40 Years After In Vitro Fertilisation

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State of the Art and New Challenges

Edited by

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FORTY YEARS OF IN VITRO FERTILISATION: A HISTORY OF CONTINUOUS EXPANSION

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Summary

Since the birth of the world's first baby conceived by *in vitro* fertilisation (IVF), the technique, initially restricted to a relatively small group of infertile women affected by tubal infertility, has expanded progressively to other types of both female and male infertility. Nowadays, IVF is used as a method of choice in virtually all cases of infertility which cannot be resolved by simpler and less invasive means. Moreover, the fact that IVF makes the fertilisation process and early embryonic development accessible to direct monitoring and targeted therapeutic interventions has led to an increasing use of IVF outside the field of infertility treatment. In particular, it can prevent different kinds of health risk and allow the birth of normal children to parents affected by various types of disease.

1. Introduction

The concept of *in vitro* fertilisation (IVF) had been born a long time before the first IVF baby was born, based on animal experimentation. According to a recent overview of IVF history (Biggers, 2012), the first attempts using rabbits and guinea pigs were described by S.L. Schenk in 1887, but the first supposedly successful IVF, followed by early embryo cleavage, was reported in rabbits in the mid 1930s (Pincus and Enzmann, 1936).

The first IVF trials in humans were made possible by advances in oocyte retrieval techniques, on the one hand, and by the availability of efficient laboratory techniques for sperm and oocyte *in vitro* treatment to enable fertilization, on the other hand. The advances in oocyte retrieval techniques were marked by three major highlights: the introduction of laparoscopy, the use of vaginal ultrasound examination, and controlled

ovarian stimulation to obtain multiple follicular development and recovery of various oocytes per attempt. The history of the application of these techniques in human IVF practice is overviewed by John Yovich in Chapter 2 of this book.

The most decisive advancement in the laboratory part of the IVF procedure was the discovery of sperm capacitation. This process which normally takes place during sperm passage through the fallopian tube (Chang, 1951; Austin, 1951), can be reproduced *in vitro* by means of extensive sperm washing from seminal plasma-derived components (Yanagimachi and Chang, 1963; Gwatkin and Andersen, 1969). It was also important to develop culture media capable of supporting the early development of *in-vitro* fertilised oocytes until the transfer of the resulting embryos to the mother. This was made possible by pioneering animal experiments carried out principally in two species: the rabbit and the mouse. These studies led to a progressive replacement of media based on ill-defined biological fluids with chemically defined media (reviewed in Summmers and Biggers, 2003), although small amounts of biological materials were usually added to optimise embryo *in vitro* development. The first human embryos resulting from IVF (Edwards et al., 1969) were achieved with the use of spermatozoa previously capacitated *in vitro* in a modified Tyrode solution (Yanagimachi and Chang 1963) and subsequent embryo culture in Bavister's medium supplemented with human or fetal calf serum (Edwards et al., 1969).

This was the beginning, which was subsequently followed by a series of discoveries, both in the clinical and the laboratory part of the IVF procedure. This discoveries progressively led to an increase in efficiency and versatility of IVF, making it a method of choice in various types of infertility (Fig. 1). This is a brief overview of these discoveries, covered in detail in individual chapters of this book.

2. The beginnings

The first human birth after IVF (Steptoe and Edwards, 1978) was achieved in a 30-year-old woman with bilateral fallopian tube occlusion. A single metaphase II oocyte, recovered by laparoscopy soon after the beginning of the natural mid-cycle surge of luteinizing hormone (LH), was inseminated *in vitro* with spermatozoa previously capacitated *in vitro* as described previously (Edwards et al., 1969). Two years later, the authors described, in more detail, the whole series of interventions leading to this first birth. These interventions were performed in 68 patients with fertilization; cleavage was achieved in 34 instances and 32 embryos were introduced

into the mother's uterus via the cervical canal, resulting in four pregnancies (Edwards et al., 1980). To identify the right moment of the preovulatory LH surge, it was necessary to analyse 3-hour samples of urine, whereas follicular growth was assessed indirectly, by measurements of urinary oestrogens in 24-hour samples (Edwards et al., 1980). To reduce laboratory workload and increase IVF efficiency, in terms of the number of oocytes obtained per attempt, important advancements were soon achieved over the following years, both at the clinical and laboratory level. We can say that each of these improvements was initiated in the clinical branch, creating new challenges requiring appropriate innovations at the laboratory level (Fig. 1).

