

Assisted Reproductive Technology (ART)

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Glossary

anovulation Absence of ovulation even though menstruation may continue.

AID Artificial insemination with donor's sperm.

AIH Artificial insemination with husband's sperm.

assisted hatching The process of helping the blastocyst hatch from its shell (zona pellucida) by making a hole in the zona pellucida; may increase the chance of embryo implantation in some patients.

asthenospermia Poor motility of sperm in the seminal fluid.

azoospermia An absence of sperm in the seminal fluid.

blastocyst The stage at which the embryo has reached 5 to 6 days after fertilization, a fluid-filled cyst has formed, and the cells have begun to differentiate into the inner cell mass (which will form the fetus) and the trophoctoderm (which will form the placenta and fetal membranes).

chromosome/chromosomal abnormalities Thread-like structures within the nucleus that contain the hereditary material DNA, where mistakes can occur either in chromosome numbers (46 in humans) or in structure.

cleavage The mitotic division of the fertilized egg (zygote) into two or more cells; usually starts at about 26 h after fertilization.

corpus luteum A small endocrine organ that develops within the ruptured follicle after the oocyte has been released from the ovary; mainly produces the hormone progesterone.

cryobiology The use of low-temperature techniques on cells, tissues, and organs, usually by freezing and storage.

cryopreservation Freezing tissues and storing them in liquid nitrogen at -196°C (e.g., sperm, oocytes, ovarian and testicular tissue, embryos).

egg donation When a woman who has no fertility problems donates her eggs (oocytes) to another woman to enable her to carry pregnancy and have a child.

egg recovery (collection, pickup) When eggs are aspirated from the ovary; performed by vaginal ultrasound-directed method or by laparoscopy.

embryo The product of fertilization; at early stage of development, usually in the uterus, but also *in vitro* in the laboratory.

embryo biopsy Taking one or more cells (blastomeres) from an embryo (often at the 8-cell stage but can be at the blastocyst stage) for genetic or other analysis.

embryo transfer/replacement Stage in *in vitro* fertilization when the embryo is transferred to the uterus.

endometrium The lining of the uterus that develops at the beginning of each cycle so that it is ready to receive an embryo; endometrium breaks down, leading to menstruation, if there is no embryo.

endometriosis The presence of endometrium outside the uterus.

fallopian tubes A pair of small, fine, delicate tubes where fertilization usually takes place; transport ova and sperm to the fertilization site and transport the developing embryo to the uterus.

fertilization The penetration of the oocyte by the sperm, a process that results in the formation of an embryo.

follicle-stimulating hormone (FSH) A hormone produced by the pituitary gland in the brain; in women, stimulates ovulation and the production of estrogen; in men, stimulates the production of sperm.

gamete intra-fallopian transfer (GIFT) When eggs retrieved (usually under laparoscopy) are mixed with a prepared semen sample and introduced into the fallopian tube.

gametes Male and female reproductive cells (sperm and oocyte).

gonadotropin-releasing hormone (GnRH) Responsible for initiating the production of FSH and luteinizing hormone from the pituitary.

GnRH agonist Drugs that have a similar effect to GnRH and that activate the receptor sites in the gonadotropin secretory cells of the pituitary (e.g., buserelin, triptorelin, leuprolide) and finally suppress (down-regulate) the pituitary.

GnRH antagonist Drugs that compete with natural GnRH on its receptor but do not activate it, thereby blocking its effect (e.g., ganirelix, cetrorelix).

human chorionic gonadotropin (hCG) The hormone produced by placental cells in pregnancy; detection of this hormone in blood or urine is the basis of pregnancy testing; this hormone mimics the activity of luteinizing hormone and so is used for triggering ovulation.

hysterosalpingogram (HSG) An X-ray study in which a radio-opaque fluid is injected into the uterus so that the uterus and the fallopian tubes are visualized.

hysteroscopy The use of a fine optical instrument to view the inside of the uterus via the cervix.

implantation The embedding of an embryo in the endometrium of the uterus.

infertility Arbitrarily defined as the inability of a couple to produce a pregnancy after 1 year of intercourse with no contraception; one in six couples affected; can be described as primary or secondary infertility.

intrauterine insemination (IUI) Insemination of a prepared sperm sample into the uterus through the cervix.

intra-cytoplasmic sperm injection (ICSI) Injecting the sperm directly into the egg; major advance in male factor infertility.

