First in vitro fertilization baby—this is how it happened

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On July 25, 1978, the first human was born following extracorporeal fertilization, an event that opened up a new medical science: expanding our knowledge of and developing novel treatments for infertility, radically changing the opportunities for families with inherited monogenic disorders, generating the new discipline of clinical embryology, and paving the way for studies into stem cell biology. In vitro fertilization (IVF), as it became known in its simplest form, went even further: it engaged the myriad of minds in human society. Not only were books written on the moral status of the human embryo, the ethics of IVF practice, and exercising governments on appropriate—which turned out to be disparate—regulation, it redefined family life! The prediction I made in 1985 that one day we may see five "parents" for one child became a reality quicker than we could have imagined at the time. The new medical science marched inexorably on in almost all countries of the world—a universal human plight at last had an opportunity for remedy: more than 10 million couples seeking a resolution to their infertility became parents, men who had no option to have their own genetic child became genetic fathers, and ever-increasing monogenic conditions were not being passed on to the next generation. The future may well bring to bear the opportunity for in vitro–developed viable gametes to generate successful pregnancies, and other "futuristic" opportunities for IVF science. But its story began over a century ago with seeking an understanding on how an egg matures and how to achieve successful fertilization—a fundamental scientific inquiry. It took one man to go beyond that scientific endeavor, to take head on a society unprepared and unwilling to accept human fertilization in vitro and unempathetic to the plight of the infertile; one man to see what prospects lay ahead for humanity should IVF become a reality, and for that man to battle every step of the way for nearly 2 decades to achieve that dream. (Fertil Steril® 2018;■:■–■. ©2018 by American Society for Reproductive Medicine.)

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EX OVO OMNIA

Pincus and Enzmann in 1935 (1), were, as far as I am aware, original in asking whether the first oocyte maturation division is an essential prelude to fertilization; although 30 years earlier Heape (2) observed the presence of polar bodies 9 hours after copulation in rabbits (rabbits only ovulate after copulation). Heape was, according to Pincus and Enzmann, only "partially correct"! Pincus and Enzmann assessed oocyte maturation in the rabbit in detail and accurately concluded on the process of maturation of rabbit oocytes, and especially that the oocyte contained a nucleus before copulation and that 2 hours later the initiation of diakinesis occurred, at another 2 hours the polar spindle is formed with dissolution of the nuclear membrane, and by 8 hours after copulation the first polar body was formed. It was another hour for the second spindle to be formed, and ovulation occurred between 9.5 and 10.5 hours after copulation; but this remained intact with only the single polar body until fertilization took place. These catalogued events of over 80 years ago will resonate with mammalian embryologists today. Although their report was entitled "mammalian," it was by far from certain that the timing was representative of all mammalian species. However, this important paper was not only the first to assess accurately the process of oocyte maturation in a mammal; the authors also correctly concluded that removal of the oocyte from its mature follicle can bring about maturation to the state where such oocytes can be normally fertilized. What they inaccurately assessed was that the period for human oocyte maturation was 12 hours.

Little interest was shown in human oocytes until 1944 and 1948 with the publications of Rock and Menkin (3, 4) and in 1955 with Shettles (5) ostensibly recording fertilization and the subsequent development of human embryos, although Edwards always maintained this work was "almost certainly erroneous" due to no clear evidence of oocyte maturation and the events associated with what we understand about fertilization and early cleavage. Without a record of such events Edwards stated that there was always the risk it was parthenogenetic activation and cleavage. (Although this criticism is tenuous too, because today

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we know that only metaphase-2 oocytes will readily become parthenogenetically activated or fertilize with subsequent cleavage. Indeed, successful fertilization may occur over a wide timeframe. Trounson et al. [6] believed that a delay of 5–6.5 hours before insemination was required for maximizing the incidence of fertilization and pregnancy, which we now know is erroneous—successful fertilization may occur immediately after ovulation or much later without compromising results [7].) It remained for another 10 years following the Shettles paper for the inquiry of Edwards finally to elucidate and document precisely the detailed events of human oocyte maturation following release from the follicle: germinal vesicle breakdown up to 24 hours; diakinesis duration, 25–28 hours; metaphase-1, 36–43 hours; and metaphase-2 and polar body release, 36–43 hours (8). That the elucidation of oocyte maturation of human eggs took so long portrays in part the beginnings of the enormous hurdles Edwards had to overcome finally to achieve his dream.

