulty establishing a claim to "their child" if the surrogate carrier decides to keep the child. To protect all participants in new technologies which are designed to promote the right to procreate, laws should be adapted to these new scientific developments.

SUMMARY

The new reproductive technologies such as IVF, GIFT, ZIFT, and micromanipulation have had a profound influence on the therapeutic and diagnostic management of infertility, and in turn have resulted in better understanding of human fertilization and embryo development. While the clinical pregnancy rates in GIFT and ZIFT procedures are comparable to the natural fecundity in the population at large, pregnancy rates in IVF have been generally lower. Further investigations should be directed to improve the implantation rates, and to develop better controlled methods of multiple follicle development. Although more studies are needed, a recent report of potential utiliztion of nonstimulated oocytes for donor programs as well as IVF-cryopreservation was a promising new development (Cha *et al.* 1989). Other exciting prospects on the horizon are the possibilities of gene transfer for the treatment of certain genetic diseases and diagnostic applications of embryonal biopsy.

These new technologies have also generated

serious ethical and legal issues. Any ethical or legal guidelines affecting new reproductive technologies should be developed to protect all participants only when the need for regulation is clear. Ethical quidelines and appropriate legislations with contributions from the medical and scientific community are gradually being established worldwide.

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