Textbook of Assisted Reproductive Techniques
The editors (from left to right: Colin M. Howles, Ariel Weissman, David K. Gardner, and Zeev Shoham) at the annual meeting of ESHRE, Stockholm, July 2011
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The beginnings of human in vitro fertilization

Robert G. Edwards

In vitro fertilization (IVF) and its derivatives in preimplantation diagnosis, stem cells, and the ethics of assisted reproduction continue to attract immense attention scientifically and socially. All these topics were introduced by 1970. Hardly a day passes without some public recognition of events related to this study, and clinics spread ever further worldwide. Now we must be approaching 1.5 million IVF births, it is time to celebrate what has been achieved by so many investigators, clinical, scientific, and ethical.

While much of this chapter “Introduction” covers the massive accumulation of events between 1960 and 2000, it also briefly discusses new perspectives emerging in the 21st century. Fresh advances also increase curiosity about how these fields of study began and how their ethical implications were addressed in earlier days. As for me, I am still stirred by recollections of those early days. Foundations were laid in Edinburgh, London, and Glasgow in the 1950s and early 1960s. Discoveries made then led to later days in Cambridge, working there with many PhD students. It also resulted in my working with Patrick Steptoe in Oldham. Our joint opening of Bourn Hall in 1980, which became the largest IVF clinic of its kind at the time, signified the end of the beginning of assisted human conception and the onset of dedicated applied studies.

INTRODUCTION

First of all, I must express in limited space my tributes to my teachers, even if inadequately. These include investigators from far-off days when the fundamental facts of reproductive cycles, surgical techniques, endocrinology, and genetics were elicited by many investigators. These fields began to move in the 20th century, and if one pioneer of these times should be saluted, it must be Gregory Pincus. Famous for the contraceptive pill, he was a distinguished embryologist, and part of his work dealt with the maturation of mammalian oocytes in vitro. He was the first to show how oocytes aspirated from their follicles would begin their maturation in vitro, and how a number matured and expelled a first polar body. I believe his major work was done in rabbits, where he found that the 10 to 11-hour timings of maturation in vitro accorded exactly with those occurring in vivo after an ovulatory stimulus to the female rabbit.

Pincus et al. also studied human oocytes (1). Extracting oocytes from excised ovaries, they identified chromosomes in a large number of oocytes and interpreted this as evidence of the completion of maturation in vitro. Many oocytes possessed chromosomes after 12 hours, the proportion remaining constant over the next 30 hours and longer. Twelve hours was taken as the period of maturation. Unfortunately, chromosomes were not classified for their meiotic stage. Maturing oocytes would be expected to display diakinesis or metaphase-I chromosome pairs. Fully mature oocytes would display metaphase-II chromosomes, signifying they were fully ripe and ready for fertilization. Nevertheless, it is well known that oocytes can undergo atresia in the ovary involving the formation of metaphase-II chromosomes in many of them. These oocytes complicated Pincus’ estimates, even in controls, and were the source of his error which led later workers to inseminate human oocytes 12 hours after collection and culture in vitro (2,3). Work on human fertilization in vitro, and indeed comparable studies in animals, remained in abeyance from then and for many years. Progress in animal IVF had also been slow. After many relatively unsuccessful attempts in several species in the 1950s and 1960s, a virtual dogma arose that spermatozoa had to spend several hours in the female reproductive tract before acquiring the potential to bind to the zona pellucida and achieve fertilization. In the late 1960s, Austin and Chang independently identified the need for sperm capacitation, identified by a delay in fertilization after spermatozoa had entered the female reproductive tract before acquiring the potential to bind to the zona pellucida and achieve fertilization. In the late 1960s, Austin and Chang independently identified the need for sperm capacitation, identified by a delay in fertilization after spermatozoa had entered the female reproductive tract (4,5). This discovery was taken by many investigators as the reason for the failure to achieve fertilization in vitro, and why spermatozoa had to be exposed to secretions of the female reproductive tract. At the same time, Chang reported that rabbit eggs that had fully matured in vitro failed to produce normal blastocysts, none of them implanting normally (6).