in vitro fertilization (IVF) Fertilizing the egg in the laboratory for subsequent embryo transfer; requires egg retrieval/pickup; can be done in a natural or simulated cycle (when the ovaries are made to produce more eggs); after recovery, eggs placed in a plastic dish with a specially prepared semen sample, where fertilization may take place; pre-embryo then transferred to the uterus, where it may implant and develop further into a fetus and eventually a baby.

laparoscopy Procedure allowing visualization of abdominal organs using an optical instrument introduced through the abdominal wall.

luteinizing hormone (LH) A pituitary gonadotropic hormone that stimulates maturation and rupture of the follicle in women.

micromanipulation Procedures used (as an extension of IVF) to manipulate the embryo by blastomere biopsy, zona drilling, and the like.

microsurgical epididymal sperm aspiration (MESA) When sperm cells are aspirated from the epididymis and used in the IVF procedure.

ovarian hyperstimulation syndrome (OHSS) A complication of gonadotropin therapy in which there is excessive stimulation of the ovaries; women having treatment undergo frequent scans and hormone testing to prevent OHSS.

oligospermia A condition in which there are fewer sperm cells in the ejaculate than are considered "normal."

oocyte Female gamete or egg.

polycystic ovarian disease (PCOD) A condition where multiple small follicles appear in the ovary; abnormal imbalance can arise, causing problems in ovulation and infertility.

progesterone A hormone produced by the corpus luteum after ovulation has occurred; also produced by the placenta in pregnancy.

teratospermia A condition in which less than 50% of the sperm are morphologically normal.

testicular sperm aspiration (TESA) Obtaining sperm directly from the testis.

ultrasound scanning A procedure in which high-frequency sound waves are emitted into the pelvis and, as they bounce back, are used to build up a picture; used in many areas of medicine; can be performed abdominally or via the vagina in reproductive medicine; many organs (e.g., kidneys, liver, heart, uterus, ovaries) can be visualized.

Y chromosome One of the chromosomes (X or Y) that determine the sex of an individual; XX determines that the embryo will be female; presence of the Y chromosome determines that it will be male.

zygote intra-fallopian transfer (ZIFT) Similar to GIFT except that the embryo is transferred into the fallopian tube rather than sperm and eggs, where the advantage is that fertilization is established before transfer.

zona drilling The process of making a hole (by acid tyrode solution or laser) in the zona pellucida (the egg shell) to assist in the hatching process.

On July 25, 1978, Louise Joy Brown (Fig. 1), the world's first *in vitro* fertilization baby, was born in Great Britain. Infertility affects millions of couple worldwide. Before the birth of Louise Brown, those women who were found to have blocked or ruined fallopian tubes had no hope of becoming pregnant if surgical procedures failed to correct the situation.

INTRODUCTION

Patrick Steptoe and Robert Edwards (Fig. 2), two physicians from the United Kingdom, had been actively working on finding an alternative solution for conception since 1966. Although Steptoe and

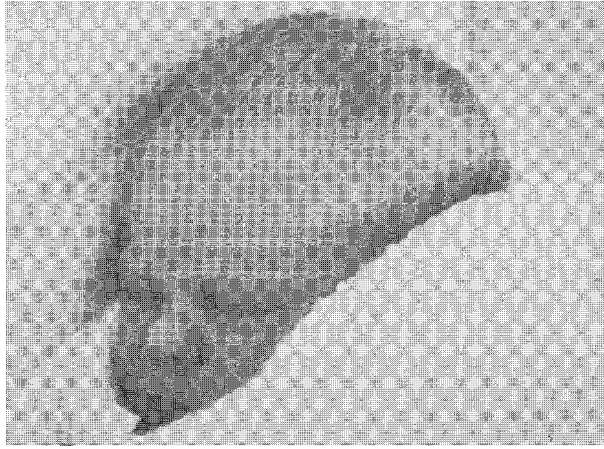


Figure 1 Louise Joy Brown, the world's first *in vitro* fertilization baby.

Edwards had successfully found a way in which to fertilize an oocyte outside a woman's body, they were still troubled by problems after replacing the fertilized egg back into the woman's uterus. By 1977, all of the pregnancies resulting from their procedure (approximately 80) had lasted only a few short weeks.

Lesley Brown, Louise Brown's mother, changed that when she successfully passed the first few weeks of pregnancy. This amazing achievement opened a new era in reproductive medicine, an era of rapid developments and achievements.

