REPRODUCTIVE
BIOLOGY

A clinician's personal view of assisted reproductive technology over 35 years

John L Yovich[1]
PIVET Medical Centre, Perth, Western Australia, and Cairns Fertility Centre, Cairns, Queensland, Australia

Received: 6 September 2011; accepted: 30 November 2011

SUMMARY

This invited presentation is intended to cover clinical developments in the evolution of assisted reproductive technology (ART), a process which was attempted during the 1940's and 50's and culminated in the first fruition in 1978. The first *in vitro* fertilisation (IVF) child ensued following the partnership by a scientist with a focussed ambition (Nobel laureate Robert Edwards) joining with the gynaecologist who introduced laparoscopy to Britain in the late 60's (Patrick Steptoe). My journey commenced in 1976 as a clinician who became immersed in the embryological and endocrinological science, whence most progress in ART emanates, and continued into a medical directorship position from which this personal view is documented. Several clinical advances have been important developments in the understanding and management of sub-fertile patients. However evolution of the various laboratory sciences has been the major key essential to meeting both the immediate as well as the long-term needs for human

[1]Address for correspondence: Pivet Medical Centre, 166-168 Cambridge Street, Leederville WA 6007, Australia; e-mail: jlyovich@pivet.com.au

reproduction. The future requires a much better understanding and control over gametogenesis and a laboratory process which much more closely duplicates intrinsic reproductive physiology, avoiding gamete and embryo exposure to the atmosphere. *Reproductive Biology 2011* ***Suppl. 3****: 31-42.*
Key words: Assisted Reproductive Technologies, IVF history, human embryos, infertility treatments

INTRODUCTION

Recent Nobel Prize winner Robert Edwards was the first to demonstrate fertilisation and successful embryo development in humans when he teamed with gynaecologist Patrick Steptoe in the UK in 1968. Edwards described the first human blastocyst resulting from *in vitro* fertilisation (IVF) in 1970, and the first human offspring, Louise Brown was born in July 1978.

I had travelled to London in 1976 mainly to learn Laparoscopic Surgery which had been introduced to the UK by Patrick Steptoe who, in turn, learnt the techniques and art from Raoul Palmer in Paris. How fortunate for me being in London 1976-80 to work under Professor Ian Craft, himself a leading surgeon in the fields of hysteroscopy and laparoscopy [1] but also interested in the emerging science of extracorporeal fertilization; ECF as it was then known, later to become known as *in vitro* fertilisation, IVF – he opened every door available. This included Embryology in Cambridge, Microsurgery (with Robert Winston) and Ultrasound (with Stuart Campbell) in London, Laparoscopy workshops in Europe with exposure to pioneers Kurt Semm from Kiel and Maurice Bruhat from Clermont-Ferrand (themselves students of Raoul Palmer) as well as finding funds to establish a working laboratory at the Royal Free Hospital (RFH) in London, at the edge of Hampstead Heath. What an exciting period from every respect.

The year I arrived in London, 1976, Edwards and Steptoe reported their first human pregnancy – unfortunately a tubal ectopic requiring excisional surgery. Following this, Steptoe advised clipping the fallopian tubes to avoid ectopics (advice not generally followed), and the team changed location (from Oldam General Hospital to Kershaw Cottage Hospital). They also changed

