To understand science, it is necessary to know its history Auguste Compte (1798–1857; said to be the first philosopher of science) It could be said that the first instance of assisted reproductive technology (ART) was when an eminent surgeon, John Hunter (1728–1793) of London (Figure 1.1), assisted a woman in becoming pregnant by taking a semen sample produced by her husband, who had hypospadias, and inseminating 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 intra-fallopian transfer (GIFT) – now rarely practiced
- zygote intra-fallopian transfer (ZIFT) – now rarely practiced
- intruterine insemination (IUI) of prepared husband/partner/donor sperm
- oocyte and embryo donation
- cryopreservation of sperm, oocytes, and embryos
- gestational surrogacy
- preimplantation genetic diagnosis and aneuploidy screening
- in vitro maturation of oocytes
- cryopreservation of testicular and ovarian tissue for future autologous use
- transplantation of ovarian tissue or whole ovaries

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 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 one hundred 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 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 onward that the science of animal and human reproduction really began to develop. However, it was not until the development of the optical microscope that researchers were able, for the first time, to study sperm, oocytes, and later, fertilization.

Anton J van Leeuwenhoek (1632–1723) (Figure 1.3), a Dutch draper and amateur scientist, was fascinated by the potential of the new science of microscopy. He built his own microscopes and, among many other specimens, he studied the sperm of different animal species; in 1677 he reported his findings to The Royal Society in London. He believed that each sperm was the beginning of an individual animal or human and, if it was “nourished” in the womb 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.

Dalenpatius in 1699 stated that he could see a miniature human within a single sperm, and the idea that humans were preformed within a sperm prevailed for more than a century, even though this report was later found to be a hoax, and Dalenpatius the fictitious name of the perpetrator of the hoax. It was Reinier de Graaf (1641–1673) 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 (1729–1799) (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, Carl 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 in 1876. Nelson observed the penetration of ascaris oocytes by spermatozoa; Newport made similar observations in amphibians, while both van Benedin and Hertwig are credited with the first observations on fertilization in mammals.

It is probable that Walter Heap (1855–1929), a physician and professor at the University of Cambridge, England, was the first scientist to transfer embryos successfully from one rabbit into another 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] gave their 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 (1908–1991) 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 (1925–2013) and published in 1965 in a landmark paper: "Maturation 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 a 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 (1913–1988) together in 1968.

Patrick Steptoe became known to Robert Edwards because he had brought laparoscopy, whereby the female pelvic organs could be visualized by this comparatively minor operation, to England from Europe where Steptoe had studied under both Raoul Palmer (1940–1995) and...
Hans Frangenheim (1920–2001). On his return to England he further developed the technique of laparoscopy, and shortly afterward wrote his famous short textbook *Laparoscopy in Gynaecology* in 1967 [5]. His first major paper “Laparoscopy and ovulation” followed in 1968 [6]. Shortly afterward, he still 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 he continued his work on immunology and oocyte maturation. He then spent a short time in the United States at Johns Hopkins University, where he collaborated with two other “greats” in the field of human ART, Doctors 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 apply it to the clinical treatment of 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 mid-piece and tail of the spermatozoon during fertilisation of human eggs in vitro” [8]; in 1970: “Laparoscopic recovery of pre-ovulatory human oocytes after priming of ovaries with gonadotrophins” [9]. They also carried out the first treatment cycles of oocyte recovery with tubal insemination, as they called the procedure, which was later to become known as GIFT.

Between the years 1968 and 1978, while they were working closely together, Steptoe was in Oldham, Lancashire, working in a National Health Service hospital, and Edwards 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 ETs, including the one ectopic pregnancy, Mrs Leslie Brown was treated and subsequently became pregnant following her first ET. 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 she finally delivered by caesarean section on July 25, 1978 (Figure 1.6). Much to the relief of everyone, baby Louise was found to be a perfectly normal fit and healthy infant. This momentous achievement was announced with a simple publica
tion as a letter in the *Lancet*: ”Birth after re-implantation 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, by 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, Doctors Howard and Georgeanna Jones had been working in Norfolk, Virginia, and Elizabeth Carr, the first baby conceived in vitro in the United States, 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 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 received a number of distinguished national and international awards over the next three years, but, in 1988, Patrick Steptoe fell seriously ill with prostate...
cancer and finally died on March 21 the same year.

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. He then went on to become the founder and editor of the journal *Reproductive Biomedicine Online* (RBMO). To “crown” his career, Robert Edwards was awarded the Nobel Prize in Physiology or Medicine for the development of IVF, and in 2011 was awarded a Knighthood by Her Majesty the Queen “for services to human reproductive biology.” He died on April 10, 2013, after a long illness. A most remarkable career for a most remarkable man.

