



Advanced Information

Human in vitro fertilization

The 2010 Nobel Prize in Physiology or Medicine is awarded to Dr. Robert G. Edwards for the development of human in vitro fertilization (IVF), a medical advance that represents a paradigm shift in the treatment of many types of infertility. The inability to conceive a child is a reproductive defect that afflicts more than 10% of all couples worldwide. During the 1950s, Edwards came to realize the potential of IVF as a treatment for this medical condition. What inspired him to take on this challenge was his research on how hormones control critical ovarian functions in mice, such as oocyte maturation and ovulation. By a brilliant combination of basic and applied medical research, Edwards overcame one technical hurdle after another in his persistence to discover a method that would help to alleviate infertility. He was the first to show that human oocytes could undergo in vitro maturation, as well as fertilization in vitro. He was also the first to show that in vitro fertilized human oocytes could give rise to early stage embryos and blastocysts. All of Edwards' accomplishments came together at 11.47 PM, on July 25 1978 with the birth of Louise Joy Brown, the worlds' first child conceived through IVF. Dr. Robert G. Edwards' research has completely transformed the field of reproductive medicine and today close to 4 million babies have been born thanks to the discovery of human IVF.

Introduction

Infertility is a widespread condition known to affect more than 10% of all couples worldwide. It is regarded as psychologically stressful by most individuals and can lead to depression, social isolation and a lower quality of life¹. Historically, little medical help has been available to infertile individuals, who were therefore forced to risk their health and even lives, by taking part in more or less obscure infertility-treatment practices. Female infertility is often

due to damage to the Fallopian tubes, obstructing a contact between the egg and the sperm (Fig. 1), whereas male infertility is linked to impaired sperm quantity and quality.