The most important clinical advancements applied in the IVF technology soon after the world's first childbirth concerned the substitution of natural ovulatory cycles with protocols of controlled ovarian stimulation and the application of vaginal ultrasound to monitor directly the progression of follicular growth. These clinical advancements led to the retrieval of multiple oocytes during each treatment cycle. Some of these oocytes had not yet reached meiotic maturity at the time of recovery, and all embryos resulting from the mature oocytes could not be transferred to the mother at a time. This led to the development of laboratory techniques to achieve *in vitro* maturation of the immature oocytes and to store the "supernumerary" embryos resulting from the IVF attempt for later transfer. Altogether, these changes enabled a substantial improvement of IVF efficiency and allowed its use in other types of infertility. According to a report on 1200 IVF cases, released by the authors of the first IVF childbirth publication 5 years later (Edwards and Steptoe, 1983), the couples treated included, in addition to tubal infertility, cases of defective spermatogenesis, prolonged unexplained infertility, and combined factors of both male and female origin.

3. Expansion of IVF indication to different types of infertility

The origin and consequences of controlled ovarian stimulation: oocyte in vitro maturation, embryo freezing and luteal phase support

Ovulation induction has been used since the early 1960s, well before the first IVF attempts, mainly to treat women with anovulation. The first ovulation protocols used clomiphene citrate to stimulate endogenous follicle-stimulating hormone (FSH) production (Greenblatt et al., 1961).

This protocol was soon adapted for use in IVF, where it was combined with the injection of human chorionic gonadotropin (HCG) to induce final oocyte maturation (Trounson et al., 1981; Edwards and Steptoe, 1983). This simplified the timing of oocyte retrieval as compared with natural cycles. However, it was the advent of direct stimulation of ovarian follicular growth by the administration of exogenous FSH which marked the major progress of IVF in the early 1980s, enabling the retrieval of multiple oocytes in a single cycle as compared with a single one in the natural cycle. Even though the first preparations of FSH were purified pituitary extractions (Gemzell et al., 1958), the use of these preparations was hardly compatible with the rapidly expanding IVF programmes. The pituitary glands from as many as 10 individuals were needed to yield sufficient quantities of gonadotropin to stimulate one patient for one cycle which made it inefficient. Moreover, while unknown at the time, some of these women were infected with Jacob Creutzfeld disease and died from this condition several years after treatment (Beall and DeCherney, 2012).

The development of the methods for extraction and purification of gonadotropins from menopausal urine led to the introduction of the so-called human menopausal gonadotropin (HMG), basically a mixture of FSH and LH, which was standardized and commercialized by Serono under the commercial name of "Pergonal" (Lunenfeld et al., 1961) and rapidly introduced into the IVF practice (Garcia et al., 1983).

Further development of ovarian stimulation protocols went through progressive standardization of the hormonal preparation composition and their purification. This trend culminated in the development of recombinant hormones, the prevailingly used form of hormonal preparations for ovarian stimulation nowadays, as resumed by Bernadette Mannaerts in Chapter 9 of this book. The application of vaginal sonography to monitor follicular growth during controlled ovarian stimulation and to guide the embryo transfer procedure marked further improvement of the efficiency of IVF. These aspects are reviewed by David Meldrum and William Schoolcraft in Chapter 5 of this book.

The use of controlled ovarian stimulation led to an increase in the availability of both mature and immature oocytes recovered in a single treatment attempt. This situation led to the necessity of developing laboratory techniques for preservation of supernumerary embryos for later transfer and for *in vitro* maturation (IVM) of those oocytes which were immature at the time of recovery (Fig. 1). The first birth after cryopreservation, thawing and transfer of an eight-cell human embryo was reported in 1983 by Alan Trounson's team at the Monash University in Melbourne, Australia (Trounson and Mohr, 1983). The history of embryo

and oocyte cryopreservation techniques is reviewed by Laura Rienzi and Alan Trounson in Chapter 3 of this book.

In the same year, an improved method for maturation and fertilisation of immature human oocytes was described (Veeck et al., 1983). Moreover, oocyte *in vitro* maturation (IVM) can also be used without ovarian stimulation in cases in which hormonal treatment required for ovarian stimulation is contraindicated for various reasons. The first successful live birth, of healthy triplet girls, which involved immature follicular oocytes harvested from unstimulated ovaries, was reported in 1991 (Cha et al., 1991). The topic of *in vitro* maturation of human oocytes is covered by Tae Ki Yoon, Jayeon Kim and Dong Ryul Lee in Chapter 4 of this book.

