

## Chapter

## 1

# Introduction

Eric R. M. Jauniaux and Botros R. M. B. Rizk

Reproduction has been a major preoccupation since the dawn of human existence and treating infertility a medical objective for centuries. Since antiquity, written records indicate that most humans have been prolific, but difficulty with conception has been and remains a real problem for many couples around the world. In many cultures it has become a taboo, and as with a silent disease, is often not discussed. The inability to conceive continues to bear a stigma, and infertility may have a profound psychological and social impact resulting in marital discord, emotional stress, and depression. The lack of an heir in Royal and upper-class families can also have far-reaching political consequences [1]. Overall, there have been millions upon millions of couples that have had to cope with infertility throughout the ages [2]. In the U.K., fertility problems affect one in seven couples [3] whereas in the U.S., around 25% of married childless women 15–44 years of age have impaired fecundity [4]. It is estimated that four million modern couples have an infertility problem at any given time. Infertility and sterility now rank third among the most life-threatening diseases of the twenty-first century, according to the World Health Organization (WHO).

In ancient times, medicine was empirical and often based on magic. Physicians were commonly priests to a local deity who was responsible for the diseases and the gods played a fundamental role in the control of human reproduction and treatment of infertility. Some of the oldest written evidence for fertility tests dates back to ancient Egypt. In Egyptian society, women were equal to men, and difficulty with conception was not considered divine punishment but an illness that had to be diagnosed and treated. As far back as 1820 BCE, there are recorded documents discussing the treatment of gynecological disorders. For example, The Carlsberg IV (Copenhagen) and Kahum 28 (London) papyri describe a test to determine if a

woman will bear children. The test was based on the use of an onion placed for the night until dawn inside her flesh (vagina). If the odor appeared in her mouth she was told that she would bear children; if not, she will never bear children [5].

The earliest known use of a modern artificial reproduction technique is the case of a couple treated by the famous Scottish surgeon John Hunter, probably in 1776 (reported by E. Home at the Royal Society in 1799). The husband, a linen draper, was suffering from hypospadias and Hunter advised the couple to have normal intercourse, then he collected the semen in a warmed syringe and injected it into the wife's vagina. The couple had their first baby within the following year [6]. The first published account of a successful human artificial insemination was performed by a doctor Girault in France on June 5, 1838. His patient, a young countess, gave birth to a normal son on March 1, 1839. This case was the first in a series of 12, which were recorded in a paper published in 1868 (*L'Abeille Medicale* 25: 409–417) [7]. The American gynecologist Dr James Marion Sims (1813–1883) published his book on sterility, which included a chapter on artificial insemination. This book generated an extremely vigorous debate around the medical, moral, and ethical issues surrounding artificial insemination.

The first published reference to donor insemination was made by an Italian, Paolo Mantegazza, in 1887 [7]. With the exception of France, the wide acceptance of artificial insemination among the medical establishment took many decades. It was still extremely controversial in the U.S. in the 1940s; donor insemination was still quite controversial in Australia in the 1970s when the first spermatozoon banks and public hospital-based donor insemination programs were established. The use of donor spermatozoa or eggs is still forbidden by many religions.

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## Chapter 1. Introduction



**Figure 1.1.** Photograph of Sir Professor Robert “Bob” Edwards in 2008 (Bourn Hall, E. Jauniaux).

Robert “Bob” Edwards (Figure 1.1) was awarded the Nobel prize in medicine in 2010 and a knighthood in 2011 for his pioneering work in the development of in vitro fertilization (IVF). Bob dedicated his career to helping couples overcome infertility and his IVF technique has touched millions of lives across the globe. He first established principles of early embryo development that served as the foundation for his later work. Louise Brown was born on July 25, 1978 at 23:47 GMT at Oldham General Hospital, Manchester, U.K. The world’s first “test-tube baby” is the result of a decade of hard work and of an unusual collaboration between a scientist, Bob Edwards, and an obstetrician – gynecologist, Patrick Steptoe. This achievement is a landmark in not only the reproductive sciences but also the history of mankind’s technological evolution. Steptoe had been facing considerable criticism over his use of laparoscopy, even being isolated at clinical meetings in London [8], yet he had done more than 1000 cases whereas committee members had not done any.