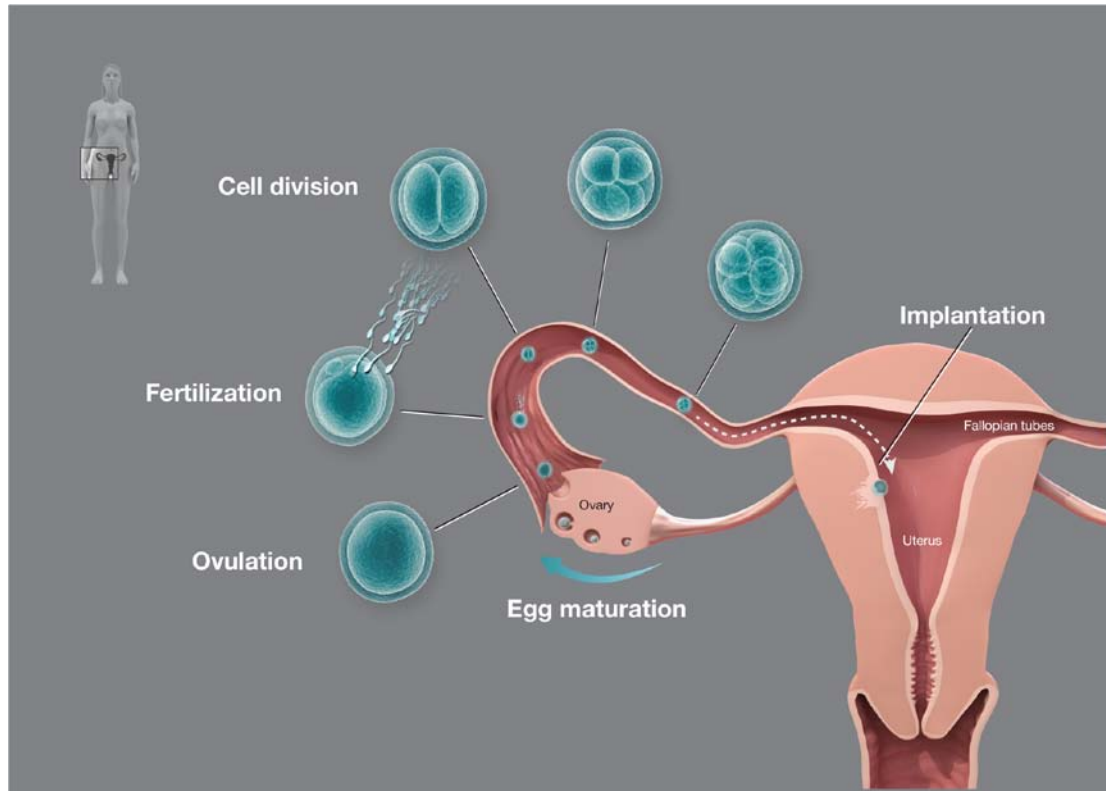


Fig. 1. The fertilization process in humans.

Female gametes are stored in the ovaries as separate follicles, i.e. each follicle contains one germ cell surrounded by one or several layers of granulosa cells. Humans (as well as other mammals) are born with a defined pool of primordial oocytes which are arrested at the dictyate stage of meiosis I. In sexually mature women, follicle stimulating hormone signaling and other factors stimulate the maturation of individual follicles on a monthly basis, generating primary and secondary follicles. In response to a rapidly increased concentration of luteneising hromone, a cascade of events are initiated, including further oocyte growth and meiotic resumption. Reactivation of meiosis completes the first meiotic chromosome segregation event and results in one set of chromosomes that become arrested at the metaphase stage of meiosis II, and a second set of chromosomes that are discarded (the first polar body). Following this, the mature follicle ruptures and ovulation ensues, a process in which the egg is is released from the ovary into the fallopian tubes (the oviduct). Sperm, entering from the uterus, will move towards the released egg from the opposite end of the fallopian tubes. Successful penetration of a single sperm through the egg coat will initiate a set of sperm-egg interaction events that relieves the meiosis II arrest of the egg. This results in the formation of two haploid sets of chromosomes, one set that will fuse with the haploid set of chromosomes contributed by the sperm, and a second set that is discarded (the second polar body). The fertilization process give rise to an embryo that undergoes a number of cell divisions while being transported through the fallopian tubes towards the uterus. Once in the uterus, the embryo (now at the blastula stage) will implant into the wall of the uterine lining, called the endometrium. Further embryo development will then take place at this location.

Early research on in vitro fertilization in mammals

The in vitro fertilization process was first studied in non-mammalian species, for example marine animals, where the fertilization process most often takes place outside the body in an aquatic environment. The first observation of sperm penetration into an egg was reported in *Ascaris* by Nelson in 1851 and subsequent studies in non-mammalian species have provided many important details of the fertilization process.

Gregory Pincus, at the Worcester Foundation for Experimental Biology in the US, described in 1935 the first experimental conditions that allowed mammalian oocytes (from rabbit) to mature in vitro, reaching the metaphase stage of meiosis II². Min Chueh Chang, at the same research institute as Pincus, showed in 1959 that in vitro-matured rabbit oocytes could be fertilized in vitro and also give rise to viable embryos³. Furthermore, when these embryos were transferred back to adult females, they gave rise to viable offspring³. Chang's findings represented a significant advancement, however, a caveat with these studies was the necessity to pre-incubate sperm in the uterus of a pregnant female prior to attempting to fertilize the oocytes³. The reason why Chang did not use strict in vitro conditions in these experiments was a general belief at this time that sperm required activation (capacitation) in vivo to contribute to fertilization in vitro^{4, 5}. Ryuzo Yanagimachi and Min Chueh Chang in 1963 showed that this dogma was incorrect, when they identified experimental in vitro conditions through which spermatozoa (from hamster) without prior in vivo activation, could fertilize oocytes and give rise to 2-cell stage embryos⁶.