MODERN BEGINNINGS OF HUMAN IVF, PREIMPLANTATION GENETIC DIAGNOSIS, AND EMBRYO STEM CELLS

My PhD began at the Institute of Animal Genetics, Edinburgh University in 1952, encouraged by Professor Conrad Waddington, the inventor of epigenesis, and supervised by Dr Alan Beatty. At the time, capacitation
was gaining in significance. My chosen topic was the genetic control of early mammalian embryology, specifically the growth of preimplantation mouse embryos with altered chromosome complements.

Achieving these aims included a need to expose mouse spermatozoa to x-rays, ultraviolet light, and various chemicals in vitro. This would destroy their chromatin and prevent them from making any genetic contribution to the embryo, hopefully without impairing their capacity to fertilize eggs in vivo. Resulting embryos would become gynogenetic haploids. Later, my work changed to exposing ovulated mouse oocytes to colchicine in vivo, in order to destroy their second meiotic spindle in vivo. This treatment freed all chromosomes from their attachment to the meiotic spindle, and they then became extruded from the egg into tiny artificial polar bodies. The fertilizing spermatozoon thus entered an empty egg, which resulted in the formation of androgenetic haploid embryos with no genetic contribution from the maternal side. For three years, my work was concentrated in the mouse house, working at midnight to identify mouse females in estrus by vaginal smears, collecting epithidymal spermatozoa from males, and practicing artificial insemination with samples of treated spermatozoa. This research was successful, as mouse embryos were identified with haploid, triploid, tetraploid, and aneuploid chromosomes. Moreover, the wide range of scientific talent in the Institute made it a perfect place for fresh collaborative studies. For example, Julio Sirlin and I applied the use of radioactive DNA and RNA precursors to the study of spermatogenesis, spermiogenesis, fertilization, and embryogenesis, and gained knowledge unavailable elsewhere.

An even greater fortune beckoned. Allen Gates, who arrived newly from the United States, brought commercial samples of Organon’s pregnant mares’ serum (PMS) rich in follicle-stimulating hormone (FSH), and human chorionic gonadotropin (hCG) with its strong luteinizing hormone (LH) activity to induce estrus and ovulation in immature female mice. Working with Mervyn Runner (7), he had used low doses of each hormone at an interval of 48 hours to induce oocyte maturation, mating, and ovulation in immature mouse females. He now wished to measure the viability of three-day embryos from immature mice by transferring them to an adult host to grow to term (8). I was more interested in stimulating adult mice with these gonadotropins to induce estrus and ovulation at predictable times of the day. This would help my research, and I was by now weary of taking mouse vaginal smears at midnight. My future wife, Ruth Fowler, and I teamed up to test this new approach to superovulating adult mice. We chose PMS to induce multifolliculation and hCG to trigger ovulation, varying doses and times from those utilized by Allen Gates. PMS became obsolete for human studies some time later, but its impact has stayed with me from that moment, even till today.

Opinion in those days was that exogenous hormones such as PMS and hCG would stimulate follicle growth and ovulation in immature female mammals, but not in adults because they would interact badly with an adult’s reproductive cycles. In fact, they worked wonderfully well. Doses of 1–3 IU of PMS induced the growth of numerous follicles, and similar doses of hCG 42 hours later invoked estrus and ovulation a further 6 hours later in almost all of them. Often, 70 or more ovulated oocytes crowded the ampulla, most of them being fertilized and developing to blastocysts (9). Oocyte maturation, ovulation, mating, and fertilization were each closely timed in all adults, another highly unusual aspect of stimulation (10). Diakinesis was identified as the germinal vesicle break-down and diakinesis were to prove equally decisive in identifying meiosis and ovulation in human oocytes in vivo and in vitro. Even as these results were gained, Ruth and I departed in 1957 from Edinburgh to the California Institute of Technology, where I switched over immunology and reproduction, a topic that was to dominate my life for five or six years on my return to the United Kingdom.

The Institute at Edinburgh had given me an excellent basis not only in genetics, but equally in reproduction. I had gained considerable knowledge about the endocrine control of estrus cycles, ovulation, and spermatozoa; the male reproductive tract; artificial insemination; the stages of embryo growth in the oviduct and uterus; superovulation and its consequences; and the use of radiolabeled compounds. Waddington had also been deeply interested in ethics and the relationships between science and religion, and instilled these topics in his students. I had been essentially trained in reproduction, genetics, and scientific ethics, and all of this knowledge was to prove to be of immense value in my later career. A visit to the California Institute of Technology widened my horizons into the molecular biology of DNA and the gene, a field then in its infancy.

