Section 1

Chapter 1

Introduction

The evolution of ART

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To understand science, it is necessary to know its history. Auguste Compte (1798–1857)

It could be said that the first instance of assisted reproductive technology (ART) was when an eminent surgeon, John Hunter (1728–93) of London (Figure 1.1), assisted a woman in becoming pregnant by taking a semen sample produced by her husband, who had hypospadias, and inseminated her with that specimen. This was an “assisted conception,” although it is not strictly within the definition of the present-day ARTs, which involve the manipulation of sperm, oocytes and embryos in vitro and include:

- in vitro fertilization (IVF)
- intracytoplasmic sperm injection (ICSI)
- gamete intrafallopian transfer (GIFT) – now rarely practiced
- zygote intrafallopian transfer (ZIFT) – now rarely practiced
- oocyte and embryo donation
- cryopreservation of sperm, oocytes and embryos
- gestational surrogacy
- in vitro maturation of oocytes
- pre-implantation genetic diagnosis.

However, although John Hunter’s treatment of his patient was one of the first instances of outside interference with the human reproductive process, man’s interest in fertility and conception in both animal species and in humans goes back thousands of years.

As early as the fifth century BC, Hippocrates (c. 460–370 BC), who is commonly thought of as the “father of medicine,” believed that both males and females produced the “liquor” which blended within the woman’s body and created babies. Some 100 years later, Aristotle (384–422 BC) proposed the theory that children are the product of “the mingling of male and female seed.” This firmly opposed the then prevailing theory that children were from the male “seed” and women were merely the receptacle for the child. This latter idea prevailed until the sixteenth century, when William Harvey (1578–1657) (Figure 1.2), having studied the behavior and fertility of the King of England’s herd of deer, wrote De Generatione Animalium in 1651, which described the egg as being

Figure 1.1 John Hunter (1728–93). The first reported person to successfully perform artificial insemination in a human. See plate section for color version.

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Figure 1.2 William Harvey (1578–1657). The first person to describe the egg as responsible for the production of all creatures. See plate section for color version.

responsible for the production of all creatures. It was from this research that his famous expression “ex ovo omnia” [from the egg everything] arose. It was from this time onwards that the science of animal and human reproduction really began to develop. However, it was not really until the development of the optical microscope that researchers were able, for the first time, to study sperm, oocytes and, later, fertilization.

Anton van Leeuwenhoek (1632–1723), a Dutch draper and amateur scientist (Figure 1.3), was fascinated by the potential of the new science of microscopy. He built his own microscopes and, among many other specimens, he studied sperm of different animal species; in 1677 he reported his findings to the Royal Society in London. He believed that each sperm was the beginnings of an individual animal or human and, if it was “nourished” in the womb, it would produce the next generation. This went against the prevailing opinion at the time that the woman produced the seed and the male merely produced the fertilizing power to produce offspring.

Figure 1.3 Anton van Leeuwenhoek (1632–1723). The first person to study animal and human sperm under microscopes, which he constructed himself. See plate section for color version.

Dalenpatius in 1699 stated that he could see a miniature human within a single sperm, and the idea that humans were pre-formed within a sperm prevailed for more than a century, even though this report was later found to be an hoax and Dalenpatius the fictitious name of the perpetrator of the hoax. It was Reinier de Graaf (1641–73) who first described the development of ovarian follicles – later to become known as Graafian follicles in his honor – but he never discovered oocytes within the follicles. He also supported the work of fellow Dutchman, van Leeuwenhoek and was aware of the importance of his work on microscopes. De Graaf died at the early age of 32 years.

Lazzaro Spallanzani (1729–99) (Figure 1.4), an Italian scientist, studied the behavior of semen microscopically and performed the first known attempts at insemination of a dog. He is also credited with the very earliest attempts at IVF in experiments with frogs; he is also said to have been the first to freeze and thaw sperm in 1776.

In 1826, Karl Ernst von Baer (1792–1876) (Figure 1.5) first identified oocytes in the ovaries of a bitch. He also finally established that mammals develop from oocytes and reported on organogenesis of early
mammalian embryos. Von Baer is credited with being the "founding father of modern embryology."

In the mid nineteenth century, extensive research was carried out on reproduction by a number of researchers who reported their observations on the process of fertilization in primitive organisms; in particular, Henry Nelson (1852), Newport (1853), van Beneden (1854) and Hertwig (1876). Nelson observed the penetration of ascaris oocytes by spermatozoa; Newport made similar observations in amphibians, while both van Beneden and Hertwig are credited with the first observations on fertilization in mammals.

