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In vitro fertilization: Four decades of reflections and promises

Yulian Zhao [a,*], Paul Brezina [a], Chao-Chin Hsu [b], Jairo Garcia [a], Peter R. Brinsden [c], Edward Wallach [a]

[a] Department of Gyn/Ob, Johns Hopkins University School of Medicine, Johns Hopkins at Green Spring Station, Lutherville, MD 21093, USA
[b] Department of Obstetrics and Gynecology, National Taiwan University Hospital, Taipei 100, Taiwan
[c] Bourn Hall Clinic, Cambridge, UK

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ABSTRACT

Background: In 2010, Robert Edwards was awarded the Nobel Prize in Medicine for his pioneering work in the development of *in vitro* fertilization, a field that has touched millions of lives across the globe. Edwards dedicated his career to helping couples overcome infertility. He first established principles of early embryo development that served as the foundation for his later work. In the 1960s, he achieved the first human fertilized oocyte *in vitro* while at the Johns Hopkins Hospital. He then continued his work at Cambridge University. In 1978, the world witnessed the birth of the first "test tube baby". This achievement is a landmark not only in the reproductive sciences but also in the history of mankind's technological evolution.
Scope of review: This article outlines the development and progression of IVF from its infancy to the refined and broadly utilized technology offered to patients today. We describe the evolution of the field and the current state of IVF, including its current technological and social challenges.
Major conclusions: We congratulate Professor Edwards for his well-deserved recognition as Nobel Laureate in Medicine.
General significance: This article is a tribute to Edwards for his exceptional accomplishments in this specific and rewarding field of modern medicine.

The announcement of the birth of Louise Brown in 1978 through in vitro fertilization (IVF) was a major milestone in the treatment of infertile couples. This historic moment was eloquently encapsulated by Howard Jones who observed: "Eleven forty-seven p.m. Tuesday, July 25, 1978, was surely a unique moment in the life of Patrick Steptoe. This was the hour and minute he delivered Louise Brown, the world's first baby, meticulously, lovingly, and aseptically conceived in the laboratory, but popularly referred to as 'the world's first test tube baby'. This midnight minute was surely a mighty moment, not only for Patrick Steptoe, but also for his scientific partner, Robert Edwards, and for their associates" [1]. The importance of this birth to scientists, clinicians and most particularly infertile patients throughout the world cannot be overstated. This milestone is considered to be one of the major medical/scientific achievements of the twentieth century. It was the culmination of many years of work that had been simultaneously carried out in a number of centers worldwide, principally the United Kingdom, the United States and Australia [2]. Most of the background originated from Robert Edwards' long and enduring efforts over the previous 20 years.

1. The story of Robert Edwards, Nobel Laureate in Medicine 2010, and the first human IVF

In vitro fertilization (IVF) may be said to have begun in the late 1800s when Walter Heap reported the first successful transfer of embryos flushed from the oviducts of an Angora doe rabbit to the uterus of a Belgian hare [3]. Other scientists soon followed suit and succeeded in achieving pregnancies, in species ranging from mice to cows, during the next 60 years [4]. *In vitro* maturation of oocytes using rabbit oocytes was accomplished in 1939 [5]. The first successful IVF was carried out with rabbit oocytes [6], followed by oocytes of the golden hamster [7], and mouse [8].

Edwards' interest in human IVF arose during his graduate studies at Edinburgh University in the early 1950s [9]. The fundamental basis of reproductive physiology intrigued Edwards, and early in his career he conducted numerous experiments in mice exploring meiosis, ovulation, fertilization, cleavage of embryos to the blastocyst stage, implantation, fetal growth to full term and studies on altering the chromosomal complements in embryos [9]. This comprehensive enthusiasm for spermatozoa and eggs later proved his real ambition, which was to work with human gametes and embryos and on human infertility [10]. An intriguing possibility emerged from his studies, namely that infertile couples might be helped to have their own children by means of IVF. However, at that time human IVF was regarded as an impossibility. The thought of bringing human

* Corresponding author at: Department of Gyn/Ob, Johns Hopkins University School of Medicine, 10755 Falls Road, Lutherville, MD 21093, USA. Tel.: +1 410 583 2762; fax: +1 410 583 2716.
E-mail address: zhao1@jhmi.edu (Y. Zhao).

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fertilization and embryology into scientific and medical practice was regarded with incredulity [11].

1.1. Basic studies that paved the way for IVF

There are at least five major prerequisites necessary to achieve successful IVF pregnancies in humans

- Obtaining oocytes that are sufficiently mature
- Obtaining suitable sperm cells which are acrosome-reacted
- Identifying suitable media for the culture of oocytes, spermatozoa and for the in vitro fertilization itself
- Ensuring the safe transfer of embryos to the uterus
- Supporting the implantation and maintenance of embryos