INDICATIONS

Pregnancy is always a matter of chance. A normal couple attempting pregnancy in their 20s has about a 20% chance of getting pregnant in any one month,

and couples in their 30s have about a 10% chance in any one month. Sooner or later, a couple will achieve pregnancy if the partners have (1) a reasonably high monthly chance of fertility (more than 5% per month) and (2) a reasonable time in which to keep trying (say, 2 years). If pregnancy has not happened within 1 or 2 years, then it is likely that the monthly chance of pregnancy will be less than 5%. Tests are then done.

For normal fertility, sperm in the vagina must swim up through the cervix, uterus, and fallopian tubes to meet an ovulated egg that has been carried from the surface of the ovary to the middle part of the fallopian tube. The embryo that results (strictly speaking, it is still a "pre-embryo") develops for 3 days in the fallopian tube. It then travels to the uterus, where it floats and develops for another 3 or 4 days before attaching to and implanting within the endometrium (lining of the uterus), thereby establishing pregnancy. A few days before the period is missed, a blood pregnancy test will be positive. It is at about this time of implantation, a week after fertilization, that the first few cells in the center of the "embryo" actually differentiate into what will be the fetus. All other cells (the majority of cells at this stage) go to form the placenta (Fig. 3).

In general, a couple may be relatively infertile (with a reduced monthly chance of conception) or completely infertile (with no chance of conception, sometimes called sterility). The following are the leading causes:

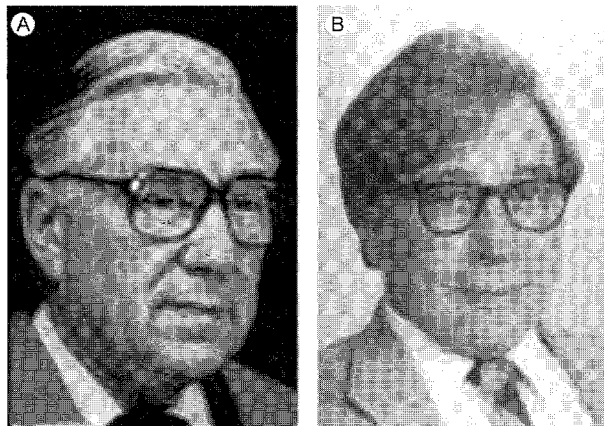


Figure 2 (A) Dr. Patrick Steptoe and (B) Dr. Robert Edwards.

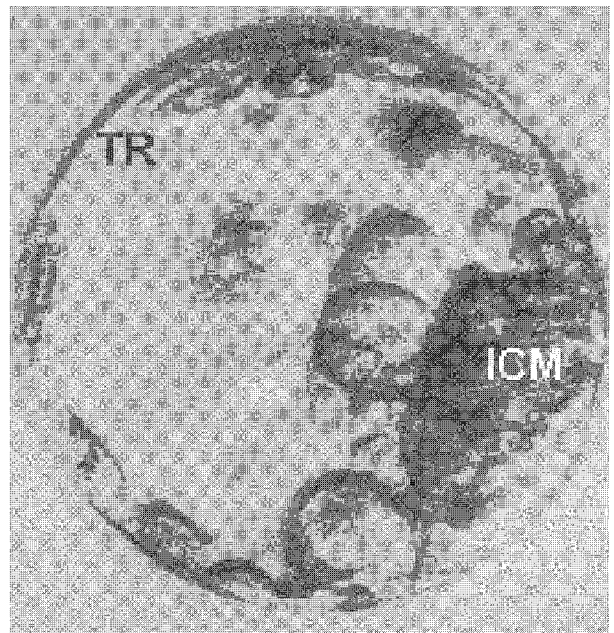


Figure 3 Embryo at the blastocyst stage. Inner cell mass (ICM) will produce the fetus itself. Trophoblast (TR) will produce the placenta and membranes.

- Problems with ovulation (the release of the egg from the ovary). There will be complete infertility if ovulation does not take place and periods are absent (amenorrhoea), although many such patients are treatable with hormones or drugs (patients with polycystic ovary syndrome). If there are no responsive eggs in the ovaries (ovarian failure), egg donation is required.

- Problems with sperm production (often not treatable except by assisted conception). There will be complete infertility if there are no sperm cells in the ejaculate (azoospermia).

