Chapter 1

A Brief Outline of the History of Human In-Vitro Fertilization

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Introduction

Any account of the history of in-vitro fertilization and embryo transfer (IVF-ET) is not complete unless descriptions of the hostility from members of the public and the media are included. Nearly all studies on mammalian fertilization and preimplantation development encountered significant opposition from the time they began in the 1930s. Immediately following the announcement in 1978 of the birth of the first IVF baby, Louise Brown, praise for a new treatment for human infertility was accompanied by loud opposition from the press and members of the public. This chapter, therefore, not only discusses the scientific underpinning of clinical IVF-ET, but also includes a brief historical perspective of the public backlash encountered along the way.

The need for an effective treatment for infertility due to blocked Fallopian tubes had been recognized for a long time, originally to help with inter-generational problems relating to the distribution of family wealth. An early attempt to unblock the tube involved passing through it a whalebone bougie (Smith, 1849). This barbarous procedure was never adopted. The discovery that an ovary can function in an ectopic site led Morris in 1895 to graft an ovary below the obstruction; however, this also was unsuccessful. Then, in 1909, Estes proposed inserting the ovary into the uterus while retaining its pedicle, leaving the blood vessels and nerves intact. This operation became known as the Estes operation, which was used occasionally until the introduction of IVF-ET, despite it being frequently unsuccessful.

The Scientific Underpinning of Clinical IVF-ET

One of the first significant discoveries in the history of IVF-ET was the embryo transfer work of Walter Heape who, in 1891, reported the successful transfer of an embryo from one rabbit to another (Heape, 1891). In this experiment, he was testing the hypothesis that phenotypic characteristics of the surrogate mother could be transmitted to the transferred embryo. Although there is no evidence that Heape thought of using this route to overcome infertility due to blocked Fallopian tubes, his work is, nevertheless, important since he demonstrated the feasibility of embryo transfer in mammals (reviewed by Biggers and Kountz, 2016).

Heape was a gentleman scientist who worked in the newly created Laboratory of Animal Morphology, University of Cambridge, under Professor Francis Balfour. He never registered as a student nor did he earn a degree. Nevertheless, he went on to become an Instructor in the Laboratory teaching embryology, which included a practical class that taught students to recover living preimplantation embryos from rabbits for observation under the microscope. The manual for this class can be found as an appendix to the second edition of *The Elements of Embryology* by Foster and Balfour (1883) [Edited by Sedgewick and Heape]. It required little for Heape to apply these techniques for the transfer of one rabbit embryo to a foster mother and obtain newborn rabbits. There is strong circumstantial evidence that this landmark work was done in Heape's private laboratory on his father's estate near Manchester in England (Biggers, 1991). Whether or not a coincidence, Manchester was where Steptoe and Edwards (1978) succeeded in producing the first IVF baby.

Perhaps Heape's more important contribution, however, concerned systematizing the description of reproductive cycles. His first studies in this area, done during his tenure of the prestigious Balfour Studentship in Cambridge, UK, involved describing the menstrual cycle in two species of monkey based on histological changes in the uterus (Heape, 1894, 1896). This work soon attracted the interest of gynecologists and resulted in invitations to speak at the Obstetric Society of London (Heape, 1898). Moreover, Johnstone

in the USA, reproduced some of his drawings in the American Journal of Obstetrics and Diseases of Women and Children (Johnstone, 1895). Heape then turned his attention to the description of mammalian reproductive cycles in general, in which he introduced such terms as oestrus, proestrous, diestrous, etc. (Heape, 1900). This work provided the basis for Marshall to demonstrate that reproductive cycles in dogs, sheep and ferrets are all under endocrine control which, in turn, paved the way for the discovery of the reproductive hormones (review: Marshall, 1910).

Heape's work on reproductive cycles had an immediate and lasting influence on our understanding of the endocrinology of reproductive cycles. By contrast, his work on the transfer of embryos between rabbits received little attention during the next 30 years, except in the field of science fiction.

Between 1930 and 1937, Gregory Pincus, an Assistant Professor in the Department of Biology at Harvard University, published several important papers, some with E. V. Enzmann, on the physiology of fertilization and preimplantation development in the rabbit. One of these papers reported successful IVF-ET in rabbits (Pincus and Enzmann, 1934) and another described meiotic maturation of rabbit oocytes in vitro (Pincus and Enzmann, 1935). The following year, results on the activation of rabbit ova were reported, and some of these parthenotes were documented to have undergone cleavage divisions (Pincus and Enzmann, 1936). Pincus (1936) summarized his work in a monograph entitled *The Eggs of Mammals* containing three chapters: "Methods employed in the experimental manipulation of mammalian ova," "Fertilization and cleavage," and "The activation of unfertilized eggs."