Between 1950 and 1960, *in vitro* maturation of oocytes was only one of the areas which Edwards studied. A series of animal studies was carried out by Edwards including ovulation induction, *in vitro* maturation, stem cells, immunology, genetics and endocrinology, which built the foundation on which Edwards pursued human IVF. Edwards described and defined the developmental timetable that exists from the release of human oocytes through the subsequent steps of oocyte maturation [12]. Edwards at this time also reviewed the prospects and probable difficulties in achieving human IVF, including embryo culture and embryo transfer [13]. He also predicted recent developments in the field such as pre-implantation genetic diagnosis (PGD) [13]. In 1965, at the invitation of Victor McKusick, Edwards travelled to Baltimore, Maryland, to collaborate with Drs. Georgeanna and Howard Jones (Fig. 1) at Johns Hopkins Hospital to obtain human oocytes and to attempt their fertilization after their maturation in vitro [14,15]. Although not readily recognized at the time, a review of the photos from this 1966 publication indicates that the first *in vitro* fertilization of a human oocyte was indeed achieved at Johns Hopkins Hospital, documented by an oocyte possessing two pronuclei [15,16]. It then became evident that the fertilization of *in vitro* matured oocytes faced the barrier of cleavage arrest in almost all animal studies [11]. Edwards was also concerned about the normality of *in vitro* matured oocytes. Thus, the detection and retrieval of mature oocytes from human ovary became the first mandatory pre-requisite. Patrick Steptoe, a pioneer of laparoscopy, was able to retrieve oocytes via laparoscopy instead of laparotomy [17]. Edwards and Steptoe pooled their individual skills to attempt IVF in 1968. They discussed in detail the safety of proposed procedures and the underlying ethical aspects [9].

From 1950 to 1960, most scientists believed that mammalian oocytes could be fertilized *in vitro* only by "capacitated" spermatozoa. Capacitation entailed exposing spermatozoa to a uterine or oviductal environment [18]. Therefore, in several early unsuccessful attempts to achieve fertilization of the human oocyte, Edwards used spermatozoa that had been recovered from the cervix several hours after intercourse [19].

The situation changed after Bavister devised a medium with high pH (~7.8) for hamster oocyte fertilization *in vitro*[7]. This medium proved successful with human eggs, yielding a high rate of fertilization [20,21]. The seminal plasma from sperm samples was washed out using a simple centrifugation technique, and no special efforts were needed to induce capacitation. They later found that media with lower pH, and other variations, would also support human fertilization [9].

However, the development of specific media for IVF and embryonic cleavage was essential. Edwards accumulated knowledge regarding the specific needs of cells and tissues growing in vitro that led to improvements in embryo cultures [11]. Fertilized oocytes were cultured in various media to enable definition of the exact conditions for achieving human embryo growth *in vitro*. Tight controls of pH, osmotic pressure, and the constituents of media were thus investigated [9,13]. Information was also gleaned from studies on non-human studies. At Pennsylvania Hospital in Philadelphia, PA, for example, the successful *in vitro* stimulation and ovulation of rabbit ovaries was achieved [22]. These *in vitro* ovulated rabbit oocytes were found to be mature and competent to undergo fertilization with embryos resulting in viable pregnancies [22]. Human fertilization *in vitro* presented several challenges. However, progress was made using Bavister's medium or standard media such as Earle's or Tyrode's, with minor modifications [23]. Fertilization and embryo growth *in vitro* proceeded smoothly as two-, four-, and eight-cell embryos, morulae, and blastocysts at 4–6 days grew *in vitro* in various media [24].

Edwards' focus then turned to determining the optimal method of replacing these embryos in the uterus. Concern was expressed that the passage of catheters through the cervical canal into the endometrium might invoke a premature decidual reaction in the uterus, cause infection, or invoke myometrial contractions that could expel the embryos [23]. Edwards soon designed catheters that minimized decidualization of the uterus and used the smallest

Fig. 1. Roberts Edwards with Howard and Georgeanna Jones (2000).

possible amount of medium to carry the embryos into the uterine lumen.

Edwards and Steptoe ultimately faced new challenges with regard to uterine physiology and early pregnancy because of the unique hormonal conditions required for embryo implantation and development. Luteal function after oocyte retrieval was carefully investigated by the team. Three years elapsed before hormonal luteal phase support was successfully employed [25–27].

1.2. Clinical IVF—the bumpy road to human IVF

The professional collaboration that began in 1968 between Patrick Steptoe, a gynecologist, and Robert Edwards brought together two very different sets of experiences and skills. Edwards regularly travelled 200 miles from Cambridge to Oldham in Lancashire. There, Steptoe carried out the laparoscopic oocyte recovery procedures, providing Edwards with the clinical material that he needed for his research. Mature human oocytes were retrieved, fertilized *in vitro*, and various stages of cleavage embryos to blastocysts were observed [10]. It was at this time that a special unit in Oldham for clinical IVF and embryo transfer was established.

After a failed bid to achieve financial support from the Medical Research Council (MRC) in the United Kingdom to establish an IVF unit in Cambridge, the Oldham authorities converted part of the small Kershaw's Hospital into the world's first IVF unit in 1970. Edwards and Steptoe had assessed in detail the teratogenic risks to babies, with a general consensus that their work was safe [28]. At that time, it was generally believed that the more oocytes retrieved the greater the chance of achieving a pregnancy. Administering human menopausal gonadotropin (HMG) during the follicular phase of the menstrual cycle stimulated the growth of several mature follicles, from which oocytes could be retrieved laparoscopically. Infertile volunteers were treated with two or three injections of HMG every 2–3 days, followed by human chorionic gonadotropin (HCG) on days 10–12 of their menstrual cycle in order to trigger the maturation of the ripening oocytes [23]. The ovaries of cycling women responded well, and each volunteer produced several large follicles as verified by laparoscopy carried out between 30 and 36 h after the injection of HCG. Actual transfer of embryos began in 1971–1972 [23].