THE SPIRIT OF DISCOVERY

Robert (Bob) Geoffrey Edwards, born in 1925 in Yorkshire, initially studied agriculture at the University of Bangor, North Wales, from 1948 to 1951 but, disenchanted, eventually switched to zoology. He then pursued a genetics course at Edinburgh University in Professor Waddington's department. Edwards left Edinburgh for a year at the California Institute of Technology having now an interest in immunology and attracted by the views of Professor Albert Tyler, who likened the "interaction between sperm and oocytes to the reaction between antigen and antibody." He returned to England in 1958 on a 5-year contract to work with Professor Alan Parkes intending to work on immunology and contraception at the National Institute for Medical Research at Mill Hill, where he also met the eminent Australian veterinary scientist and reproductive physiologist Colin "Bunny" Austin, who was a specialist on mammalian fertilization, and codiscovered "capacitation" in 1951. At this time Edwards oscillated between work on immunology of reproduction and a growing interest in embryology, particularly new work being published on human chromosomes and the recent evidence that the common compliment for humans was 46. It was in 1960 that Bob Edwards began his passion and quest for understanding the ripening of human oocytes, their fertilization, and the prospect of replacing an embryo into the woman. He first had to step back to mice to repeat the published work of others to map oocyte maturation in vitro. Previously, as mentioned above, Pincus had worked with human oocytes recovered from extracted ovaries. What was astonishing was that Pincus being first to elucidate this in rabbit and (although with flawed timing) man; nothing further had been published in the intervening 25 years!

During Edwards 5-year tenure at Mill Hill he spent time trying to persuade gynecologists to provide him with human ovarian tissue. It was Dr. Molly Rose working at the Edgware General Hospital that became his first significant collaborator in this. But all that happened in these early studies was that Edwards could not repeat Pincus's work on human oocytes. This gave Bob cause to pause and time for thought. While at the institute he obtained rhesus monkey ovarian tissue to see whether Pincus had just got the timing wrong—on the premise that primate oocyte maturation had a very different program to the one reported for rabbit. His first and apparently only attempt with a few rhesus oocytes demonstrated no germinal vesicle breakdown by 15 hours, but potentially it was happening at 18 hours—this he observed on the one remaining oocyte he studied! Molly Rose obtained some more human ovarian tissue for Bob, and from the few observations on the rhesus oocytes he decided to leave these new human oocytes much longer. The first evidence of the timing of human oocyte maturation occurred at that time—but regrettably with no record of the discovery (and that's another story!).

In 1963 Edwards moved to the Physiology Laboratory, Cambridge, rejoining Alan Parkes who in 1961 was the newly appointed Marshall professor. Bob had to make new contacts with gynecologists at the Cambridge Addenbrookes hospital to continue his work on human oocytes, but the tissue was slow in coming, and he reverted to working more on bovine, sheep, and occasionally monkey (which Bob included in his paper in *Nature* on October 23, 1965 [9]). Eventually, in 1965 Edwards had three ripening human oocytes and used his sperm in an attempt to fertilize them. At the time the conventional wisdom was that sperm need to "capacitate" in the reproductive tract before fertilization could take place. Edwards never achieved fertilization, only "one spermatozoon passed through the outer membrane of an oocyte." Perhaps conventional wisdom was right? (eventually, we knew it wasn't!).

Needing more inspiration Bob (at the advice of his wife Ruth) contacted Vicktor McKusick, a geneticist working on inherited disorders at Johns Hopkins, who suggested that Edwards takes a few weeks to work with the husband and wife gynecologist team Professors Georgeanna and Howard Jones at the Johns Hopkins hospital. Edwards secured a grant from the Ford Foundation and in July 1965 headed to the United States for 6 weeks. Obtaining ovarian tissue from the Joneses, Bob had enough human oocytes to confirm that maturation was 36 hours. However, fertilization of the oocytes still eluded Bob, even after collecting sperm from the cervix of several of Howard Jones' patients after sexual intercourse.