A decade later they made medical history but they also opened a new ethical and religious Pandora’s box. Prominent ethicists, the Vatican, politicians, Nobel prize winners, and rigid protestants decried them, forecasting abnormal babies, a rumor that is still present in the collective mind of lay people today. They announced how IVF did not cure infertility, because women remained infertile after their IVF baby, and some labeled IVF as eugenic. The birth of the first IVF baby was snubbed by some clinicians now styled as “pioneers in artificial reproduction technologies” (ART), shouting that the test-tube claim was a fake! A grant application to the U.K. Medical Research Council for an IVF clinic in Newmarket, near

Cambridge, U.K., was rejected because it was claimed that laparoscopy was dangerous and rhesus monkeys should have been used initially. Major ethical arguments in the press formed a constant background for more than a decade after Louise Brown’s birth.

At some point, Bob Edwards had to issue eight libel actions in the High Court of London, U.K., on a single day, and many have continued to oppose what they saw to be interfering with nature. When interviewed on her 30th anniversary, Louise Brown said: “The children at school used to ask questions like ‘how did you fit in a test tube?’ and things like that, but they could see I was normal; they could see I was the same as them.” The same year it was estimated that around three and a half million women worldwide had undergone IVF. In the U.K., birth country of the first IVF baby, it took more than 25 years before the government supported funding for IVF in the National Health Service (NHS), but four years down the line 94 % of trusts were still not providing the full three cycles recommended by the Department of Health guidelines [9]. Low-cost IVF programs are being developed for low-income countries to improve access to ART for infertile couples living in Africa [10], showing the universal desire of achieving a pregnancy and the worldwide contribution of IVF to the treatment of sterility.

When, on October 4, 2010, it was announced that Edwards had been awarded the 2010 Nobel prize in physiology or medicine for the development of in vitro fertilization, a Vatican official condemned the move as “completely out of order.” Edwards’ work not only provided the means to overcome many forms of infertility, but it also gave us a better understanding of the early stages of human embryonic development and preimplantation genetic diagnosis (PGD) [11,12]. His original IVF work has also opened up areas further from reproductive health such as human embryonic stem cell research. From the beginning of his career, Edwards realized the wide-ranging new therapeutic applications of embryos created in vitro for degenerative disorders and cancer [13–19], opening another ethical and philosophical Pandora’s box and a new debate [20–23], which is going to carry on for many decades to come.

For decades, rumors were spread about the long-term health of children born following IVF and other ART. However, the most common complications associated with IVF treatment have remained indirect and technical, such as the failure of treatment, ovarian

hyperstimulation syndrome, the surgical risks associated with egg collection, and the possibility of ectopic pregnancy. Probably the most controversial possible complications of IVF/ART are the risk associated with multiple pregnancies and the issue of birth defects following the use of newer ARTs such as intracytoplasmic spermatozoon injection (ICSI). These issues and other topics related to ART pregnancies are presented and discussed in this book, which we dedicate to Bob Edwards as a tribute to his constant support and friendship and to mark his unique contribution to medicine.

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## Chapter

## 2

# Assisted reproductive technology pregnancies: the historical perspective

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## Introduction

Since the birth of the world's first in vitro fertilization (IVF) baby in 1978, an estimated four million children have been born to date following artificial reproductive technology (ART) [1]. The first data on the number of ART births worldwide were collected in 1989. In that year, only about 30 000 babies were born following ART. Based on the most recent data from the International Committee for Monitoring Assisted Reproductive Technologies (ICMART) for the year 2002 collected from 53 participating countries, it has been estimated that ART now produced approximately 200 000 babies per year, representing an increase of 12% compared to the year 2000 [2]. This chapter reviews the history of ART starting with the first attempts at IVF in animals and presents the incredible journey taken by the pioneers to give us an efficient treatment for human infertility, culminating in 2010 with the award of the Nobel prize to Robert Edwards.

## How did it all begin?

Probably one of the first unpublished attempts at IVF is that of the famous British surgeon John Hunter (1728–1793) (Figure 2.1), who in the summer of 1767 attempted to artificially impregnate silkworms. He kept a female moth in confinement until she laid some unfertilized eggs, dissected a male moth to collect a sample of semen, and then combined the two in a covered box. The experiment was successful, leading to eight of the eggs hatching at the same time. “Thus then I ascertained that the eggs could be impregnated by art [sic], after they were laid” Hunter recorded [3]. Hunter's scientific approach was ahead of its time, but if most of his numerous discoveries were presented at the Royal Society in London, they remain unpublished during his life. Furthermore, his discoveries on

evolution, which were later to inspire Charles Darwin, challenging the traditional biblical story made him a pariah at the Royal Society.