Pincus's work immediately encountered controversy in the press and the general public. W. L. Lawrence wrote a conjectural op-ed in the *New York Times* on March 27, 1936:

> As rabbits and men belong to the mammalian group, the work is viewed as pointing towards the possibility of human children being brought into the world by a 'host-mother' not related by blood to the child.
>
> It is reasoned that eventually women capable of having children whose health does not permit them to do so may 'hire' other women to bear their children for them, children actually their own flesh and blood.
>
> To one who desires to speculate at this point the Harvard experiment offers another possibility. Theoretically, at least, it may become possible for a woman so inclined, particularly in a country influenced by eugenic considerations, to bring into the world twelve children a year by 'hiring' twelve 'host-mothers' to bear their test-tube-conceived children for them.
>
> Advocates of 'race-betterment' might urge such procedures for men and women of special aptitudes, physical, mental or spiritual.

The following day the *New York Times* published an emotional negative editorial under the title Brave New World. The next year, J. D. Radcliff, writing in the widely circulated *Collier's Magazine*, March 20, 1937, in an article "No father to guide them," commented:

> In the resulting world man's value would shrink. It is conceivable that the process would not even produce males. The mythical land of the Amazons would then come to life. A world where woman would be self-sufficient; man's value precisely zero.

In 1937, Pincus took a sabbatical leave at the University of Cambridge, UK, with the knowledge that his academic appointment at Harvard would not be renewed. Despite the scientific recognition of Pincus's work some believe that one of the reasons he went to Cambridge was that the Harvard administration felt its reputation was being tainted by the press (Speroff, 2009). Pincus and Enzmann's claim that they had successfully achieved IVF-ET in rabbits was accepted by the scientific community for several years until the work was challenged by later work on fertilization done in the 1950s, particularly with regard to capacitation.

While Pincus was in Cambridge, UK, Professor John Rock and his colleagues at the Boston Lying-In Hospital, an affiliate of Harvard Medical School, published a paper in the *New England Journal of Medicine* where they claimed to be able to detect an electrical sign that determined the time ovulation occurred in women (Rock et al., 1937). In the same issue, John Rock also wrote anonymously the following remarkable editorial:

> Contemplating this new discovery, one's mind travels much further. Lewis and Hartman have fertilized a monkey ovum and photographed its early cleavage *in vitro*. Pincus and Enzmann

> have started one step earlier with the rabbit isolating an ovum, fertilizing it in a watch glass, and reimplanting it in a doe other than the one that furnished the egg, and have thus successfully inaugurated pregnancy in an unmated animal. If such an accomplishment with rabbits were to be duplicated in human beings, we should, in the words of 'flaming youth', be 'going places'. The difficulty with human ova has been that those recovered from tubes have regressed beyond the possibility of fertilization *in vitro*. But by using the electric sign we may be able to obtain them from the follicle at the peak of their maturity. If the new peritoneoscope can be developed along the lines of the operating cystoscope, laparotomy may even be dispensed with. What a boon for the barren woman with closed tubes.

He wrote the above editorial anonymously, perhaps to avoid the problems that Pincus had encountered with the Harvard Administration.

Rock, with the assistance of Miriam Menkin and the advice of Pincus, went on to establish a research program to develop a method for fertilizing human ova in vitro (*Science News Letter*, 1944). They claimed to have succeeded in a paper published in *Science* in 1944 (Rock and Menkin, 1944; see also Menkin and Rock, 1948). Although the United States was fighting World War II, the potential public health importance of this work was recognized by the *Boston Globe*, which published a front page article in which belief was expressed that the work would contribute to treating serious problems of infertility.

Several of Rock's medical colleagues at Harvard hailed his work for opening up a way to treat intractable forms of human infertility (Marsh and Ronner, 2008). However, the work was not free of criticism: The President of Harvard received a letter from a Missouri woman telling him "when you interfere with the laws of Nature, you interfere with the laws of God, and when you interfere with the laws of God, you insult the intelligence of Christian people" (preserved in the Harvard Medical School Archives, quoted by Marsh and Ronner, 2008).

