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PRIMER

Human *in vitro* fertilisation and developmental biology: a mutually influential history

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ABSTRACT

This article describes the origins and development of *in vitro* fertilisation (IVF) and how it was influenced by, and influenced, basic research in developmental biology. It describes the technical and social challenges that confronted the pioneers in this field of study, and the considerable progress that has been made since those early days. It also considers how IVF has contributed, and continues to contribute, to our understanding of early human development.

KEY WORDS: Developmental biology, IVF, Embryo transfer

Introduction

In vitro fertilisation (IVF; Fig. 1) is defined as the fertilisation of an egg by a spermatozoon outside the body. Because many organisms routinely fertilise their eggs in this way, the term IVF is usually only applied to those organisms, such as mammals, in which fertilisation is naturally internal. Interest in achieving mammalian IVF and embryo transfer (ET) developed in the early to mid-20th century as part of a general awakening of interest in reproduction in mammals (Clarke, 1998). As well as responding to concerns about food self-sufficiency in large farm animals, IVF also promised to provide a means to study the early development of the human embryo, in particular to complete the series of specimens in the Carnegie collection (Hertig et al., 1956) and to study the origin of chromosomal anomalies. The value of IVF as a tool in the development of contraceptive agents and as a means of treating the infertile also became powerful drivers of research in human IVF from the mid-1960s. The challenges facing these early pioneers were considerable and involved obtaining knowledge about the basic biological processes of sperm and egg maturation, ovulation, fertilisation, early development *in vitro* and the factors affecting its success, ET techniques and implantation in mammals, about all of which very little was then known (Johnson, 2019a).

Human IVF was first achieved in 1969 by Robert Edwards and his colleagues (see Fig. 2). In this Primer, I first consider the early work on humans and animals that led up to Edwards' success, and then focus on Edwards, Patrick Steptoe and Jean Purdy and how their work led to the first IVF babies in 1978 and 1979. I then summarise the key subsequent developments in IVF in all these cases with a focus on the basic science that underpinned the clinical success, and include examples of how IVF has permitted better basic research on the early human embryo. I end with a summary of where we are now, and a general discussion about the relationship between basic science and its application to IVF and vice versa.

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The early days leading up to IVF

The first birth from fertilisation *in vitro* was celebrated just over 40 years ago, when Louise Brown was born in July 1978 (Steptoe and Edwards, 1978). Her birth was the result of the work of Bob Edwards, Patrick Steptoe and Jean Purdy, and for which Edwards was belatedly awarded the 2010 Nobel Prize for Physiology and Medicine 'for the development of *in vitro* fertilisation' (https://www.nobelprize.org/prizes/medicine/2010/summary/; Gardner, 2015), both of his collaborators having died in the previous century. However, Edwards and his colleagues were not the first to try to achieve IVF in the mammal.

The history of mammalian IVF and ET began in the late 19th century in Oldham, UK, when Heape (1891) transferred two fertilised ova recovered from an Angora doe rabbit into the Fallopian tube of a Belgian hare recipient resulting in the birth of six young – two with Angora phenotypes and four with Belgian hare phenotypes. This experiment was performed to test the idea that the phenotype of the offspring could be influenced by the uterine environment. Subsequently, after World War 2, Rowson and Moor in Cambridge, UK, attempted, and ultimately succeeded, in the transfer of embryos recovered by uterine lavage to cattle and sheep uteri, as part of a drive to improve food production (Polge, 2000).

Work on IVF itself first got underway seriously in the 1930s in the USA, at a time when little was known about the process of, and requirements for, fertilisation in mammals. Thus, Gregory Pincus famously, but controversially (Biggers, 2012), claimed to have produced rabbit offspring after fertilising rabbit eggs *in vitro* (Pincus and Enzmann, 1934). Later, in 1937, John Rock and his research collaborator, Miriam Menkin, started experimenting on both fertilised and unfertilised human eggs retrieved from patients during surgery, as part of an attempt to complete the collection of early stages of human embryos known as the Carnegie collection (Hertig et al., 1956), as well as furthering Rock's interest in treating infertility (Marsh and Ronner, 2019). In 1944, they claimed to have