Further improvement of IVF outcomes was enabled by the introduction of luteal phase support. The term "luteal-phase defect" (LPD), characterized by insufficient progesterone secretion by the *corpus luteum*, was first studied and treated by Georgeanna Seegar Jones since the late 1940s, well before the IVF era, and was considered a relatively infrequent cause of infertility (Astwood and Seegar Jones, 1941; Jones, 2008). As compared with natural reproduction, however, LPD occurs with a much higher frequency in patients treated by IVF (Garcia et al., 1981), and is particularly associated with certain specific ovarian stimulation protocols (van der Linden et al., 2015). The history and the current status of luteal phase support in IVF are fully covered by Robert Casper in Chapter 6 of this book.

Oocyte donation and surrogate motherhood

The possibility of generating embryos by IVF enabled the development of two novel treatment options: oocyte donation and surrogate motherhood. The first pregnancies and successful livebirth achieved by IVF with donated oocytes were reported at the Monash University in Melbourne (Australia) in 1983 (Trounson et al., 1983) and 1984 (Lutjen et al., 1984), respectively. Curiously, the first pregnancy (Trounson et al., 1983) was achieved with oocytes from an older woman (42 years old) with normal ovarian function donated to a younger woman (38 years old) with several previous unsuccessful *in vitro* insemination attempts. In the following years, oocyte donation was mainly practiced with oocytes donated by younger donors to recipients with premature or natural premenopause or menopause. In 1993, two cases of successful delivery after IVF with donated oocytes were reported in women at age 52 (Check et al., 1993), and four years later, a team of Southern California School of Medicine in

Los Angeles, headed by Richard Paulson, reported a successful pregnancy and a normal birth in a 63-year-old woman (Paulson et al., 1997).

Richard Paulson's group is also involved in an active programme of gestational surrogacy. Although, from the scientific point of view, gestational surrogacy does not represent a significant breakthrough, it remains the only solution for those patients in whom carrying a pregnancy is not a safe or viable option. Among these we find women with no uterus, previous hysterectomy or congenital absence, non-functional uterus, multiple fibroids, an unresponsive endometrium, and those in whom carrying a pregnancy is not advisable because of different systemic pathologies. As for oocyte donation, surrogate motherhood is subject to multiple regulatory restrictions in different parts of the world, as outlined recently (Tesarik, 2017a), encouraging the rising trend of reproductive (fertility) tourism. Chapter 7 of this book, written by Meghan Smith and Richard Paulson, deals with both of these two types of third-party parenthood.

Cell micromanipulation: a revolution in the treatment of male infertility

In the late 1980s, extensive knowledge on the function of human spermatozoa and *in vivo* and *in vitro* treatments to enhance the fertilizing ability of functionally defective spermatozoa had accumulated and made enabled the use of IVF with success in different types of male infertility (Tesarik and Testart, 1989). However, it was only during the early 1990s that the application of new cell micromanipulation techniques to assist sperm penetration into the oocyte made it possible to treat even the most severe cases of male infertility that had been impossible to resolve so far.

The first attempts at assisting fertilisation by means of a micromanipulation technique were carried out by subzonal insemination (SUZI) whereby several spermatozoa were deposited in the space between the oocyte plasma membrane (oolemma) and the inner surface of the zona pelucida (perivitelline space) (Fishel et al., 1990). However, it was the introduction, two years later, of a direct injection of a single spermatozoon into a single oocyte – intracytoplasmic sperm injection (ICSI) - which allowed fertilisation with spermatozoa affected by a great variety of morphological and functional defects. The ICSI technique was developed at the Centre for Reproductive Medicine of Vrije Universiteit Brussel, and the world's first pregnancies were reported by this group in 1992 (Palermo et al., 1992). The ICSI technique made it possible to achieve fertilisation even in cases of completely immotile spermatozoa, and marked an enormous progress in the treatment of cases of azoospermia, in which a

few spermatozoa could be retrieved surgically from the epididymis or from the testis (Devroey et al., 1995). Most of the pioneers of IVF in the era of gamete and embryo micromanipulation developments are shown in a snapshot taken, in 1997, at Bourn Hall, the headquarters of Robert G Edwards' clinic (Fig. 2).