After a year in California, London beckoned me, to the National Institute for Medical Research to work with Drs Alan Parkes and Colin (Bunny) Austin. I was fortunate indeed to have two such excellent colleagues. After two intense years in immunology, my curiosity returned to maturing oocytes and fertilization in vitro. Since they matured so regularly and easily in vivo, it should be easy to stimulate maturation in mouse oocytes in vitro by using gonadotropins. In fact, to my immense surprise, when liberated from their follicles into culture medium, oocytes matured immediately in vast numbers in all groups, with exactly the same timing as those maturing in vivo following an injection of hCG. Adding hormones made no difference. Rabbit, hamster, and rat oocytes also matured within 12 hours, each at their own species’ specific rates. But to my surprise, oocytes from cows, sheep, and rhesus monkeys, and the occasional baboon, did not mature in vitro within 12 hours. Their germinal vesicles persisted unmoved, arrested in the
stage known as diffuse diplotene. Why had they not responded like those of rats, mice, and rabbits? How would human oocytes respond? A unique opportunity emerged to collect pieces of human ovary, and to aspirate human oocytes from their occasional follicles. I grasped it with alacrity.

MOVING TO HUMAN STUDIES

Molly Rose was a local gynecologist in the Edgware and District Hospital who delivered two of our daughters. She agreed to send me slithers or wedges of ovaries such as those removed from patients with polycystic disease, as recommended by Stein and Leventhal, or with myomata or other disorders demanding surgery. Stein–Leventhal wedges were the best source of oocytes, with their numerous small Graafian follicles lined up in a continuous rim just below the ovarian surface. Though samples were rare, they provided enough oocytes to start with. These oocytes responded just like the oocytes from cows, sheep, and pigs, their germinal vesicles persisting and diakinesis being absent after 12 hours in vitro.

This was disappointing, and especially so for me, since Tjio and Levan, and Ford had identified 46 diploid chromosomes in humans, while studies by teams in Edinburgh (Scotland) and France had made it clear that many human beings were heteroploid. This was my subject, because chromosomal variations mostly arose during meiosis and this would be easily assessed in maturing oocytes at diakinesis. Various groups also discovered monosomy or disomy in many men and women. Some women were XO or XXX; some men were XYY and XYYY. Trisomy 21 proved to be the most common cause of Down’s syndrome, and other trisomies were detected. Monosomy or disomy in many men and women. Some women were XO or XXX; some men were XYY and XYYY. Trisomy 21 proved to be the most common cause of Down’s syndrome, and other trisomies were detected. All this new information reminded me of my chromosome studies in the Edinburgh mice.

For human studies, I would have to obtain diakinesis and metaphase I in human oocytes, and then continue this analysis to metaphase II when the oocytes would be fully mature, ready for fertilization. Despite being disappointed at current failure with human oocytes, it was time to write my findings for Nature in 1962 (11). There was so much to write regarding the animal work, and describing the new ideas then taking shape in my mind. I had heard Institute lectures on infertility, and realized that fertilizing human oocytes in vitro and replacing embryos into the mother could help to alleviate this condition. It could also be possible to type embryos for genetic diseases when a familial disposition was identified. Pieces of tissue, or one or two blastomeres, would have to be excised from blastocysts or cleaving embryos, but this did not seem to be too difficult. There were few genetic markers available for this purpose in the early 1960s, but it might be possible to sex embryos by their XX or XY chromosome complement by assessing mitoses in cells excised from morulae or blastocysts. Choosing female embryos for transfer would avert the birth of boys with various sex-linked disorders such as hemophilia. Clearly, I was becoming totally committed to human IVF and embryo transfer.

