Human In Vitro Fertilization

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ABSTRACT

Human in vitro fertilization has become a widely accepted method of treatment for infertile couples. The list of indications for this technique is rapidly growing and includes oligospermia, endometriosis, and unexplained infertility. The techniques of follicular recruitment, ovum maturation, and follicular aspiration, as well as laboratory culture techniques, are described. The various factors to be taken into consideration when trying to assess possible success of in vitro fertilization and embryo transfer are discussed in this paper.

Physiology of Fertilization

During the prenatal period, oocytes are arrested in the prophase of the first meiotic division. As the graafian follicle matures, the oocyte contained within it is reactivated and meiosis is resumed. With the influence of luteinizing hormone (LH), a meiosis inhibiting factor (MIF) is overcome and meiosis resumed. Excess chromatin material is abstracted from the oocyte as the first polar body. Upon ovum release, spermatozoa, which have already completed their meiotic division and have undergone a process of "capacitation", traverse the cumulus oophorus, corona radiata, zona pellucida, and finally enter the vitellus. At this point, a second polar body is formed. Once fertilization has occurred, male and female pronuclei are formed by decondensation of the compacted haploid chromosomes derived from the male and female nuclei. In the human, the female and male pronuclei join together in a process of syngamy and a normal chromosome complement of 46 chromosomes is realized. In the absence of fertilization, cytolysis of the oocyte occurs in approximately 15 to 18 hours.

Three days following ovulation, the conceptus completes its journey through the fallopian tube and enters the uterine cavity. Implantation is thought to occur on approximately the seventh postovulatory day. The techniques for extracorporeal fertilization utilized in our laboratory simulate these natural events in as precise a manner as is technically possible.

Follicular Recruitment

Oocytes are collected following either a spontaneous or stimulated menstrual cycle. In a spontaneous cycle, timing for laparoscopic ovum recovery is best accomplished by hormonal monitoring of the patient's estrogen (E2) with concomitant ultrasound examination of the
developing follicle. Patients are usually admitted to the hospital when the follicular diameter is greater than 16mm (figure 1). Laparoscopic ovum recovery is performed 28 hours after the onset of the LH surge with a final ultrasound examination carried out one to two hours prior to laparoscopy in order to detect possible premature ovulation.\textsuperscript{1,3}

The majority of In Vitro Fertilization/Embryo Transfer (IVF/ET) programs advocate a method of control follicular hyperstimulation. Although various treatment regimens have been employed at various institutions (i.e., gonadotrophine releasing hormone (GnRH), follicle stimulating hormone (FSH), FSH/LH, human menopausal gonadotropin (HMG), clomiphene citrate (CC), and CC and HMG), the present authors now utilize, almost exclusively, either CC or HMG.\textsuperscript{5}

In clomiphene citrate stimulated cycles, clomiphene citrate, 50 mg, is administered orally on days 5 through 9 of the menstrual cycle. Ultrasonography is begun on day 10 of the menstrual cycle and continued until the mean diameter of a dominant follicle has reached 2.0 to 2.2cm and the estrogen level has plateaued. Five thousand IU of human chorionic gonadotropin (HCG) is administered 24 hours prior to the anticipated ovum retrieval.\textsuperscript{3}

In HMG stimulated cycles, three ampules per day are administered intramuscularly beginning on day 3 of the menstrual cycle. Human menopausal gonadotropin is continued throughout and inclusive of day 7. Ultrasonography of the ovary is begun on day 8 and is continued until the day prior to ovum recovery. In addition, HMG is continued in a previously mentioned regimen until two follicles have reached a mean diameter of 1.5 cm in three planes, at which time HMG is discontinued. Human chorionic gonadotropin, 5,000 IU, is administered when the estrogen levels remain constant, ovum recovery being performed 34 hours later.\textsuperscript{2} Monitoring for the LH surge and ultrasonography on the day of ovum recovery are not routinely employed since a rise in estradiol following the injection of HCG has not been associated with ovulation in our hands. The current authors have found that the main advantage of HMG is a statistical increase in the number of follicles and ova obtained, fertilized and transferred.