clinical approach, pursuing natural cycle egg collection using the HiGonavis kit to detect the LH surge. At this point they had experienced several disappointments including failure to gain Medical Research Council funding and receiving medico-scientific criticism, hence they appeared reluctant to reveal their "secrets" to us at the RFH unit. In 1976, therefore, there was no written manual on how to go about human IVF, hence it was traditional research for myself, embarking on an MD thesis (i.e. PhD in Medicine) which was presented and accepted by my *alma mater* the University of Western Australia in 1985 [5]. That thesis resides in the Reid Library and details 135 pregnancies achieved by IVF with 102 infants born to March 1985. The journey was difficult however and included training in embryology which was achieved in Cambridge attached to Chris Polge (pioneer of bull sperm freezing and researching pig egg cryopreservation) at the Animal Research Station (ARS) in Huntingdon Road. This gave me exposure to the ideas of several prominent embryologists at the time including Steen Willadsen (cloning by nuclear transfer in sheep), Cyril 'Dub" Adams (who advised the rabbit oviduct as a good site to conduct human IVF and which I pursued for a period), Bob Moore who taught tight adherence to lab culture systems (controlling pH, osmolality and temperature control), Bill 'Twink' Allen attempting horse cloning and Ian Willmut (who later achieved somatic cell cloning with Dolly the sheep). There were regular combined Wednesday meetings at Bob Edwards department of Physiology but, at the time, the focus was on large animal reproduction rather than the nebulous human pursuit. The ARS was founded by John Hammond in 1932 and dedicated to pioneer embryologist Walter Heape with many famous workers conducting research including Gregory Pincus, MC Chang and Australia's own Colin R 'Bunny' Austin. It was unceremoniously closed down in 1986 following animal rights agitation and a period of austerity cuts during Margaret Thatcher's period of government.

Further experience in embryology was gained from Anne McLaren and David Whittingham at Wolfsson house in London who assisted me to develop a small animal Laboratory within the Academic Department of O&G at the RFH. I soon became adept at mouse IVF and culture through to hatched blastocysts, but the human process was much more challenging.

I would acknowledge also advice from Lynne Frazer who was researching toxicology on mouse reproduction at Chelsea house, King's College and John Marsden researching embryo transfer and synchrony aspects on Rhesus monkeys in Birmingham, noting that his unit was closed down following the 'smallpox disaster' at the University in 1978 and potential risk related to primates.

A further acknowledgement is due to Ian Ferguson, a gynaecologist attempting IVF on his tubal surgery patients at St Thomas' Hospital in London between 1976-78. He had gained some advice from Steptoe and Edwards but had difficulty generating embryos of sufficiently good quality. He did share his work with me and introduced ideas of sperm and egg transfer to the fallopian tube as well as embryos, and appears unlucky not to have gained at least the first gamete intra-fallopian transfer (GIFT), pregnancies before closing his laboratory from frustration.

STEPTOE & EDWARDS PRESENTATION AT RCOG JANUARY 1979

At this historic meeting [2] attended by interested Medical and Scientific luminaries from all over the world, Steptoe revealed the clinical aspects and Edwards the laboratory and scientific aspects of their collaborative work. It emerged that they had admitted 79 women to Kershaw Cottage Hospital for HiGonavis monitoring. This was to detect the LH surge in natural cycles where women had been collecting 24 hour urine for oestrogen tracking to identify a potential ovulatory cycle. Laparoscopies were conducted on 68 women where an LH surge was identified and 11 women were sent home deemed anovulatory after prolonged testing over several days. The ovaries proved inaccessible in three women and no follicle was identified in nine others; a further eleven cases had follicles aspirated without finding an oocyte. The aspirator was a self-made apparatus involving a Y-piece switch, using finger closure to direct a suction pressure of around 100 mm Hg. An oocyte was recovered from 45 women but ten had failed fertilisation and a further three failed to demonstrate cleavage, leaving 32

women undergoing embryo transfer. This was undertaken using a double-catheter transfer technique. Pregnancy was diagnosed in four women being 5% of initiated cycles, 6% of laparoscopies, 9% of successful collections and 12.5% of transfers. Of the four pregnancies, Louise Brown was born near term 25 July 1978; a miscarriage occurred in the second at 11 weeks gestation with a triploidy XXX fetus identified; a third female infant named Courtney Cross was born severely premature at 21 weeks on 16 October 1978 and succumbed as a post-amniocentesis loss; the fourth pregnancy proceeded to term with the first IVF male infant, Alistair MacDonald born 14 January 1979.