Similarly, Anton Philips van Leeuwenhoek (1632–1723), who recorded the first microscopic observations of human spermatozoa in 1677, had a lot of trouble with his discoveries and in particular with Dutch theologians. Most of his microscopic observations were initially met with scepticism, including by the Royal Society. At around the same time (1779), Lazzaro Spallanzani (1729–1799), an Italian physiologist, described the role of semen in fertilization, and showed that spermatozoa have to make physical contact with the egg for fertilization to take place. He is also credited with having noted that frog oocytes only develop into tadpoles after contact with semen, with having performed the first artificial insemination of a mammal (dog), and with having attempted IVF in frogs. However, these early experiments were ignored by the scientific establishment, and before the nineteenth century, precisely how fertilization took place and the role played by the male and female gametes led to numerous fruitless debates among European scientific societies.

The first observation of spermatozoon penetration into an ovum was reported in a non-mammalian species (*Ascaris mystax*) by Henry Nelson at the Royal Society in 1851 and published the following year in the *Philosophical Transactions* [4]. Newport (1853) made similar observations in amphibians, and van Beneden (1854) and Hertwig (1876) are credited with the first observations on fertilization in mammals. In 1890, Walter Heape (1855–1929), who had performed research on reproduction in numerous animal species, became the first known scientist to successfully transfer embryos in a mammal (rabbit). In his first experiments, probably performed at a laboratory at his home in Manchester (U.K.), he flushed two embryos from the

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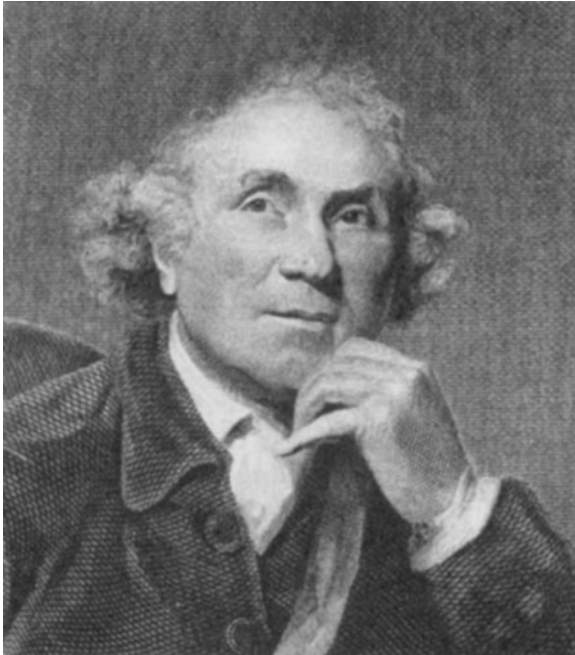
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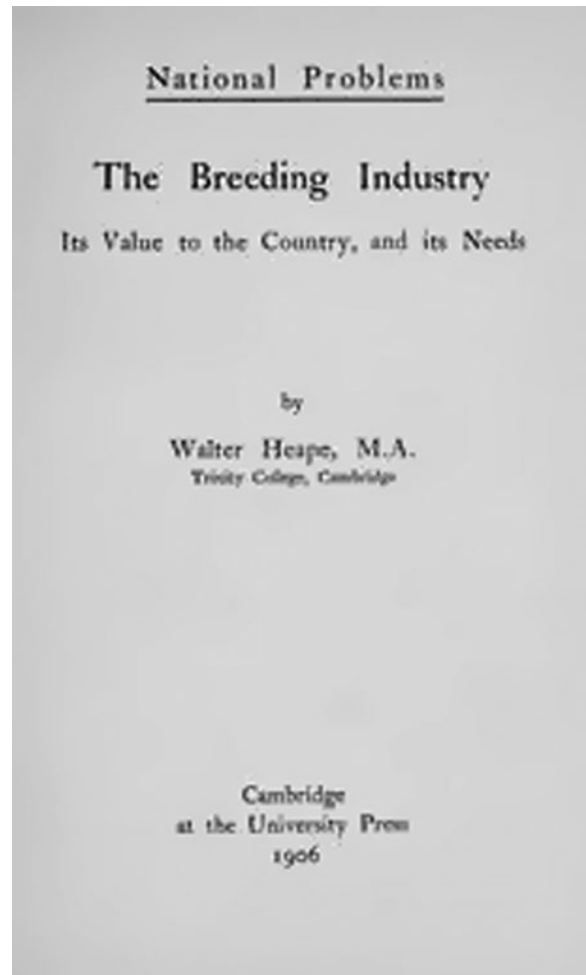
## Chapter 2. Assisted reproductive technology pregnancies: the historical perspective