Although the data from that trip, eventually published in the *Lancet* in 1965 (8), seem indispensable to those of us in reproductive biology, the *Lancet*'s response to Edwards was that they "could not see the point of the work"!

IN SEARCH OF HUMAN FERTILIZATION IN VITRO

A further 2 years on, toward the end of 1967, and as yet unable to achieve fertilization of matured eggs, Edwards learned of laparoscopy and how this may make it easier to get the material he needed. He contacted Patrick Steptoe, who during the early 1960s was pioneering laparoscopy in the United Kingdom, having learned from the father of gynecological laparoscopy, the Parisian Raoul Palmer. During that time John Webster had joined Steptoe, in 1963—a pivotal appointment for what lay ahead! The progress of Palmer's work was made possible by the development of instrumentation by Hans Frangenheim of Koln, particularly an improved

insufflator to facilitate the installation of CO_2 into the abdomen. Steptoe also visited Frangenheim for training. Back in the UK, Steptoe met with considerable establishment opposition for the acceptance of laparoscopy.

Edwards and Steptoe actually met for the first time in 1968 at a Royal Society of Medicine meeting. Following their meeting, Steptoe agreed to try to find room at his hospital in Oldham (near Manchester) as Edwards would have to come to where the patients were. Eventually, in April 1968, following a small room being given for Steptoe's use at the Oldham General Hospital, they started a small research program to extract mature human oocytes and attempt to fertilize them. Edwards would have to travel to Oldham from Cambridge to recover oocytes from the ovarian tissue that Steptoe extracted. Edwards had to transport basic equipment, microscopes, culture fluid, and so on, for this next phase in his endeavors to achieve fertilization. He was helped by his assistant at the time, Clare Jackson.

Another problem that needed resolving was the funding of this work, and all the traveling to and from Oldham. Although the clinical costs were apparently borne by the Oldham and District General Hospital, it was both the Ford Foundation and philanthropic funding that allowed Edwards to continue his work. The 1968 Ford Foundation Endowment of $240,000 created the Ford Foundation Reader (associate professor) in Physiology that funded Edwards' salary, overheads, and most likely the salary of Jean Purdy, who was appointed as Bob's assistant when Clare Jackson left. This endowment was achieved by Bunny Austin through the support of the then Vice Chancellor of Cambridge University, Professor Matthews. Bunny Austin had also moved to the Physiology Department by then as the University of Cambridge had created a special Charles Darwin Chair for him in 1967. Bunny—a charming and engaging man—was a constant supporter for Bob's work. Ford made a further endowment in 1988, the Ford Foundation Professorship in Human Reproduction, to which Edwards was appointed. Effectively Ford funded Bob's salary for 26 years in addition to funding a series of grants. Of great significance was the benefactor Miss Lillian Lincoln Howell (1921–2014), a pioneer of early American television. Her contribution, allegedly because "herself having suffered problems similar to those patients now being treated" (10), of approximately $100,000 between 1968 and 1978 was vital. Without this it is uncertain whether all the trips to and from Oldham, and the funding of equipment and culture media and so on, could have been achieved.

During 1968 Edwards' Ph.D. student Barry Bavister was using new culture media to achieve good rates of success fertilizing hamster eggs. This media was bicarbonate based and contained among other things bovine serum albumin and penicillin. Edwards wanted to use Bavister's culture system. Edwards did not succeed with the medium in Oldham and began to bring the eggs back to the Cambridge lab, keeping them in a container strapped to his body!

Ironically, it was while back in Cambridge that a phone call from Molly Rose in Edgware in March 1968 provided the decisive tissue from one of her oophorectomies—the last tissue Bob was to obtain from Edgware General Hospital. This resulted in 12 oocytes, and working with Barry Bavister, the eggs matured through the now established timely events. Using their own sperm, nine of the eggs were inseminated, with the remainder left as controls. Not only did they achieve fertilization with Bavister's culture media, this result determined that it was no longer necessary to recover sperm from a patient's fallopian tube/reproductive tract. Edwards then repeated this work in the coming months in Oldham, with Steptoe providing ovarian tissue.