While looking in the library for any newly published papers relevant to my proposed Nature manuscript, I discovered those earlier papers of Pincus and his colleagues described earlier. They had apparently succeeded 30 years earlier in maturing human oocytes cultured for 12 hours, where I had failed. My Nature paper (11) became very different from that originally intended, even though it retained enough for publication. Those results of Pincus et al. had to be repeated. After trying hard, I failed completely to repeat them, despite infusing intact ovaries in vitro with gonadotropin solutions, using different culture media to induce maturation, and using joint cultures of maturing mouse oocytes and newly released human oocytes. Adding hormones to culture media also failed. It began to seem that menstrual cycles had affected oocyte physiology in a different manner than in nonmenstruating mammalian species. Finally, another line of inquiry emerged after two years of fruitless research on the precious few human oocytes available. Perhaps the timing of maturation in mice and rabbits differed from that of those oocytes obtained from cows, baboons, and humans. Even as my days in London were ending, Molly Rose sent a slither of human ovary. The few oocytes were placed in culture just as before. Their germinal vesicles remained static for 12 hours as I already knew, and then after 20 hours in vitro. Three oocytes remained, and I waited to examine them until they had been in vitro for 24 hours. The first contained a germinal vesicle, so did the second. There was one left and one only. Its image under the microscope was electrifying. I gazed down at chromosomes in diakinesis and at a regressing germinal vesicle. The chromosomes were superb examples of human diakinesis with their classical chiasmata. At last, I was on the way to human IVF, to completion of the maturation program and the onset of studies on fertilization in vitro.

This was the step I had waited for, a marker that Pincus had missed. He never checked for diakinesis, and apparently confused atretic oocytes, which contained chromosomes, with maturing oocytes. Endless human studies were opening. It was easy now, even on the basis of one oocyte in diakinesis, to calculate the timing of the final stages of maturation because the postdiakinesis stages of maturation were not too different from normal mitotic cycles in somatic cells. This calculation provided me with an estimate of about 36 hours for full maturation, which would be the moment for insemination. All these gaps in knowledge had to be filled. But now, my research program was stretching far into the future.

At this wonderful moment, John Paul, an outstanding cell biologist, invited me to join him and Robin Cole at Glasgow University to study differentiation in early mammalian embryos. This was exciting, to work in biochemistry with a leading cell biologist. He had heard that I was experimenting with very early embryos, trying to grow cell lines from them. He also wanted to grow stem cells from mammalian embryos and study them in vitro. This began one of my most memorable 12 months of research. John’s laboratory had facilities unknown outside, with CO₂ incubators, numerous cell lines in
constant cultivation, cryopreservation facilities, and the use of media droplets held under liquid paraffin. We decided to start with rabbits. Cell lines did not grow easily from cleaving rabbit embryos. In contrast, stem cells migrated out in massive numbers from cultures of rabbit blastocysts, forming muscle, nerves, phagocytes, blood islands, and other tissues in vitro (12). Stem cells were differentiating in vitro into virtually all the tissues of the body. In contrast, dissecting the inner cell mass from blastocysts and culturing it intact or as disaggregated cells produced lines of cells which divided and divided, without ever differentiating. One line of these embryonic stem cells expressed specific enzymes, diploid chromosomes, and a fibroblastic structure as it grew over 200 and more generations. Another was epithelioid and had different enzymes but was similar in other respects.

The ability to make whole-embryo cultures producing differentiating cells was now combined with everlasting lines of undifferentiated stem cells which replicated over many years without changing. Ideas of using stem cells for grafting to overcome organ damage in recipients began to emerge. My thoughts returned constantly to growing stem cells from human embryos to repair defects in tissues of children and adults.

Almost at my last moment in Glasgow, with this new set of ideas in my mind, a piece of excised ovary yielded several oocytes. Being placed in vitro, two of them had reached metaphase II and expelled a polar body at 37 hours. This showed that another target on the road to human IVF had been achieved as the whole pattern of oocyte maturation continued to emerge but with increasing clarity.

Cambridge University, my next and final habitation, is an astonishing place. Looking back on those days, it seems that the Physiological Laboratory was not the ideal place to settle in that august university. Nevertheless, a mixture of immunology and reproduction remained my dominant theme as I rejoined Alan Parkes and Bunny Austin there. I had to do immunology to obtain a grant to support my family, but thoughts of human oocytes and embryos were never far away. One possible model of the human situation was the cow and other agricultural species, and large numbers of cow, pig, and sheep oocytes were available from ovaries given to me by the local slaughterhouse. Each species had its own timing, all of them longer than 12 hours (13). Pig oocytes were closest to humans, requiring 37 hours. In each species, maturation timings in vitro were exactly the same as those arising in vivo in response to an hCG injection. This made me suspect that a woman ovulated 36–37 hours after an injection of hCG. Human oocytes also trickled in, improving my provisional timings of maturation, and one or two of them were inseminated, but without signs of fertilization.