- A blockage between the uterus and the ovary, preventing fertilization (Fig. 4). The most common site of blockage is the fallopian tubes, sometimes treatable with microsurgery or assisted conception.

- Endometriosis, a common condition in which the endometrium grows outside the uterus, disturbing a number of events essential to conception and implantation of the embryo in the uterus. Treatment can be medical, surgical, or with assisted conception. Infertility is usually relative rather than complete.

Fertility tests involve the following:

- Progesterone level in the second half of the menstrual cycle (luteal phase) to establish ovulation. Other hormonal tests (e.g., prolactin, luteinizing hormone [LH], follicle-stimulating hormone [FSH], thyroid-stimulating hormone [TSH], testosterone, estradiol) are also often done to assess endocrine integrity and ovarian reserve.

- Sperm count (or a postcoital test looking for sperm in the mucus of the cervix).

- X ray of the uterus and fallopian tubes (a hysterosalpingogram) or a laparoscopy to look at the pelvic organs.

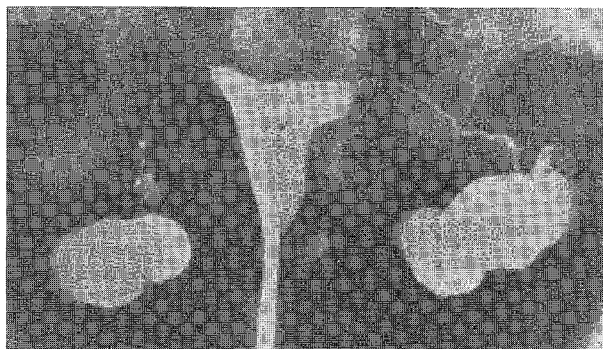


Figure 4 Mechanical infertility. Both fallopian tubes are greatly dilated and blocked.

These tests may show the following:

- Complete infertility (“sterility”): (a) ovarian failure, with no chance of inducing ovulation; (b) complete absence of sperm (“azoospermia”); or (c) complete obstruction of the fallopian tubes.

- Relative infertility: (a) infrequent ovulation or absent ovulation, resolved partly by treatment; (b) a decrease in the sperm count; (c) partial blockage of the tubes or the presence of scar tissue around the tubes or ovary; (d) endometriosis of any degree; (e) an abnormality of the uterus such as fibroids, polyps, or scarring of the lining; (f) an abnormality of the cervix such as a previous cone biopsy or inflammation (“cervicitis”); and/or (g) an immune reaction against sperm cells (“anti-sperm antibodies”) in either partner.

- Unexplained infertility: sometimes no abnormality is obvious.

Some causes of infertility can be overcome with drugs or surgical intervention. Otherwise, assisted reproductive technology (ART) is needed, with *in vitro* fertilization (IVF) being the cornerstone of this treatment.

SUPEROVULATION FOR IVF

The first step of the IVF procedure involves stimulation of egg growth. Spontaneously, a cycling woman will ovulate only one egg each month. This single egg (Fig. 5) may be used for IVF. In fact, the first IVF baby was produced in this way. However, the procedure is

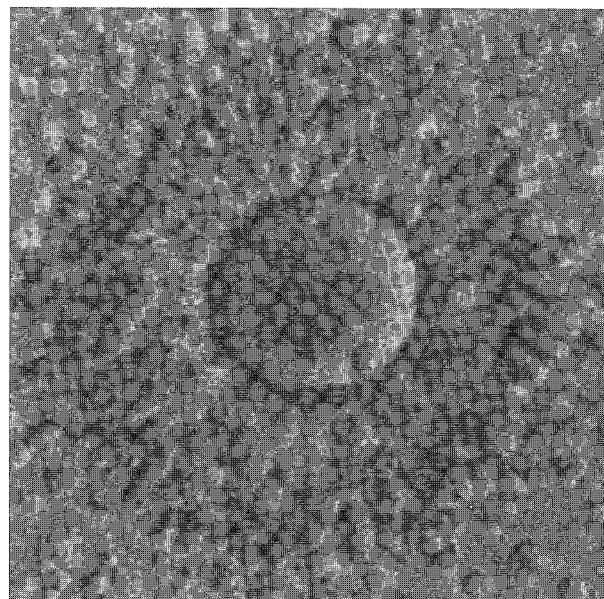


Figure 5 Mature oocyte surrounded by cumulus cells.