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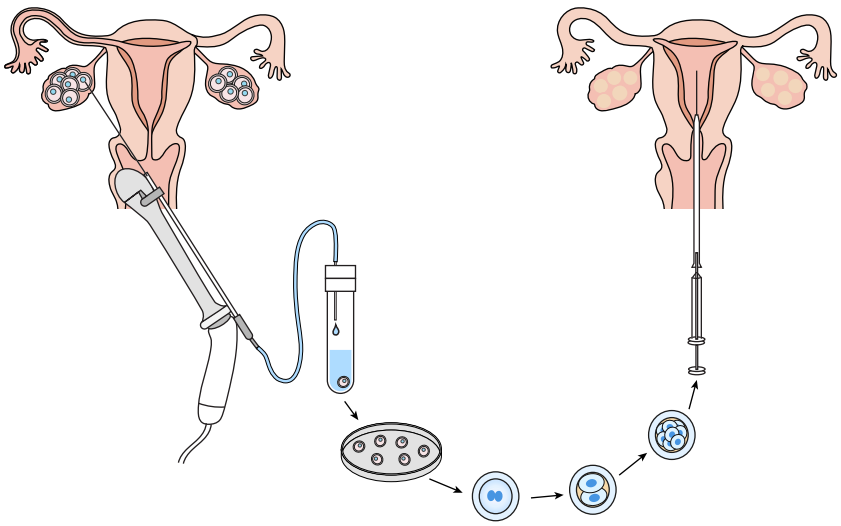

Fig. 1. Summary of IVF procedure. Egg recovery is achieved laparascopically by inserting a needle under ultrasound guidance into each follicle, which is then flushed with warm culture medium, and the oocyte collected by aspiration with its cumulus cells into a sterile receptacle. It is then transferred to a drop of capacitated sperm for fertilisation and early cleavage after which it is transferred at the 8-cell or blastocyst stage to the uterus via a cannula inserted through the vagina and cervix. Adapted from Johnson (2018b).

achieved fertilisation and cleavage *in vitro* of three human ova out of 138 attempts (Rock and Menkin, 1944), although it is unclear whether this was really fertilisation or parthenogenetic activation. Then, in 1951, Min Chueh Chang (1951) and Colin 'Bunny' Austin (1951) independently discovered capacitation, the requirement for sperm to undergo a series of surface changes in the uterus before they are capable of fertilising the egg. The discovery of sperm capacitation led Austin (1962) to re-evaluate the previous claims of IVF and produce five criteria that had not until that stage been satisfied for the human: namely, that (1) capacitated sperm be used, (2) use of aged ova be avoided, (3) there be clear evidence that sperm had entered the ooplasm, (4) the possibility of parthenogenetic activation be excluded, and, ultimately, (5) the birth of young genetically identified as related to the transferred embryo(s) be demonstrated. Based on Austin's criteria, the first definitive proof of mammalian IVF had been provided 2 years earlier by Chang (1959), who built on earlier work by Dauzier and Thibault (1959) and Moricard (1950) by removing unfertilised ripe ova from a rabbit, fertilising them with capacitated sperm, incubating them, and transferring the resultant embryos to another rabbit, which gave birth to viable offspring. This demonstration was followed after 4 years by the successful fertilisation of the hamster egg, though onward culture past the 2-cell stage failed (Yanagimachi and Chang, 1963, 1964). It was not until 1968 that the mouse egg was successfully fertilised *in vitro* (Whittingham, 1968). Moreover, in a small number of cases fertilised mouse eggs cultured to the 2-cell stage and transferred to the oviducts of recipient mice went on to produce viable male and female fetuses that were genetically distinct from the host mother (Whittingham, 1968). These early successes revealed that not only was IVF possible but also the culture of embryos *in vitro* was likely to prove difficult, with most embryos blocking at the 2-cell stage.

During the 1950s and 1960s, a small number of scientists continued to pursue the elusive and controversial goal of IVF in humans, but none was considered to be successful (e.g. Petrov, 1958; Petrucci, 1961; Hayashi, 1963; Yang, 1963; *The New York Times*, July 16, 1974, reports an informal comment by Dr Douglas Bevis). Among these was Landrum Shettles, a gynaecologist at Columbia University (NY, USA) who claimed to replicate the techniques of Rock and Menkin in a series of experiments with