More oocytes were urgently needed to conclude the timings of oocyte meiosis. Surgeons in Johns Hopkins Hospital, Baltimore performed the Stein–Leventhal operation, which would allow me to collect ovarian tissue, aspirate oocytes from their follicles, and retain the remaining ovarian tissues for pathology if necessary.
never be very far away. These issues were all acceptable, since I was confident that studies of human conception were essential for future medicine, and correct ethically, medically, and scientifically. The increasing knowledge of genetics and embryology could assist many patients if I could achieve human fertilization and grow embryos for replacement into their mothers.

Few human oocytes were available in the United Kingdom. Despite this scarcity, one or two of those matured and fertilized in vitro possessed two nuclei after insemination. But there were no obvious sperm tails. I devised a cow model for human fertilization, using in vitro matured oocytes and insemination in vitro with selected samples of highly active, washed bull spermatozoa extracted from neat semen. It was a pleasure to see some fertilized bovine eggs, with sperm tails and characteristic pronuclei, especially using spermatozoa from one particular bull. Here was a model for human IVF and a prelude to a series of events, which implied that matters in my research were suddenly changing. A colleague had stressed that formalin fixatives were needed to detect sperm tails in eggs. Barry Bavister joined our team to study for his PhD and designed a medium of high pH, which gave excellent fertilization rates in hamsters. We decided to collaborate by using it for trials on human fertilization in vitro.

Finally, while browsing in the library of the Physiological Laboratory, I read a paper in *The Lancet* which instantly caught my attention. Written by Dr P C Steptoe of the Oldham and District General Hospital (18), it described laparoscopy, with its narrow telescope and instruments and the minute abdominal incisions. He could visualize the ampulla and place small amounts of medium there, in an operation lasting 30 minutes or less and maybe even without using anesthesia. This is exactly what I wanted, because access to the ampulla was equivalent to gaining access to ovarian follicles. Despite advice to the contrary from several medical colleagues, I telephoned him about collaboration and stressed the uncertainty in achieving fertilization in vitro. He responded most positively, just as Molly, Howard and Georgeanna, and Victor had done. We decided to get together.

Last but by no means least, Molly Rose sent a small piece of ovary to Cambridge. Its dozen or more oocytes were matured in vitro for 37 hours, when Barry and I added washed spermatozoa suspended in his medium. We examined them a few hours later. To our delight, spermatozoa were pushing through the zona pellucida, into several of the eggs. Maternal and paternal pronuclei were forming beautifully. We saw polar bodies and sperm tails within the eggs. That evening in 1969, we watched in delight virtually all the stages of human fertilization in vitro (Fig. I.1). One fertilized egg had fragments, as Chang had forecast from his work on oocyte maturation and fertilization in vitro of rabbit eggs. This evidence strengthened the need to abandon oocyte maturation in vitro and replace it by stimulating maturation by means of exogenous hormones. Our 1969 paper in *Nature* surprised a

![Figure I.1](https://example.com/image1.png)
world unaccustomed to the idea of human fertilization in vitro (19).

Incredibly fruitful days followed in our Cambridge laboratory. Richard Gardner, another PhD candidate, and I excised small pieces of trophectoderm from rabbit blastocysts and sexed them by staining the sex chromatin body. Those classified as female were transferred into adult females and were all correctly sexed at term. This work transferred my theoretical ideas of a few years earlier into the practice of preimplantation diagnosis of inherited disease, in this case for sex-linked diseases (20). Alan Henderson, a cytogeneticist, and I analyzed chiasmata during diakinesis in mouse and human eggs, and explained the high frequencies of Down’s syndrome in offspring of older mothers as a consequence of meiotic errors arising in oocytes formed last in the fetal ovary, which were then ovulated last at later maternal ages (21). Dave Sharpe, a lawyer from Washington, joined forces to write an article in *Nature* (22) on the ethics of IVF, the first ever paper in the field. I followed this up with a detailed analysis of ethics and law in IVF covering scientific possibilities, oocyte donation, surrogacy by embryo transfer, and other matters (22). So the first ethical papers were written by scientists and lawyers and not by philosophers, ethicists, or politicians.