The massive use of ICSI in the treatment of severe male infertility has unmasked new, previously largely unknown, sperm abnormalities. Especially those related with the use of morphologically abnormal spermatozoa for fertilization and the developmental consequences of sperm DNA fragmentation. Spermatozoa with severe morphological abnormalities can hardly penetrate oocytes with the use of conventional IVF methods, but they can easily do so when ICSI is used, often leading to abnormal embryo development (Tesarik, 2005). To cope with this problem, the technique of motile sperm organelle morphology examination (MSOME), making it possible to isolate living spermatozoa in real-time at high magnification (up to x6600), (Bartoov et al., 2001), was developed and applied in ICSI, giving rise to intracytoplasmic morphologically selected sperm injection IMSI (Fig. 3). The use of IMSI has been shown to improve implantation and pregnancy in cases with high frequency of sperm nuclear abnormalities (Berkovitz et al., 2005).

Fertilisation with spermatozoa with fragmented DNA can usually be achieved by ICSI and is compatible with apparently normal early embryonic development, in most cases. However, transfer of the resulting embryos often leads to implantation failure or miscarriage (Tesarik et al., 2004). In some of these cases, IMSI can also be of help (Hazout et al., 2006). As can other techniques, such as those based on the affinity of healthy spermatozoa for hyaluronic acid (Parmegiani et al., 2010), which ease selection of spermatozoa without DNA damage. A guideline for the optimal use of the available sperm selection techniques in different clinical scenarios involving sperm DNA fragmentation has been published recently (Tesarik and Galán Lázaro, 2017).

Understanding of the cell signaling pathways starting embryonic development

The massive use of ICSI in the treatment of male infertility in the mid 1990s opened new horizons related to the understanding of the biological mechanisms underlying the activation of the early embryonic development by the fertilising spermatozoon. It was soon noted that some oocytes, into which a spermatozoon had been successfully injected, did not start embryonic development and remained arrested at metaphase II stage,

while the injected spermatozoon remained unchanged in the oocyte cytoplasm. This condition is known as a failure of oocyte activation. Oocyte activation is a complex sequence of cell signaling events, triggered by the entry of extracellular calcium ions. Normal oocyte activation requires repeated raises of intracellular calcium ion concentration, referred to as calcium oscillations. Calcium oscillations in human sperm-injected oocytes (Fig. 4) show specific temporal and spatial characteristics (Tesarik et al., 1995) and are mediated by exchanges of calcium ions between free cytoplasm and two types of intracellular calcium stores, mainly endoplasmic reticulum (Tesarik et al., 1994; Tesarik and Mendoza, 1999). The ability of the calcium stores to generate ongoing calcium oscillation is dependent on the action of a sperm protein (Parrington et al., 1996), originally termed "oscillin" and later identified as a phosphoinositide-specific form of phospholipase C (PLC) named PLCζ (Saunders et al., 2002). The sperm-induced calcium oscillations activate a series of cell signaling events during subsequent preimplantation embryo development, including protein kinase C redistribution and endoplasmic reticulum reorganisation in embryonic cells (Sousa et al., 1996), and their abnormalities are suspected to cause some of the *de novo* chromosomal abnormalities arising during cleavage divisions after ICSI (Tesarik, 1995).

Use of immature germ cells for fertilization

The possibility to achieve fertilization by injecting individual spermatozoa into the oocyte cytoplasm led to further development of even more advanced techniques to be used in cases of extremely severe abnormalities of male germ cell development, including a complete block at different stages of spermatogenesis. The progress in this field was pioneered by animal experiments, carried out by Ryuzo Yanagimachi's research group, in which immature male germ cells, spermatids (Ogura and Yanagimachi, 1993; Ogura et al., 1993) and secondary spermatocytes (Kimura and Yanagimachi, 1995a) were introduced to mouse and hamster oocytes by electrofusion or microinjection. Live offspring were obtained after fertilisation of mouse oocytes with round spermatids (Ogura et al., 1994), secondary spermatocytes (Kimura et al., 1995b) and primary spermatocytes (Ogura et al., 1998).

In 1993, I organized a symposium on male factor in human infertility at the American Hospital of Paris, resulting in the publication, by Ares-Serono Symposia, of a book with contributions of the world's leading scientists and clinicians of that period (Tesarik, 1994). During extensive discussions with Ryuzo Yanagimachi and other specialists in the field of

mammalian gamete biology (Fig. 5), the idea of investigating the possibility of obtaining normal human embryos by round spermatid injection (ROSI) into oocytes was born. We started working on this subject in 1994, and one year later we achieved the first two human births with the use of ROSI (Tesarik et al., 1995, 1996 Tesarik and Mendoza, 1996a). Soon thereafter, the ROSI technique was used with success in Israel (Barak et al., 1998). One year later, the birth of the first baby after fertilising oocytes with *in vitro* matured round spermatids was reported (Tesarik et al., 1999).

