Regular Review

Research in human in-vitro fertilisation and embryo transfer

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The present procedures for in-vitro fertilisation were derived from the studies of several groups working in the late 1960s and 1970s, who provided credence for Rock and Menkin's fascinating proposition that human fertilisation and cleavage could be obtained in vitro.[1] Most of the early technical advances in man were made by Edwards and Steptoe, including the demonstration that spermatozoa would penetrate oocytes in vitro,[2] the use of laparoscopy for recovery of oocytes,[3] and the growth of embryos to the blastocyst stage of development in vitro.[4] Soupart and Strong provided ultrastructural evidence of sperm penetration of oocytes, removing any doubt that human oocytes could be fertilised in vitro.[5][6] These reports raised the probability of successful in-vitro fertilisation as previously shown in animals by several biologists.[7]

Studies on human in-vitro fertilisation were terminated in the United States in the mid-1970s,[8][9] leaving Edwards and Steptoe in Britain and Wood and associates in Australia to continue the research. In 1973 the Australian group reported a transient rise of human chorionic gonadotrophin in one patient after transfer of an eight-cell embryo fertilised in vitro,[10] and the English group reported a tubal pregnancy in 1976 resulting from in-vitro fertilisation and embryo transfer.[11] The physiological basis for the in-vitro fertilisation technique was described by Edwards in 1973,[12] and various modifications then refined and improved the procedure. The birth of the first baby after in-vitro fertilisation was announced by Steptoe and Edwards in 1978,[13] reporting their procedures at a conference held in 1979 at the Royal College of Obstetricians and Gynaecologists in London. Their techniques were published in 1980.[14][15] Their four pregnancies and two liveborn babies marked the start of a rapid growth in research and clinical use of the technique for the treatment of human infertility. The Australian group was the first to repeat the success of the British group,[16] and major alterations to the procedure at that time led to a substantial increase in success rate.[17][18] By 1981 in-vitro fertilisation had been established as a clinical procedure[19][20] and pregnancies have now been reported in many other centres throughout the world.[21-25]

Three choices are available for the treatment of patients, depending on whether gonadotrophins or clomiphene citrate are used to stimulate the development of multiple follicles and whether or not the time of recovery of the oocytes is controlled by administering human chorionic gonadotrophin.

The first three babies born after in-vitro fertilisation were conceived by fertilisation carried out in the natural cycle.[14][16] In this approach patients are not treated with any drugs or hormones, and usually a single follicle will spontaneously develop each ovulatory cycle. The time of recovery of the oocyte is usually determined by daily assay of oestrogen concentration in the urine or plasma, and twice-daily assay of urine or plasma samples for luteinising hormone.[14][16][17][26-29] Ultrasound may also be used to aid the prediction of approaching ovulation.[16][17][29][30]

When in-vitro fertilisation is attempted in the natural ovulatory cycle,[17][31] the disadvantages include the need for protracted monitoring for the surge of luteinising hormone and for facilities and staff to be available 24 hours a day, seven days a week; furthermore, usually only one preovulatory follicle is present. The overall pregnancy rate in patients treated in this way is likely to be lower than in those receiving ovarian stimulation, in whom multiple follicles, oocytes, and embryos for transfer are potentially available.

In the stimulated ovulatory cycle patients are given clomiphene citrate,[17] human menopausal gonadotrophin,[22][32] or combinations of both.[32][33] Treatment may begin from the second to the fifth days after the start of menstruation and continue for five days, depending on the growth and development of follicles for each individual patient. The growth and development of follicles can be accurately assessed only by changes in daily plasma or urine samples with some help from ultrasound.[33-35] No human chorionic gonadotrophin is given to the patients in this group, and the spontaneous luteinising hormone surge has to be detected by twice-daily assay of urine or plasma samples.[17][34][35] This system improves the chances of obtaining oocytes, embryos for transfer, and pregnancies because of the development of multiple follicles,[17][18] but the other disadvantages of the natural ovulatory cycle still exist.