Despite the acclaims given to Rock's work, the independent discovery of capacitation by Austin (1951) and Chang (1951) raised questions about the criteria required for unequivocal proof that fertilization in vitro had been achieved. In 1962, Austin, after reviewing about 30 claims of IVF in various mammals (including the rabbit, guinea-pig, sheep and human), listed the following four required criteria: (1) use of capacitated spermatozoa, (2) avoidance of aged ova, (3) confirmation that a spermatozoon had entered the ovum, and (4) conditions that exclude parthenogenesis. None of the claims fulfilled Austin's criteria. The ultimate convincing proof of successful IVF-ET is the birth of young whose origin can be identified by phenotypic by or genotypic characteristics, preferably the latter. Eight years elapsed before Chang (1959) reported that he had achieved fertilization in vitro in rabbits, having met Austin's four criteria. However, it was Whittingham who unequivocally demonstrated the in-vitro fertilization of mouse ova using a genetic marker (eye color) to identify native and in-vitro produced offspring (Whittingham, 1968).

The independent discovery of capacitation by Austin and Chang raised doubts that fertilization in vitro had been attained by Pincus in the rabbit and Menkin in the human. A letter, preserved in the Harvard Medical School Archives, was written on June 6, 1954, to John Rock by Carl Hartman, one of the doyens of mammalian reproduction. It reads:

> I don't believe you ever got *in vitro* fertilization. . . Have a dozen reasons to question your conclusions, chief of which is the simultaneous and independent discovery by Chang, Austin and Blandau [Braden?] that 'raw' sperms won't fertilize any egg even *in vivo*! Sperms must be 'capacitated'(Austin) in the female tract, either in the uterus or the tube.
>
> Now, I want you to go back to the problem and clean it up and really immortalize yourself, inject 50,000,000 sperm into a woman's uterus, in 2 h take out the sperms and add to the ovarian egg (but only from a 16-18 mm follicle, eggs in lesser ones are N.G.). I'm betting heavy odds on the outcome of the experiment.

Neither Rock nor Menkin took up Hartman's suggestion. They discontinued their studies after 1948. Rock may have been discouraged by the low success rate of Menkin's experiments and by the objections of antagonists at Harvard (McLaughlin, 1982). He and Pincus left this area of research to begin their major contributions to the development of the oral contraceptive pill. Menkin moved to another institution where she had no possibility of carrying the work further.

John Rock's ideas were not completely abandoned, for others tried to repeat his experiments. The best

known clinician was Landrum Shettles, at Columbia University in New York City, who, in 1954, claimed to have successfully fertilized human ova in vitro. Further results were reported in 1958 at a meeting of the New York Obstetrical Society, which was attended by John Rock who was excited by the work. Rock commented:

> The time may be rapidly approaching when the poor woman whose tubes had been excised, yet who still wants a baby, will rejoice that Dr Shettles will be able to extract an ovum from her ovary, probably not by laparotomy, but through an operating telescope (which can be done – we have done it); then fertilize the egg *in vitro* by the husband's spermatozoa; and finally put it back in the uterus. Thus will he impregnate the woman in spite of the fact she has no tubes.

In the years that followed, Shettles' work was sharply criticized and caused controversy.

The 1950s and 1960s saw major advances in the field of developmental reproductive biology as investigations focused on identifying the conditions needed to achieve in-vitro fertilization. In addition to discovery of capacitation (Austin, 1961; Chang, 1951), and identification of the evidence needed to prove fertilization in vitro (Austin, 1962) as discussed above, the following key milestones were achieved: (1) the demonstration that mouse preimplantation embryos could develop in chemically defined media (Whitten, 1956, 1957); (2) the demonstration that cultured mouse preimplantation embryos would develop in surrogate mothers, using differences in coat color to distinguish in the young native and cultured offspring (McLaren and Biggers, 1958); and (3) the demonstration that the maturation of mouse oocytes and the early cleavage of mouse preimplantation embryos in vitro required pyruvate in the medium (Biggers et al., 1967).

Between 1964 and 1971, Sir Robert Edwards and his colleagues published the following four key papers, two in *Nature* and two in *The Lancet*, that paved the way to successful in-vitro fertilization in women.