In the beginning, only a few clinics used ROSI, mainly because of the unknown risk of gene imprinting abnormalities in the offspring (Tesarik and Mendoza, 1996b). Nowadays, these fears appear unsubstantiated, since 90 births after ROSI were reported in Japan (Tanaka et al., 2018) and 17 in Spain (Tesarik et al., 2018). No disease related to abnormal genomic imprinting was detected. The biological and clinical issues related to the use of immature male germ cells for human reproduction are reviewed by Ryuzo Yanagimachi and Atsushi Tanaka in Chapter 10 of this book.

New horizons opened by nuclear transfer techniques

The first human births achieved by fertilisation of oocytes with immature male germ cells encouraged further development of gamete micromanipulation techniques and their use in new therapeutic indications. Experience gained by introducing cell nuclei into immature and mature oocytes was a source of so far inaccessible information about the cellular mechanisms underlying oocyte activation and triggering early embryo development. These new data were used for further development of nuclear transfer techniques applicable in the treatment of some oocyte and embryo abnormalities, as specified in the following section.

4. Expansion of IVF indication beyond infertility treatment

Prevention of disease transmission by gamete and embryo selection

The first successful use of IVF in a case unrelated to infertility was reported in 1990 by a group of investigators working at Hammersmith Hospital in London (UK) and headed by Alan Handyside (Handyside et al., 1990). The authors performed a series of IVF attempts followed by cleaving embryo biopsy and genetic analysis to determine the sex of the

embryos, with the aim to prevent transmission of adrenoleukodystrophy and X-linked mental retardation (Handyside et al., 1990). This was the first case of preimplantation genetic testing (PGT). Two years later, the same group achieved the birth of a normal girl after IVF and PGT for cystic fibrosis by avoiding the transfer of affected embryos through a direct targeting of a three-nucleotide deletion (ΔF508) responsible for the disease (Handyside et al., 1992). The history and current state of PGT is reviewed by Alan Handyside in Chapter 8 of this book.

In the following years, PGT was applied, as a less invasive alternative to prenatal diagnosis, to avoid the transmission of a number of severe Mendelian disorders, structural chromosome abnormalities or mitochondrial diseases (Geraedts and De Wert, 2009).

The use of PGT in these conditions is fully justified, since the anomaly searched is usually present in all, or at least a vast majority of embryonic cells and each cell can thus be considered a reliable representative of the whole embryo. On the other hand, the use of preimplantation detection of *de novo* chromosomal abnormalities, typically accumulating in the oocytes with increasing female age, has been widely criticized in the past few years. This technique, also called preimplantation genetic screening (PGS) or preimplantation genetic testing for aneuploidies (PGT-A), appears to be reliable when performed with the first and the second polar body, whose chromosomes are exact counterparts of those of maturing oocytes. Polar body biopsy was originally developed for single gene mutations (Verlinsky et al., 1990), but it was soon adapted for the use in preimplantation diagnosis of aneuploidy (Munné et al., 1995).

As compared with the analysis of the polar bodies, the use of blastocyst trophectoderm biopsy to yield embryonic cells for the analysis of ploidy has recently been seriously criticized because of the inability to obtain reliable data about the inner cell mass (future embryo) from trophectoderm cells (future placental component), due to a high degree of mosaicism in the trophectoderm (Gleicher et al., 2017; Murtinger et al., 2018; Tesarik et al., 2018). Recently developed non-invasive genetic screening (NIGS) methods (Xu et al., 2016; Kuznyetsov et al., 2018) using soluble DNA obtained by liquid biopsy (spent medium after blastocyst culture) appear to reflect better the embryo chromosomal status as compared with solid trophectoderm cell biopsy and may become the first-choice methods to be used in human PGS/PGT-A in the near future (Tesarik et al., 2018)

Prevention of disease transmission by gamete and embryo therapy

The development of PGT techniques made it possible to prevent transmission of a variety of genetic diseases, from the parents to the offspring, without the need for late disease detection by prenatal diagnosis and induced abortion of affected fetuses. However, as for prenatal diagnosis, the avoidance of disease transmission is based on the destruction of affected embryos. Apart from ethical issues related to embryo destruction, making this solution unacceptable for some persons, there is also a purely pragmatic problem with the destruction of abnormal embryos. In fact, if we sum the clearly affected embryos, those with a doubtful outcome (e.g. different degrees of chromosomal mosaicism), false positive results and the risk of embryo destruction during the manipulation, only a few or no embryos may remain available for transfer in some couples, especially when the technique is performed in older women or poor responders. To cope with these problems, another strategy can be imagined, that of considering the affected embryos as full-right patients in whom all effort should be done to correct the anomaly detected. This sounds like a purely futuristic fantasy nowadays. However, cases in which abnormal embryos were cured instead of being destructed have been reported in the literature since the end of 1990s.