**Figure 2.1.** Extract of the portrait of John Hunter by Sir J Reynolds PRA, 1786.

Fallopian tubes of an Angora doe rabbit and placed them into the uterus of a recently mated Belgian hare doe, resulting in the birth of a litter of six young, four of them Belgians and two of them Angoras. His experiments proved conclusively that it was possible to transfer embryos to a gestational carrier without affecting their development [5]. In his book *The Breeding Industry* (Figure 2.2), published in 1906, he criticized the British Government for not supporting the scientific study of animal breeding and improvement [6]. The same criticism could be applied to the fact that it took nearly 30 years after the birth of the first IVF baby for the British Government to provide funding for IVF treatment within the National Health Service (NHS).

Many scientists, inspired by Heape's results, started culturing eggs and embryos in laboratories around the world. Gregory Pincus (1903–1967), working at Harvard University, was the first to show that eggs of various animals could be matured when released from their follicles and cultured in vitro. In 1935 he described the first experimental condition that allowed rabbit oocytes to mature in culture, reaching the metaphase stage of meiosis II [7]. He claimed to have achieved successful mammal birth from the result of in vitro fertilization of rabbit eggs. As nobody could repeat his experiments at the time, doubts were



**Figure 2.2.** Front cover of Walter Heape's book *The Breeding Industry*, published in 1906.

cast over the authenticity of the claim. It was in 1959 that Min Chueh Chang (1908–1991), who was working at the Worcester Foundation for Experimental Biology in Shrewsbury, MA (U.S.) that Pincus co-founded in 1944, reported that in vitro-matured black rabbit oocytes could be fertilized in vitro and also give rise to viable embryos. Furthermore, when these embryos were transferred back to adult females, they resulted in viable offspring [8]. This was the sort of evidence attesting to the feasibility of in vitro fertilization (IVF) for which many scientists had been searching. In the years that followed, Chang and his colleagues conducted further research to determine specific conditions of successful IVF and performed the technique on other mammals such as hamsters, mice, and rats. It was on the basis of Chang's findings

## Chapter 2. Assisted reproductive technology pregnancies: the historical perspective

that the first IVF of human eggs was done. As these experiments were very controversial at the time, Pincus and Chang remained best known for having invented the combined oral contraceptive pill.

Robert Edwards started his career studying genetics at Edinburgh University (U.K.), obtaining his PhD in 1956 for his work on inducing heteroploidy in mouse preimplantation embryos. He became interested in working with human eggs at the end of the 1950s. At the National Institute of Medical Research, London (U.K.) in 1960, he became interested in alleviating infertility and tried maturing animal and human oocytes in vitro. The animal experiments were very successful, but Edwards worked for two years trying to induce human egg maturation without success, because human eggs did not mature in vitro when released from the follicle, as Pincus had reported in 1939. Finally, Edwards discovered that human oocytes required 37 hours to polar body extrusion, and having timed each stage of human oocyte maturation, he opened the way to human IVF [9,10]. After a couple of years at Glasgow University, working on embryonic stem cells, he moved to Cambridge University (U.K.) in 1963. In 1968 he and Barry Bavister, his then research student, achieved the first human fertilization in vitro using a high-pH medium, which proved unnecessary in later studies. Reading the *Lancet*, Edwards learned about Patrick Steptoe (1913–1988) and laparoscopy. Steptoe was criticized for developing this technique by almost every gynecologists because they thought it was dangerous. Edwards and Steptoe set up a small laboratory in a room next to the operating theater in Oldham District General Hospital in the suburbs of Manchester, 180 miles from Cambridge. This meant that Edwards would have to travel three to four hours one way to get to collect oocytes retrieved by laparoscopy by Steptoe at Oldham. Women stimulated with low-dose human menopausal gonadotrophin/human chorionic gonadotrophin (HMG/hCG) ovulated at 37 hours post-hCG; thus Steptoe aspirated eggs at 35–36 hours to gain 5–6 proovulatory oocytes on average before they ovulated [11–14]. Their attempts met significant hostility and opposition in the U.K. including a refusal by the British government (Medical Research Council) to fund their research because laparoscopy was considered too dangerous, and a number of lawsuits followed.