In 1969 Edwards, Bavister, and Steptoe published in *Nature* (11) their early work on oocytes recovered from ovarian tissue. It was a relatively low-key, technical paper, but it was enough, the media interest was sparked—"Life in a Test Tube" hit the press (and as we say today—went viral!), and it was accompanied by the beginnings of vociferous objections from all walks of life!

From 1969, and the successful, repeatable fertilization of human eggs, the work picked up pace, with Bob Edwards assisted then by Jean Purdy. They began making more frequent visits to Oldham. Steptoe and Edwards started to work on oocytes aspirated from follicles following ovarian stimulation and hCG. The important next stage was to observe cleavage and then, hopefully, development to blastocyst. By the end of 1970, growth to the blastocyst in culture had been achieved. (Looking through the "retroscope," once this had been achieved one would only assume that by harvesting the oocyte from a follicle where it was matured in vivo a successful pregnancy could not be far off!)

FROM SCIENCE TO MEDICINE

The year 1971 was a major year—Edwards and Steptoe began their clinical work, for which they moved to Dr. Kershaw's Cottage Hospital, Royton, Oldham. As Edwards had to keep traveling to Oldham, they were hoping to move to the Newmarket General Hospital, which was closer to Cambridge. This move relied on anticipated support from the Medical Research Council to fund the program, but in April, Edwards received a crushing letter, which stated, "in considering your application made jointly with Mr P C Steptoe for long-term support for a programme of research in the field of human reproduction … I am sorry to have to tell you … the Council … could not agree to your request for long-term support since they had serious doubts about ethical aspects of the proposed investigations in humans." They did, however, go on to say they would consider the type of work proposed if they were to reapply for it to be done on primates! No amount of further attempts by Edwards and Steptoe in their response to the Medical Research Council could change their mind—they were resolute in their rejection. A blow, yes, but a block to progress? Not for Bob and Patrick!

Between 1971 and 1972 Steptoe and Edwards undertook their first ETs, using ovarian stimulants and hCG, but it was not until 1975, some 150 laparoscopic oocyte recoveries (LORs) later, that the first pregnancy resulted, which was an ectopic (and questionably from IVF as there remained several uncollected oocytes). I personally heard about this from Bob when asking about his work, having recently joined him as a Ph.D. student at the Marshall lab to work on fertilization, embryo cleavage, and implantation. With

no success to date, during 1976–77 Edwards and Steptoe tried bromocriptine with ovarian stimulation and hCG mix, still to no avail.

BACK TO NATURE

A step change occurred in 1977 when they considered using no ovarian stimulation—to watch rather than control spontaneous ovulation. This was made possible by a suggestion from a London colleague to try a new assay, called HI-Gonavis, to measure urinary LH in naturally cycling women. A paper was published by Völker in 1978 comparing this to serum radioimmunoassay of LH (12). The Japanese company Mochida Pharmaceuticals marketed this hemagglutination assay kit, based on the use of erythrocytes sensitized against anti-hCG antibody. It required the women to produce regular urine samples (later defined as 8/24 hours, 6 during the day and 2 at night) and took 2 hours to produce a result. Titrated against the sample, the multiwell dish would produce an ever-increasing intensity of precipitation depending on the concentration of LH in urine. By observing the density of the rings of precipitate, the start of the LH could be pinpointed. Due to the presumed clearance rate, laparoscopy was performed 24–26 hours after the start of the LH surge. This proved a turning point, and the method was used in the early years post-1978.

By the time the switch to natural cycle and monitoring of LH occurred, over 300 LORs had been performed without success. On November 10, 1977, Lesley Brown underwent LOR 26 hours after the LH surge was detected in her urine. The left ovary contained a single follicle—recorded by Steptoe as ~3 cm but "difficult to access due to adhesions." The follicle was aspirated and the fluid carried by a nurse to Jean Purdy in the lab, who then passed the tube on to Bob Edwards, who, sitting at the microscope under the laminar flow hood (class I in those days—also known as horizontal flow clean workbench), identified the cumulus mass containing the egg in the follicular aspirate. Bob was very keen for the embryo to reach the 8-cell stage before transfer, but to proceed as soon as possible thereafter. Following the embryo's development, the time for transfer of Lesley and John Brown's embryo came at midnight on the November 12, 1977. A late-night transfer of an 8-cell embryo, conceived in the natural cycle that resulted in the first successful IVF delivery made an impact on how Edwards and Steptoe would practice IVF going forward!