Key Paper (1) Maturation In Vitro of Human Ovarian Oocytes. *The Lancet* (Edwards, 1965)

Edwards' initial work on the meiotic maturation of human oocytes in vitro used the technique for maturing human oocytes, originally described by Pincus who estimated the time for maturation as 12 h. Edwards' work proceeded slowly because human oocytes were hard to obtain in the UK. Fortunately, he received an invitation from Howard Jones who worked at the Woman's Clinic, Johns Hopkins Hospital, to conduct the tests in Baltimore, where human oocytes were easier to obtain. Edwards' main result showed that Pincus and Saunders had underestimated the time for meiotic maturation to occur in vitro in human oocytes and that the required time was 36–43 h. The results were described in the first of the four key papers, which was published from Johns Hopkins Hospital. Unfortunately, attempts to fertilize these matured ova in vitro failed (Edwards, 1966).

Key Paper (2) Fertilization and Cleavage In Vitro of Preovular Human Oocytes. *Nature* (Edwards et al., 1970)

This advance, made by Edwards and his colleagues, was the production of human blastocysts by exposing human ova to human spermatozoa in vitro. At least five media were tested: two modified physiological salines developed for the study of animal fertilization and three that were used in general cell culture. All allowed modest development only if they contained pyruvate. Two of the media were modifications of physiological salines. The first medium to be tested was a modified Tyrodes solution used by Barry Bavister to study capacitation in the hamster (Bavister, 1969). Later, a modified Krebs–Ringer bicarbonate solution was tested by David Whittingham to study in-vitro fertilization in the mouse. The medium used by Whittingham was a medium developed by Whitten and Biggers (1968) for the culture of mouse preimplantation embryos. Both Bavister and Whittingham were working in the same department, the Physiological Laboratory in Cambridge, as was Edwards.

Key Paper (3) Laparoscopic Recovery of Preovulatory Human Oocytes after Priming of Ovaries with Gonadotropins. *The Lancet* (Steptoe and Edwards, 1970)

The notion of an "operating telescope" envisioned by John Rock was shown to be possible in France by Klein and Palmer (1961) who used a cystoscope to recover an oocyte from a single human follicle. Steptoe and Edwards adapted the laparoscope to recover human oocytes from patients hyper-stimulated with

gonadotropins. However, finding optimal doses of the gonadotropins that did not interfere with implantation was difficult, so Edwards and Steptoe reverted to collecting oocytes from a natural cycle without ovarian stimulation; this was the approach they used to achieve the first successful IVF-ET birth.

Key Paper (4) Birth after the Reimplantation of a Human Embryo. *The Lancet* (Steptoe and Edwards, 1978)

The birth of the first in-vitro human baby was achieved after innumerable failures. Indeed, through December 1980, the live birth rate per embryo transfer was only 5.4% (three babies born from 56 embryo transfers; Lopata, 1980). However, this rate was likely considerably lower if cycle start is used as the denominator.

Without doubt, Steptoe and Edwards' achievement of the first IVF-ET success was remarkable and marked the beginning of what was to become a major advance that has resulted in the successful treatment of millions of cases of human infertility. The persistent work done by Edwards and Steptoe over many years is a superb example of translational research.

The Public Backlash to Louise Brown's Birth

The day after the announcement of the birth of the first in-vitro baby, there was worldwide and often frenzied coverage by the media, with commentaries ranging from those heralding a major scientific achievement to those that maintained it was a dangerous or amoral procedure which should be outlawed. This is not surprising since similar comments had been made when artificial insemination was introduced at the end of the twentieth century (Schellen, 1957), and similar reactions were evoked by the experiments of Gregory Pincus and John Rock and Miriam Menkin.

Edwards and Steptoe met praise and resistance to their work in the United Kingdom before and after the birth of Louise Brown. This history has been summarized in a book by Edwards and Steptoe in 1980 entitled *A Matter of Life*, and an article by Edwards written a decade later entitled "A bumpy road to human in vitro fertilization," in which he states that "popes were critical and rigid Protestants were sometimes vicious" (Edwards, 2001). Edwards acknowledges being influenced by a particular friend, Gordon Dunstan, a leading senior ethicist of the Church of England, who wrote a book entitled *The Artifice of Ethics* with four chapters on IVF-ET and a "penetrating and ethical analysis" (Dunstan, 1974). Edwards and Steptoe had other setbacks. For example, the Medical Research Council did not approve an application to support setting up an IVF-ET clinic in Cambridge and they had to seek private funds to set up the famous clinic at Bourn Hall, near Cambridge. Importantly, Edwards and Steptoe made a major contribution to the many public policy debates surrounding IVF-ET in the years that followed that, collectively, resulted in the gradual adoption of IVF-ET as an acceptable medical procedure.