THE OLDHAM YEARS

Patrick and I began our collaboration six months later in the Oldham and District General Hospital, almost 200 miles north of Cambridge. He had worked closely with two pioneers, Palmer in Paris (23) and Fragenheim in Germany (24). He improved the pneumoperitoneum to gain working space in the abdominal cavity and used carbon fibers to pass cold light into the abdomen from an external source (25). By now, Patrick was waiting in the wings, ready to begin clinical IVF in distant Oldham. We had a long talk about ethics and found our stances to be very similar.

Work started in the Oldham and District General Hospital and moved later to Kershaw’s Hospital, set up by my assistants, especially Jean Purdy. We knew the routine. It was based on my Edinburgh experiences with mice. Piero Donini from Serono Laboratories in Rome had purified urinary human menopausal gonadotropins (hMG) as a source of FSH and the product was used clinically to stimulate follicle growth in anovulatory women by Bruno Lunenfeld (26). It removed the need for PMS, thus avoiding the use of nonhuman hormones. We used low-dosage levels in patients, that is, 2–3 vials (a total of 150–225 IU) given on days 3 and 5, and 5000–7000 IU of hCG on day 10. Initially, the timing of oocyte maturation in vitro was confirmed, by performing laparoscopic collections of oocytes from ovarian follicles at 28 hours after hCG to check that they were in metaphase I (27). We then moved to 36 hours to aspirate mature metaphase II oocytes for fertilization. Those beautiful oocytes were surrounded by masses of viscous cumulus cells and were maturing exactly as predicted. We witnessed follicular rupture at 37 hours through the laparoscope. Follicles could be classified from their appearance as ovulatory or nonovulatory, this diagnosis being confirmed later by assaying several steroids in the aspirated follicular fluids (Fig. I.2).

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**Figure I.2** Eight steroids were assayed in fluids extracted from human follicles aspirated 36–37 hours after the human chorionic gonadotropin (hCG) shot. The follicles had been classified as ovulating or nonovulating by laparoscopic examination in vivo. Data were analyzed by cluster analysis, which groups follicles with similar features. The upper illustration shows data collected during the natural menstrual cycle. Note that two sharply separated groups of follicles were identified, each with very low levels of within-group variance. Attempting to combine the two groups resulted in a massive increase of within-group variation, indicating that two sharply different groups had been identified. These different groups accorded exactly with the two groups identified by means of steroid assays. The lower figure shows the same analysis during stimulated cycles on fluids collected 36–37 hours after injecting hCG. With this form of stimulation, follicle growth displays considerable variation within groups. Attempts to combine all the groups result in a moderately large increase in variation. This evidence suggests that follicles vary considerably in their state of development in simulated cycles using human menopausal gonadotropin (hMG) and hCG.
It was a pleasure and a new duty to meet the patients searching for help to alleviate their infertility. We did our best, driving from Cambridge to Oldham and arriving at noon to prepare the small laboratory there. Patrick had stimulated the patients with hMG and hCG, and he and his team led by Muriel Harris arrived to prepare for surgery. Patrick's laparoscopy was superb. Ovarian stimulation, even though mild, produced five or six mature follicles per patient, and ripe oocytes came in a steady stream into my culture medium for insemination and overnight incubation. The next morning, the formation of two pronuclei and sperm tails indicated fertilization had occurred, even in simple media, now with a near-neutral pH. Complex culture media, Ham's F10 and others, each with added serum or serum albumin, sustained early and later cleavages (28), and, even more fascinating, was the gradual appearance of morulae and then light, translucent blastocysts (Fig. I.3) (29). Here was my reward—growing embryos was now a routine, and examinations of many of them convinced me that the time had come to replace them into the mothers' uterus. I had become highly familiar with the teratologic principles of embryonic development, and knew many teratologists. The only worry I had was the chance of chromosomal monosomy or trisomy, on the basis of our mouse studies, but these conditions could be detected later in gestation by amniocentesis. Our human studies had surpassed work on all animals, a point rubbed in even more when we grew blastocysts to day 9 after they had hatched from their zona pellucida (Fig. I.4) (30). This beautifully expanded blastocyst had a large embryonic disc which was shouting that it was a potential source of embryonic stem cells.

When human blastocysts became available, we tried to sex them using the sex chromatin body as in rabbits.