In 1976, after 8 laborious years, accompanied by many disappointments, their first pregnancy was achieved. However, this pregnancy proved to be a tubal ectopic pregnancy and therefore was not viable [29]. Many other treatment regimens were subsequently developed and studied over the following 2 years. The team of Steptoe and Edwards investigated different forms of ovarian hormonal stimulation, performed the first gamete intra-fallopian tube transfers, cryopreserved oocytes and embryos, and performed oocyte donation to a recipient. They finally embarked on natural-menstrual-cycle IVF by closely following the urinary luteinizing hormone surge to time the oocyte retrieval in their patients [25–27].

Lesley Brown was the second patient they treated in a natural cycle; her single oocyte was aspirated laparoscopically, inseminated quickly and transferred precisely when it reached the eight-cell stage. Edwards hoped that earlier transfer would benefit from the embryos spending less time *in vitro*. After a successful pregnancy, Louise Brown was born on 25 July 1978 on a momentous evening in Oldham [30]. In the words of Edwards, "It is hard to put into words what the occasion of her birth meant to me, and to our wonderful supportive team." The emotional feeling was vividly described in the subject title of "Going It Alone", depicting the long struggles with his laboratory and clinical trials and the overwhelming stress caused by ethical criticism and vilification by his peers [9,31]. Each advance they achieved in IVF was made in the face of mounting ethical concerns from society. It had been a painful, long, and rocky road, the full trauma of which is admirably revealed in their book [25]. Births at other centers throughout the world shortly thereafter served to document IVF as an acceptable form of treatment for infertile couples [32,33].

1.3. IVF is perfected in Bourn Hall Clinic and spreads around the world

Soon after the birth of Louise Brown, Steptoe retired from the National Health Service. He and Edwards then perceived the need for a new clinic in which they could continue their pioneering work. At that time, neither the National Health Service, the Medical Research Council, nor for that matter any major academic department in the United Kingdom would support them. There was a belief that IVF was a treatment that would never receive medical or ethical acceptance [34]. This lack of economic support halted their work for two and half years after their initial success. Finally, venture capital was obtained and they established Bourn Hall Clinic near Cambridge. This clinic, which opened its doors in September of 1980, was the world's first centre for IVF treatment. Steptoe was its Medical Director until his death in 1988, and Edwards was its Scientific Director until his retirement. Gynecologists and cell biologists the world over trained at Bourn Hall, where the methodology for IVF was continuously refined. By 1986, a total of 1000 children had been born as a result of IVF performed at Bourn Hall. At that time, approximately half of all children born in the world following IVF had been conceived at Bourn Hall [17,35]. At Bourn Hall Clinic, research proliferated including studies on innovative hormonal preparations, embryo transfer instruments, the implantation process, pregnancy outcomes, and early embryologic growth and development [35].

Today, IVF is recognized as an established therapy throughout the world. To date it is estimated that some four million children have been born as a result of this procedure. Louise Brown and several other IVF children have become mothers themselves, to-date all conceiving naturally, thus providing evidence of the safety and success of IVF therapy. Today, Robert Edwards' vision is a reality which continues to bring joy to infertile couples all over the world. It is regrettable that Patrick Steptoe was not able to share the honor of receiving the 2010 Nobel Prize in Medicine together with his colleague Robert Edwards.

2. Highlight of IVF milestones

The path to achieving the first successful human IVF procedure was paved with a rich history of novel scientific achievements. Once established as a viable procedure, IVF technology advanced rapidly. The next decade witnessed many robust innovations. Cryopreservation was introduced in the early 1980s. The birth of the first cryopreserved embryo baby was reported in 1983 [36]. During the same year, the first successful delivery following embryo donation occurred [37,38]. By 1985, there were many other "firsts" in the field, including the first pregnancy by IVF using sperm aspirated from the epididymis [39], the first description of culture media (first published as a formula entitled Human Tubal Fluid) designed to mimic the *in vivo* environment to which the embryo is exposed [40], and the first delivery resulting from gestational surrogacy [41].

Micromanipulation of human oocytes and embryos was introduced in the late 1980s, leading to the first reported pregnancy after intracytoplasmic sperm injection (ICSI) [42]. Subsequent technologies included:

- The first successful human cleavage-stage embryo vitrification followed by a successful delivery [43].
- Pregnancies from biopsied human pre-implantation embryos sexed by Y-specific DNA amplification [44].
- *In vitro* maturation of oocytes in an unstimulated cycle, resulted in pregnancy in a donor oocyte program [45].
- Sequential media that allowed embryo culture to the blastocyst stage, facilitating the practice of single embryo transfer [46].

Additional technologic advances shaping the field of IVF today are highlighted below.