It is probable that Walter Heape (1855–1929), a physician and professor at the University of Cambridge, England, was the first scientist to successfully transfer embryos into rabbits in the early 1890s. Only one pregnancy and delivery was reported, but this experiment showed for the first time that it was possible to remove embryos from one animal and transfer them to another, without interfering with their development.

In their reviews on the early history of IVF, both Bavister [1] and Clark [2] give the opinion that 1951 was probably the "critical boundary" defining the beginning of the modern era of IVF. Both Colin "Bunny" Austin (1914–2004) and M. C. Chang (1908–91) discovered the need for spermatozoa to undergo capacitation and the acrosome reaction before they are able to penetrate the zona pellucida of the oocyte. Later, in 1963, Yanagimachi and Chang, were able to achieve the first live births after transfer of hamster oocytes fertilized in vitro using spermatozoa capacitated in vitro [3]. Work continued, more or less successfully, over the next decade attempting to achieve fertilization in vitro and live births of various other mammalian species.

Some of the earliest observations on fertilization of human oocytes were made by Robert Edwards (1925–) and published in 1965 in a landmark paper: "Matura- tion in vitro of human ovarian oocytes" [4]. It was not possible for Edwards to progress further with efforts to achieve IVF of human oocytes for clinical use without close collaboration with clinical colleagues, who were able to provide a supply of human oocytes – usually from patients having ovarian wedge resections for polycystic ovary disease. It was the need for Edwards to be able to obtain these supplies of pre-ovulatory human oocytes that brought him and gynecologist Patrick Steptoe (1913–88) together in 1968.

Patrick Steptoe became known to Robert Edwards because he had brought laparoscopy, whereby the female pelvic organs could be visualized by a relatively minor operation, to England from Europe where Steptoe had studied it under both Raoul Palmer (1940–95) and Hans Frangenheim (1920–2001). On his return to England, he further developed the technique of laparoscopy, and shortly afterwards he wrote his famous short textbook Laparoscopy in Gynaecology in 1967 [5]. His first major paper; "Laparoscopy and ovulation" followed in 1968 [6]. Shortly afterwards, he further developed the laparoscopic technique to enable aspiration of oocytes from follicles under direct vision.

Robert Edwards started his career in reproductive biology at the Institute of Animal Genetics and Embryology, Edinburgh, in 1951, having just been demobbed...
from the British Army. There, under the supervision of Professor Alan Parkes, he did his PhD on reproductive genetics. He moved on to the Institute of Medical Research, London, and then to the University of Cambridge in 1963 to join two well-known researchers in reproductive physiology: Professors Alan Parks and "Bunny" Austin. There Edwards continued his work on immunology and oocyte maturation. He then spent a short time in the USA at Johns Hopkins University, where he collaborated with two other "greats" in the field of human ART, Drs. Howard and Georgeanna Jones. On his return to Cambridge, he continued his work on human oocytes. In 1968, Edwards, who had heard about Steptoe’s work in retrieving pre-ovulatory human oocytes laparoscopically, contacted him and met with him at a Royal Society of Medicine meeting in London. They quickly struck up a working relationship and friendship which, between 1968 and 1978, led them to further develop the techniques of human IVF and applied it to the clinical treatment of intractably infertile women.

Steptoe and Edwards soon started to produce landmark papers together – in 1969: “Early stages of fertilisation in vitro of human oocytes and matured in vitro” [7]; and also in 1969: “Identification of the midpiece and tail of the spermatozoon during fertilisation of human eggs in vitro” [8]; and in 1970: “Laparoscopic recovery of preovulatory human oocytes after priming of ovaries with gonadotrophins” [9]. They also carried out the first treatment cycles of oocyte recovery with tubal insemination (ORTI), as they called the procedure, which was later to become known as gamete intrafallopian transfer (GIFT).