Figure I.3 Successive stages of human preimplantation development in vitro in a composite illustration made in Oldham in 1971. (Upper left) 4-cell stage showing the crossed blastomeres typical of most mammals. (Upper middle) 8-cell stage showing the even outline of blastomeres and a small piece of cumulus adherent to the zona pellucida. (Upper right) A 16 to 32-cell stage, showing the onset of compaction of the outer blastomeres. Often, blastocelic fluid can be seen accumulating between individual cells to give a “striped” appearance to the embryo. (Lower left and middle) Two living blastocysts showing a distinct inner cell mass, single-celled trophectoderm, blastocelic cavity, and thinning zona pellucida. (Lower right) A fixed preparation of a human blastocyst at 5 days, showing more than 100 evenly sized nuclei and many mitoses.

Figure I.4 A hatched human blastocyst after 9 days in culture. Notice the distinct embryonic disc and the possible bilaminar structure of the membrane. The blastocyst has expanded considerably, as shown by comparing its diameter with that of the shed zona pellucida. The zona contains dying and necrotic cells and its diameter provides an estimate of the original oocyte end embryo diameters.
Unfortunately, they failed to express either sex chromatin or the male Y body so we were unable to sex them as female or male embryos. Human preimplantation genetic diagnosis would have to wait a little longer.

During these years there were very few plaudits for us, as many people spoke against IVF. Criticism was mostly aimed at me, as usual when scientists bring new challenges to society. Criticism came not only from the Pope and archbishops, but also from scientists who should have known better, including James Watson (who testified to a U.S. Senate Committee that many abnormal babies would be born), and Max Perutz, who supported him. These scientist critics knew virtually nothing about my field, so who advised them to make such ridiculous charges? Cloning football teams or intelligentsia was always raised by ethicists, which clearly dominated their thoughts rather than the intense hopes of our infertile patients. Yet one theologian, Gordon Dunstan, who became a close friend, knew all about IVF from us, and wrote an excellent book on its ethics. He was far ahead of almost every scientist in my field of study. Our patients also gave us their staunch support, and so did the Oldham Ethical Committee, Bunny Austin back home in Cambridge, and Elliott Philip, a colleague of Patrick’s.

Growing embryos became a routine, so we decided to transfer one each to several patients. Here again we were in untested waters. Transferring embryos via the cervical canal, the obvious route to the uterus, was virtually a new and untested method. We would have to do our best. From now on, we worked with patients who had seriously distorted tubes or none whatsoever. This step was essential, since no one would have believed we had established a test-tube baby in a woman with near-normal tubes. This had to be a condition of our initial work. Curiously, it led many people to make the big mistake of believing that we started IVF to bypass occluded oviducts. Yet we already knew that embryos could be obtained for men with oligozoospermia or antibodies to their gametes, and for women in various stages of endometriosis.

One endocrinological problem did worry me. Stimulation with hMG and hCG shortened the succeeding luteal phase, to a very short time for embryos to implant before the onset of menstruation. Levels of urinary pregnanediol also declined soon after oocyte collection. This condition was not a result of the aspiration of granulosa and cumulus cells, and luteal support would be needed, preferably progesterone. Csapo et al. stressed how this hormone was produced by the ovaries for the first 8–10 weeks before the placenta took over this function (31). Injections of progesterone in oil given over that long period of time seemed unacceptable since it would be extremely uncomfortable for patients. While mulling over this problem, my attention turned to those earlier endocrinologists who believed that exogenous hormones would distort the reproductive cycle, although I doubt they even knew anything about a deficient luteal phase.

This is how we unknowingly made our biggest mistake in early IVF days. Our choice of Primolut (Sigma Chemical co., St Louis, USA) depot, a progestogen, meant it should be given every five days to sustain pregnancies, since it was supposed to save threatened abortions. So, we began embryo transfers to patients in stimulated cycles, giving this luteal phase support. Even though our work was slowed down by having to wait to see whether pregnancies arose in one group of patients before stimulating the next, enough patients had accumulated after two to three years. None of our patients was pregnant, and disaster loomed. Our critics were even more vociferous as the years passed, and the mutual support between Patrick and me had to pull us through.