Following the above unique experience at Kershaw, Steptoe and Edwards found themselves without a working facility and were effectively retired until the establishment of Bourn Hall where they were able to resume their amazing work in 1980 and where I later had a direct personal association as Medical Director of sister clinic Hallam Medical Centre in 1989-91, being part of the Bourn-Hallam Group owned by Ares-Serono.

KEY MILESTONES FROM THE LITERATURE

In developing the IVF lab within the labour ward facility of RFH in 1977, I studied the literature utilising the excellent libraries of the Royal Society of Medicine and the RFH. The relevant literature concerned IVF attempts in the 30's and 40's by Gregory Pincus and his assistant Miriam Menkin who later joined (and married) gynaecologist John Rock with the couple reporting the generation of 2 early human embryos from 138 eggs collected from hysterectomy specimens; the work being published in the American Journal of Obstetrics & Gynaeolcogy (AMJOG) in several papers over the period 1946-48. Subsequently in the early 1950's Landrum Shettles (later joined by Pincus) duplicated the Rock-Menkin work and also published in AMJOG 1951-53. I personally duplicated their described work at RFH, identifying their egg-spinning descriptions which related to excessive sperm numbers used for fertilisation, in the order of 1-5 million! Embryo descriptions were clearly 'suboptimal'. They did not describe transfers or pregnancies.

Subsequent dates of interest include the NIH Moratorium on IVF in 1971 following the del Zios case in New York when patients sued after their developing embryos were discarded by a non-supportive laboratory technician. Two years later in Australia in 1973 a team lead by Carl Wood described in the Lancet the world's first 'sign' of pregnancy following IVF and embryo transfer (ET). This was really just a luteal phase biochemical blip but enough to maintain hope and optimism. I have already mentioned Steptoe and Edwards' ectopic gestation in 1976 and their four famous pregnancies with two healthy children in 1978-9. In that latter year my thesis records two pregnancies following clomid/hCG but both miscarried in the first trimester. The following year 1980 again in Melbourne, Australia a successful IVF pregnancy was achieved using the natural cycle and LH surge detection method – a healthy female Candice Reed. The team was again under the Carl Wood leadership but he had established a dual competitive model whereby gynaecologist Ian Johnston and medical scientist Alex Lopata pursued the natural cycle, resulting in Candice Reed, and a second model with gynaecologist John Leeton teamed with veterinary scientist Alan Trounson to pursue programmed cycles using clomiphene citrate and hCG trigger. Although this second team were slower off the mark, when pregnancies occurred in 1981 they described the first proper rate being 14 pregnancies from 115 initiated cycles and nine births; thereafter with gradually improving rates. Thereafter the natural cycle system faded from extremely poor rates and more than 80 further laparoscopies by the Ian Johnston group without further pregnancies! In that same year 1981, Howard Jones reported from Norfolk, Virginia USA his first IVF infant, Elizabeth Carr following so- called controlled ovarian hyperstimulation (COH); using Pergonal/hCG and Proluton luteal support [3].

In 1982 several other teams reported their first IVF children including my small team from Perth, Western Australia; Wilfred Feichtinger from Vienna, Austria; Seigfried Trotnow from Erlangen, Germany; Rene Frydman from Paris, France; and Paul Devroey from Brussels, Belgium – we all used some form of ovarian stimulation and hCG triggers. Also in 1982 my former unit at RFH led by Ian Craft described pregnancies and infants following the transfer of gametes (sperm and eggs) to the uterine cavity.

During 1983-84, Zielmaker from Holland and Mohr from Carl Wood's team in Melbourne described successful embryo freezing, and in 1984 the miraculous achievement of pre-implantation genetic diagnosis (PGD) by Alan Handyside at Robert Winston's Hammersmith unit was an advance ahead of its time. During the mid-eighties, ultrasound-guided egg recoveries introduced a major clinical advance and Asch's GIFT method provided a period of successes whilst many fledgling units were finding difficulty establishing satisfactory pregnancy rates with conventional IVF. Other tubal transfer methods such as pro-nuclear stage transfer (PROST), zygote intrafallopian transfer (ZIFT) and tubal embryo stage transfer (TEST), including trans-cervical TEST were also more successful at this time.