Between the years 1968 and 1978, while they were working closely together, Steptoe was in Oldham, Lancashire, England, working in a National Health Service hospital, and Edwards was at the University of Cambridge. When the clinical treatment of infertile women started, there followed a number of very difficult years in which none of the first 40 patients they treated by IVF and embryo transfer (ET) became pregnant. In 1976 they did achieve their first pregnancy following transfer of a single blastocyst, but this subsequently turned out to be an ectopic pregnancy [10]. After 102 failed embryo transfers, including the one ectopic pregnancy, Leslie Brown was treated and subsequently became pregnant following her first embryo transfer. This was achieved in a “natural” IVF cycle, with no stimulation; one oocyte was collected and a single eight-cell embryo was transferred. There followed a difficult pregnancy for Mrs Brown, but her baby, Louise Brown, was delivered by cesarian section on July 25, 1978 (Figure 1.6). Much to everyone’s relief, baby Louise was found to be a perfectly normal, fit and healthy infant. This momentous achievement was announced with a simple publication as a letter in the *Lancet*: “Birth after reimplantation of a human embryo” [11]. The arrival of Louise Brown was heralded as “The baby of the century.” This was in spite of considerable criticism and opposition by clinical and
scientific colleagues, the lay press and by many representatives of different religious faiths.

Work on human IVF was also being conducted elsewhere in the world, particularly in Melbourne, Australia, where the team of Professor Carl Wood and Dr. Alan Trounson achieved the birth of the world’s fourth IVF baby, Candice Reed, in June 1980 [12]. At the same time, Drs. Howard and Georgeanna Jones had been working in Norfolk, Virginia, USA, and Elizabeth Carr, the first US in-vitro conceived baby, was born on December 28, 1981 [13].

In England, meanwhile, Patrick Steptoe and Robert Edwards were unable to continue their work on human IVF, since neither the UK’s National Health Service nor any of the Universities or the Medical Research Council were willing to provide funding to help them to continue their work. They eventually found Bourn Hall, an old Jacobean manor house in the Cambridgeshire countryside, where they founded the World’s first IVF treatment and research center – Bourn Hall Clinic – which opened in September 1980 (Figure 1.7). There, Steptoe and Edwards continued their research and, by 1986, they had achieved 500 live births [14]. Steptoe and Edwards achieved a number of distinguished national and international awards over the next 3 years, but, in 1988, Patrick Steptoe fell seriously ill with prostate cancer and died on March 21, 1988. Robert Edwards continued to work as Scientific Director of Bourn Hall and as Editor of the newly formed journal Human Reproduction, which he cofounded. In 1994, he retired from working at Bourn Hall.

In the early years following the first IVF births in England, Australia and the USA, other teams were successful in achieving births: in 1982 in France from the group of Professors Frydman and Testart; and in Sweden from the group of Professor Lars Hamberger. In 1982, in England, both Dr. Brian Lieberman’s group and Professor Ian Craft’s group also achieved live births, followed by, in Austria, Professors Feichtinger and Kemeter, and later that year births also occurred in Finland, Germany and the Netherlands.

An interesting historical point is the suggestion that the world’s second IVF baby was achieved in India, following work by Dr. Subhash Mukhopadhyay. A baby was born on October 3, 1978 following IVF and ET, but his achievement was never officially recognized; indeed, he was derided by his colleagues and officials in India at the time. He eventually committed suicide in 1981. However, following a close investigation of his claim some 27 years later, he was officially accepted as being the first Indian and second in the world to achieve a live birth following IVF.

In 1983, the first IVF baby was born following the transfer of frozen-thawed embryos in Australia [15]. Oocyte donation, as a treatment option in IVF programs, also developed from about the mid 1980s, with the first successful live birth reported from Australia [16]. Originally developed to treat women with premature menopause, this was extended to treat women with inherited diseases and, increasingly now, is being used in the treatment of women in their mid to late 40s, or even older, to help them to have children...
late in their lives. In a few countries, treatment using donated embryos has been permitted and, in some countries, treatment using gestational surrogates has become available to treat women without a uterus or with other reasons meaning that they are unable to carry a child. Utian et al. published the first report of an IVF birth in the USA through gestational surrogacy in 1985 [17].

The need to develop more “user friendly” techniques to obtain oocytes, other than by the relatively invasive technique of laparoscopy, was developed by Lenz and Lauritsen in 1982, who described the technique of abdominal ultrasound-guided needle oocyte recovery [18]. Gleicher et al. further developed this technique in 1983, approaching the ovaries transvaginally but using an abdominal probe [19]. Later, in 1985, Mats Wikland in Sweden developed the now almost universally used transvaginal ultrasound probe-guided needle aspiration of pre-ovulatory follicles [20]. The techniques of intrauterine insemination (IUI) and GIFT also developed over the following years as a more simplified variant of standard IVF.