2.1. Hormonal management of IVF cycles and oocyte retrieval

2.1.1. Controlled ovarian stimulation

At the time of the birth of Louise Brown, IVF attempts were primarily performed using monitored natural ovulatory cycles [17]. Gonadotropins had been used in the context of infertility treatment for conditions such as hypogonadotropic hypogonadism since the 1930s, when the medications had been extracted from animal sources [47]. Gonadotropins from the human pituitary and the urine of menopausal women had been established as a safer source by the middle of the 20th century [45,48,49]. The advent of controlled ovarian hyperstimulation (COH) using urinary derived gonadotropins was pioneered first by Howard and Georgeanna Jones in the United States and then by Alan Trounson in Australia [17,50]. At the same time, others achieved success in ovarian follicular stimulation using clomiphine citrate [51].

Many of today's IVF protocols utilize injectable gonadotropins, containing follicle stimulation hormone (FSH) and luteinizing hormone (LH), followed by an injection of human chorionic gonadotropin (hCG). These protocols expose the oocytes housed within the ovary to supraphysiologic levels of hormones that promote follicular development and oocyte maturation. This development made it possible to time oocyte retrieval more predictably and to obtain multiple oocytes (often 10–20) from a single IVF cycle, rather than one or two oocytes, as occurs in a natural cycle. Retrieval of multiple oocytes enhances the patient's ability to achieve pregnancy in fewer cycles and in less time [52]. Furthermore, excess embryos not selected for uterine transfer may be cryopreserved, thawed, and transferred in subsequent cycles, increasing the overall pregnancy rate associated with any given IVF cycle [53]. Therefore, gonadotropin therapy ushered in a new era for the field. The following years would witness modifications of stimulation protocols [17]. Ovarian stimulation still remains a fundamental tool in managing IVF treatment cycles, now with a variety of regimen options to choose from.

2.1.2. Ovulation induction

Despite the ability to induce the simultaneous development of multiple follicles successfully, significant challenges remained. Ovulation occurs 10 h, plus or minus 5 h, after the time of the LH surge peak [54]. Testart et al. in France developed a plasma luteinizing hormone LH assay which could detect the initial LH rise, allowing accurate prediction of the ideal time for the retrieval of oocytes [55]. Early IVF cycles relied on determining the LH surge to time surgical retrieval of oocytes [15]. This approach required evaluation of LH hormone levels up to four times daily; surgical staff and facilities needed to be available around the clock [50]. Needless to say, this protocol required significant resources and also resulted in considerable inconveniences for IVF patients.

The gonadotropin-releasing hormone agonist (GnRHa) is a small decapeptide whose continuous administration results in an initial increase but then a precipitous fall in endogenous sex steroid levels [53]. In the 1980s, GnRH agonists were first used to suppress the endogenous LH surge in IVF cycles [53,56]. This technique allowed physicians to recruit more follicles, with more aggressive controlled ovarian stimulation protocols, to perform oocyte retrieval procedures at more predictable times, and to minimize the number of follicles lost due to premature ovulation. More recently, GnRH antagonists have been used to inhibit the LH surge in IVF cycles [53]. GnRH antagonists took almost 30 years of research and development to produce a clinically efficacious and safe medication [53]. Unlike GnRH agonists, GnRH antagonists have an immediate suppressive affect on the hypothalamus [53]. There is not a difference in pregnancy rates, birth rates, and neonatal outcomes between the use of GnRH agonists and antagonists in IVF cycles [53].

2.1.3. Ultrasound guided oocyte retrieval

Another limiting factor in early IVF procedures was the reliance on laparoscopy to obtain follicles [15]. In the early 1970s the concept that follicular growth could be followed via ultrasound monitoring was introduced [57]. Lenz and Lauritsen described trans-abdominal oocyte aspiration using an ultrasound-guided needle [58]. In the early 1980s, the practice of retrieving oocytes vaginally from ovarian follicles under ultrasound guidance was described [16]. This minimally invasive approach to retrieving oocyte has become the gold standard for performing IVF procedures.

2.2. Embryo cryopreservation

The term cryopreservation refers to the storage of viable cells at low temperature, normally at −196 °C in liquid nitrogen. Cryopreservation in assisted reproduction is primarily used for preserving surplus embryos after IVF, gamete or embryo donation, postponement of fertility or pregnancy, and fertility preservation [59,60]. Successful cryopreservation of human reproductive tissues was initially achieved in 1954 with spermatozoa [61], and then with embryos [36] and with oocytes in 1986 [62]. Cryopreservation of human sperm and embryos has now become an integral part of IVF treatment procedures.

Techniques used for cryopreservation of human reproductive tissues must ensure high survival and viability after thawing. However, the process of cryopreservation, through the formation of intracellular ice crystal spikes and other mechanisms, may damage embryos [63,64]. The two major technologies currently used to accomplish cryopreservation are slow-freezing and vitrification protocols. Slow-freezing protocols involve a programmed cooling process coupled with low concentrations of cryopretectants aimed at preventing ice crystal formation [64]. Although the technique has been shown to have inconsistent results, slow-freezing has been the most widely used method for freezing human gametes and embryos for years [65]. Vitrification is accomplished using high concentrations of cryoprotectants and extremely rapid cooling rates (15,000–30,000 °C per minute), resulting in the solidification of water into a glass-like state without ice crystal formation [64]. Recent data suggest that vitrification may result in a better clinical outcome compared to slow-freezing protocols [64]. However, concerns about toxicity and the risk of contamination need to be addressed in the future [64]. Additionally, several recent trials have described successful pregnancies following cryopreservation, through vitrification, of retrieved non-fertilized oocytes [66]. The ability to freeze oocytes successfully in this manner has significant implications for the application of this technology in the future.