Human in vitro fertilization - a monumental challenge

In the first part of the 20th century, researchers studying reproduction began to discuss the possibility of defining conditions that would allow human oocytes to be fertilized in vitro. The immense complexity of the fertilization process was a challenge and despite advances in animal reproductive research, no progress had yet been made regarding IVF of human oocytes in the early 1960s. Several technical advances and discoveries would be required before successful human IVF could be achieved; the ability to control the oocyte maturation process, the ability to retrieve oocytes at a developmental stage suitable for IVF, the ability to

activate sperm in vitro, the ability to define conditions that would promote fertilization as well as early embryo development in vitro and finally, a method through which early embryos could be transferred back to the uterus of the mother.

Robert G. Edwards, working at the National Institute for Medical Research in London in the late 1950s, was committed to develop a method that would alleviate human infertility. Edwards had an exceptionally broad knowledge of the fertilization process, gained through many years of basic research on animal reproductive physiology, and he was therefore well prepared for this challenge⁷⁻¹⁴. The first problem that he had to solve was to find a method that provided access to mature oocytes suitable for IVF. His first choice was to try to identify conditions that would promote maturation of human oocytes in vitro. He knew from the work of Pincus that mammalian oocytes seemed to require only a few hours of cultivation in vitro before they assumed meiotic maturation². Starting from immature human oocytes that had been released from ovarian tissues, Edwards tried for several years to find in vitro conditions that would activate these dormant oocytes. Edwards' work was rewarded in 1965 when he discovered that human oocytes, in contrast to the prevailing dogma, required 24 hours of incubation in vitro, before they would initiate their maturation process^{15, 16}. Importantly, the in vitro maturation method also provided oocytes at a late developmental stage (the metaphase stage of meiosis II), suitable for IVF. Edwards' next research challenge was to find conditions that would promote fertilization of oocytes in vitro. Barry D. Bavister, a graduate student of Edwards at Cambridge University, had recently identified buffer conditions to support in vitro activation of hamster sperm¹⁷. Edwards, using these buffer conditions, showed in 1969 that activated human spermatozoa could promote fertilization of in vitro matured oocytes¹⁸. This result represents an important discovery and a milestone in reproductive research as it opened the way for the development of a method to treat infertility.

The Breakthrough

Edwards' research on in vitro maturation of human oocytes, however, had given him a crucial insight. He had found that while human oocytes that had matured in vitro could be fertilized, they failed to progress beyond the 2-cell stage. This failure could be attributed to the lengthy periods that in vitro matured oocytes had to spend outside the body. Edwards now instead decided to try to use oocytes that had completed their maturation process in vivo. Edwards postulated that if mature human oocytes could be retrieved from the ovary prior to the onset of ovulation, these oocytes would be more competent to undergo IVF and early embryo development (Fig. 1 and Fig. 2).