A further major development occurred in 1992 when Gianpiero Palermo and the Brussels team demonstrated successful intra-cytoplasmic sperm injection (ICSI) and this subsequently enabled fertilisation using sperm collected from the epididymis or even testicular sperm in several reports in the mid-nineties. In 1998, Gardner's introduction of the serum-free sequential media culture system proved a key to the modern-day success for blastocyst culture, single embryo transfers along with generally improved clinical and laboratory systems using solid-state incubators for embryo culture and vitrification proving to be the better method of cryopreservation. Clinical improvements include tempered ovarian stimulation, targeting the oversensitive polycystic ovary with high antral follicle count (AFC), and high associated serum anti-mullerian hormone (AMH) for minimal dosage of recombinant follicle stimulating hormone (rFSH). There has also been the recent the introduction of Cabergoline and the appropriate use of both GnRH agonists and antagonists to minimise the risk of ovarian hyperstimulation syndrome (OHSS) and virtually exclude the life-threatening severe forms [10].

ESTABLISHING AN IVF LAB AT RFH 1977-78

Professor Craft attracted donations, endowment monies and generated personal revenues to enable me to create a human IVF laboratory within

a small 'facility' room of the labour ward on the 5th floor of the RFH based in Hampstead, London. This included a Wild-Leitz laborlux-II compound microscope for semen analysis, a Wild M7 stereomicroscope for gamete and embryo handling and to which was attached a Leica camera for time-lapse photography. Other equipment included a Mettler balance, an osmometer, a water-jacketed incubator, dessicator jar, Dreschel flasks, small fridge, a small oven for heating and sterilising equipment, a water bath for deactivating serum and a heating stage (histology slide warmer) within the workstation area. This was personally constructed using perspex on a light timber frame through which the stereomicrosope was inserted. Tubes, pipettes and Bunsen burner were a part of the amenity and I had already learnt the technique of pulling pipettes over a Bunsen flame and was comfortable using a mouth pipette. Triple gas (O_2 5%, CO_2 5% & N_2 90%) was the preferred gassing system, as used by Edwards.

The first problem encountered related to the character of human oocytes. In the first instance, we were collecting at around 32 hrs after estimated onset of LH surge (for natural cycles) or post-hCG trigger for Clomid cycles. Unlike ovulated mouse oocytes, human retrieved oocytes were surrounded by a dense coronal coat of 3-5 cell layers and this entity was semi-concealed within a large cumulus mass of cells, sometimes with part of the denser granulosa sheet attached to one end. At any rate the follicle fluid contained a lot of apparent debris, mostly constituting granulosa sheets which had to be carefully lifted or washed to reveal oocytes underneath. As we moved away from natural cycles and extended the hCG trigger-recovery interval to 34-35 hrs, the cumulus was noted to be less dense and oocytes easier to identify although the better quality still had their coronal coat. When oocytes were 'naked', I eventually realized, they were unfavourable for fertilisation or embryo development and undoubtedly already partially atretic. I learnt that sperm preparations dispersed the cumulus and this could be artificially achieved with hyaluronidase, but the coronal coat was persistent, eventually learning to remove it by adjusted fine-tipped pipette dissection assisted by fine 30 French gauge needles.

Early sperm preparations were rather dense, >1 million/ml but with good effect on cumulus dispersal, gradually reducing sperm numbers to

<100,000 then experimenting with lower concentrations as little as 10,000/ml but finding reduced fertilisation rates. Much work was also done on adding albumin to the sperm preps in the range of 4 mg, 8 mg, 12 mg, 16 mg and 32 mg with rising fertilisation rates but settling for lower concentrations to avoid stickiness and clumping.