The procedure in the controlled ovulatory cycle differs from the stimulated cycle only by the injection of human chorionic gonadotrophin to control the final stage of maturation of the follicles and oocytes,[17] so reducing the need for staff and facilities being available 24 hours a day and for prolonged tracking of patients for the endogenous luteinising hormone surge. Controlled follicular maturation is very successful for obtaining pregnancies by in-vitro fertilisation[17][33] if the correct time is chosen for injection of human chorionic gonadotrophin.[34][35] Ultrasound cannot be used as the sole monitor to determine the time of injection of human chorionic gonadotrophin because it does not adequately assess the functional capacity of the follicles.[34] Success rates using ultrasound alone are likely to be unacceptably low, and few pregnancies have been reported from many cases attempted.[36][37]

Treatment regimens for ovarian stimulation are variable and depend on the experience of the clinic concerned. Generally 100 to 150 mg clomiphene citrate is given daily for five days beginning the third to the fifth days after the start of menses.[17]

Treatment with human menopausal gonadotrophin needs to be very carefully monitored because of the risk of hyper-stimulation. Doses of 75 to 225 international units of follicle-stimulating hormone may be given each day or every second day depending on the results of oestrogen assays in the urine or plasma and on the number of growing follicles responding to treatment with gonadotrophins.[32] Treatment with a combination of clomiphene and human menopausal gonadotrophin has also been successfully used in our own studies.[32]

In our programme blood sampling for oestradiol-17-beta begins on the seventh to ninth days of the menstrual cycle. An ultrasonic examination of the ovaries is made when plasma oestradiol concentrations rise above 1·8 nmol/l (500 pg/ml). Treatment with human menopausal gonadotrophin and clomiphene is varied according to the number of follicles larger than 1·0 cm. Three-hour urine samples are collected when total plasma oestrogen concentrations correspond to an amount of 1·5 nmol/l (400 pg/ml) for each large follicle present or when a follicle is seen to be 1·8 cm or larger. Usually only one ultrasonic examination is made unless all the follicles are less than 1·2 cm diameter. Urinary luteinising hormone is determined twice daily by rapid radioimmunoassay.[34 38] The start of the luteinising hormone surge is determined by a significant rise above the mean plus two standard deviations of the previous baseline.[34 35] Oocytes are recovered 24 to 26 hours after the start of the spontaneous luteinising hormone surge. Most patients are given human chorionic gonadotrophin before the endogenous luteinising hormone surge, provided the oestrogen concentrations continue to increase.[33 34] An oestrogen concentration of about 1·8 nmol/l should be present for each large follicle.

The amounts of progesterone in the plasma will usually begin to rise the day before the spontaneous luteinising hormone surge and may be used as an indicator for human chorionic gonadotrophin injection.[32 39] If oestrogen concentrations fall without a coincident luteinising hormone surge attempts at in-vitro fertilisation are discontinued for that cycle because of the poor results obtained in these circumstances: if human chorionic gonadotrophin is given the follicles are usually atretic, the oocytes rarely become fertilised, and pregnancy is very unlikely. The amounts of steroid in the follicular fluid are intimately related to success of in-vitro fertilisation,[40] and continued secretion of oestrogens is essential for the correct follicular response to luteinising hormone or human chorionic gonadotrophin.[39] Premature injection of human chorionic gonadotrophin leads to abnormal oocyte maturation, and oocytes obtained from small follicles have a reduced capacity for fertilisation and development into a normal embryo.[34]

Recovery of oocytes

Oocytes may be recovered by aspirating the follicles at laparoscopy[3] or under ultrasonic guidance[41] between 24 and 28 hours after the start of the urinary luteinising hormone surge[14 17] or 28 to 37 hours after the plasma luteinising hormone surge[26 42] or human chorionic gonadotrophin injection.[17] The technique of recovery of oocytes has been described in detail.[43-46] Recovery rates obtained by experienced laparoscopists are very high—usually of the order of 90% or more of the follicles aspirated. A range of aspiration needles may be used,[43 46] and a Teflon-lined needle and collecting kit are commercially available. Special precautions are required to ensure viability if laparoscopy is done with 100% carbon dioxide pneumoperitoneum[33]—indeed, nitrogen gas mixture may be preferable on those grounds, but there is an increased risk of embolism from the nitrogen gas.[44]

Fertilisation

A dramatic increase in the fertilisation rate can be achieved if it is delayed for five to six hours to allow completion of the maturation of the oocytes.[47 48] During this time the oocytes are incubated at 37°C in culture medium without spermatozoa. Semen obtained two hours before insemination from the husband is washed, prepared,[47] and added to the oocytes, so that a final concentration of 10 000 to 100 000 motile spermatozoa per ml of culture medium is obtained.[33 49] If necessary the husband's semen may be stored frozen and used for insemination after thawing and washing. In our own studies frozen semen gives similar fertilisation rates to those with freshly ejaculated semen. The spermatozoa may penetrate the oocytes within three to six hours,[50 51] and pronuclei may be readily identified by 12 hours.[47] The cumulus cells may be removed 12 to 20 hours after insemination for identification of pronuclei and confirmation of fertilisation.[47] Ultrastructural details of the events at fertilisation have been described.[5 6 52 53] Twenty to 25 hours after insemination the two pronuclei fuse at syngamy, which restores the normal diploid chromosome state[39] completing the process of fertilisation.