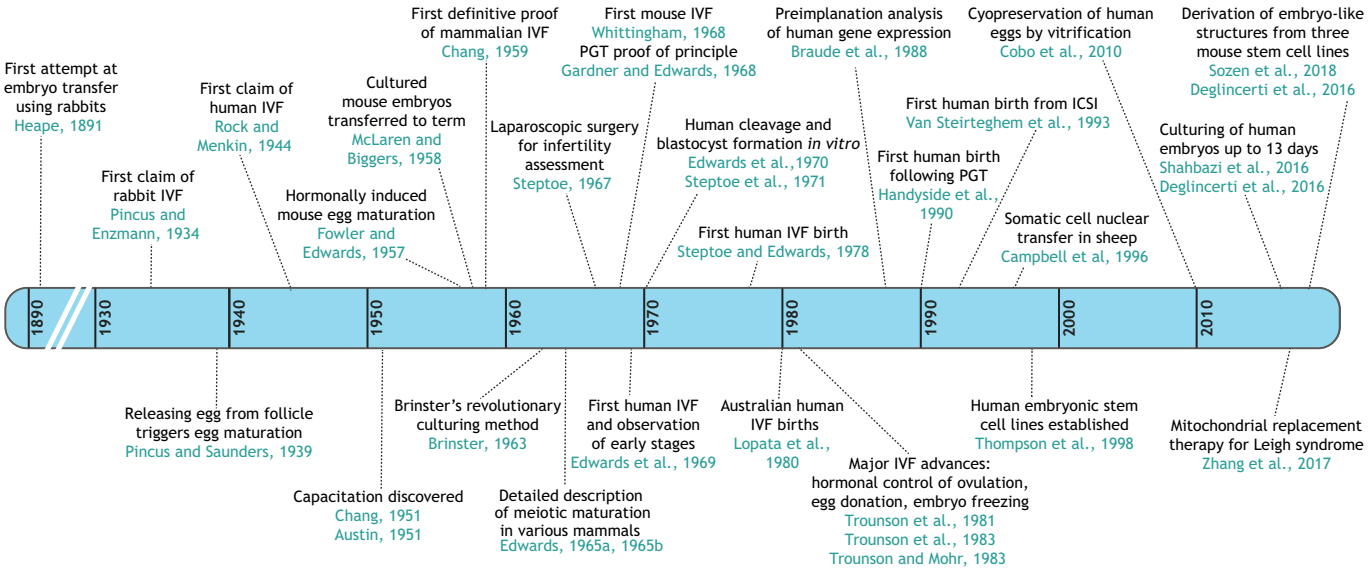

Fig. 2. Timeline showing key events in IVF, as discussed in the text.

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retrieved human eggs, but convincing evidence of his success was never published (Shettles, 1955). It was only the report of the fertilisation of human eggs by Edwards et al. in 1969 that, together with his subsequent follow-up work, finally convinced the general scientific population that IVF had finally been achieved in humans.

The early days of egg maturation *in vivo* and *in vitro*

The early motivation underlying Bob Edwards' research that led to IVF was to understand the origins of, and to possibly avoid, chromosomal anomalies such as Turner or Down syndromes, which were first characterised in 1959 (Ford et al., 1959; Lejeune et al., 1959). Thus, Edwards, working in the 1950s in Edinburgh, UK, studied the chromosomal dance that the meiotic mouse egg displayed between receiving the endocrine signal to ovulate and ovulation (Edwards and Gates, 1959). He was able to time this process precisely because, working with his wife Ruth (Fowler and Edwards, 1957), he had shown that it was possible to stimulate egg maturation *in vivo* by the appropriate hormonal administration to female mice, foreshadowing induced ovulation in women half a decade later (Gemzell, 1962). After stimulating egg maturation in female mice and inseminating them with mouse spermatozoa, Edwards recovered *in vivo* fertilised early mouse embryos from the female tract and used drugs such as colchicine, trypaflavine, Toluidine Blue, triethylenemelamine and nitrogen mustard, or alternatively used exposure to X-rays and UV light, to disturb the chromosomal balance of the eggs, sperm and embryos and examine the developmental consequences (Edwards, 1954, 1957a,b, 1958a, b,c,d; Sirlin and Edwards, 1957; Cattanach and Edwards, 1958). Research in model organisms, often carried out with different aims in mind, was a continuing influence on the development of IVF in humans.

After Edwards left Edinburgh to work at the National Institute for Medical Research, Mill Hill, UK, in 1958, he rediscovered earlier findings that simply releasing the mouse egg from its follicle triggered the same meiotic maturation programme (Pincus and Saunders, 1939; Chang, 1955a); this suggested that the follicle exercised a restraining influence on the egg, now known to be exerted by cAMP and cGMP (Gilchrist et al., 2016). This observation meant that, were the human egg to show the same spontaneous maturation on release from its follicle, then the opportunity to study this otherwise inaccessible process was a possibility. Edwards spent the next six or so years trying to get eggs of various species, including human, to mature *in vitro* after their release from ovarian biopsies. It took a long time in part because no one then knew how long the interval was between the rise in the level of the hormone inducing the initiation of ovulation (luteinising hormone or LH) and the re-entry of the egg into meiosis in humans. It in fact takes considerably longer – around 36 h in women compared with the 12 h then known for mice and rats. However, in 1965 Edwards, who had relocated to the Physiology Department in Cambridge, UK, in 1963, was able to publish two papers describing detailed time courses of the meiotic maturation of eggs in mouse, cow, pig, sheep, rhesus monkey and human (Edwards, 1965a,b). Indeed, the paper (published in the Lancet) describing the human results sets out the possibilities and difficulties that flow from his work with astonishing foresight and imagination (see Box 1). The discussion in this paper also clearly identifies his primary interest as not being the alleviation of infertility but the ability to study, and thereby to avoid, genetic disease. Indeed, within 2-3 years he had demonstrated proof of principle of preimplantation testing for genetic disease (preimplantation genetic testing or PGT) in the rabbit embryo. This involved taking trophoblastic biopsies from embryos and sexing them by staining for the presence (or absence) of the Barr body (indicating X-chromosome inactivation), and transferring the embryos to recipient does: they achieved 100% correct sexing of the resultant fetuses (Gardner and Edwards, 1968). This was 20 years before PGT was achieved in the human (Theodosiou and Johnson, 2011). Thus, Edwards' early research programme led to two technologies that changed the face of human reproduction: IVF and PGT.