On the July 25, 1978, Louise Brown was delivered at 23:47 by Patrick Steptoe assisted by John Webster. The world's first IVF baby was conceived and delivered in Oldham, near Manchester, within the National Health Service system. A further group of patients continued to be treated from November 1977 and into the summer of 1978. A second live birth for the team, Allastair MacDonald, was delivered January 14, 1979, in Stobill Hospital, Glasgow. The tally of work from January 9, 1969, to August 1, 1978, was 250 patients, 457 cycles, 112 ETs, five clinical pregnancies, and two live births. Should this be measured against contemporary success rates it represents a 1.8% live birth/ET, 0.44%/cycle started, 0.8%/patient.

It is worth a brief pause and thought on how such endeavors could ever be considered today. Could medical scientists be permitted to continue so long with so many failures? How did the resolve of Steptoe and Edwards, with the unfailing support of Jean Purdy, continue in the face not just of failure but with so little support from colleagues and the community? One has to admire not just the courage to continue through persistent failure, but, even in the face of imminent closure of their Oldham work due to Steptoe's impending retirement, the desire to continue never abated—even in the knowledge of continued failure from other groups around the world—and still against a backdrop of widespread criticism. They had no intention of resting on their laurels. Yes, they had achieved the first IVF birth in human history—but now the science of infertility and human embryology had to begin!

UNDERSTANDING A HISTORIC MOMENT

Still today, it is hard for many to understand the animosity that this enormously significant event stirred up, and the welter of vociferous objections, rather than the ability to see the potential good to come of it. Hostility came from the international community, including Nobel laureates, and none more significant than James Watson, stating it was akin to "infanticide"; many accused Edwards of creating a world of defective babies. And closer to home, Fellows of the Royal Society and even the likes of Professor Anne McLaren publicly stated that she "feared Dr Edwards will go too far too fast"—but surely, not far or fast enough for those suffering from infertility! Even once the first births were truly established, none other than Robert Winston, then a less well-known tubal surgeon at London's Hammersmith Hospital, was pontificating vehemently against IVF. And even closer to home still, among his very own students, as Martin Johnson once put it, "Both Richard Gardner [later Professor Sir Richard Gardner, embryologist and geneticist] and I were very unsure about whether what Bob was doing was appropriate and we didn't want to get too involved in it."

I recall when Bob asked me to join him at Bourn Hall once it finally opened, a very senior member of the Physiology Department, where I had worked under Bob since 1975, stopped me on the steps of the department's third floor as I was on my way up to Bob's office and said, "Fishel, I've been wanting to have a word—don't throw away a potentially brilliant research career to work with the devil; between you and me I'm not sure he'll be at the university much longer!" As a young, newly appointed postdoc, this certainly gave me cause for thought and to slow down my step—but not for long! I knew this was what I wanted to do, and believed myself, as did Bob, what was doable and successful in other species should also be so for humans. And with so much unknown and to be learned, then what better way than to begin to learn! I remember thinking at the time that the Medical Research Council spent vast sums of money giving grants to scientists working on mice—was there ever any indication that what we learned on mice could be extrapolated back to humans? Even though I shared an office in the Marshall Lab with Ruth Fowler (Bob's wife) for 5 years, it was not until

much later did I appreciate that she held the same views when fully supporting her husband in his work. But if one lives long enough, one sees the oscillations of life's influences and how the world changes; both Martin and Richard eventually became involved more I expect than they could ever have predicted in those early days. While Richard and his wife, as publicly stated, were beneficiaries of IVF, Martin, tragically due to Edwards' ill health, delivered an oration on behalf of Bob in acceptance, as the sole recipient, of the 2010 Nobel Prize for Physiology and Medicine. And how consoling it is today to see the notable plaque on the wall of the very department where antagonism and distrust pervaded the corridors: "In this building Bob Edwards succeeded in fertilizing a human egg in vitro. This work revolutionised treatments for infertility and laid the foundations for human stem cell research." Eventually, I was to get a taste of the animosity directed toward Bob over the years, for when my 1982/3 work on embryo secretion of hCG was published in *Science* (13) Bob and I received a writ for murder!