The injection of donor oocyte cytoplasm into patients' oocytes (Cohen et al., 1997, 1998) and the transfer of metaphase chromosomes from the patients' oocytes into previously enucleated donor oocytes (Tesarik et al., 2000) were originally developed to help patients with unexplained repeated poor preimplantation development and implantation failure, supposed to be caused by oocyte cytoplasmic deficits. However, it was soon noted that these techniques can be useful even in fertile couples at risk for the transmission of mitochondrial DNA diseases from mother to child (Malter and Cohen, 2002).

Several tens of babies were born after enriching maternal oocytes with donor oocyte cytoplasmic components in different countries, between 1998 and 2001, and the presence of donor mitochondria was demonstrated in some of them (Barrit et al., 2001). Soon thereafter, the 'United States Food and Drug Administration' issued a negative report on this technique, based on a deep misunderstanding of its real nature, leading to the suspension of the human trials in that country. Unfortunately, health authorities of many other countries followed this example. It was only in 2016 when a team of Mexican and US authors carried out the "banned technique" in Mexico, this time using metaphase chromosome transfer (Tesarik et al., 2000; Tesarik, 2017b) instead of oocyte cytoplasmic injection, to prevent the transmission of Leigh syndrome, a severe

mitochondrial DNA disease (Zhang et al., 2017). Obviously, this was not the world's first "3-parent" baby, although it was claimed to be so by lay press. Oocyte cytoplasmic donation issues are reviewed by Henry Malter, one of the pioneers of this technique in the late 1990s, in Chapter 11 of this book.

Oocyte cytoplasm donation is the first example, in the history of IVF, of treating preimplantation embryos as patients. However, the restriction of this technique merely to mitochondrial DNA mutations would reduce its use to a very limited number of cases. In fact, it is likely to be useful in many other types of oocyte cytoplasmic deficiencies, as considered in the period of the late 1990s and early 2000s, when the technique began to be used in human clinical trials (Malter and Cohen, 2002). The extension of this technique beyond the scope of mitochondrial anomalies is warranted.

However, future developments of preimplantation embryo therapy are likely to employ the rapidly expanding technique of gene editing, based on the technology known as CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats). The CRISPR system, coupled with Cas genes, coding for nucleases capable of cutting the DNA sequence and representing a natural defence system acting against viruses in prokaryote cells (Barrangou et al., 2007), can be modified to incorporate guiding RNAs which can direct the CRISPR/Cas system to cut and paste (edit) specific parts of genomes of eukaryotic cells (Mali et al., 2013). A CRISPR/Cas-based gene editing method has been apparently successfully applied to correct a pathogenic gene mutation in human embryos. However, by precaution, the authors had not transferred any of the treated embryos to the mother yet (Ma et al., 2017).

Even though the gene editing techniques are promising, they still need some time and much experimental work to prove their efficiency and safety in the treatment of human preimplantation embryos. Because of their versatility, the gene editing techniques can be expected, one day, to be able to correct monogenic disorders of both nuclear and mitochondrial DNA, as well as, structural chromosomal aberrations and aneuploidies. The time required for their clinical application is not expected to be so long as to exclude the currently generated abnormal embryos from eventual future therapeutic intervention. Consequently, some clinics have already started to cryopreserve embryos diagnosed as abnormal by PGT for their eventual future correction, especially in cases in which no other embryos are available for the couple and there are serious medical reasons against the repetition of assisted reproduction attempt to create new ones (Tesarik and Mendoza, 2017). I will resume the issues related to the treatment of embryos as patients, as well as, other challenges for future

developments of the IVF and IVF-derived techniques, both inside and outside the field of infertility treatment, in the concluding chapter of this book.