Box. 1. Key points in the programme of research discussed in Edwards' 1965 Lancet paper

(1) Studies on non-disjunction of meiotic chromosomes as a cause of aneuploidy in humans*
(2) Studies on the effect of maternal age on non-disjunction in relation to the origins of trisomy 21*
(3) Use of human eggs in IVF to study fertilisation
(4) Study of culture methods for human eggs fertilised *in vitro*
(5) Use of priming hormones to increase the number of eggs per woman available for study/use
(6) Study of early IVF embryos for evidence of (ab)normality – especially aneuploidies arising prior to or at fertilisation*
(7) Control of some of the genetic diseases in man*
(8) Control of sex-linked disorders by sex detection at blastocyst stage and transfer of only female embryos*
(9) Intra-cervical transfer of IVF embryos into the uterus
(10) Use of IVF embryos to circumvent infertility‡
(11) Avoidance of a multiple pregnancy (as observed after hormonal priming and *in vivo* insemination) by transfer of a single IVF embryo

*Aims relating specifically to genetic disease.
‡Aim relating specifically to infertility relief.

The partnership with Steptoe, and the successful fertilisation and development *in vitro* of human eggs

It was only after meeting Patrick Steptoe in the autumn of 1968 that Edwards was persuaded that IVF was a means of treating infertility for many couples. The fact that Edwards had not come to this idea strongly beforehand was unsurprising at that time: in the 1960s, little was known about the incidence and causes of infertility, and reproductive sciences were instead focussed on the perceived population problem and the potential role of novel contraceptives in reducing it. Indeed, Edwards was spending most of his time trying to induce immunity to spermatozoa as a potential form of novel contraceptive (Johnson, 2011) in work funded by the Ford Foundation, which also agreed to fund his work on IVF as a means of opening up the process to novel approaches to fertility control (Johnson and Elder, 2015b). This scientific focus contrasted with Steptoe's long-term interest in treating the infertile (Edwards, 1996). Thus, Steptoe had trained as a senior registrar at the Whittington hospital in Highgate, north London, where he was greatly encouraged in the treatment of infertility by Kathleen Harding, his immediate chief. She taught Steptoe that infertility was a problem of two people resulting in a desperately serious complaint causing lifelong unhappiness that could destroy an otherwise amiable relationship. Indeed, one of the main reasons that Steptoe had developed and pioneered use of laparoscopic (or keyhole) surgery in the UK was to see into the abdomen relatively non-invasively and thereby assess the likely cause of, and thus prognosis for, infertility in women (Steptoe, 1967). In 1951, Steptoe became a consultant obstetrician and gynaecologist at Oldham District Hospital, where he had established a fine reputation amongst both the staff and patients (Edwards, 1996).

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Edwards' partnership with Steptoe confronted massive technical and basic scientific challenges (Table 1). The partnership was initially based on the idea that Steptoe could help him to overcome the problem of sperm capacitation, with which Edwards had been struggling for 4 years (Johnson, 2011). Steptoe could recover sperm laparoscopically from the oviduct, and Edwards realised that such recovered sperm could fertilise his *in vitro*-matured eggs. However, this idea was rendered irrelevant by Barry Bavister's discovery that capacitation of hamster sperm could be achieved simply by exposure to raised pH (Bavister, 1969). The 1969 Nature paper describing the first IVF of human eggs (Edwards, et al., 1969) thus did not involve any laparoscopically derived sperm.

Moreover, at that time, the idea was resurfacing that the maturation of eggs *in vitro*, while allowing the chromosomal dance to proceed, did not result in cytoplasmic maturation sufficient to support normal development (Chang, 1955b). So the attention of Edwards and Steptoe, together with Purdy, who had also joined the team in 1968 after training as a nurse (Johnson, 2019b; Johnson and Elder, 2015b), turned to the development of the laparoscopic recovery of almost matured eggs from the follicles, thereby avoiding this problem. Edwards allied his extensive knowledge of the timing of oocyte maturation *in vivo* with the key technical innovation made by Steptoe – namely surgical use of the laparoscope. Use of this instrument enabled the collection of multiple oocytes, induced by injection of gonadotrophins (Gemzell, 1962), from the intact ovaries of patients under anaesthesia (Steptoe and Edwards, 1970). The laparoscopic approach enabled superior visualisation of the inner abdomen by using cold light conducted through a flexible fibre optic tube. The surface of the ovary could thus be clearly observed and the follicles containing eggs punctured with a thin hollow needle passing through the abdominal wall, enabling egg collection by suction of the follicle contents – a process known as aspiration.