2.3. Micromanipulation to enhance fertilization of oocyte and embryos implantation

2.3.1. Intracytoplasmic sperm injection

Intracytoplasmic sperm injection (ICSI) is a technique in which a single spermatozoon is inserted directly into the oocyte using micromanipulation. This approach bypasses the requisite penetration steps, including capacitation followed by the acrosome reaction, wherein the sperm enters the egg during natural fertilization [67]. ICSI was developed to treat couples of whom the male partner had very poor semen parameters. In 1985, Hosoi and colleagues accomplished the first ICSI offspring at the Johns Hopkins Hospital in the rabbit after transfer of sperm-injected eggs into the oviduct of a pseudopregnant female [68,69]. Fertilization of human oocytes through microinjection of a single sperm under the zona pellucida was reported by Laws-King and colleagues [70]. In 1992, Palermo, Joris, Devroey, and Van Steirteghem accomplished the first successful ICSI human pregnancy

[42]. The first human pregnancies with ICSI occurred in 1992 [42]. Since then, this technique has been integrated into routine clinical practice in fertility centers offering assisted reproductive technology throughout the world. Hundreds of thousands of ICSI babies have been born. Employing ICSI, patients with a traditionally poor prognosis, such as women who are poor responders to gonadotropins or severely subfertile males, may achieve pregnancies, with birth rates similar to those of patients treated by conventional IVF [71]. Additionally, ICSI is useful in human immunodeficiency virus (HIV) discordant couples [71].

Recent years have witnessed a broader application of ICSI. In many centers worldwide, ICSI is the predominant *in vitro* insemination technique in all patient populations. In the United States for the year 2007, 63% of all IVF cycles performed utilized ICSI to accomplish fertilization (CDC). Advocates of this broad application of ICSI point to excellent fertilization rates, greater than 70%, achieved through ICSI [71]. The broad application of this technology has been criticized by some who point to increased costs and safety concerns associated with the universal application of ICSI [72]. A consensus regarding the defined role of ICSI has yet to be realized.

2.3.2. Assisted hatching

Embryo hatching is a process in which the cells destined to form the placenta herniate through the wall of the zona pellucida of a blastocyst embryo to interact with the uterine endometrium [73]. This hatching process is necessary for endometrial implantation and necessarily has to occur in all successful pregnancies [73]. The failure of embryos to hatch is thought to be a core reason for pregnancy failure in IVF [74,75]. Cohen et al. in 1990 described the use of micromanipulation, a technique known as “assisted hatching” (AH), to promote hatching following IVF by introducing an artificial incision in the zona pellucida of embryos just prior to transfer to the uterus [74]. Since this time, multiple methods for AH have been explored. Protocols have been described that either create a full thickness hole through the entire zona or thin out the zona using mechanical manipulation (either a glass micro-needle or piezo-micromanipulator), chemical application (acidified Tyrode's solution), or laser [73,75]. Among the various AH protocols available, many feel that the laser method represents the least invasive method of accomplishing AH [76]. Laser AH allows the operator to complete the hatching procedure with non-contact manipulation of the embryo while minimizing mechanical or chemical injury [76].

The merits of AH have been a controversial subject. While some centers have adopted the practice of routinely performing assisted hatching, others do not believe that this technology confers a significant clinical benefit [77]. Numerous recent studies indicate an advantage to assisted hatching in properly selected cases [78]. Circumstances in which AH may be of benefit include advanced maternal age, repeated implantation failure, poor embryo quality, thickened zona, and embryo transfers following cryopreservation and thaw [76,79].

2.4. Pre-implantation genetic diagnosis and preimplantation genetic screening

Preimplantation genetic diagnosis (PGD) and preimplantation genetic screening (PGS) involve obtaining one or more cells from the developing embryo and evaluating the genetic composition of this cell (s) for either a specific genetic defect known to exist in the parents (PGD) or to screen for the presence of embryo aneuploidy (PGS) [44,80]. The results of this information then guide the decision as to which embryos are appropriate for embryo transfer [80].

PGD was developed first in England in the 1990s [44]. Handyside et al. reported the first established pregnancies using this procedure in two couples known to be at risk for transmitting adrenoleukodystrophy and X-linked mental retardation [44]. Since then, advancements in molecular biology and IVF techniques have enabled the perfecting of PGD. PGD has successfully detected the presence of numerous genetic based disorders, such as sickle cell anemia and retinoblastoma [81,82]. PGD and PGS require close collaboration between obstetricians, fertility specialists, an IVF laboratory and geneticists.