![Figure 1](image-url)
Ovum Retrieval

Laparoscopy is performed under general anesthesia, utilizing an 8 mm laparoscope and two puncture sites situated beneath the pubic hairline. The first puncture site is used for placement of a 55 mm atraumatic grasping forceps, which is used for traction on the utero-ovarian ligament and subsequent stabilization of the follicles against the pelvic sidewall. The aspirating needle, constructed after the design of Renou in Australia, utilizes a closed system for aspiration. A teflon tubing is passed throughout the stainless steel 12 gauge needle, connected to a 10 ml culture tube, and subsequently connected to a 120 mg Hg vacuum. During the course of the laparoscopy, the individual follicles are identified (figure 2), punctured, and aspirated into a 10 ml culture tube, (figure 3) which is immediately labelled, brought to the laboratory, and examined for the presence of oocytes. Since oocytes are not present in a fair percentage of initial follicular aspirates, the follicles are immediately reflushed with a solution consisting of a modified Ham’s F-10 medium, 7.5 percent serum, and heparin. The “follicular flush” is sent to the laboratory and examined accordingly. No more than five healthy oocytes are obtained from any patient.

Laboratory

Oocytes are classified as immature oocytes when the corona layer, cumulus and granulosa cells enveloping the imma-

Figure 2. Laparoscopic view of human follicles: (1) preovulatory follicle, (2) puncture of follicle with aspirating needle, (3 and 4) aspiration of follicle.
ture oocytes are aggregated in a compact fashion (figure 4). These oocytes are inseminated 24 hours after aspiration. On the other hand, preovulatory or mature oocytes are characterized by loosely aggregated granulosa cells and are inseminated six to eight hours after retrieval. Hypermature oocytes, characterized by a markedly expanded corona layer with granulosa cells showing marked cellular expansion and intercellular spacing, are inseminated within one to two hours of aspiration.7

Sperm are collected by masturbation. Semen is allowed to liquify to room temperature for 30 minutes and washed in a modified solution of Ham's F-10 medium. The sperm suspension is centrifuged at $20 \times g$ for five minutes, and the supernatant discarded. Capacitation of the sperm theoretically occurs when the intact pellet is layered with one ml of sperm washing medium and incubated for one hour in five percent CO$_2$. The uppermost layer of motile sperm are placed in a separate test tube, counted, and the egg inseminated with 50 to 100,000 motile sperm per each inseminated egg. The inseminated egg is incubated at 37°C in a humidified atmo-
sphere of five percent CO₂ and air for 18 to 21 hours.

Approximately 18 to 21 hours after insemination, the egg is observed under the dissecting and inverted stereoscopic microscope for the presence of pronuclei and polar bodies. Usually, the cumulus mass surrounding the egg is manually dispersed in order that the presence of pronuclei and their number can be ascertained. Abnormal eggs containing three or more pronuclei (polypronuclear) are not transferred.⁸⁻¹⁰ “Normal” development occurs in a humidified atmosphere of five percent CO₂ and air for another 22 to 24 hours, at which time the zygotes are again observed. Generally, two to eight cell embryos with even sized (figure 5) blastomeres are seen. The concepti are then transferred into the uterus using a transfer catheter which permits the placement of the embryo in 50 ml of transfer media approximately 0.5 cm from the uterine fundus (figure 6).