cumbersome and laborious because the natural cycle must be followed very carefully to time egg retrieval. To increase the chance of obtaining pregnancy, an effort is made to recruit 10 to 12 eggs, with an eye on patient safety to prevent ovarian hyperstimulation syndrome. This goal is achieved by stimulating the ovaries with drugs (e.g., Pergonal, Puregon, Humegon, Metrodin, Gonal-F, Menogon). These drugs contain gonadotropins produced from urine of postmenopausal women (e.g., Pergonal, Humegon, Menogon, Metrodin) or recombinant human FSH (e.g., Puregon, Gonal-F). Because spontaneous ovulation might occur, leading to cycle cancellation, other drugs are used to prevent the endogenous production of LH, which triggers ovulation.

Gonadotropin-releasing hormone (GnRH) agonists are a group of drugs (e.g., Decapaptyl, Leuprolide, Buserelin, Nafarelin) that activate the pituitary GnRH receptors. Following an initial burst of gonadotropins, prolonged exposure of these receptors to the GnRH agonist leads to pituitary down-regulation, with prevention of endogenous LH surge. Newer compounds act as GnRH antagonists (e.g., Orgalutran, Antagon, Cetrotide). They bind to the receptors but do not activate them (competitive inhibition).

The growth and development of the ovarian follicles are closely monitored by repeated ultrasound studies and blood tests for hormone levels (estradiol and progesterone). Based on the information obtained from these tests, the optimal timing for ovulation is determined. Ovulation itself is triggered by an injection of human chorionic gonadotropin (hCG) (e.g., Chorigon, Profasi, Pregnyl), after which the egg retrieval is scheduled (usually after 34–36 h).

THE LABORATORY

Egg Retrieval

The procedure itself is performed under general anesthesia or sedation at the hospital by a transvaginal route. A needle, guided by ultrasound imaging, is inserted through the vaginal wall into the ovaries, where the follicles containing the eggs are punctured and aspirated. The released eggs are transferred to the lab, where their developmental stage is assessed under the microscope.

Sperm Preparation

Sperm is obtained by masturbation. It then undergoes a series of lab procedures to prepare it for interaction

with the egg. During these procedures, the sperm is washed and resuspended in special medium.

Fertilization

Eggs are kept in dishes, to which sperm is added at the proper concentration. The dishes are kept in an incubator where the environment (temperature, humidity, and gas composition) is carefully monitored. In given time intervals, the eggs are assessed for fertilization and subsequent cleavage (divisions). This assessment is based on morphology; hence, the detailed chromosomal composition of the embryos cannot be addressed without further tests. Approximately 18 h after fertilization, the eggs are examined for the appearance of 2 pronuclei (maternal and paternal) (Fig. 6A). If the number of pronuclei is greater than 2, the eggs are discarded (Fig. 6B). On day 2 postfertilization, the eggs are examined for cleavage. The number

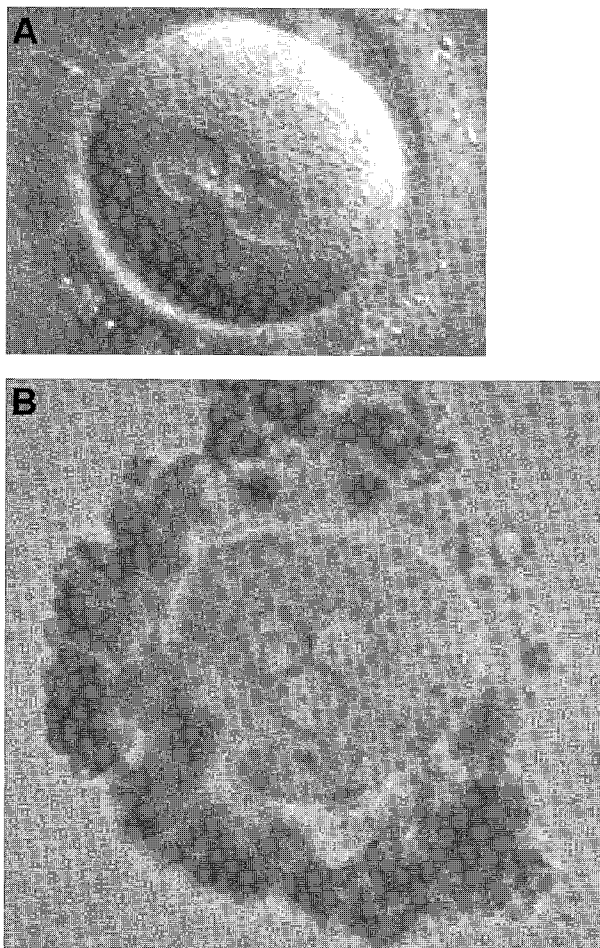


Figure 6 (A) Fertilized oocyte with 2 pronuclei clearly visible, indicating normal fertilization. (B) Fertilized oocyte with 3 pronuclei visible, indicating abnormal fertilization.