Determining the optimal timing of laparoscopic egg collection was a challenging but crucial component of successful IVF, but this phase of the research was accomplished fairly rapidly. The two important initial goals were to aspirate oocytes from their follicles just before ovulation was expected, and to have more than one pre-ovulatory oocyte available for aspiration. Between 1969 and 1971, Edwards, Steptoe and Purdy then successfully achieved cleavage and blastocyst formation *in vitro* (Edwards et al., 1970; Steptoe et al., 1971), providing for the first time (aside from their rare recovery by uterine lavage) a potential source of embryos that could be made available for studying. Indeed, Edwards examined the eggs recovered from the first 50 treatment cycles to check that they were chromosomally normal, the first evidence of fertilisation being on 21st October 1969 (Elder and Johnson, 2015a). The early embryos recovered were also analysed to assess the normality of their development up to December 1971, when transfer of embryos to women was commenced, mostly at the 8- to 16-cell stage, to try to achieve pregnancy (Elder and Johnson, 2015a).

The problem of implantation: faulty embryos or wrongly phased uteri?

For the next 7-8 years, the problem that confronted the three pioneers was getting the embryos to implant. This problem was then, and remains so today, a difficult one to crack, because it is difficult to know whether the failure to implant resided with defective embryos or the uterus that was to receive them, or a combination of both. With regard to the first uncertainty, they had considerable experience with animal embryo culture by which to be both encouraged and alarmed. Thus, by the 1950s it had been established that the culture of mouse embryos from the 8-cell stage to the blastocyst required glucose (Hammond, 1949; Whitten, 1956) and that their transfer to recipient uteri could result in live young (McLaren and Biggers, 1958). By the early 1970s, it had become clear that mouse 2-cell embryos recovered from the female tract could also be cultured successfully *in vitro* to the blastocyst stage and then transferred to the uterus with the production of live young (Cholewa and Whitten, 1970). Some mouse embryos could even be cultured to the blastocyst from the pronuclear stage with live young resulting (Whitten and Biggers, 1968). It had also been established that culture from the 2- to the 8-cell stage required lactate or pyruvate (Whitten, 1957; Brinster, 1965a,b). Brinster (1963) had also introduced a revolutionary method for culturing mouse embryos, using small drops of medium under paraffin oil rather than the larger volume test tubes as had been done previously, an approach adopted by Edwards from January 1970 (Elder and Johnson, 2015b).

Edwards could thus build on these advances from animal models (though, in fact, aside from adopting a modified version of Bavister's medium for the fertilisation phase, he chose to largely ignore the work on mouse embryo culture in his choice of media,

Table 1. Some of technical challenges that had to be overcome before the first successful live birth following IVF and embryo transfer was achieved

Challenge	Solution
Ovarian stimulation to induce oocyte maturation and ovulation	Ultimately used natural ovulation after having tried human menopausal gonadotrophin or clomid (*vice* FSH) and HMG (*vice* LH).
Technical aspects of follicle aspiration	Initially used a syringe to withdraw fluids from follicles but then developed and used a new suction gadget (Elder and Johnson, 2015b).
Timing of laparoscopy in order to collect the eggs at the optimum time	Used the time for *in vitro* maturation of eggs on release from their follicles as a guide. Initially, went in at 28.75-29.50 h after the hCG injection but eventually settled on 32-33 h post-hCG injection or spontaneous rise.
Cycle monitoring	Extensive urine samples, and some blood and follicular fluid samples, taken to measure the levels of various steroid and gonadotrophic hormones as described by Elder and Johnson (2015b).
Oocyte culture	Details of how they varied the oocyte culture media are described by Elder and Johnson (2015b).
Sperm preparation, including capacitation	Details of how they varied the preparation of sperm samples are described by Elder and Johnson (2015b).
Insemination procedure: culture medium, timing	Details of how they varied the insemination process are described by Elder and Johnson (2015b).
Culture for embryo cleavage: culture medium, assessment	Details of how they varied the media are described by Elder and Johnson (2015b).
Technical aspects of embryo transfer, including route of transfer, medium and timing	Details of how they varied the embryo transfer are described by Elder and Johnson (2015b).
Luteal support	Details of how they varied the luteal support are described by Elder and Johnson (2015b).