**Indications for In Vitro Fertilization**

Originally, IVF-ET was designed for patients with severe tubal disease, although the technique has expanded to include treatment of severe male factor infertility (oligozoospermic male) as well as of couples who have not had an explanation for their infertile state (the so-called idiopathic infertility group). Success rates with these groups have not been established (table I).⁶

Alternatively, recovery of oocytes can

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**Figure 5.** Fertilized human ova: (a) embryo with two pronuclei, (b) two cell human embryo (c and d) three and four cell human embryo, respectively.
be attempted using ultrasonographically guided follicular aspiration. When follicles are considered mature, an ultrasound transistor is placed on the patient's abdomen and is used to guide the needle that is introduced under local anesthesia through a distended filled bladder to the precise location of the mature follicle. By this method, oocytes may be recovered without laparoscopy. This type of ovum aspiration may be complicated by perforation of the pelvic viscera and possible initiation of tubo-ovarian abscesses.

Encouraging results are being reported with the use of IVF for treatment of infertility owing to male subfertility and endometriosis. Other groups as well as our own group are exploring various manipulations of laboratory protocols and sperm culture media in the hope that fertilization rates using sperm of suboptimal characteristics will be improved.

Because the number of infertile couples in the United States is increasing, the need for IVF-ET programs is apparent. The main concern at the present time is determining what qualifications and types of facilities are necessary to provide optimal chances for successful outcome. Ideally, IVF-ET should be performed and managed in a hospital setting. Whether the procedure is carried out in a university research center or in private community hospitals, rigid standards and quality control must be maintained.

Predicting the Success Rate

There are no reliable criteria to predict which patient is more likely to achieve pregnancy as a result of in vitro fertilization procedures. Maternal incremental changes in estradiol levels seem to correlate with higher successful pregnancy rates. An increase in estradiol after HCG administration has a much better prognosis than those patients with a decreasing estrogen levels following HCG administration. Likewise, "high" responders with estradiol levels over 600 pg per ml have pregnancy rates higher than patients with estradiol levels lower than 300 pg per ml. A high estrogen/testosterone ratio in follicular fluid is likewise a sign of ovum maturity.

Pregnancy rates may be related to the number of embryos replaced in an in vitro fertilization cycle. (table II).

At present, there is no clear morpho-
 logic criteria which may be used to differentiate oocytes which will result in pregnancy from others that will not. Other favorable prognostic indices derived from follicular fluid analyses are high levels of alpha-antitrypsin and increased vacuolization of the granulosa cells of the cumulus.

The IVF-ET pregnancy rate has shown a steady increase since the first IVF-ET pregnancy occurred in England. In 1983 in the United States, using multiple oocyte transfer, pregnancy rates ranged from 20 to 30 percent per transferred cycle. In Australia and England, the pregnancy success is about the same as that in the United States.

At least six pregnancies have been achieved using eggs fertilized in vitro, frozen, and then thawed prior to embryo transfer. The technique employed consists of in vitro fertilization and culture of the conceptus to an eighth cell stage. The embryos are incrementally slowly cooled to temperatures of −80° with liquid nitrogen in the presence of 1.5 molar dimethylsulfoxide in phosphate buffered saline. At an appropriate, receptive time in the women’s cycle, the embryos are then thawed and replaced in the uterus. The success of pregnancy following this protocol represents an initial success rate of 25 percent, following 24 embryo transfers. This technique avoids the necessity for repeated laparoscopies to retrieve ova and has the added advantage of perform-

ing the transfer of embryo during menstrual cycles when the woman has not had surgery and induction of ovulation. It must be stressed that the use of frozen thawed embryos, although successful, must still be considered experimental.

In addition, the use of IVF/ET could conceivably be used for women who, for various reasons, are unable to produce their own oocytes but who still have a normal uterus which would allow the development of embryos. Conversely, women with normal ovaries but without uteri could conceivably produce a conceptus, having her genetic characteristics, and nurture the same in a surrogate uterus. The ethical issues inherent in this form of treatment must be resolved.

Indeed, any couple entering the IVF-ET process must undergo extensive counseling enumerating the risks and benefits of the procedure before beginning an IVF-ET cycle. Research and treatment involving IVF-ET raise many questions of ethical importance now being widely debated by the public as well as within the medical and legal communities. A discussion of these issues will be made available by the American College of Obstetricians and Gynecologists in the near future.

References


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