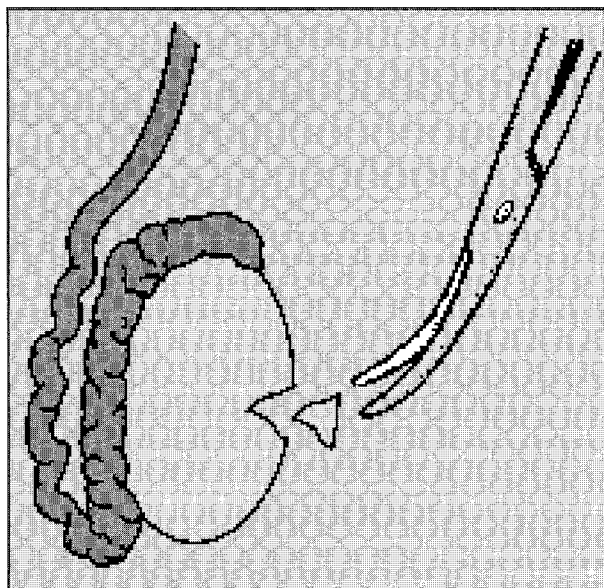


Figure 9 Testicular sperm extraction: A piece of testicular tissue is surgically removed, and sperm cells will be obtained from this tissue to be used in ICSI.

Microsurgical Epididymal Sperm Aspiration

Microsurgical epididymal sperm aspiration (MESA) involves obtaining immature sperm cells from the epididymis (which joins the testicle to the vas) in cases where obstruction in the genital tract leads to absence of sperm in the ejaculate. The recovered sperm can be used for ICSI, either immediately or after cryostorage.

Testicular Sperm Aspiration

Testicular sperm aspiration (TESA or TESE) (Fig. 9) is a surgical procedure to obtain sperm from within the testicular tissue in azospermic patients.

Preimplantation Genetic Diagnosis

In preimplantation genetic diagnosis (PGD), a single blastomere is obtained by embryos biopsy (Fig. 10) at the 6- to 8-cell stage (3 days postfertilization). The chromosomal composition of this cell is assessed by DNA hybridization technology, or a potential abnormal gene is targeted by polymerase chain reaction (PCR). If found to be normal, the embryo is transferred to the uterus. Abnormal embryos are discarded.

Blastocyst Culture

The first stage, when embryo differentiation is observed, is the blastocyst stage. Embryos reach that stage 5 to 6 days after egg retrieval (Fig. 11). In “natural pregnancy,” the embryo reaches the uterus at this stage. In some cases (e.g., patients after repeated IVF failure),

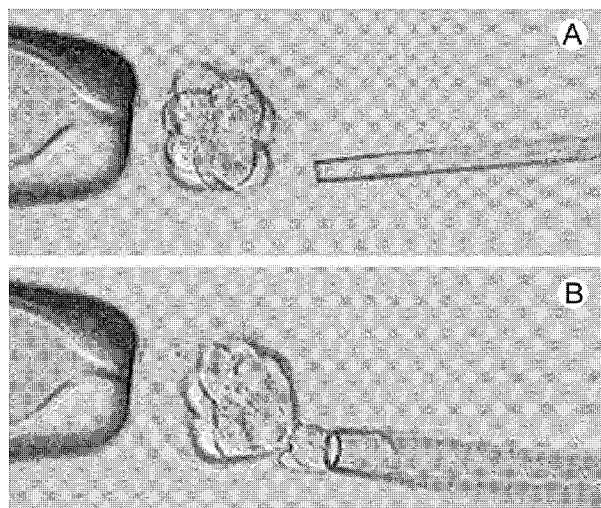


Figure 10 (A) Embryo biopsy: An 8-cell embryo is held, while a biopsy capillary is introduced through a hole in the zona pellucida. (B) Embryo biopsy: A single cell is gently removed from the embryo.

embryo culture up to the blastocyst stage should be considered. In addition, this technology may help to minimize the multiple pregnancy rate because the implantation potential of embryos at the blastocyst stage is higher than that of embryos at the cleavage stage (days 2–3); therefore, 1 or 2 transferred blastocysts have a good chance of achieving pregnancy.