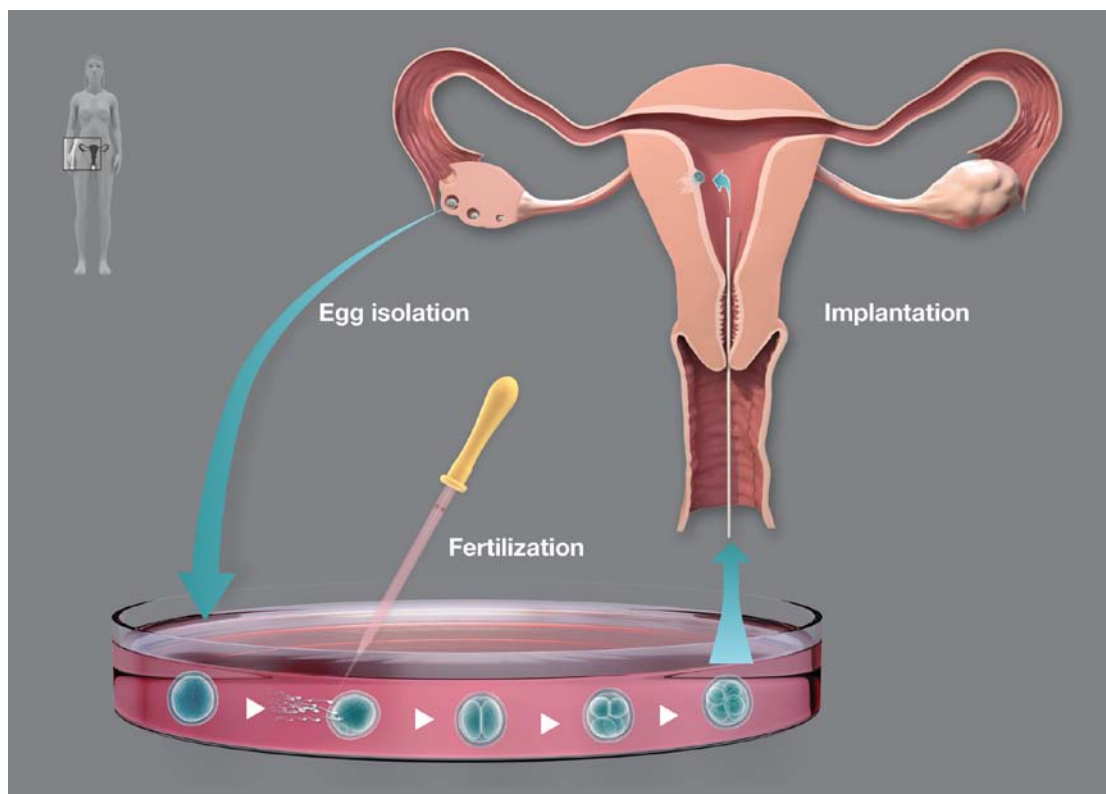


Fig. 2. The principle for IVF as developed by Edwards. Oocytes arrested at the metaphase stage of meiosis II are retrieved prior to ovulation from the ovary by laparoscopy. The oocytes are placed in a culture dish with medium and mixed with sperm. The medium condition promotes sperm activation in vitro, a necessary requirement for the fertilization process. The egg-sperm interactions relieve the meiosis II arrest of the egg. This results in the formation of two haploid sets of chromosomes, one set that will fuse with the haploid set of chromosomes contributed by the sperm, and a second that is discarded (the second polar body). The fertilization process results in the formation of an embryo that undergoes a number of cell divisions in vitro. The embryo is transferred back to the uterus at the eight-cell stage (2.5 days after onset of fertilization) using a thin needle. The embryo will divide further in the uterus until it reaches the blastula stage and thereafter implant into the wall of the uterine lining, the endometrium. Further embryo development will take place at this location.

Edwards' new approach arose from his previous work on the reproductive biology of the mouse. He had shown that initiation of meiotic maturation of oocytes could be controlled by externally provided gonadotrophins, i.e. hormones that mimicked the function of the intrinsically acting hormone (luteneising hormone)^{12, 13}. He also knew that it took an equally long time in vitro and in vivo to mature mouse oocytes to the metaphase II stage of meiosis, and from his in vitro studies of human oocytes, also the timing of the meiotic maturation process in humans was known. However, Edwards' (now at Cambridge University) new strategy raised an important technical problem; no method known to him could retrieve a sufficient number of human oocytes from the ovary at the correct stage of development. In the late 1960s, access to human oocytes required the surgical removal of a small part of the ovary from infertile women, an approach not suitable for IVF. Reading a scientific article written by Dr. Patrick C. Steptoe¹⁹, Edwards became aware of a new method called laparoscopy. Laparoscopy allowed the human female reproductive tract to be visualized by a fiber-optic endoscope inserted through an incision near the navel. Steptoe (working at the Oldham and District General Hospital) was a skilled surgeon and obstetrician, who had introduced and developed the use of laparoscopy in England and shown that it was possible to aspirate oocytes from the ovary. Edwards immediately realized that this method could be used to retrieve oocytes at the metaphase stage of meiosis II from the ovary during a suitable period of the menstrual cycle. Edwards therefore contacted Steptoe and they showed in 1970 that mature preovulatory oocytes at the metaphase II stage of meiosis could indeed be retrieved from infertile women after priming ovaries with gonadotrophins²⁰.