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preferring to use his own complex formula). He was also fortunate that that two limiting features of animal embryo cultures – developmental arrest at the time of maternal-to-zygotic transition (which occurs at the 2-cell stage in the mouse and hamster and the 8-cell stage in humans) and transfer of the embryo to the uterus at a premature time point (i.e. younger than the late morula and early blastocyst) – did not occur in humans (Braude et al., 1988; Goddard and Pratt, 1983; Marston, et al., 1977). Indeed, their success in culturing embryos to the blastocyst stage caused the team to turn their attention to the endocrine conditions prevailing during the second half (luteal phase) of the cycle. They decided that the problem of the lack of pregnancies lay in poor uterine receptivity, possibly arising from the use of exogenous hormones to override the natural human endocrine secretory programme. They concentrated initially on finding a way to support the uterus through the luteal phase of the cycle, eventually resorting to abandoning the use of exogenous hormones in favour of using a natural cycle by monitoring the levels of the ovulatory stimulating hormone LH, and collecting the single egg laparoscopically just before it was due to ovulate. This was quite a technical feat given that the assay developed and marketed only recently for detecting the presence of LH present in 2-3 hourly collected urine samples was not particularly reliable, and the whole team of largely volunteer staff had to dance to the tune of the women's natural cycles, rather than having planned egg collections according to a controlled schedule (Johnson and Elder, 2015a). Nevertheless, this approach worked and they got two pregnancies to full-term live births, namely Louise Brown on 25th July 1978 (Steptoe and Edwards, 1978) followed by Alistair MacDonald on January 14th, 1979 (Steptoe et al., 1980) – the ultimate and final proof of their successful attempts at IVF. Having succeeded in achieving two live births from 242 women volunteers treated (Elder and Johnson, 2015a; Johnson, 2019a), they then had to close down their operation in Oldham due to the enforced retirement of Steptoe from the NHS. The team of three resumed treating patients again at Bourn Hall outside Cambridge after a 2-year break.

The Australian competition

During Edwards and colleagues' enforced period of experimental quiescence between 1978 and late 1980, two teams of scientists and clinicians in Melbourne, one led by Carl Wood with Alan Trounson at the Queen Victoria Hospital and Monash University and the other led by Ian Johnstone with Alex Lopata at the Royal Womens' Hospital and Melbourne University, moved centre stage, and the next group of IVF births occurred there, the first using the natural cycle approach successfully used by Edwards and his colleagues (Lopata et al., 1980). Indeed, it was the Melbourne teams, which had both been stimulated in the early 1970s to try to achieve IVF after learning about Edwards' successful fertilisation of human eggs at conferences, that claimed the first (unsuccessful) pregnancies following the transfer of human embryos to the uterus in 1973, when they claimed a rise in human chorionic gonadotropin (hCG), produced by the trophoblast of the developing embryo – the earliest sign of a 'chemical pregnancy' – following transfer of a fertilised egg to the uterus of a woman (De Kretzer et al., 1973). However, their claim is somewhat undermined by the fact that the same woman gave birth spontaneously several years later, suggesting that she was not infertile after all.

The Australians largely followed the same path as Edwards, trying first induced ovulation and switching to natural cycles after learning of the success of this approach with the birth of Louise Brown. However, it was the arrival in 1977 of Alan Trounson that marked the beginning of the really flourishing phase of Melbourne's pre-eminence. Trounson was a graduate of Sydney University who had undertaken post-doctoral training in Cambridge at the ARC Animal Research Station. Here, he had learnt about IVF in rabbits, and egg maturation, embryo transfer and freezing in cows and sheep, and had also met Edwards, who was well aware of the Australian teams' attempts. It was largely due to Trounson's drive, together with his experience in the hormonal stimulation of large farm animals that the next major advances in IVF occurred. These advances included: the successful hormonal control of ovulation via clomiphene citrate with or without a dose of hCG (Trounson et al., 1981; this was soon followed by the successful use in the USA of human menopausal gonadotrophin (HMG) and hCG by Coddington and Oehninger, 2018); the first use of egg donation (Trounson et al., 1983); and the first successful use of embryo freezing to generate a pregnancy (Trounson and Mohr, 1983). However, this period of Australian dominance was cut short by the 1984 introduction of ambiguous and restrictive legislation by the state of Victoria that was influenced heavily by the Catholic church and was aimed at curbing the activities of local teams. This legislation, combined with political pressure from the State government, inhibited the inventive environment that had existed hitherto and led some of the key players to leave Victoria and commence research in other states and countries, thereby breaking up the local teams (Leeton, 2013; Wilton, 2018).

Where are we now?

So where are we now? With over 8 million IVF babies born worldwide, IVF has clearly been a remarkable reproductive technology. Since the original research of Edwards and Trounson, it has also advanced technically, influenced various other aspects of reproductive health and fertility, and helped our understanding of basic human development.