UNCERTAIN FUTURE

Patrick Steptoe retired from the NHS in September 1978, and his work with Edwards ceased at Oldham. No more IVF occurred in the UK until 1980. So, after nearly a decade of hard clinical slog, only right at the end did the success arrive. The mind boggles at the timing: just a few more months of failure and it is highly probable Steptoe and Edwards would never have continued after Patrick's retirement. However, the closure of the work at Oldham provided opportunity, the chance for Bob and Jean to work with Patrick closer to home! They eventually succeeded in acquiring Bourn Hall, a grade 11 listed Heritage building with a history dating back to the site of a wooden castle burned down during the Peasants' Revolt in the 14th century. A timber-framed house was built there early in the 16th century and was acquired by the Hagar family and enlarged in 1602. Bourn itself, a small civil parish some 8 miles from Cambridge, was a little closer than Oldham!

Acquiring Bourn Hall was no different from much else that Bob and Patrick dreamed—it was not straightforward! Associated Newspapers were to acquire Bourn Hall. At dinner one evening at Churchill College late in 1979 Bob said to me, "Simon, I'm not unsure if the Bourn project will go ahead." Associated Newspapers withdrew their interest over potential consequences should any child be born with an abnormality. Eventually, thanks to Bob and Patrick being introduced to Alan Dexter, an independent health care management consultant, and his work with Industrial and Commercial Finance Corporation (now the venture capital firm 3i), they were able to acquire Bourn Hall.

FROM MAKING HISTORY TO PROVING A NEW MEDICAL DISCIPLINE

Early in 1980 Steptoe, needing a second in command for the clinical work and sought the support of John Webster who agreed to move to Bourn from where he lived near Oldham in October 1980; I confirmed with Bob that I would also join the team to work with him. In late 1980 the opening of Bourn Hall as the world's first private IVF clinic enabled the continuation of the work of Bob, Patrick, and Jean, with a team of nurses, admin, catering, hormone lab, and maintenance staff. The medical work was undertaken by Patrick Steptoe, Medical Director, and John Webster, Deputy Medical Director; and the clinical embryology work by Bob Edwards, Scientific Director, and myself as Deputy Scientific Director, with Jean Purdy having several functions as the Laboratory Assistant but also helping with patient coordination in those early days. Early practice continued with the natural cycle, marshalling the teams for LOR at any time of the day and night. From our early work, 70% of women began their surge by 09:00 (LOR being 26 hours later) and 15% after 16:00. Some of the variation depended on where in the world the women came from and how long they had been in the UK before we started taking measurements—the relationship between the pineal and hypothalamus! Tonic and surge levels of LH were evaluated in over 400 spontaneous cycling patients and hundreds of Clomid and Clomid and Pergonal cycles using spontaneous ovulation detection. The prediction of ovulation was highly accurate—this is truly where natural cycle and "mild" IVF was cemented in practice (14). ETs commenced only at night, and Patrick insisted on the knee-chest position—there was much still to evolve! (15).

Jean Purdy's role during all the previous years and during those early months at Bourn, or indeed in the previous decade, cannot be overlooked or understated. Jeannie (as she was affectionately known) ensured quality control in the IVF lab (probably the world's first IVF quality manager!), which, similar to the wards and theater, were all constructed in Portacabins—the main building being used for patient admin, dining services, and consulting. Culture media, very much Jeannie's department following instruction from Bob in the earlier years, had changed over time from modified Tyrode solution with 15% human serum, Ham's F10, with various modifications and 15% human serum, Hoppe and Pitts medium, Earle's with 7.5% human serum, and culture media plus serum and follicular fluid, to the early days at Bourn Hall using Earle's balanced salt solution plus 8% maternal serum for fertilization and 15% for cleavage. We also tried (among many investigations) the use of 25% and 50%–100% serum for ET. All media was prepared very carefully from 10× concentrate excluding bicarbonate powder, which was added last. Water was provided as purchased Analar (British Drug Houses) water, although we also had our own double distilled system—especially for washing glassware, particularly as Cambridgeshire had hard water. In Oldham where the water was soft Jean Purdy used tap water.