In the early years following the first IVF births in England, Australia, and the United States, 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 Brian Lieberman’s group and Professor Ian Craft’s group also achieved live births, followed by, Feitchinger and Kemeter in Austria, and later that year births also occurred in Finland, Germany, and the Netherlands.
Introduction

An interesting historical point is the suggestion that the world’s second IVF baby was achieved in India, following the work by Dr Subash Mukhopadhyay. A baby was born on October 3, 1978, following, it was stated, IVF and ET, but his achievement was never officially recognized; indeed, he was derided by his colleagues and officials in India at the time as being a fraud. He eventually committed suicide in 1981. However, following a close investigation of his claim some 27 years later, he was officially accepted by his Indian colleagues 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 forties, and 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 for other reasons are unable to carry a child. Utian et al. published the first report of an IVF birth in the United States 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]. Gleischer 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 IUI, GIFT, and ZIFT also developed over the following years as more simplified variants of the 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 menopausal gonadotropin (HMG)
- Gonadotropin-releasing hormone agonists (GnRH-a) + urinary-HMG
  “Flare” protocol
  Ultrashort and short protocols
  Long luteal phase or Follicular phase start protocols
- 1990s GnRH-a + urinary follicle stimulating hormone (uFSH) (im)
  GnRH-a + high purity-FSH (sc)
  GnRH-a + high purity-HMG (sc)
  GnRH-a + recombinant-hFSH (sc)
- Late 1990s – 2000+
  GnRH-antagonists + r-hFSH ± r-LH
  Recombinant-LH (r-LH)
  Recombinant HCG (r-HCG)
- “Fill by mass” vs. IU recombinant-FSH
- “Patient-friendly” sc injections and use of “pens” for self-injection
- Minimum stimulation IVF or “IVF lite.”

As can be seen earlier, follicular 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 and Craft from 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 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, the recombinant gonadotrophins were developed, producing the purest forms of 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 (r-FSH).
More recently still, there has been an enthusiasm for the concept of “IVF lite.” This 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.

IVF 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 micro-injection 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 and colleagues of the Free University of Brussels [26]. This technique, for the first time, 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 cycles.

Other firsts include, in 1989, Handyside and his colleagues from London, who first showed it is possible to take a single blastomere from an embryo, perform preimplantation 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 ET 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 of oocytes and reported their first pregnancy using this technology [29].

Silber et al. in 1994 reported the first cases of testicular sperm extraction, combined with intracytoplasmic injection [30] for men with obstructive and nonobstructive 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 oligoasthenozoospermia had deletions on the Y chromosome [31]. The whole understanding of male factor infertility has developed dramatically in the last 20–25 years.

The freezing and storage of sperm for post-pubertal boys and men who are about to start chemotherapy or radiotherapy for malignancies is a well-established option to help them to achieve pregnancies in the future. This option however, for obvious reasons, is not available for prepubertal boys and infants. Current research suggests that it may now or soon be possible to take and store testicular tissue from these children and to be able to produce viable spermatozoa in the future from the tissue [32].

The preservation of human oocytes by vitrification has greatly increased the chances of future success for women freezing their oocytes, either for medical or for social reasons. However, oocyte vitrification does delay any chemotherapy or radiotherapy that may be necessary for about 14 days or more and the patients must be post-pubertal. For prepubertal girls and women requiring an immediate start to chemotherapy or radiotherapy, the only choices are to rely on the cryopreservation of their ovarian tissue or to rely on donor oocytes in the future.

In 1990, Gosden and colleagues, in their pioneering work, achieved viable pregnancies in mice by transferring primordial ovarian follicles [33] and later in sheep by transferring frozen/thawed ovarian autografts [34]. This early work led to the first autologous transplantation of frozen/thawed ovarian tissue in humans, conducted by Oktay et al. in 2001 [35], and, in 2004 Donnez et al. reported the first live birth after orthotopic transfer of frozen/thawed ovarian tissue [36].

Major progress has been achieved in recent years with the preservation of ovarian tissue for future use – either by slow freezing or by vitrification – followed by reimplantation of the ovarian tissue at a later date. Ovarian activity has been re-established and more than 100 live births have been achieved (as of June 2017) by natural conception. Pregnancies and live births have also been achieved after transplanting ovarian tissue from a normal fertile twin to her identical sister with premature ovarian failure [38].
These advances will do much in the future to improve the reproductive outcomes for young men and women who require chemotherapy or radiotherapy for malignancies. There is even now the possibility of both male and female gametes being produced from stem cells, and even from skin cells.

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 by the treatment. 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 toward making the majority of ETs in an IVF program single ETs – be it at the day 2, 3 or blastocyst stages. This change in practice was originally mainly led by the Northern European countries, where multiple rates have been reduced to less than 10%, and even in some practices to less than 5%. 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 [39]. The latest report from the European IVF-monitoring Consortium (EIM) published in July 2018 [40] shows that in 2013, single ET was performed in 31.4% of cycles, double ET in 56.3%, three ET in 11.5%, and four or more embryos transferred in only 0.7% of cycles. These resulted in singleton, twin and triplet delivery rates of 82.5%, 17.0%, and 0.5%, respectively – a very considerable change since the 2009 report [39].

One of the most important developments in ART worldwide during the last 40 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 United Kingdom was the first country to develop a full regulatory process and regulatory body. This started with an initial review by a government appointed committee, 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 United Kingdom. The Human Fertilisation and Embryology Act was 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 United Kingdom. The rules and regulations are set out in a “Code of Practice,” which is reviewed regularly, and the ninth edition of the HFEA Code of Practice was published in June 2018 (www.hfea.gov.uk/media/2609/june-2018-code-of-practice-9th-edition-draft.pdf) [41].

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 three-yearly publication produced by the International Federation of Fertility Societies (IFFS); the last edition was published in 2016 [42].

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 6–8 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
The Evolution of the ARTs

References


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