My first 'eureka day' involved turning over a coronal ball about 50 hrs after insemination in a Tyrode B culture medium containing 10% deactivated Maternal Serum (dMS) – low and behold, the most perfect 6-cell embryo I have ever seen slipped out of an opening in the coat. The year was 1977 and I was not conducting pronuclear checks at that time. The following year, 1978, I had a second 'eureka night' spending the evening in the lab watching a blastocyst hatching and the hatched embryo "crawling" around the Falcon dish well away from its empty zona shell. I had changed the culture medium on day 2 from Tyrode B+10% dMS to Ham's F10 in microdroplets under British Drug Houses (BDH) paraffin oil, refreshing second daily to observe this event on day 6 and subsequently repeated it several times. I later recall Bob Edwards telling of his first hatched blastocyst emerging from under its coronal coat in 1970 – clearly these events were rare in the 1970's and probably remained so until Gardner's work 20 years later (late 1990's). I undertook routine early embryo dissection in 1979 to commence pronuclear checks once I was confident with dissection techniques; two early failed pregnancies resulted from 4-cell transfers following pro-nuclear (PN) checks.

KING EDWARD MEMORIAL HOSPITAL (KEMH), WESTERN AUSTRALIA

In December 1979 I enjoyed returning to my birthplace and hometown of sunny Perth where lifestyle aspects are superb, but I soon realized that the laboratory facility I had established with Ian Craft in London would be difficult to reproduce. None-the-less on a shoestring budget and relying on community lamington drives, I did put together a laboratory in allocated space near the theatre suite at KEMH, but for logistic reasons was only able to access the theatre for laparoscopic egg recovery on a Tuesday morning i.e.

my allocated gynaecological operative list. I employed a young high-school graduate Alison Pusey to assist in the lab and boldly stimulated 42 women with clomiphene tablets, triggering with hCG 5,000 iu and undertaking my own laparoscopic collections. It was a bizarre struggle in the theatre, asking the anaesthetist to keep the patient asleep whilst I un-gowned and carried out checks of the follicle fluids to determine if the eggs were recovered, re-gowning for further aspirations and flushings if the news was negative. During 1981, I undertook 42 attempts at KEMH, achieving three pregnancies one of which proceeded to term, the others failing in the first trimester.

Western Australia's first IVF baby Jarrad Carter was born 13 July 1982 at KEMH with birth weight 2340 grams [8]. His mother Linda had lost both fallopian tubes from separate ectopic pregnancies requiring salpingectomies as life-saving procedures. In October 1981, she commenced Clomid 50 mg tds (i.e. 150 mg/day) on day two for five days; last menstrual period (LMP) being October 13. On day 12 with ultrasound detecting two follicles of 18 mm she was triggered with hCG 5000 iu. I recovered two oocytes at laparoscopy and inseminated with 50,000 sperm 3-4 hrs later (after completing several other listed operations). Fertilisation was undertaken in modified Tyroid medium (T6) and PN check confirmed fertilisation the next morning (18 hrs post-insemination). Further culture was undertaken in Hams F10 with 7.5% dMS and 44 hrs post-insemination two embryos were transferred – a 4-cell and 8-cell – using a double polyethylene catheter system. This case was tracked closely with serum βhCG along with progesterone and oestradiol measurements beginning 10 days post follicle aspiration and reported in the Lancet. Because of vaginal bleeding in the luteal phase, medroxyprogesterone (Provera) support was added for several days then ceased when pregnancy appeared well established; however resumed again when progesterone levels were seen to be falling and continued until luteal day 100, in keeping with the Howard Jones formula of luteal support. Jarrad Carter, birth weight 2340 g was born on July 13 1982, by elective caesarean section because of signs of fetal growth retardation in the latter weeks of the pregnancy. He has thrived, now developed into a strapping young man who attained tertiary education, married in 2005 and spontaneously conceived his own child, a daughter born in September 2008!