For LOR Steptoe and Edwards also had to design the best aspiration system. This consisted of a simple aspirator and collecting chamber, described in detail by Edwards and Steptoe (16). Oocytes were first collected by the follicular aspirates being collected into petri dishes. Where possible the cumulus mass was placed in 100% follicular fluid then washed through. Where follicular fluid was not available, the cumulus masses were washed thoroughly through heparinized culture media; removal of traces of blood was important (see below). The cumulus mass was transferred to a small (5 mL) plastic tube with a plastic cap. Every time the cap was removed, a

gas mix of 5% CO_2, 5% O_2, and 90% N_2 was bubbled into the tube until the added phenol red turned pale to indicate the appropriate Ph, and the cap snapped closed. The manipulation of the oocyte from these tubes, their handling for pronuclear assessment, and retransfer to a petri dish was a tricky operation. The tube (and later petri dishes) were placed inside a glass desiccator, which was flooded with the same gas mix—this was added protection should the cap on the tube become loose. The rim of the desiccator—separating the top from the base—plus the top release valve had to be greased to ensure they were hermetically sealed. The glass desiccator was placed in a warming cabinet to maintain 37°C, and the desiccator valve had to be bled over the next couple of hours to ensure the lid did not become displaced under rising pressure. This was particularly important when using petri dishes; should the lid pop away from its seal for a fraction of a second the gas mix would dissipate and the culture medium rapidly become alkaline—hardly close to the incubators of today, let alone time-lapse systems!

The tubes were held in a small dish at an angle, and hence the cumulus mass invariably settled adherent to the side, approximately 2 cm from the bottom. A blunt ended, sterile pipette and a normal sterile aspiration pipette were prepared and laid beside the microscope. The tube was gently rotated under microscopic visualization, and, as the tubes were not designed for this, the view of the cumulus mass was most often blurred, and the dense layer of corona radiata (CR) cells was sometimes difficult to locate. Once located it was important to work quickly. The cap was removed with the thumb of the holding hand, the blunt pipette was collected, and, all the while maintaining visual contact with the CR, the pipette was carefully inserted through the neck of the tube and followed down to the edge of the CR. It is at this point that any blood-stained cumulus mass would cause havoc as the cumulus would contract around the pipette and retract from the edge in a mass of coagulated cells. (When this happened, our recovery contingency was another story!) Hopefully avoiding this, the CR was nudged free from the cumulus and then followed, gently rotating the tube, as it gradually descended to the base. Keeping an eye on the settled CR, the aspirating pipette was loaded into the tube and the CR gently aspirated. Working quickly, especially in those days before culture drops were overlaid with paraffin oil to minimize increasing pH (which I introduced once we started using Clomid), the CR was transferred to a dish of culture medium—sometimes the same medium was tipped into a dish from the tube (we did not have a heated stage so modulations in osmolarity were less of an issue). Again, working rapidly but very carefully, two 27-gauge injection needles were used gently to peel away the CR to visualize the oocyte and, hopefully, the pronuclei. It was one of those procedures where you had to remind yourself not to hold your breath! I likened it to trying to remove the skin of an orange with two axes without damaging the pith! Once visualized, the zygote was quickly returned to culture conditions. Thank goodness, we only worked with the natural cycle—having the occasional twin follicles and cumulus masses was hard enough; imagine using tubes with today's stimulation protocols!

For so many years, and through the ups and downs, Jeannie was key to the continuation of the work of Edwards and Steptoe. Her role has been evaluated and published previously (17). I first met Jeannie in 1975 in the lab next to Bob's first, small office on the top floor of the Physiology Department, as it was then. I saw her at the long right-angled bench organizing papers—many dozens of them—for Bob's publications (and eventually his enormous, in size and stature, text book: *Conception in the Human Female* [18]). Jeannie was never involved in the practice of embryology, or what we now know as clinical embryology, during the time I knew her. We were regularly together in the IVF lab at Bourn, the two of us working on cases as Bob traveled. And, on several occasions asking her if she'd like to swap roles, I'd pass the tubes, or hand her the sperm solution, and she would say "I don't handle the gametes or embryos, never did; that's for Bob and now you." Bob and Jean were never in any doubt about the significance of those embryos!