Embryo culture

Fertilisation and embryo culture may be carried out in the same medium, though more usually the cumulus cells are removed, and the fertilised oocyte is washed and placed in fresh culture medium.[47] Fertilisation and embryo growth may be obtained in droplets of culture medium under oil[54] or in test-tubes.[55] The cleavage intervals for human embryo development have been reported,[47 56 57] and the more rapidly fertilised and dividing embryos seem to be the ones which result in pregnancy after transfer.[47 58] The viability of the embryo is a complex matter; it depends on cellular integrity and biochemical capacity and is related to gross morphology and the cleavage rate.[39 47 58] There appears to be no association between the development of normal embryos or in the pregnancy rate and the different media used by various groups to culture embryos,[34 39] but the follicular steroid concentrations at the time of oocyte recovery appear to have a very substantial effect on embryo viability and capacity for implantation.[40] These follicular steroids, particularly oestradiol-17-beta and progesterone, are associated with the degree and normality of oocyte maturation.[34 35]

Quality control is essential for routine preparation of culture media,[20 59] as is the maintenance of osmolarity and pH during the handling of embryos and during culture in vitro. Culture conditions and media should have the potential for supporting the growth of embryos to the hatched blastocyst stage of development.[47 60] The fine structure of human embryo development of the later cleavage stages is essentially similar to that of other mammalian species.[60 61]

Embryo transfer

The procedures and results of embryo transfer have been

reviewed recently.[20] [59] [62] A fine catheter is passed through the cervical canal to deposit the embryos close to the uterine fundus. This does not require any anaesthesia, and the ease of transfer depends on the parity of the patient, the type of catheter used,[62] and the skill of the operator. Several types of catheter and procedures have been described.[15] [16] [62–64] The number of embryos transferred influences the pregnancy rate; indeed, the transfer of two or three embryos increases the rate by a factor of three above that obtained by the transfer of a single embryo.[39] Pregnancies have been obtained from the transfer of one-, two-, three-, four-, eight-, and 16-cell stage embryos[14] [17] [25] [39] [47] [59] to the uterus of the oocyte donor. There are not enough data from properly controlled trials to decide whether the pregnancy rate is related to cell stage, though in one study transfer of embryos of the two-cell to three-cell stage resulted in higher pregnancy rates than transfer of one-cell or four-cell stages.[39] The cell stage, combined with age after insemination, appears to be important for implantation.[39] [58] Treatment of patients at the time of embryo transfer with antiprostaglandins such as mefenamic acid or progestogens to reduce uterine contractility has not increased the pregnancy rates.[17] [65]

There have been few attempts to transfer human embryos into the uterine cavity through the wall of the uterus instead of the transcervical route, and none have been successful.[59] [62]

Pregnancy and fetal normality

Clinical aspects of the establishment and outcome of pregnancy have been reported for some of the early successes.[15] [65] [66] Abnormalities have included the abortion of a triploid fetus[15] and the birth of a child with transposition of the major vessels of the heart.[66] Until such time as more data are available to compare with the rate of abnormalities in spontaneous abortions[67] and natural births no one can say whether in-vitro fertilisation leads to any increase in abnormal fetuses. None of the aborted material in our own studies has been chromosomally aberrant.

There is an apparently higher incidence of abortion in pregnancy after in-vitro fertilisation, especially in new clinics establishing the technique. Miscarriage among our own patients has decreased to the level expected for patients with infertility (12% to 18%).

Ectopic pregnancies have been reported after in-vitro fertilisation[9] [68]; this phenomenon may be associated with incorrect placement of embryos in or near the Fallopian tube. In women with disease or abnormality of the tubes the normal embryo-transport mechanisms may be defective.