Edwards subsequently reported that IVF of preovulatory oocytes using in vitro activated sperm could give rise to 8-cell stage human embryos²¹. This was a seminal finding in two respects, it was the first time that in vitro activated sperm had been shown to be capable of contributing to embryo development beyond the 2-cell stage in a mammalian system and it was the first time human embryos had been shown to undergo cell divisions in vitro (Fig. 3). Following this, he then showed in 1971 that human oocytes fertilized in vitro could undergo further

cleavage generating 16-cell stage embryos and forming blastocysts in vitro²². The series of discoveries made by Edwards during 1969-1971 represent important milestones in IVF research and set the stage for the next phase to come.

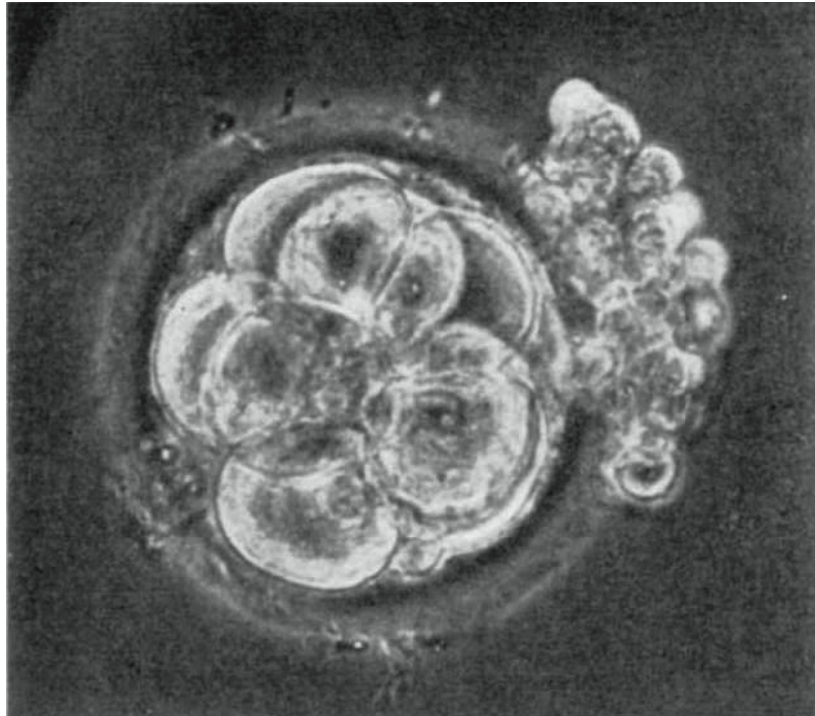


Fig. 3. Picture of 8-cell stage human embryo resulting from IVF (Fig. 2., in Edwards et al (1970) Nature, vol 227:1307-1309).

In the early 1970s, Edwards and Steptoe started to transfer the early embryos that resulted from IVF back into women. After more than one hundred attempts that all led to short-lived pregnancies, they realized that the hormone treatments given to women to induce oocyte maturation disturbed implantation of the embryo in the uterus, resulting in spontaneous abortions. Finally, after a change in the hormone treatment protocol, the first successful pregnancy was achieved in 1976²³. Unfortunately, the embryo had implanted ectopically in the Fallopian tube and the pregnancy had to be terminated. Edwards and Steptoe then decided to abandon the ovarian stimulation protocol altogether and instead rely on the natural menstrual cycle of the patients, although this meant that they would have

access to only one egg per cycle. Based on the concentration of luteinizing hormones in the urine of the women, they could predict when the maturing oocyte would reach the metaphase stage of meiosis II in vivo. They hoped that they then would be able to retrieve the egg by laparoscopy before ovulation occurred. Steptoe and Edwards succeeded in their efforts and in 1978 they made the historic announcement that a normal, fit and healthy baby, Louise Joy Brown, had been born through successful IVF of a human oocytes^{24, 25}. Edwards' long-term vision and persistence had finally come to fruition, opening up a new era in the treatment of infertility (Fig. 4).