Having seen the results of Edwards and Steptoe's experiments, another research team followed their tracks and the first IVF pregnancy, which resulted in early miscarriage, was reported in 1973 by the Monash

research team of Carl Wood in Melbourne (Australia) [15]. In 1976 Steptoe and Edwards published a case of an ectopic pregnancy following transfer of a human embryo at the early blastocyst stage [16]. After this adverse outcome, they decided to abandon ovarian stimulation and instead rely on the natural menstrual cycle of the patients. Based on the concentration of luteinizing hormones in the women's urine, they could predict when the maturing oocyte would reach the metaphase stage of meiosis II in vivo and then proceed to egg retrieval by laparoscopy before ovulation occurred. They managed to aspirate a single oocyte in a natural menstrual cycle without using any fertility medication. In addition, they proceeded to an earlier embryo transfer, at the eight-cell stage, in order to compensate for the inadequate culture conditions in vitro [17].

The birth of Louise Brown, the world's first "test tube baby," at 11:47 P.M. on July 25, 1978 at the Oldham General Hospital, Manchester (U.K.) made medical history (Figure 2.3). Her mother Lesley had failed to conceive over a nine-year period due to her



**Figure 2.3.** Front cover of the *Evening News* of July 27, 1978, two days after Louise Brown was born at Oldham General Hospital, Manchester, U.K.

## Chapter 2. Assisted reproductive technology pregnancies: the historical perspective

bilateral Fallopian tube obstruction and had been referred to Steptoe in 1976. A single oocyte was aspirated from one of Lesley's ovaries during laparoscopy, fertilization in vitro was performed, and a few days later the developing embryo was transferred into Lesley's uterus [18].

On December 20, 2006, Louise gave birth to her own child, Cameron John Mullinder, without the need for IVF treatment. Following the birth of Louise Brown, Edwards and Steptoe founded an infertility clinic at Bourn Hall, in Cambridge, U.K. (Figure 2.4) where the second and third children in the world were born after IVF [19]. In 1984 Edwards was elected as a Fellow of the Royal Society (London), in 2001 he was awarded the Albert Lasker Clinical Medical Research Award by the Lasker Foundation (New York), and on October 4, 2010 it was announced that Edwards had been awarded the 2010 Nobel prize in physiology or medicine for the development of in vitro fertilization. A Vatican official condemned the move as "completely out of order."

The first IVF birth in Australia was achieved in 1980 by the Victorian Monash–Melbourne team, also using the natural cycle [20]. Nevertheless, the achievement of pregnancies was still sporadic, mainly due to the limitations of the natural cycle.

A major breakthrough that increased the achievement of pregnancies in a consistent way and led to the worldwide application of IVF was the re-introduction in 1981 of ovarian stimulation in IVF by Trounson et al. [21]. In vitro fertilization started to spread over the world and one year later the first IVF baby in the U.S. was announced by Howard and Georgianna Seegar Jones in Norfolk, VA [22]. In 1982, the first French IVF birth was reported in Clamart, Île-de-

France, by Testart and Frydman [23]. The same year the first IVF birth was announced in Sweden [24] and in Austria [25]. By 1986 about 1000 additional births had been reported in different countries.

### Further assisted reproductive technology developments and pregnancies

#### Gamete intra-Fallopian transfer and zygote intra-Fallopian transfer

Intratubal transfer procedures were attempted as an alternative to IVF. Although the technique of gamete intra-Fallopian transfer (GIFT) for all forms of non-tubal infertility was introduced in 1979 by Shettles [26], it was only four years later that the first pregnancy was reported by Tesarik et al. [27]. The original concept was that transferring gametes back into the Fallopian tube would benefit from the protective tubal environment. If fertilization occurred, transfer of embryos in the uterus would take place at a more appropriate time, and finally, the avoidance of uterine cavity trauma, which might occur during a transcervical intrauterine transfer procedure, would lead to higher implantation rates. However, the main disadvantage of the GIFT technique was the lack of control with regard to fertilization.