For PGD/PGS, one or two blastomeres or a few trophectoderm cells are performed at 3 or 5–6 days after fertilization and are genetically analyzed. This methodology is based on the concept that nearly all blastomeres or trophectoderm cells comprising the embryo are genetically identical and therefore allow deduction of the genotype of the entire embryo according to the analysis of a single or a few biopsied cells from an embryo. Because the procedure involves randomly obtaining cells, the presence of low-level mosaicism in the embryonic complex can confuse the diagnosis, thus posing a risk for which couples need to be appropriately counseled. Two common technologies used for analyzing PGD are sequencing or genotyping for monogenic disorders and fluorescence *in situ* hybridization analysis for diagnosing limited numbers of structural or numerical chromosomal aberrations [83]. Recently DNA microarray technology has been employed in PGS, allowing all 23 pairs of chromosomes to be evaluated for numerical and structural imbalances [84–86]. These technological advances suggest that there will be a wider implementation of PGD/PGS in the future.

2.5. IVF laboratory advancements and their impact on better understanding basic principles of developmental biology/physiology

Since the early days of IVF, dramatic improvements in pregnancy rates per cycle have been realized. Much of this improvement has been attributed to refinements in the growth media used to culture oocytes and embryos prior to uterine transfer [87]. The earliest IVF culture medias used simple salt solutions [87]. Since this time, embryo culture media has been refined [87,88]. Improved understanding of the mechanisms that govern early embryologic development has driven these refinements [87,88]. The metabolic needs of developing embryos to successfully accomplish proper patterns of mitotic division are fundamentally different from those of other fully differentiated somatic cells [87]. For example, instead of glucose, the early mammalian embryo utilizes carboxylic acids pyruvate and lactate as its primary energy [87,89]. Other research has shown the requirement of certain key amino acids for the development of mammalian embryos in culture [90].

Currently, culture media has a complex composition, containing specific concentrations of a series of amino acids, glucose, ammonium, and chelators such as EDTA [87]. The specific composition of IVF culture media also differs from the early stages of development, the cleaving embryo, to later developmental stages at day 5 or so of development, the blastocyst stage [87,91]. Defining optimal culture media composition also has given insights into the key metabolic demands of the developing embryo immediately post fertilization. However, IVF culture medias do not possess more aggressive cellular modulators, such as growth factors. This is notable, given that growth factors and other cellular modulators are commonly employed in the growth and manipulation of cells in other fields within cellular biology such as stem cells [92].

The ability to successfully cryopreserve and thaw embryos with resultant pregnancies has also been largely due to laboratory innovations. Prior to initiating cryopreservation, embryos are exposed to low concentrations of glycerol and propanediol, supplemented with sucrose [93]. These compounds result in decreasing the intracellular water content of embryonic cells, minimizing the risk of intracellular ice crystal formation that may be associated with cryopreservation [93]. The newer technique of vitrification requires decreased concentrations of these compounds and therefore, in theory, may minimize theoretical concerns surrounding possible embryonic toxicity [93]. The chemical cocktails that are employed to

accomplish embryonic cryopreservation are dynamic and constantly changing as our understanding of cellular demands evolves.

Detailed analysis of the culture media for the byproducts of embryonic growth, a practice known as metabolomics, is a rapidly evolving field [94]. As embryos develop, classes of metabolites, such as glycolytic intermediates and amino acids, are produced and may be detected and measured in the culture media [94]. Recent study into this field suggests that specific differences exist in the metabolomic signature of genetically normal versus abnormal embryos [94,95]. These signatures have been shown to be able to predict pregnancy success independent of morphologic grading of embryos, the traditional measure of determining optimal embryos for uterine transfer [96]. Measuring these end products, such as amino acids, gives insights into transcription events in early embryologic development [94]. These insights may prove to further the broader fields of developmental biology and genetics [94].

3. Current IVF status

3.1. IVF success rate

Worldwide, more than 70 million couples are afflicted with infertility [97]. Since the first successful IVF procedure in 1978 [30], the use of this and related technologies has expanded to become commonplace around the globe. Over the past decade, the use of ART services has increased at a rate of 5–10% annually [98,99].

In 1996, approximately 60,000 IVF cycles were initiated in the United States with approximately 17,000 clinical pregnancies and 14,000 live births [100]. Currently, approximately 1% of births in the United States occur in women who conceived through IVF [100]. In 2007 these numbers had grown to approximately 100,000 IVF cycles with live birth rates per cycle ranging from approximately 40% in women under the age of 35 to 5–11% in women 41 years of age or older [101]. An additional 15,000 transfers were performed using donor oocytes [101]. Similar success rates have been realized in some other countries. Approximately one-half of all IVF cycles are performed in Europe [102]. In 2006, IVF clinics in 32 European countries reported to national registries [99]. That year, approximately 458,000 cycles were reported, with clinical pregnancy rates of approximately 32% per embryo transfer procedure [99].

Pregnancy rates per individual, particularly in younger women, are further enhanced with the addition of repeated IVF cycles. Data have shown a cumulative live-birth rate following up to six IVF cycles of greater than 85% in women younger than 35 and 42% in women 40 years of age or older [103]. Furthermore, some studies suggest that up to 21% of women during their lifetime may achieve pregnancy spontaneously following discontinuation of unsuccessful IVF cycles [104]. However, the emotional investment that couples expend in repeated IVF cycles limits continued utilization of IVF after failed cycles [105].