The British Government established a committee in 1982, under the chairmanship of Dame Mary Warnock called The Committee of Inquiry into Human Fertilisation and Embryology. The general conclusion of their Report, published in 1984, recommended that the human embryo should be protected, but that research on human embryos and IVF-ET would be permissible given appropriate safeguards. A regulatory committee was established and eventually the British Parliament passed the Human and Fertilisation Embryology Act (1990), which has led to countless worthwhile studies on human preimplantation development.

The birth of the first test-tube baby had immediate individual reactions in the United States, which can only be illustrated by individual experiences. At the time, one of us (JDB) was Program Director of a grant at Harvard Medical School from the National Institutes of Health (NIH), which had a specific aim to study human oocyte maturation, under the direction of Melvin Taymor at the Peter Bent Brigham Hospital. A day after Louise Brown's birth, JDB received a call from the NIH informing him that the NIH was immediately freezing the funds allotted to the human oocyte maturation work. Shortly thereafter, a conference was held at the NIH in Bethesda, MD under the co-chairmanship of JDB and Luigi Mastroianni on Fertilization and Embryonic Development In Vitro and its proceedings were published the following year, in 1981. The book contains no papers on human in-vitro fertilization because officials at the NIH instructed the co-chairmen to disallow discussion of the subject at the meeting and in the published papers.

President Carter activated on September 15, 1978, a dormant Ethics Advisory Board of the Department of Health, Education and Welfare, to consider whether or not research using human ova and preimplantation embryos should be controlled or forbidden. The

members of the Committee represented comprehensive scientific, medical, legal and philosophical interests and an infertile couple gave testimony of their struggles with infertility. JDB was scientific advisor to this committee and CR also attended these hearings. Among the many topics discussed, in addition to the incidence of infertility in the US population and the likely efficiency of the procedure, was the safety of the procedure particularly regarding the production of abnormal fetuses, and the conflicting views on the morality of IVF-ET. A report was finally produced and it recommended that human embryos used for research could be kept in culture no longer than 14 days and not replaced in a human patient. Further, the ova could only be obtained from married couples. No grants for research on human preimplantation embryos or oocytes, either exposed to sperm or induced to undergo activation, has since been funded by the NIH.

The formation of the first IVF clinic in the USA at the Eastern Virginian Medical School, Norfolk, Virginia, was a particularly turbulent process, as is well documented by the late Dr. Howard W. Jones in his book *In Vitro Comes to America* (2014). Starting a clinic involved getting a Certificate of Need that necessitated a public hearing as required by the State of Virginia. JDB testified at the public hearing, which lasted approximately six hours, where right-to-lifers on one side of the hall shouted insults at infertile couples who shouted back "you want to prevent me having a baby." Some testifiers opposing the Certificate of Need were particularly objectionable and rude to Dr. Jones and his wife, Dr. Georgiana Jones, who were trying to set up the IVF Clinic. A few weeks later JDB was a speaker at a symposium on IVF held by the Virginia Bar Association. The meeting was held at Virginia Beach, home of the Christian Broadcasting Network and Regent University founded by the southern Baptist televangelist Pat Robertson, who at one time ran for the republican nominee for President of the United States. On the day of the symposium, a hostile crowd formed at the entrance to the hotel causing the speakers to be taken into the hotel through the kitchens! Fortunately the Certificate of Need was granted, which allowed the Joneses to begin forming what became the renowned Norfolk Clinic.

Concluding Remarks

As is usually the case when a remarkable medical treatment is established, much precedes the first success. The establishment of clinical IVF-ET is no exception. Mammalian reproduction entails a remarkably complex system of processes involving not only the production of fully developmentally competent gametes, but also a uterus that is receptive to implantation and to maintenance of pregnancy. However, reproduction in the human is unique in that it is remarkably inefficient. Therefore, it is of no surprise that rigorous and exhaustive scientific investigations in a multitude of species were required to fill the necessary knowledge gaps for the first clinical IVF-ET success. Indeed, it took more than eighty years of painstaking research before Louise Brown's birth. However, the remarkable team of Edwards and Steptoe must be given all the credit in the world for their accomplishment and for the joy that they have brought to millions of couples worldwide. This is all the more noteworthy in light of the ethical and religious hurdles that not only needed to be overcome during the decade leading up to their success, but also during subsequent years as research on human reproduction continues to advance.

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