Twenty or more different factors could have caused our failure, for example cervical embryo transfers, abnormal embryos, toxic culture dishes or catheters, inadequate luteal support, incompatibility between patients’ cycles and that imposed by hMG and hCG, inherent weakness in human implantation, and many others. We had to glean every scrap of information from our failures. I knew Ken Bagshawe in London, who was working with improved assay methods for gonadotropic hormones. He offered to measure blood samples taken from our patients over the implantation period using his new hCG-® assay. He telephoned: three or more of our patients previously undiagnosed had actually produced short-lived rises of hCG-® over this period. Everything changed with this information. We had established pregnancies after all, but they had aborted very early. We called them biochemical pregnancies, a term that still sticks today. It had taken us almost three years to identify the cause of our failure, and the finger of suspicion pointed straight at Primolut. I knew it was luteolytic, but it was apparently also an abortifacient, and our ethical decision to use it had caused much heartache, immense loss of work and time, and despair for some of our patients. The social pressures had been immense, with critics claiming our embryos were dud and our whole program was a waste of time; but we had come through it and now knew exactly what to do next.

We accordingly reduced the levels of Primolut depot, and utilized hCG and progesterone as luteal aids. Suspicions were also emerging that human embryos were very poor at implanting. We had replaced single embryos into most of our patients, rarely two. Increasingly we began to wonder whether more should be replaced, as when we replaced two in a program involving transfer of oocytes and spermatozoa into the ampulla so that fertilization could occur in vivo.

This procedure was later called gamete intrafallopian transfer (GIFT) by Ricardo Asch. We now suspected that single embryo transfers could produce a 15–20% chance of establishing pregnancy, just as our first clinical pregnancy arose after the transfer of a single blastocyst in a patient stimulated with hMG and hCG (32). Then came the fantastic news—a human embryo fertilized and grown in vitro had produced a pregnancy. Everything seemed fine, even with ultrasound images. My culture protocols were satisfactory after all. Patrick rang: he feared the pregnancy was ectopic and he had to remove it sometime after 10 gestational weeks. Every new approach we tested seemed to be ending in a disaster, yet we would not stop, since the work itself seemed highly
ethical, and conceiving a child for our patients was perhaps the most wonderful thing anyone could do for them. In any case, ectopic pregnancies are now known to be a regular feature with assisted conception.

I sensed that we were entering the final phase of our Oldham work, seven years after it began. We had to speed up, partly because Patrick was close to retiring from the National Health Service. Four stimulation protocols were tested in an attempt to avoid problems with the luteal phase: hMG and hCG; clomiphene, hMG, and hCG to gain a better luteal phase; bromocriptine, hMG, and hCG because some patients had high prolactin concentrations; and hCG alone at mid-cycle. We also tested what came to be known as GIFT, calling it ORTI (oocyte recovery with tubal insemination, by transferring one or two eggs and spermatozoa to the ampulla) (Fig. I.5). Natural-cycle IVF was introduced, based on collections of urine samples at regular intervals eight times daily, to measure exactly the onset of the LH surge, using a modified HiGovanis assay (Fig. I.6). Cryopreservation was also introduced, by freezing oocytes and embryos that looked to be in good condition when thawed. A recipient was given a donor egg fertilized by her husband’s spermatozoa, but pregnancy did not occur.

Lesley and John Brown came as the second entrants for natural-cycle IVF. Lesley had no oviducts. Her egg was aspirated in a few moments and inseminated simply and efficiently. The embryo grew beautifully and was

![Figure I.5](image-url) The first attempts at gamete intrafallopian transfer (GIFT) were called oocyte recovery with tubal insemination (ORTI). In this treatment cycle, using human menopausal gonadotropin (hMG) and human chorionic gonadotropin (hCG), including additional injections of hCG for luteal support, a single preovulatory oocyte and 1.6 million sperm were transferred into the ampulla. Abbreviations: LMP, last menstrual period; ODGH, Oldham and District General Hospital return to menstruation (RTM) indicates stages of the menstrual cycle.

![Figure I.6](image-url) Recording the progress of the human natural menstrual cycle for in vitro fertilization (IVF). Three patients are illustrated. All three displayed rising 24-hour urinary estrogen concentrations during the follicular phase and rising urinary pregnanediol concentrations in the luteal phase. Luteinizing hormone (LH) levels were measured several times daily and the data clearly reveal the exact time of onset of the LH surge.