Letters to the Editor

BIRTH AFTER THE REIMPLANTATION OF A HUMAN EMBRYO

SIR, — We wish to report that one of our patients, a 30-year-old nulliparous married woman, was safely delivered by caesarean section on July 25, 1978, of a normal infant girl weighing 2700 g. The patient had been referred to one of us (P.C.S) in 1976 with a history of 9 years' infertility, tubal occlusions, and unsuccessful salpingostomies done in 1970 with excision of the ampullæ of both oviducis followed by persistent tubal blockages. Laparoscopy in February, 1977, revealed grossly distorted tubal remnants with occlusion and peritubal and ovarian adhesions. Laparotomy in August, 1977, was done with excision of the remains of both tubes, adhesiolysis, and suspension of the ovaries in good position for oocyte recovery.

Pregnancy was established after laparoscopic recovery of an oocyte on Nov. 10, 1977, in-vitro fertilization and normal cleavage in culture media, and the reimplantation of the 8-

cell embryo into the uterus 2¹/₂ days later. Amniocentesis at 16 weeks' pregnancy revealed normal α -fetoprotein levels, with no chromosome abnormalities in a 46 XX fetus. On the day of delivery the mother was 38 weeks and 5 days by dates from her last menstrual period, and she had pre-eclamptic toxæmia. Blood-pressure was fluctuating around 140/95, œdema involved both legs up to knee level together with the abdomen, back, hands, and face; the blood-uric-acid was 390 μ mol/l, and albumin 0.5 g/l of urine. Ultrasonic scanning and radiographic appearances showed that the fetus had grown slowly for several weeks from week 30. Blood-œstriols and human placental lactogen levels also dropped below the normal levels during this period. However, the fetus grew considerable during the last 10 days before delivery while placental function improved greatly. On the day of delivery the biparietal diameter had reached 9.6 cm, and 5 ml of amniotic fluid was removed safely under sonic control. The lecithin: sphingomyelin ratio was 3.9:1, indicative of maturity and low risk of the respiratory-distress syndrome.

We hope to publish further medical and scientific details in your columns at a later date.

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Fig. 4. A copy of the Letter to the Editor in Lancet (1978) 2:366, documenting the birth of the first IVF baby, Louise Joy Brown.

Further developments of IVF

Following the birth of Louise Brown, Edwards and Steptoe founded an infertility clinic at Bourn Hall, in Cambridge, UK, where they continued to develop the IVF

method. The second and third child in the world conceived through IVF was born at Bourn Hall Clinic²⁶. Bourn Hall rapidly became a center for IVF research and successful modifications were made to the experimental protocols used for hormonal ovarian stimulation and embryo cultivation at Bourn Hall Clinic^{27, 28}, resulting in 139 births by 1983 and 1000 births by 1986. Rapid advances in IVF methodology now also began to take place outside the Bourn Hall Clinic and by 1986 about 1000 additional births had been reported in other countries. Today, close to 4 million babies have been born worldwide as a result of IVF²⁹. A large majority of infertile women can now be helped to conceive a baby as a result of IVF³⁰. The first generation of children conceived through IVF, including Louise Joy Brown, are now of reproductive age. Several of them have had children of their own, without the need for IVF.

Further medical developments

Edwards' seminal achievements attracted many other researchers to the field of reproductive medicine, resulting in rapid technical development. The laparoscopic recovery of oocytes was replaced by a vaginal ultrasound-guided oocyte recovery method³¹ and cryopreservation of surplus human embryos was introduced³². Successful IVF of in vitro matured human oocytes was reported in 1994³³, a method important to women that are sensitive to ovarian hormone stimulation protocols and to women that risk losing their ovarian pool of oocytes due to treatment of cancer. The development of intra-cytoplasmic sperm injection (ICSI), in which single sperm are microinjected into the cytoplasm of the mature egg, represented a technological breakthrough, making it possible to also to treat many categories of male infertility³⁴.