In 1986 zygote intra-Fallopian transfer (ZIFT), a technique in which pronuclear-stage embryos were transferred into the Fallopian tube, was introduced by Devroey et al. [28] and the first report of a pregnancy was announced the same year. Zygote intra-Fallopian transfer combined both the advantages of the GIFT



**Figure 2.4.** Outside view of Edwards and Steptoe's Bourn Hall infertility clinic.

## Chapter 2. Assisted reproductive technology pregnancies: the historical perspective

technique with those of IVF, as normal fertilization could be confirmed, excluding polyploid embryos and incubating immature oocytes.

### Intracytoplasmic spermatozoon injection

Although IVF had been successfully applied in couples with male infertility, it became apparent that the results of conventional IVF were significantly decreased when the semen characteristics of the male partner were well below the World Health Organization (WHO) criteria. This was due to the significantly lower percentage of oocytes than would normally have been fertilized, resulting in the formation of fewer embryos available for transfer [29]. Several procedures of assisted fertilization were developed and used in cases of severe semen deficiencies instead of conventional IVF, such as partial zona dissection (PZD) and subzonal insemination (SUZI). However, both were associated with low fertilization, pregnancy, and delivery rates that precluded their routine clinical use.

The first direct injection of a single spermatozoon into the ooplasm, after passage through the zona pellucida and the membrane of the oocyte, was reported by Lanzendorf et al. [30], but was abandoned because of disappointing results. However, the team at the Free University of Brussels (Belgium) continued to experiment with this technique and obtained the first successful fertilization and pregnancy, which was delivered on January 14, 1992. In 1992 Palermo et al. reported the first pregnancies obtained by this novel technique of assisted reproduction, which they called intracytoplasmic spermatozoon injection (ICSI), in couples with infertility caused by severely impaired spermatozoon characteristics and for which IVF and SUZI had failed [31]. Hence, from 47 metaphase-II oocytes, 38 oocytes remained intact after injection, 31 became fertilized, and finally, 15 embryos were transferred. After eight treatment cycles, four pregnancies occurred, two singletons and one twin pregnancy, that resulted in four healthy babies.

### Spermatozoon recovery techniques and assisted conception for azoospermia

Various procedures for spermatozoon recovery have made it possible to help men with both obstructive and nonobstructive azoospermia to achieve genetic fatherhood. Human pregnancy by IVF using spermatozoa by percutaneous epididymal spermatozoon aspiration

(PESA) was first reported in 1985 [32]. The first two babies born after microsurgical epididymal spermatozoon aspiration (MESA) from men with congenital bilateral absence of the vas deferens (CBAVD) were reported in 1988 [33]. However, the overall results of IVF and MESA in cases of obstructive azoospermia were poor, with low conception rates [34].

The introduction of ICSI not only significantly improved the fertility prospects after assisted reproduction by using spermatozoa recovered from the seminal tract, but has also allowed extension of the spectrum of recovery techniques. For example, in the case of obstructive azoospermia, it became possible to use not only spermatozoa obtained by MESA, but also spermatozoa obtained from the testicle by means of testicular biopsy (TESE), or spermatozoa percutaneously aspirated from the testicle/epididymis by minimally invasive techniques (TESA, PESA, FNA [fine needle aspiration]). Within this context, ICSI with epididymal or testicular spermatozoa has revolutionized the treatment of patients who were infertile because of congenital disorders causing obstruction of the excretory ducts. The first successful fertilization by testicular spermatozoa from a man with CBAVD and ICSI was in 1993 by Schoysman et al. [35], while the first pregnancies using TESE and ICSI were reported by Silber et al. [36] and Tournaye et al. [37] in 1994. The first pregnancies after TESE and ICSI in cases of nonobstructive azoospermia were reported by Devroey et al. in 1995 [38].