Variables, including differences in the patient populations, types of ART/IVF treatments offered, access to care, percentage of fertility clinics which comply with comprehensive and accurate reporting of data, make direct comparisons of the success rates among different nations difficult. The use of technologies to enhance pregnancy outcomes is variably applied to IVF cycles. For example in the United States for the year 2007, ICSI was performed in 63% of all cycles and PGD/PGS was used in 5% of all cycles [101]. Since these technologies are not applied universally or consistently further complicates the comparison of IVF data. The continual improvement of IVF pregnancy rates, coupled with the rapidly expanding indications for this technology, suggests that IVF will be increasingly utilized in the foreseeable future. At the same time, associated effects, such as multiple gestation pregnancies, will likely lead to increased oversight of IVF.

3.2. Social, ethical and regulatory aspects of IVF

Through centralized reporting registries, general estimates of IVF activity are available in many nations. In an effort to define current IVF statistics and to make this information more transparent and available to patients, the Fertility Clinic Success Rate and Certification Act of 1992 was created in the United States [106]. This law requires clinics providing IVF in the United States to report specific information regarding IVF cycles, including pregnancy rates [100]. In other countries, similar national registries exist [99], making it possible to evaluate data from IVF cycles on both a national and international scale.

The transfer of multiple embryos in a single cycle increases the rates of multiple births [107]. Because of the increased social costs and health risks associated with multiple births, legislation or guidelines from professional societies have been introduced in many countries restricting the number of embryos that may be transferred per IVF cycle in an effort to limit the incidence of multiple gestations [107–109]. In the United States in 2007, the number of embryos transferred per cycle ranged from 2.2 in women under 35 to 3.1 in women over 40 years of age (CDC). Multiple birth rates in the United States in 2007 ranged from approximately 35% in women under 35 to 15% in women over the age of 40 [101]. In Europe the approximate number of embryos transferred in the year 2006 was one (22%), two (57%), three (19%), or four (1.6%) [89]. In 2007, 79.2% of European births were singletons, with a twin rate of 19.9% and a triplet rate of 0.9% [99].

However, there are a host of complexities and confounders regarding much of the data obtained from IVF cycles. Variability of legislation regulating IVF exists in different countries and even states/provinces within a single nation [100]. For example, some laws place limits on the number of embryos that may be transferred, cryopreserved, or fertilized per IVF cycle [99,100,110,111]. Other countries mandate that the identity of gamete donors (sperm and egg) be made available to offspring upon request [111]. In some cases, these regulations or fiscal pressures result in couples traveling across international border to obtain treatments that are unavailable in their native country [112]. This practice, known as cross-border reproductive care (CBRC), is thought to account for as much as 10% of the total IVF cycles performed worldwide [112,113].

3.3. Financial aspect for IVF treatment

The funding structure for IVF/assisted reproductive technology (ART) is highly variable among different nations. For example, no federal government reimbursement exists for IVF in the United States, although certain states have insurance mandates for ART [98,114,115]. Many other countries provide full or partial coverage through governmental insurance [98,107]. In many instances, long wait times for IVF through these government programs encourage couples to seek treatment in private fertility centers that accept remuneration directly from the patients [98,116]. In the United Kingdom for example, only approximately 25% of all IVF cycles performed are funded by the National Health Service [107].

The fact that significant economic barriers to IVF exist in many countries results in the preferential availability of these technologies to couples in a position of financial strength [114]. The cost of performing ART per live birth varies among countries [98]. The average cost per IVF cycle in the United States is USD 9266 [117]. However, the cost per live birth for autologous ART treatment cycles in the United States, Canada, and the United Kingdom ranged from approximately USD 33,000–41,000 compared to USD 24,000–25,000 in Scandinavia, Japan, and Australia [98]. The total ART treatment costs as a percentage of total healthcare expenditures in 2003 ranged from 0.06% in the United States to 0.25% in Australia [98]. Some have maintained that the cost for these cycles pales in comparison to the social advantages yielded by the addition of productive members of

society [118]. This is especially true in societies that have a negative or flat population growth rate coupled with an aging population [118].

4. Future of IVF

The future of ART/IVF is encouraging. Pregnancy rates associated with IVF are high compared to those seen in the early days of the procedure. The current efficiency of IVF is more cost-effective and efficacious in achieving pregnancy than other modalities, such as injectable gonadotropins coupled with intra uterine insemination (IUI), which traditionally some have preferred [119]. The increased efficiency of IVF has also resulted in an increased rate of multiple gestations. Recent data suggest that single embryo transfer, coupled with subsequent frozen embryo transfer, results in equivalent pregnancy rates compared with the transfer of multiple embryos, without an increase in multiple pregnancy rates [109]. Therefore, a trend toward single embryo transfer is likely to increase in the future.

4.1. Advancement of technologies

In addition to refining and improving existing applications in technologies, novel uses for IVF are being developed that have the potential to significantly impact modern medicine. Currently, tests, such as antral follicle count and the measurement of follicular stimulating hormone (FSH) in the early follicular phase, coupled with physical history, diagnostic imaging, male evaluation, and maternal age are the hallmarks of infertility evaluation. Other more recently developed tests such as anti-mullerian hormone (AMH) evaluation are likely to add diagnostic accuracy [120,121]. The future will likely bring new testing modalities that will aid in the diagnosis and determination of optimal therapeutic protocols for infertile couples. With the continued progress made in genetic diagnostics, one could envision the advent of tests that could determine specific defects, perhaps dealing with sperm–egg binding or endometrial receptivity, in the future.