Luteal-phase defects in secretion of progesterone and oestradiol-17-beta have been reported in primates[69] and in patients[70] after follicular aspiration. Other studies, however, have shown no effect of follicular aspiration on these steroids[71] [72] —in agreement with our own observations.[39] No advantage has been reported for the establishment or maintenance of pregnancy by support of the luteal phase or early pregnancy with progesterone, progestogens, or human chorionic gonadotrophin.[14] [17] [65]

Indications for in-vitro fertilisation

In-vitro fertilisation was developed as a treatment for defective tubal function as a result of abnormality, disease, or sterilisation. In such cases it is necessary to have laparoscopic access to the ovaries, and this will depend on the skill of the laparoscopist. The development of ultrasonically guided aspirations[41] may increase the number of patients suitable for in-vitro fertilisation because recovery of the oocytes will not necessarily depend on laparoscopic visualisation of the ovaries.

In addition, unexplained infertility may be treated by in-vitro fertilisation because, contrary to earlier reports,[55] [73] there is no difference in the rate of fertilisation and embryo development or in the rate of implantation after embryo transfer between patients with tubal infertility and those with unexplained (idiopathic) infertility.[18] [74] Defects of gamete transport, particularly sperm transport, may influence fertility in this group of patients.

Successful fertilisation and pregnancy have been obtained in our studies in couples with the husband with severe agglutination of spermatozoa (sperm antibodies) and in some cases of severe oligospermia. The success of fertilisation, however, depends on the presence of at least some morphologically normal spermatozoa with forward progressive motility.

The age of the infertile woman also influences the success of in-vitro fertilisation. Most patients aged 45 years or older cannot be superovulated, and there are frequently abnormalities of the oocytes obtained. Our policy is not to treat women aged over 45.

Future of in-vitro fertilisation

The technique of in-vitro fertilisation could be applied to women with absent or non-functional ovaries, with known genetic disease, or who have completely inaccessible ovaries due to a pelvic condition, postoperative adhesions, or abnormality. In such cases donation of an oocyte would be required so that it may be fertilised by the husband's spermatozoa. This is similar to artificial insemination by donor semen and subject to similar legal and ethical considerations. Our group has ethical approval to study this procedure. In Australia, because of legal considerations associated with adoption, embryos cannot be transferred to surrogate mothers in cases of women with active ovaries who do not have a uterus or have an abnormal uterus.

Preservation of embryos or oocytes by deep freezing may be incorporated into the procedure of in-vitro fertilisation because it provides a method for preserving embryos for the patient. The ethical arguments have been presented.[75] In fact, there may be an obligation on in-vitro fertilisation clinics to develop freezing so that in circumstances where embryos cannot be transplanted—because of illness or unexpected difficulty—or where excess embryos develop they are not disposed of or used for other purposes. Preliminary results of freezing studies[58] [76] show that human embryos may survive freezing and thawing and continue growth and development in culture. There is currently no indication that the human oocyte can survive freezing and thawing, and there may be an increased chance of chromosomal abnormality because of the arrangement of chromosomes at metaphase; donor oocyte banks seem unlikely to be established in the immediate future. All embryos which are frozen are thawed and transferred to the original donor within a few months—or after birth if she is pregnant.

The manipulation, cloning, or the insertion of DNA by techniques of genetic engineering in human embryos is not considered ethical at present, though there may be arguments presented for the use of these techniques to identify and treat

genetic disease. These debates are presented elsewhere.[75] [77]

Treatment of various forms of male infertility by in-vitro fertilisation is an important prospect in the future[33] [75] and is the subject of research in several in-vitro fertilisation clinics.

Ethics of in-vitro fertilisation

Much has now been published on the ethics of in-vitro fertilisation.[20] [77-82] The major concern of opponents of in-vitro fertilisation is the manipulation and survival of human embryos and the concept of a human person and when this begins. Some groups consider that fertilisation is the beginning of a person, despite the ability of the embryos to divide into identical fetuses at a later time and the high loss of fertilised embryos which occurs in natural conception.[83] [84] With this view the process of in-vitro fertilisation cannot be easily accepted. Others believe, however, that an embryo conceived outside the body is not subject to the same considerations as one fertilised

in vivo. Others consider that implantation or some other stage during fetal development is the beginning of a person. The real difficulty is that there is no universal agreement on the matter. It is essential for scientists and clinicians working on the subject to do their utmost to preserve the uniqueness and developmental potential that in-vitro fertilisation initiates. There is now very little doubt that the technique of in-vitro fertilisation has a very important place in the treatment of human infertility, and the increasing success rates in the major clinics will ensure its further development—even though a few years ago this was considered unlikely.[85] [86]

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