But she was more than capable of identifying the cleavage stages of course; this she had been doing during those formative years of the 1970s. And what she did do was invaluable: apart from the culture media, she ordered supplies and did the quality control, recorded data, and passed on her knowledge to and trained her next lab assistant, Helen Izzard, as well as being a constant support.

Although we stuck with using the word "embryo" in our communication and publications, as had been used by embryologists for nearly 100 years, during the early years of IVF some will recall the sensitive debate about what the embryos should appropriately be called—"preembryo," or "conceptus" rather than what was deemed the much more emotive term "embryo"; the United States adopted "conceptus"!

WHY SO MANY FAILURES?

A penultimate thought—and one that struck me in 1981. Given what we eventually learned about doing human IVF, it is somewhat astounding that with the attempts made, in different labs around the world, on the back of what was being achieved in animal embryology, why did it take so long to achieve success? In the early1980s when we moved on from the natural cycle to improve success rates I remarked that we can stimulate the female, perform surgery under anesthesia to recover oocytes—undoubtedly inducing significant physiological stress on an already psychologically stressed patient—recover a preovulatory oocyte, bring about its fertilization and cleavage (evidently in suboptimal culture conditions), and transfer the embryo to the uterus within that same woman's cycle (and deploying a tenaculum at ET!)—and achieve a viable pregnancy; in which other mammalian species could we do that? The fact was, we didn't; almost all mammalian work was done using donors and recipients, yet here we were getting a reasonable live-birth rate in humans—why did success take so long? (A good exam question for a student of our field I suspect!)

A final word should be given to the parallel work elsewhere in the world; but for various circumstances one can never be sure of whether the historic event on July 25, 1978, would indeed have been the world's first IVF birth. It

is documented that during the early to mid-1970s other attempts were being made in the United States, India, and Australia. However, I wish to conclude on …

THE STRANGE CASE (AND TIMING) OF MR. JOHN AND MRS. DORIS DEL ZIO

In 1973 Dr. Landrum B. Shettles, a Mississippi native who had published some of the first photographs of human oocytes and embryos, and who in 1954 received the Markle Prize from Columbia University, was trying to achieve the world's first test tube baby at the Columbia-Presbyterian Hospital in New York City. Indeed, Dr. Shettles had been a visitor to Kershaw's hospital in Oldham.

On September 12, 1973, Shettles placed the sperm and oocytes of Mr. and Mrs. Del Zio into a test tube in the hope of achieving an IVF pregnancy. However, the new chairman of the Obstetrics and Gynecology Department, a Belgium Dr. Vande Wiele, ordered Shettles to dispose of the test tube and its contents! This resulted in an angry conflict between the two men, and Shettles resigned under pressure 1 month later (not to mention the loss of a chance of pregnancy for the Doris and John Del Zio!).

Doctors at Columbia Presbyterian encouraged the Del Zios to sue because they wanted to continue IVF research, and this case effectively resulted in a voluntary moratorium on IVF in the United States. Eventually the case came to trial in July 1978 (*Del Zio v. Columbia Presbyterian Hospital*, U.S. Dist. Lexis 14550 (1978)).

Remarkably Louise Brown was born on the seventh day of the Del-Zio trial, and the judge instructed the jury to disregard that fact! But no longer was IVF deemed "impossible, preposterous, dangerous"—predictions from eminent scientists as mentioned above (James Watson also decried IVF at a 1974 Congressional Hearing using demonizing terminology) and a position relied upon by the defense. The case for the defense, initially, was that IVF was the wild idea of Dr. Shettles and could never come to fruition. The birth of Louise Brown could not have been timelier, and the defense had to quickly resume another tack! Nevertheless, the jury found for the Del-Zios; Vande Wiele, Columbia, and Columbia-Presbyterian had engaged in behavior "utterly intolerable in a civilized community." The jury had awarded $50,000 for Doris Del Zio's damages, and $3 to her husband John!

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