### Embryo and oocyte cryopreservation

The introduction of superovulation treatment as part of ART usually results in the development of multiple embryos. Replacement of more than one embryo increases pregnancy rates to a certain extent, but at the same time leads to multiple pregnancies, with increased pre- and postnatal risks (see Chapter 8). In this respect, cryopreservation of human embryos has been considered as the method to reduce multiple pregnancy rates. Furthermore, success with frozen embryo transfer cycles increases the cumulative pregnancy rate per retrieval. The first report on human pregnancy following cryopreservation, thawing, and transfer of an eight-cell embryo was reported by Trounson and Mohr in 1983 [39], who used a slow freezing protocol with dimethyl sulfoxide (DMSO). Unfortunately, this pregnancy was terminated at 24 weeks' gestation, because of the development of a



## Chapter 2. Assisted reproductive technology pregnancies: the historical perspective

septic *Streptomyces agalactiae* chorioamnionitis after premature rupture of the membranes. The first live birth of twins following the transfer of intact frozen-thawed embryos was subsequently reported by Zeilmaker et al. in 1984 [40].

Although pregnancy rates related to the use of frozen-thawed oocytes are improving, they still remain well below those that can be achieved with established IVF procedures. The first successful attempt of deep freezing and thawing of a human oocyte was reported by Chen in 1986 [41], while a twin pregnancy was achieved after insemination and replacement in utero of frozen-thawed oocytes one year later by Van Uem et al. [42].

Slow-freeze protocols using controlled rate freezers that decrease the temperature to below  $-30^{\circ}\text{C}$  have traditionally been used for embryo cryopreservation in the laboratory. Additionally, diverse cryoprotectant solutions have been used for embryo dehydration, depending on the cell stage. Recently, a lot of attention has been given to an ultra-rapid method of cryopreservation or vitrification, whereby the embryo is transitioned from  $37^{\circ}\text{C}$  to  $-196^{\circ}\text{C}$  in less than 1 sec. Vitrification has shown great promise for cryopreservation of human embryos and oocytes, because it minimizes cryo-injuries by preventing the formation of intracellular ice crystals. The first successful human cleavage-stage embryo vitrification followed by a successful delivery was reported by Gordts et al. in 1990 [43], while the first live births after vitrification of oocytes from a stimulated IVF cycle were reported in 2003 by Yoon et al. [44].

### Oocyte donation

The first successful delivery following oocyte donation was announced by Buster et al. in 1983 [45]. The original technique involved intracervical artificial insemination of a volunteer with spermatozoa from the partner of the infertile woman, uterine lavage during the preimplantation interval, and finally, transfer of the developed embryo to the uterus of the infertile woman, who received a hormone replacement regimen in order to synchronize endometrial and embryo development. The same year, the first pregnancy after ovum donation, IVF, and transfer in a woman without ovaries was reported by Trounson et al. [46]. Within a year, the first pregnancy resulting from oocyte donation in a woman with ovarian failure was also reported [47].

### Preimplantation genetic diagnosis

When Robert Edwards moved to Cambridge (U.K.), he suggested to one of his students, Richard Gardner, that he follow-up on the Glasgow work on stem cells. Their first approach was to inject one or several mouse inner cellular mass cells or cultured embryo stem cells into the blastocoelic cavity of recipient blastocysts, using specific genetic markers for host and donor cells. The method worked as chimerism and was established in literally all tissues of many offspring. They also worked jointly to test if operations on rabbit blastocysts would permit the excision of a few cells, which could be used for establishing the gender (“sexing”) by staining them for the sex chromatin body expressed in female but not male embryos. After transferring the “sexed” blastocysts to recipient females, all offspring were correctly sexed – the first example of preimplantation genetic diagnosis (PGD) [48].

Preimplantation genetic diagnosis was only introduced clinically at the beginning of the 1990s as an alternative to prenatal diagnosis, in order to select healthy embryos, thus reducing the risk of having a child affected by a sex-linked genetic disease. The first embryos obtained in vitro were tested to determine their gender, and only female embryos were transferred. Handyside et al. [49] were the first to describe pregnancies from biopsied human preimplantation embryos that were selected for gender by Y-specific DNA amplification in order to avoid the transmission of a sex-linked disease to boys, adrenoleukodystrophy, and X-linked mental retardation. Since then, techniques for genetic analysis at the single-cell level, involving assessment of first and second polar bodies, fluorescence *in situ* hybridization (FISH) for the analysis of chromosomes, and polymerase chain reaction (PCR) for the analysis of genes in cases of monogenic diseases, have been introduced. The first report on polar body biopsy, transfer of the embryo, and achievement of pregnancy was by Verlinsky et al. in 1990 [50]. Pregnancy after embryo biopsy and coamplification of DNA from X- and Y-chromosomes was reported by Grifo et al. [51]. Munné et al. published the first report of aneuploidy testing in 1995 [52]. The first live birth following blastocyst biopsy and PGD analysis was reported in 2002 by De Boer et al. [53]. The same year, the first clinical application of comparative genomic hybridization and polar body testing for PGD of aneuploidy was performed by Wells et al. [54]. The first clinical experience of