Most IVF cycles do not now use the natural cycle method but rather induced ovulation, as originally attempted by Edwards, but now in which the reproductive endocrine state of the woman is first closed down completely by use of gonadotropin-releasing hormone analogues or antagonists, and then exogenous recombinant follicle-stimulating hormone (FSH) and LH are used to control the ovarian response (Mochtar et al., 2017). However, we still have no way of knowing if the embryos we place back into the uterus are those with the best chance of producing a pregnancy, although we do know from studying biopsied IVF blastocysts that between 40 and 70% of human preimplantation embryos from women under 35 years of age have a chromosomal abnormality, and are therefore more likely to fail developmentally (Irani et al., 2018). We know from experiments conducted first in the mouse (Bolton et al., 2016) that many embryos that are chromosomally mosaic initially can nonetheless give rise to a chromosomally normal baby, the abnormal cells evidently being lost by overgrowth or by their segregation to the extra-embryonic membranes, and a similar finding has been reported in humans (Grati et al., 2018). Moreover, even chromosomally normal embryos that result from induced ovulation and culture *in vitro* often show epigenetic changes that may lead to metabolic diseases later in life, as has been shown in mice, sheep and more recently in humans (Fleming, et al., 2018). Part of the problem is damage by oxygen free radicals (Johnson and Nasr-Esfahani, 1994), and various ways of reducing damage have been described, initially in mice and then subsequently in humans, including the incubation of embryos in low oxygen to mimic the situation *in vivo* (Ma et al., 2017; Gomes Sobrinho et al., 2011; Bontekoe et al., 2012; Wale and Gardner, 2010; Gardner and Lane, 1998; Burton et al., 2017), and inclusion of

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anti-oxidants in the media (Li et al., 1993; Wdowiak and Wdowiak, 2015; Akarsu et al., 2017). However, even today not all clinics use low-oxygen incubations.

Other developments of IVF have come thick and fast. After the successful freezing of spermatozoa, which was achieved in the 1950s, cryopreservation of embryos, and more recently eggs, has been achieved. Traditional approaches to embryo cryopreservation used slow-freeze-and-thaw methods, in which time was allowed for the penetration and equilibration of cryoprotectants in order to avoid damage by ice crystals. Recently, successful cryoprotection by vitrification, which involves flash-freezing with higher levels of cryoprotectant and produces a glassy appearance owing to a lack of ice crystals, has allowed embryos, and particularly oocytes, to be frozen more rapidly and in the case of eggs with much greater success (Cobo et al., 2010). This procedure allows women to electively freeze oocytes for later use – often long after their peak egg quality period (in their mid to late 20s, but declining rapidly from about 35 years). Again experiments on mouse eggs and embryos (Johnson, 1989; Vincent et al., 1990; Vincent and Johnson, 1992; George et al., 1994) were followed by experiments on human eggs and embryos (Pickering et al., 1991), emphasising how important mouse models have been in the development and refinement of IVF.

In 1992, the first report of a successful birth using intra-cytoplasmic sperm injection (ICSI) was published (Van Steirteghem et al., 1993). Initially used as a route for circumventing male subfertility, this variant IVF technique, in which a single spermatozoon is picked up in a micropipette and injected directly into the egg cytoplasm, is now used routinely in many clinics, having in general a greater success rate than IVF, though this claim has recently been disputed (Li et al., 2018). However, this technique was only later applied to mouse eggs, which are much more difficult to inject with maintained viability than human eggs, meaning that this is one technology for which the mouse has not proved a good model for the human – in fact, rather the reverse!

Despite the proof of principle for PGT being established in rabbits by Gardner and Edwards in 1968, it was not until 1986 that clinical interest increased, with a corresponding increase in the pace of research (Theodosieu and Johnson, 2011), the first PGT pregnancies being reported in 1990 (Handyside et al., 1990). Only over the past 10-15 years has PGT taken off clinically, especially in the USA and driven largely by experiments on human embryos rather than those of animals. Now, PGT has been authorised by the Human Fertilisation and Embryology Authority for nearly 400 genetic mutations (see https://www.hfea.gov.uk/pgd-conditions/), controversially including some that encode genes that predispose to certain illnesses rather than unambiguously causing them. PGT can also be used for tissue-typing an early embryo so that one can be selected for transfer with a view to using its umbilical stem cells to help an existing sibling in need of compatible cells by transplantation (Verlinsky et al., 1997, 2001). Finally, PGT is used somewhat more controversially (Gianaroli et al., 1997; Kang et al., 2016; Kushnir et al., 2016; Gleicher et al., 2018) to detect and eliminate chromosomally abnormal embryos from older women or from women who have repeatedly lost pregnancies, but double-blind prospective trials have not revealed any advantage of such an approach thus far (Verpoest et al., 2018).