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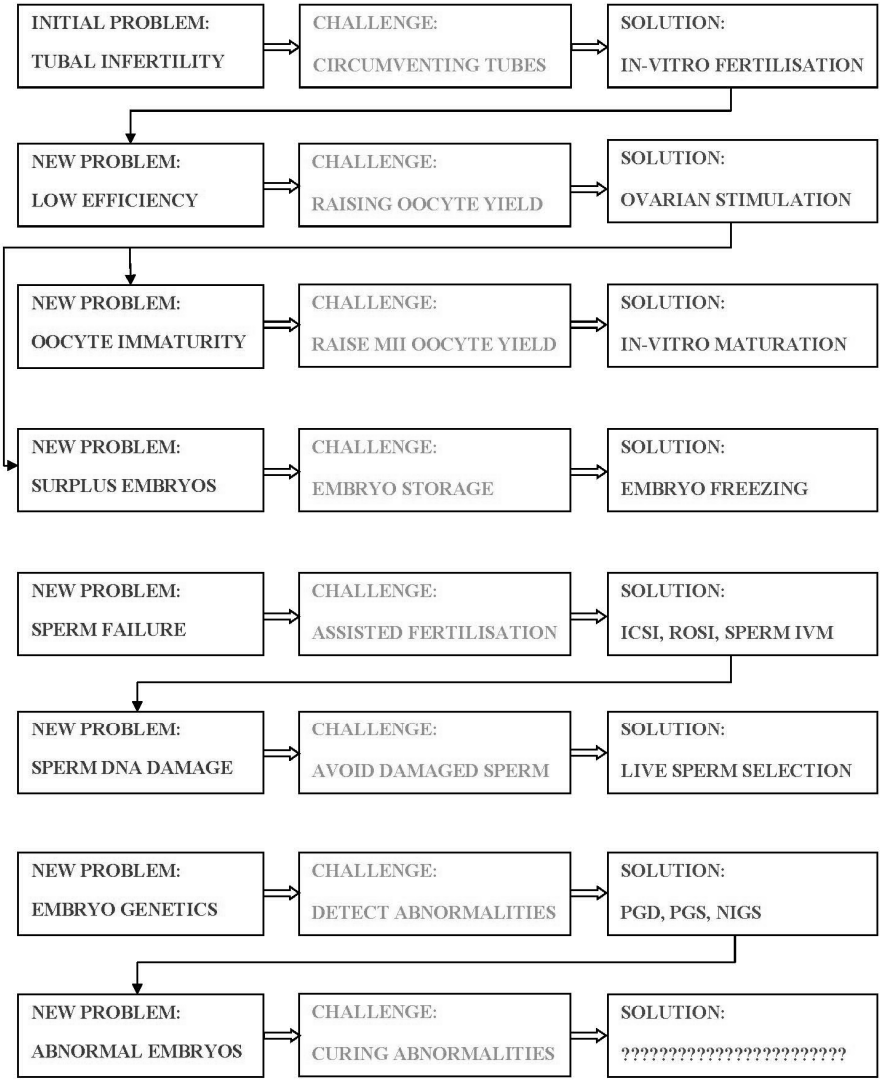
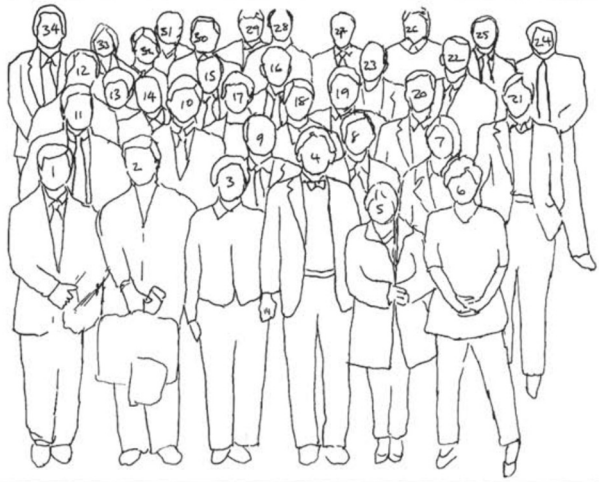


Figure 1. The main highlights of IVF evolution, represented as traffic lights turning repeatedly between the left, middle and right, showing how the solution of each particular problem was at the origin of a new challenge.



1 Dr Bruce Lessey	2 Dr Jan Tesarik	3 Dr Georgeanna Jones	4 Dr Howard Jones	5 Dr Jacqueline Mandelbaum	6 Dr Michelle Plachot	7 Dr Karen Leibu
8 Prof Basil Tarlatzis	9 Prof Klaus Diedrich	10 Prof Carlos Simon	11 Dr Scott Chappell	12 Dr J Russell	13 Dr Carol Warner	14 Dr Arnaud Ythier
15 Prof Markku Seppala	16 Prof Lars Hamberger	17 Dr Anne Chandley	18 Dr Rene Fanchin	19 Dr Jean Cohen	20 Dr Ernest Loumaye	21 Prof Steve Smith
22Prof Bart Fauser	23Dr Mats Wikland	24 Dr Alan Trounson	25 Dr Peter Brinsden	26 Prof Bob Edwards	27 Dr Jacques Salat-Baroux	28 Dr Sergio Oehninger
29 Dr Paul Devroey	30 Pro Andre Van Steirteghem	31 Dr Jacques Cohen	32 Dr David Gardner	33 Dr Kay Elder	34 Dr Colin Howles	

Figure 2. Attendees of the 1997 Bourn Hall Meeting with a drawing identifying each person in the picture.