OUTCOME

In general, it is recommended that the patient should refrain from physical exertion following embryo

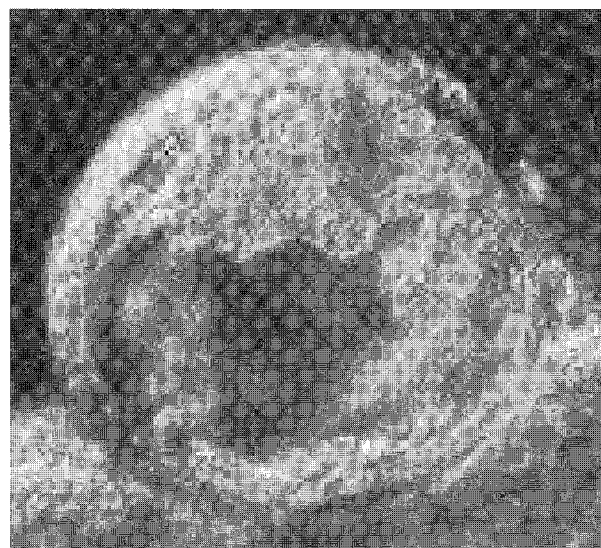


Figure 11 An embryo at the blastocyst stage.

transfer; however, complete bed rest does not seem to be necessary. The presence of pregnancy is established by using a sensitive test for hCG, which is the hormone secreted by the placenta. The test is done 14 days after egg retrieval. If pregnancy is established, monitoring is continued until ultrasound imaging allows a direct visualization of the developing fetus. Success depends on many variables. The most important predictor of success is the age of the female patient. Current IVF technology can deliver approximately a 20% implantation rate (the number of gestational sacs that develop in the uterus divided by the number of transferred embryos). This is close to the natural implantation rate.

Complications

Multiple Pregnancy

Usually, 1 to 3 embryos are transferred to the uterus in a given cycle, with the hope that 1 or at most 2 will implant and develop. However, occasionally the procedure “over-succeeds” and it is found that a patient has a triplet pregnancy. The outcome of these pregnancies is not optimal given that premature delivery is the rule, with potential severe impact on the newborns. In these cases, selective fetal reduction should be considered.

Ovarian Hyperstimulation Syndrome

Fertility drugs override the natural process of egg development for the purpose of obtaining many eggs. Occasionally, a patient may develop a large number of eggs, a process that can give rise to a clinical syndrome known as ovarian hyperstimulation syndrome (OHSS). The syndrome is characterized by lower abdominal pain, ovarian cysts, and (in its severe form) accumulation of fluid within the abdomen. Occasionally, hospitalization is required, and in extreme cases, termination of pregnancy is necessary.

Increased Risk of Cancer?

About 1 in 10 women will develop cancer of the breast at some stage of their lives. About 1 in 90 women who live to their 70s will develop cancer of the ovary. Because IVF has become a common procedure, it is understandable that quite a number of cancers will develop among women once they are treated in this way. However, like pregnancy itself, stimulating the ovaries during IVF causes the ovarian hormones to reach high levels, and this may accelerate the development of a breast cancer (or an ovarian cancer) that is already present but that has not been detected. It is not known whether the long-term risk of breast

cancer is increased after repeated IVF treatments, although studies are currently under way. There is a strong presumption that repeated IVF cycles, especially if there has been no pregnancy, are likely to increase the risk of cancer of the ovary during later life. There are two reasons for this. First, some studies have implied that the use of fertility drugs is associated with an increase in risk. Second, it is known that anything that stops ovulation and menstruation, such as having been on birth control pills for a number of years or having been pregnant and having breast-fed, is rather protective against later cancer of the ovary. Ovulating 10 eggs in 1 month in an IVF program may have the same effect as 10 months of normal ovulation in terms of risk.

THE FUTURE

Current efforts focus on technologies that will let us know which embryo in the lab has the potential to implant and become a healthy baby. Routine use of techniques such as PGD and assessing biochemical markers will improve implantation rates.