Another area of human fertility in which Edwards and colleagues' work has been influential is mitochondrial replacement and gene therapy. In 2017, a mitochondrial disease called Leigh syndrome was treated by the transfer of the second meiotic spindle from an afflicted oocyte to an enucleated healthy oocyte, and the successful fertilisation, transfer and development of the zygote to a full-term baby (Zhang et al., 2017). This success depended upon the study of similar strategies in mouse and primate eggs (Liu et al., 1999; Tachibana et al., 2009). The possible wider treatment of genetic diseases is being studied using modern techniques of gene editing, such as CRISPR/Cas9, which have been successfully applied in mouse eggs and zygotes (Wu et al., 2013; Wang et al., 2013) as well as in human triploid eggs (Liang et al., 2015) and reportedly, and controversially, in eggs allowed to go to birth (Ilic, 2018). Recently, a research paper applying the approach to the human zygote has been published, and has led to the re-evaluation of the role of OCT4 (also known as POU5F1) in early human development (Fogarty et al., 2017). This work benefitted from human zygotes donated from couples undergoing IVF, and was undertaken with the aim of understanding how the patterning of the early human differed from that in mice. The increased understanding of early human development – without a necessary link to fertility – has been another profound outcome of IVF technology. For example, early studies in mouse on the timing of embryonic gene expression (mid-late 2-cell stage; Flach et al., 1982) set the pattern for similar experiments in the human (8-cell stage; Braude et al., 1988). Later approaches to the same question have likewise built upon work originally undertaken in mouse (Dobson et al., 2004; Niakan and Eggan, 2013; Stirparo et al., 2018) as have approaches to other developmental questions (Petropoulos et al., 2016; Zhu et al., 2018; Wang et al., 2018; Popovic et al., 2019).

IVF also permitted in 1998 the production of human embryonic stem cells (ESCs) from spare blastocysts donated by couples for research (Thompson et al., 1998), following their successful derivation in rabbits by Edwards (Cole et al., 1965, 1966) and in mice by Evans and Kaufman (1981) and Martin (1981). In combination with somatic cell nuclear transfer (SCNT, first described successfully for sheep in 1996; Campbell et al., 1996), the use of ESCs allows the production of pluripotent cell lines matched genetically to the nuclear donor. This approach opened the possibility of tissue repair and cell therapy that otherwise involved the use of long-term immunosuppressants. However, the use of other sources of pluripotent cell lines, such as induced stem cells (Takahashi and Yamanaka, 2006), has turned out to be ethically more acceptable and practically less demanding.

Advances in culture conditions have also increased the accessibility of the study of IVF embryos. Recently, human embryos have been cultured *in vitro* in a matrix that has allowed them to develop for up to 12-13 days, approaching the legally permitted limit of 14 days in the UK and subsequently adopted in many other countries (Pera, 2017). Surprisingly, the embryos seem to undergo normal post-implantation morphogenesis, forming a proamniotic cavity in the absence of any maternal input, suggesting that the pattern of early development resides entirely within the embryo itself (Shahbazi et al., 2016; Deglincerti et al., 2016). Such *in vitro*-implanted embryos may be able to develop for another 1-2 weeks thereby permitting the study of the generation of the neural plate and gamete (germ line) formation *in vitro*, but this would be subject to a change in the law. Even if the 14-day limit is not extended, the study of *in vitro* implantation over the period of gastrulation by use of human embryo-like entities may be possible. Recently, aggregates of mouse ESCs have been found to function in many respects comparably to embryos up to pregastrulation stages (Bedzhov and Zernicka-Goetz, 2014). When trophoblast stem cells are also added, better development is observed (Harrison et al., 2017; Rivron et al., 2018), and the addition of a third cell line, equivalent to the hypoblast in the mouse, has taken the embryos

through a quasi-gastrulation with the formation of germ cells (Sozen et al., 2018). For humans, for which, until recently, only ESC lines have existed, development is at least equivalent to that observed with purely mouse ESCs (Taniguchi et al., 2015; Shao et al., 2017a, b), meaning that discovery of a human hypoblast cell line, and confirmation of a recently described trophoblast stem cell line (Okae et al., 2018), are imperative. The need for IVF embryos aid our to understanding of early human development may therefore soon be lessened, other than as a comparator for the stem cell-derived embryo-like structures, in addition to use of the limited samples from the Carnegie collection for comparative purposes (Hertig, 1935, 1945).

Conclusions

From its early contested beginnings, IVF now offers a range of technical possibilities that theoretically allow us to control the reproductive process in many ways. This modern technology was often controversial (Johnson, 2018a) and has changed the meaning of families; thus, dead mothers and fathers can still produce genetic offspring, two mothers or two fathers can parent a child, indeed the meaning of the terms father and mother has changed and multiplied. IVF has also become so normalised compared with the early days, when its use was considered shameful, that now it sets the standard for natural methods of reproduction (Franklin, 2013). Truly, Edwards, Steptoe and Purdy started a scientific and social revolution with their pioneering discovery of IVF, and much of this discovery rested on work that had been initiated on animal developmental biology, as I have described in this article. But the advent of IVF has allowed the human embryo itself to be studied for its own interest and not simply as an adjunct to IVF. Indeed, Edwards himself always saw the role of basic science and the scientist as pivotal in the development of IVF, hence his insistence that the European Society for Human Reproduction and Embryology had the specification of embryology in its name, and that the Society's constitution mandates a key role for scientists.

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Competing interests

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