MODERN DAY IVF

Following the birth of Jarrad Carter, I nominated my IVF clinic as PIVET, being an acronym *for **P**rogrammed **IV**f and **E**mbryo **T**ransfer* indicating a preference for controlled ovarian stimulation. My efforts using HiGonavis and natural cycle tracking in London proved time-consuming, highly frustrating and a serious drain on the emotional energy of myself, patients and co-workers. I saw no future in the methodology as a serious treatment method for infertile couples. In fact, the recent trend towards natural cycle and minimum stimulation systems is, in my opinion, flawed when compared with more efficient advances [7] sometimes incorporating Growth Hormone [9] and recently updated in the PIVET FSH Algorithm [10].

In Australia, IVF units function under an accreditation system conducted by Reproductive Technology Accreditation Committee (RTAC) which defines a Code of Practice. All units are obliged to provide their data in a virtually real-time basis to Australia & New Zealand Assisted Reproduction Database (ANZARD) which categorises performance of the 77 fertility clinics of Australia and New Zealand into quartiles. The report of 2010 details 61,929 initiated ART treatment cycles undertaken in 2008 and tracks the delivered infants through 2009. It can be seen that for women under 35 years, the success rate of IVF units ranges from a low of 4.5% to a high of 36.0% for fresh transfers and 4.5% to 31.5% for frozen transfers [4]. Pleasingly PIVET functions at the highest level of the top quartile with 35.6% for fresh and 30.5% for FET cycles in that very data set i.e. "*Top of the Wazza*" to use an Australian expression!

THE FUTURE

Both clinical and laboratory developments are required to improve assisted reproductive technology (ART) outcomes. Certainly the technology is impacting significantly in some countries with nearly 4% of infants currently arising from ART procedures and another estimated 4% from associated fertility management processes arising from Fertility clinics in Australia.

Future clinical benefits should arise from better understanding and control over gametogenesis focusing on both the oocyte and sperm production, particularly with *in vitro* spermatogenesis. Identifying and selecting the optimum sperm for fertilisation is also required. From the laboratory one expects the evolution of a system of IVF and embryo culture which more closely resembles the natural process. This will require an automated system with perfect and continuous control over pH, osmolality, temperature, pO_2 and pCO_2 along with the application of automated metabolomic adjustments [10]. Identification and selection of the optimum blastocyst for transfer is thereafter a universal expectation.

REFERENCES

1. Craft I, Senanayake F, Yovich JL **1978** Laparoscopy, clip occlusion and uterine cancer. *Lancet* **ii** 208.
2. Edwards RG, Steptoe PC, Purdy JM **1980** Establishing full term pregnancies using cleaving embryos in vitro. *British Journal of Obstetrics and Gynaecology* **87** 737-742.
3. Jones HW Jnr **2011** Seven roads travelled well and seven to be travelled more. *Fertility and Sterility* **95** 853-856.
4. Vajta G, Rienzi L, Cobo A, Yovich JL **2010** Embryo culture: can we perform better than nature? *Reproductive Biomedicine Online* **20** 453-469.
5. Wang YA, Chambers GM Sullivan EA **2010** Assisted Reproductive Technology in Australia and New Zealand 2008. Australian Institute of Health and Welfare, Australian Government, Canberra Australia. 58p
6. Yovich JL **1985** MD Thesis: Human pregnancies achieved by in-vitro fertilisation. Reid Library, University of Western Australia 409p.
7. Yovich J, Grudzinskas G **1990** The management of infertility: a manual of gamete handling procedures. Heinemann Medical Books 276p.
8. Yovich JL, Puzey A, De Atta R, Roberts R, Reid S, Grauaug A **1982** In-vitro fertilization pregnancy with early progestagen support. *Lancet* **ii** 378-379.
9. Yovich JL, Stanger JD **2010** Growth hormone supplementation improves implantation and pregnancy productivity rates for poor-prognosis patients undertaking IVF. *Reproductive Biomedicine Online* **21** 37-49.
10. Yovich JL, Stanger JD, Hinchliffe P **2011** Targeted gonadotrophin regimen using the PIVET Algorithm markedly reduces the risk of ovarian hyperstimulation syndrome. *Reproductive Biomedicine Online* 2012 (in press).