Edwards' work on human embryonic cells and blastocysts^{21, 22} was also instrumental for later work that resulted in the derivation of human embryonic stem cells³⁵, which has been important for our understanding of cellular differentiation, and may become important in regenerative medicine in the future. The IVF method has also been instrumental for the development of preimplantation genetic diagnostics (PGD). PGD is a procedure performed in vitro on in vitro fertilized early embryo cells to reduce the risk that parents

transmit a severe genetic disorder or a chromosomal abnormality to their children^{36,37}.

Health status of offspring conceived through in vitro fertilization

Children born after IVF are in general as healthy as children born after natural conception according to several long-term follow-up studies³⁸⁻⁴². There is, however, a higher frequency of multiple births associated with IVF treatments compared to normal pregnancies³⁹⁻⁴². This is largely due to the practice at some infertility clinics of transferring two or more embryos back into the mother. Multiple births are associated with an increased risk for preterm birth, low birth weight and cesarean sections, factors that could give rise to perinatal and postnatal health problems. Many European countries have introduced mandatory or voluntarily regulatory procedures that insist on single embryo transfers, which have dramatically reduced the incidence of multiple births after IVF³⁸⁻⁴². Despite the introduction of the single embryo transfer policy, a two-fold increased risk for preterm birth for women undergoing IVF treatment remains⁴². This could be explained by the older age of these women or by factors related to the underlying cause of their infertility. It has been shown that the use of IVF slightly increases the frequency of two imprinting disorders, Beckwith-Wiedemann Syndrome (BWS) and Angelman Syndrome (AS), although the absolute risk is still small as both diseases are very rare in the general population⁴³. Meta-analyses of controlled studies have reported an increased risk of major malformations (defined as a condition that causes functional impairment or requires surgical correction) in children conceived through IVF, however, the underlying studies lack appropriate control groups and have significant methodological limitations^{39,44}.

Ethical considerations

Edwards realized from the onset that IVF research would raise many important ethical concerns that had to be addressed. He wrote, together with the lawyer David Sharpe, a visionary key paper that initiated a debate on many of the

complicated issues related to reproductive medicine that lay ahead⁴⁵. They argued that research on human germ cells and embryos should be conducted under strict ethical guidelines. Edwards himself acted forcefully on these issues, as he ensured that an Ethics Committee for IVF was created at Bourn Hall Clinic. Since 1978 Edwards has taken a very active part in ethical discussions on many different aspects of human reproductive research. Despite Edwards' persistent attention to ethical and safety questions, his work on IVF initially met with strong opposition from religious leaders saying that this was morally wrong, from government officials who felt it was more important to limit fertility than to treat infertility and from scientific colleagues whose criticism was based on embryo safety issues, the latter aspect being one of the reasons why the Medical Research Council in the UK rejected an application submitted by Edwards and Steptoe in 1971 for IVF research ⁴⁶⁻⁴⁸. In retrospect, it is amazing that Edwards not only was able to respond to the continued criticism of IVF, but that he also remained so persistent and unperturbed in fulfilling his scientific vision.

Conclusions

Robert G. Edwards has developed a method to treat human infertility. This discovery represents a monumental medical advance that can truly be said to confer the "greatest benefit to mankind". Human IVF has radically changed the field of reproductive medicine. Today, 2-3% of all newborns in many countries are conceived with the help of IVF and many individuals that turn to an infertility clinic can be helped. IVF has also opened up new ways to treat many forms of male infertility. The development of IVF recognized by this year's Nobel Prize in Physiology or Medicine has touched the life of millions of infertile people, giving them an opportunity to have children.

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