## Chapter 2. Assisted reproductive technology pregnancies: the historical perspective

preimplantation human leukocyte antigen (HLA) matching without PGD of a causative gene was reported by Verlinsky et al., demonstrating the feasibility of this novel approach for stem cell transplantation in siblings with bone marrow failure [55]. Recently, Fishel and colleagues from CARE Fertility, Nottingham (U.K.) reported a live birth after polar body array comparative genomic hybridization [56].

### In vitro maturation

The application of IVF in combination with ovarian stimulation has proved to be a successful treatment for infertile couples. However, several drawbacks of this technique, for example, the risk of ovarian hyperstimulation syndrome, the high cost of medication, the inconvenience of daily gonadotropin injections, and their side effects, have stimulated researchers to develop new treatment strategies. The idea of retrieving immature oocytes from the unstimulated ovary, to mature them in vitro, and finally to fertilize the resulting mature oocytes in order to create multiple embryos seemed to be an attractive idea and a solution that could compensate for the disadvantages of IVF. The first human pregnancy following in vitro maturation (IVM) in an unstimulated cycle in a donor oocyte program was reported by Cha et al. [57], 13 years after the birth of Louise Brown. In 1994 the first live birth as a result of IVM in a polycystic ovarian syndrome (PCOS) patient following transvaginal ultrasound-guided oocyte collection was reported by Trounson et al. [58]. A first report of fertility preservation for cancer patients using IVM and oocyte vitrification was published by Rao et al. [59]. The team at McGill Reproductive Center (Canada) has reported a series of deliveries after transfer of human blastocysts derived from oocytes matured in vitro, showing an implantation rate of 26.8% and a clinical pregnancy rate of 51.9% [60].

### Ovarian tissue cryopreservation

Cryopreservation of ovarian tissue is the only option available for prepubertal girls and women who have to undergo chemotherapy without delay and wish to preserve their fertility. Several attempts were made to cryopreserve ovarian cortex strips and reimplant them after thawing. With regards to autotransplantation, Oktay and Karlikaya were the first to report on ovarian tissue transplants after frozen storage [61]. However, the first pregnancy was reported a few years later by Donnez et al.

[62]. In 1997, they took biopsy samples of ovarian cortex from a woman with stage IV Hodgkin's lymphoma, which were cryopreserved before chemotherapy was initiated. After her cancer treatment, the patient developed premature ovarian failure. In 2003, after thawing, orthotopic autotransplantation of the ovarian cortical tissue was realized by laparoscopy. Eleven months after reimplantation, a viable intrauterine pregnancy was confirmed, which resulted in a live birth. Since this first live birth after autotransplantation of cryopreserved ovarian tissue, orthotopic reimplantation has led to the birth of 13 healthy babies from cancer patients and women treated with high doses of chemotherapy for benign diseases [63]. Pregnancies have also been obtained after heterologous transplantation of fresh or cryopreserved ovarian cortical tissue between twins discordant for premature ovarian failure [64,65]. Despite the births from ovarian tissue transplantation, the overall success rate remains low and further research is needed in order to define the optimal conditions for ovarian tissue transplantation. Alternatives, such as whole ovary or isolated follicle transplantation, require further investigation but are likely to be successful in humans in the future.

### Conclusion

The road to finding a cure to human infertility has been bumpy, but since the birth of Louise Brown, the first IVF baby, substantial progress has been made. As technology and outcomes improved, ART became a successful treatment for an increasing number of infertile couples worldwide. The advent of those techniques greatly contributed to the rise in the number of children born from ART. Robert Edwards forced the doors open but we are still at the end of the beginning of the development of ART.

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