Over the years since the beginning of human IVF, many changes have occurred in ovarian stimulation protocols for IVF. The major developments have been:

- **1970s**
  - natural cycle IVF
  - clomiphene alone

- **1980s**
  - clomiphene + urinary human menopausal gonadotropin (HMG)
  - gonadotropin-releasing hormone (GnRH) agonists + urinary HMG
  - “flare” protocol
  - ultra-short and short protocols
  - long luteal phase or follicular-phase start long protocols

- **1990s**
  - GnRH agonist + urinary-follicle stimulating hormone (FSH) intramuscularly (im)
  - GnRH agonist + high purity FSH subcutaneously (sc)
  - GnRH agonist + high purity HMG (sc)
  - GnRH agonist + recombinant human follicle stimulating hormone (rhFSH) (sc)

- **Late 1990s–2000+**
  - GnRH antagonists + rhFSH ± recombinant human luteinizing hormone (rhLH)
  - recombinant LH
  - recombinant human chorionic gonadotropin (hCG)
  - “fill by mass” versus IU recombinant FSH
  - “patient friendly” sc injections and use of “pens” for injection

As can be seen above, stimulation protocols have undergone many changes. In the early days, IVF was conducted in natural cycles or with clomiphene-only stimulation. It was in 1984 that Porter et al. in London first developed the use of GnRH agonists in IVF stimulation protocols to prevent premature LH surges [21], which, over the following years, became the “gold standard” for use in IVF stimulation protocols; indeed, it remains so for many practitioners. Introduction of the GnRH antagonists in stimulation protocols, first reported by Frydman et al. in 1991 [22], increasingly has become used, allowing a more “natural” cycle and being more “patient friendly,” since treatment does not last as long as do GnRH-agonist protocols. Also from the mid 1990s, there were major developments in the production and use of gonadotrophins. These were produced originally from human menopausal urine and injected intramuscularly. High purity FSH and HMG were a great improvement and could be injected subcutaneously by patients themselves after training. Also from about the mid 1990s, recombinant gonadotrophins were developed, producing the purest FSH, LH and hCG. It was in 1992 that Germond et al. [23] and Devroey et al. [24] reported the first pregnancies using the new recombinant FSH (rFSH).

More recently still, there has been an enthusiasm for the concept of “IVF lite.” This has introduced the concept of a milder stimulation strategy for IVF in order to reduce the risk of complications of stimulation, particularly of ovarian hyperstimulation syndrome. It is also thought to improve the chance of implantation by reducing interference of the development of the endometrium that may occur in some high dose gonadotrophin stimulation protocols.

In vitro fertilization was never really successful in the treatment of severe male factor infertility, and techniques were developed to try to improve the outcome for men with this diagnosis, for whom the
only real option then was the use of donor sperm. In 1987, Laws-King et al., in Australia, first reported the microinjection of spermatozoa under the zona pellucida of oocytes [25]. However, it was not until 1992 that the first pregnancy after ICSI of oocytes with single spermatozoa was reported by Palermo et al. of the Free University of Brussels [26]. For the first time, this technique allowed men with the most intractable infertility problems to achieve pregnancies with their partners. This treatment option has been the most important development in ART since human IVF first started. Most ART units worldwide now treat 40–50% or more of their ART cycles by ICSI, and some even advocate using it for all ART cycles.

Other firsts include, in 1989, Handyside and colleagues from London who first showed it is possible to take a single blastomere from an embryo, perform pre-implantation genetic diagnosis (PGD) and to sex the embryo by DNA amplification [27]. This technology has led to the development of a whole new subspecialty of techniques used to diagnose not only the sex of an embryo but to detect a multitude of genetic abnormalities, including single gene defects, and also to perform screening for aneuploidy. In 1990, Verlinsky et al. reported the first polar body biopsy, with a subsequent embryo transfer and pregnancy [28]. This has proved to be most useful in countries which do not allow embryos to be manipulated or biopsied, such as Germany and Switzerland.

In 1991, Cha et al. developed the technique of in vitro maturation (IVM) of oocytes and reported their first pregnancy using this technology [29]. Silber et al. in 1994 reported the first cases of testicular sperm extraction (TESE) combined with intracytoplasmic injection [30] for men with obstructive and non-obstructive azoospermia. Other landmark developments were made in the diagnosis of male factor infertility, particularly in 1996 when Reijo et al. showed that some men with severe oligo-o-thenoospermia had deletions on the Y chromosome [31]. The whole understanding of male factor infertility has developed dramatically in the last 12–15 years.