Figure 12 Dolly, the cloned sheep.

The ability to produce embryos *in vitro* has introduced us to cloning and stem cell technology. In short, cloning is based on enucleation of a mature unfertilized oocyte and injection of a nucleus taken from an adult diploid cell. The oocyte cytoplasm has the capacity to reprogram and de-differentiate the donor nucleus to be followed by cleavage and embryo development. Healthy newborns have been produced in domestic animals (with Dolly the cloned sheep being the first [Fig. 12]), although with low efficiency and high rates of abortion, congenital malformations, and neonatal deaths.

The inner cell mass detected at the blastocyst stage is composed of cells that have the potential to differentiate to any tissue. These cells can be kept undifferentiated in culture for a long time, and can serve as a source for any needed tissue, once the signal for differentiation is known. Theoretically, it will be possible to produce any type of tissue or organ to suit the individual patient's needs. A major advantage of "therapeutic cloning" is that immunity-based rejection will be avoided because the genetic material of the implanted cells/organs will be identical to that of the patient.

See Also the Following Articles

Endometriosis • Fertilization • Gonadotropin-Induced Ovulation • In Vitro Fertilization (IVF) • Infertility, Overview • Ovarian Failure Treatment Strategies: Egg Donation • Pregnancy Endocrinology • Premature Ovarian Failure • Superovulation and Intrauterine Insemination

Further Reading

Bolton, V. N., Wren, M. E., and Parsons, J. H. (1990). Preimplantation genetic diagnosis. *Brit. Med. J.* **301**, 1277.

- Bolton, V. N., Wren, M. E., and Parsons, J. H. (1991). Pregnancies after *in vitro* fertilization and transfer of human blastocysts. *Fertil. Steril.* **55**, 830–832.
- Brinsden, P. R. (ed.) (1999). "A Textbook of *in Vitro* Fertilization and Assisted Reproduction: The Bourn Hall Guide to Clinical and Laboratory Practice," 2nd ed. Parthenon, Pearl River, NY.
- Kol, S., and Itskovitz-Eldor, J. (2000). Severe OHSS: Yes, there is a strategy to prevent it! *Hum. Reprod.* **15**, 2266–2267.
- Nygren, K. G., and Andersen, A. N. (2001). Assisted reproductive technology in Europe, 1997: Results generated from European registers by ESHRE, European IVF-Monitoring Programme (EIM), for the European Society of Human Reproduction and Embryology (ESHRE). *Hum. Reprod.* **16**, 384–391.
- Out, H. J., Mannaerts, B. M. J. L., Driessen, S. G. A. J., *et al.* (1995). A prospective, randomized, assessor-blind, multicentre study comparing recombinant and urinary follicle stimulating hormone (Puregon versus Metrodin) in *in vitro* fertilization. *Hum. Reprod.* **10**, 2534–2540.
- Palermo, G., Jpriss, H., Devroey, P., *et al.* (1992). Pregnancies after intracytoplasmic sperm injection of a single spermatozoon into an oocyte. *Lancet* **340**, 17–18.
- Porter, R. N., Smith, W., Craft, I. L., *et al.* (1984). Induction of ovulation for *in-vitro* fertilization using buserelin and gonadotropins. *Lancet* **2**, 1284–1285.
- Silber, S. J., Van Steirteghem, A. C., Liu, J., *et al.* (1995). High fertilization and pregnancy rate after intracytoplasmic sperm injection with spermatozoa obtained from testicle biopsy. *Hum. Reprod.* **10**, 148–152.
- Society for Assisted Reproductive Technology and American Society for Reproductive Medicine. (2000). Assisted reproductive technology in the United States: 1997 results generated from the American Society for Reproductive Medicine/Society for Assisted Reproductive Technology Registry. *Fertil. Steril.* **74**, 641–654.
- Stephens, P. C., and Edwards, R. G. (1978). Birth after the re-implantation of a human embryo. *Lancet* **2**, 366.
- Trounson, A., and Mohr, L. (1983). Human pregnancy following cryopreservation, thawing, and transfer of an eight cell stage human embryo. *Nature* **305**, 707–709.
- Wilmut, I., Schnieke, A. E., McWhir, J., *et al.* (1997). Viable offspring derived from fetal and adult mammalian cells. *Nature* **385**, 810–813.