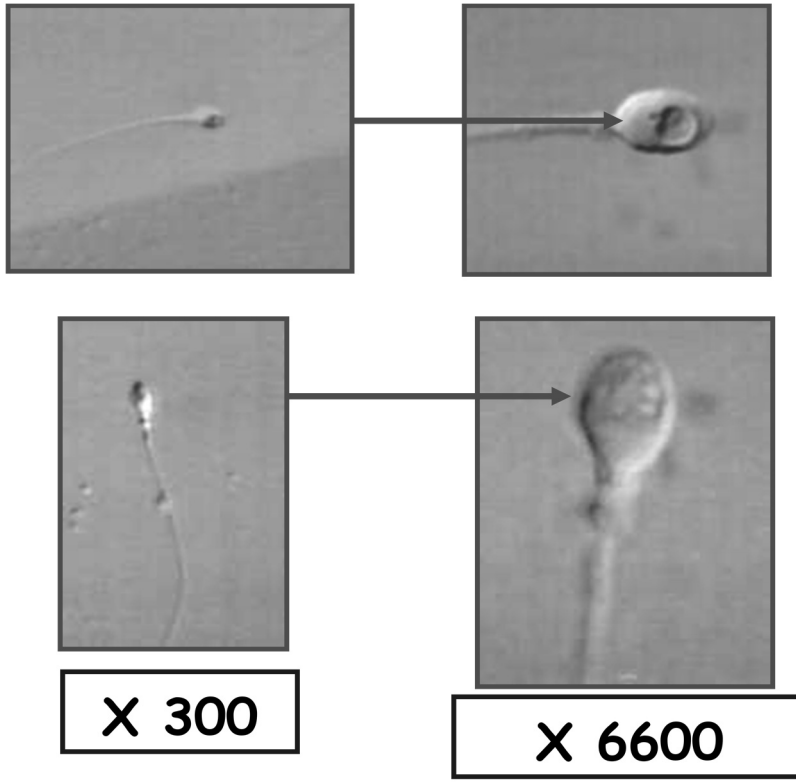


Figure 3. Pictures of the same sperm heads taken at magnifications of x300 (conventional ICSI) and x6600 (IMSI), respectively.

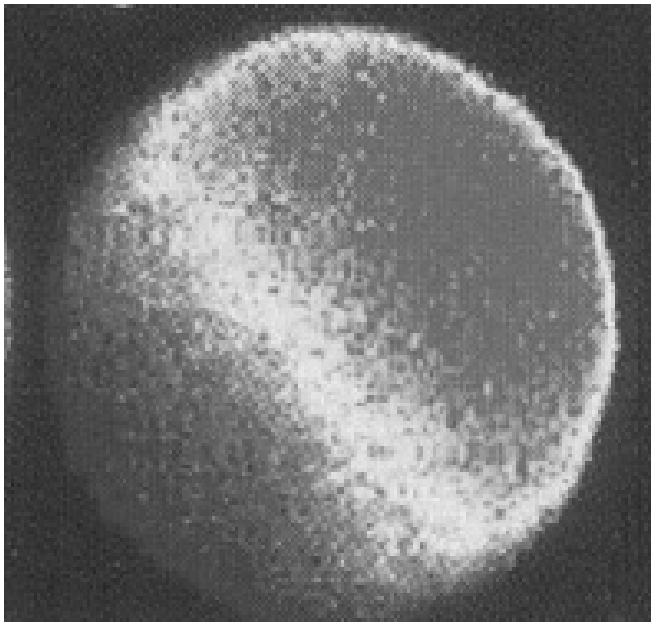


Figure 4. The beginning of calcium oscillations in human sperm-injected oocytes visualized by confocal microscopy and fluorescent calcium ion indicator fluo-3 as described (Tesarik et al., 1994).

Figure 5. A snapshot showing (from left to right) Drs. Jan Tesarik, Carmen Mendoza, Alain Gougeon and Ryuzo Yanagimachi during the Ares-Serono Symposium on the Male Factor in Human Infertility, held at the American Hospital of Paris in 1993.