The first autologous transplantation of frozen–thawed ovarian tissue was conducted by Oktay et al. in 2001 [32] and, in 2004, Donnez et al. reported the first live birth after orthotopic transfer of frozen–thawed ovarian tissue [33]. These advances will do much in the future to improve the reproductive outcomes for young women who require chemotherapy or radiotherapy for malignancies.

One of the major hurdles still to be overcome by clinicians and scientists practicing the ARTs is to reduce the number of multiple pregnancies created. It is considered now to be unacceptable that some 40–50% of children born as a result of IVF and related procedures are from multiple births, with the consequent major increase in complications, both for the babies and for the mothers. There is a very positive move now towards making the majority of ETs in an IVF program single embryo transfers – be it at day 2, 3 or blastocyst stages. This change in practice is largely being led by the Northern European countries, where multiple rates have been reduced to <10% and even, in some practices, to <5% [34]. However, in certain countries, transfer of four or more embryos is occurring in some 25–35% of cycles, producing twins in 25–35% of cycles and triplets in anywhere between 2.7 and 5.7% of deliveries. This is in spite of large numbers of fetal reductions being performed. However, these alarming figures are slowly reducing, year on year.

One of the most important developments in ART worldwide during the last 32 years has been the evolution of guidelines or regulatory systems to govern the practice of the ARTs. The state of Victoria, in Australia, was the first state to pass legislation on IVF in 1984; this became known as the “Infertility (Medical Procedures) Act 1984.” The UK was the first country to develop a full regulatory process and regulatory body. This started with an initial review by a Government appointed body, which produced a report in 1984 known as the “Warnock Report.” This proposed a UK regulatory system which would cover clinical and scientific practices of:

- all treatment involving the creation of human embryos outside the body
- all treatment involving donated gametes
- all storage of human gametes and embryos
- all research on human embryos

The report also recommended that all clinics providing ART services should be licensed by a regulatory authority. Following publication of this report in 1984, voluntary and then interim licensing authorities were set up to monitor ART practice in the UK. The Human Fertilisation and Embryology Act finally passed through Parliament in 1990, which led to the establishment of the Human
Fertilisation and Embryology Authority (HFEA) in 1991. This body is responsible for the licensing, regulation and monitoring all units practicing ART in the UK. The rules and regulations are set out in a “Code of Practice,” which is reviewed regularly, and in 2009 the eighth edition of the Code of Practice was produced, following passage of an updated Human Fertilisation and Embryology Act in 2008 [35].

Most countries in 2010 have some form of regulation, more or less strict, while other countries have guidelines, but there are still countries that have no regulation or guidelines at all. The state of regulation and practice worldwide is summarized in a 3-yearly publication produced by the International Federation of Fertility Societies (IFFS) and published by the American Society for Reproductive Medicine (ASRM); the last edition was published in 2007 [36].

The study of fertility, both animal and human, has fascinated clinicians and scientists for more than two millennia. Research into fertility and infertility led us, via many important milestones, to being able to treat women and men with hitherto untreatable infertility by IVF and related techniques. These treatments – the ARTs – are now very well established as “mainstream” treatments, almost universally accepted and practiced. Worldwide, there are now an estimated 4–5 million babies who have been born since human IVF was first successful in 1978. Although the early pioneering days of IVF are over, there is still a limitless amount of research to be done in the field of ART, particularly in genetics and stem cell research. It is also to be hoped that IVF and related ARTs will become still more simple and “patient friendly” and, in particular that they may become much cheaper, so that ART can be provided in the less developed countries, where presently infertile couples are unable to obtain treatment because of cost. It has been impossible to cover the whole story of the development of ART over so many years in one short chapter, but the present status of ART worldwide is built upon the fundamental achievements of the early scientific and clinical pioneers of our specialty. Their story bears more in-depth study and understanding, to better appreciate what we all struggle to achieve for our patients – families.

A thorough comprehension of the history of IVF would improve the depth of appreciation of challenges we are facing today, hopefully resulting in improved outcomes of future treatments [37].

Just as this book was going to press, the Nobel Prize Committee made the following announcement on the 4th of October 2010:

“Robert G. Edwards, the 2010 Nobel Laureate in Physiology or Medicine, battled societal and establishment resistance to his development of the in vitro fertilization procedure, which has so far led to the birth of around 4 million people.”

The many friends and colleagues of Robert “Bob” Edwards are delighted at this very happy and hugely well-deserved accolade and send him our most sincere congratulations.

References


Further recommended reading


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