Refinements in the embryological laboratory materials and procedures, including culture media, ICSI, and assisted hatching, have had a profound effect on improving pregnancy rates associated with IVF [122,123]. The use of incubators with time lapse photography/video and the use of proteomics that evaluate specific chemical ratios within the fluid of developing embryos are being developed that may further the ability to choose optimal embryos for transfer [124–126]. Additionally, these technologies will further our understanding of early embryologic development.

PGS and PGD offer the unique ability to characterize the genetic composition of embryos prior to embryo transfer. Given the recent successes of these technologies, the broader implementation of this technology in the future is likely.

4.2. Fertility preservation

Female fertility is well documented to decrease with age [127,128]. Consequently, much research has been conducted aimed at preserving female fertility before advanced age is realized. Additionally, fertility preservation for individuals afflicted with cancer has important implications as often the chemo-therapeutic agents used to treat cancer are toxic to the ovary and result in diminished ovarian reserve and reduced fertility. While techniques for freezing sperm and embryos are well established, techniques for freezing oocytes and ovarian tissue are still considered experimental [129]. Multiple techniques including oocyte cryopreservation and preservation of strips of ovarian cortex with subsequent reimplantation and stimulation have been described, with some pregnancy success [59,66,130,131]. Fertility preservation for cancer patients using *in vitro* maturation (IVM), oocyte vitrification and the freezing of intact human ovaries with their vascular pedicles has also been reported [132]. As of 2008, more than 5 babies had been delivered through IVF following ovarian tissue transplantation [133]. Many have suggested that, prior to being treated for cancer, women should be offered fertility preservation measures as outlined above [132].

Recently, several laboratories have demonstrated the ability to successfully cryopreserve oocytes following an IVF cycle, using vitrification protocols, with increased rates of successful thaws, fertilization, and clinical pregnancies. The pregnancy rates obtained in these laboratories were comparable to fresh cycles [66]. These developments have profound implications. As the birth control pill gave women the ability to prevent pregnancy, oocyte cryopreservation may give women the flexibility to preserve their fertility potential, starting at a young age, while postponing childbearing.

4.3. Concerns and challenges

Advances in the arena of assisted reproductive technologies (ART) are accompanied by ethical and societal concerns. Legislation and professional societies have attempted to address these concerns for some time. For example, in 1986, the American Fertility Society first published guidelines for the ethical implementation of ART in the United States [134]. However, the dynamic nature of ART and the rapid evolution of the field result in constant paradigm shifts that require frequent and comprehensive evaluation by professional organizations and society alike. Although controversial, using PGD to choose embryos solely on the basis of gender is currently being practiced [135,136]. In the near future, with refinements in microarray technology and the defining of genetic sequences associated with certain physical characteristics, it is conceivable that specific physical or mental characteristics may be evaluated to guide the decision as to which embryos to transfer. This possibility raises concerns on both ethical and practical levels. Of more concern is the possibility that in the future, technology will permit the manipulation of genetic material within an embryo. Rigorous public and scientific oversight of these technologies is vital to ensure that scientific advances are tempered with the best interests of society in mind.

The majority of couples suffering from infertility reside in developing countries where access to ART is scarce [97]. Much of the infertility in these regions is caused by tubal obstruction following pelvic infections [137]. While strategies concentrating on prevention of these diseases are important, offering ART to this population will likely also be important in the future [97].

There are questions that remain outstanding regarding the use of IVF. Conflicting data exist about the risks of IVF on the developing embryo. Multiple studies have failed to find a clinically relevant association between IVF or embryo cryopreservation and adverse maternal or fetal effects [138–140]. Other studies have suggested that infants of IVF pregnancies may be at a small but statistically significant increased risk for rare epigenetic and other abnormalities [141–143].

Despite this controversy, there is a general consensus that IVF confers a small but measurable increased risk for a variety of congenital abnormalities including anatomic abnormalities and imprinting errors as compared to the general population [144]. Some maintain, however, that this is secondary to an increased baseline risk for these problems in the population of infertile patients [144]. Regardless of the cause, this small increased risk, while statistically significant with extremely large sample sizes, will likely not be a powerful enough factor to dissuade infertile couples from pursuing parenthood through IVF.

5. Conclusions

In 1978 when Edwards and Steptoe performed the first successful IVF procedure, a new science was born. Previously described only in the context of science fiction, IVF has made possible the birth of countless individuals and changed a multitude of lives for the better.

As this technology improves, the implications and applications for IVF continue to expand. Innovations such as PGS/PGD and fertility preservation promise to change the field in new and exciting ways and offer great benefit to many individuals. However, this technology also carries with it the potential for inequitable distribution and unethical abuses. Currently, IVF is preferentially offered to those with economic means. It is ethically incumbent upon societies around the world to make IVF more universally available to the populations in need. Additionally, scientific advances, particularly in the realm of genetics, have made possible the evaluation of the human embryo on a genetic level. As this technology advances, one could imagine more detailed evaluations and even manipulations being possible. Although the development and implementation of IVF have offered the hope to infertile couples the world over, it is vital that we as a global community strictly monitor the development of applications of this technology to ensure that ethical abuses do not emerge.

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