Review Article

Assisted Reproductive Technology after the Birth of Louise Brown

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Abstract

Background: Public interest in Assisted Reproductive Technology (ART) has remained high since the birth of the world's first *in vitro* fertilization baby, Louise Brown, in the United Kingdom. ART allows scientists to manipulate the fertilization process in order to bypass some pathological obstacles such as blocked fallopian tubes and non-functioning ovaries in the females, and blocked vas deferens and low sperm count in the males. The purpose was to provide a historical outline and identify the researches that most contributed to ART.

Methods: A review of published experimental and clinical studies of assisted reproduction carried out at the University of Bristol library website (MetaLib®). A cross-search of seven different medical databases (AMED-Allied and Complementary Medicine Database, BIOSIS Previews on Web of Knowledge, Cochrane Library, Embase, and the Medline on Web of Knowledge, OvidSP and PubMed) was completed by using the key words to explore the major milestones and progress in the development and implementation of ART.

Results: A speedy advancement in the development of different assisted reproductive techniques makes infertility problem more treatable than it ever had been.

Conclusion: Although no other field in the medicine has integrated new knowledge into the daily practice more quickly than ART yet, there is a need for social research to counterbalance the dominance of biomedical one, in particular the people's actual experiences and expectations of ART.

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Introduction

The beginning of *in vitro* fertilization (IVF) was an inspiring event. Lesley Brown and her husband John, from Bristol city in the United Kingdom had failed to conceive naturally throughout nine years of their continuous marriage. Lesley Brown had bilateral tubal blocks. Bilateral salpingostomy was done without success. In 1976, she was referred to Dr. Patrick Christopher Steptoe, a gynaecologist in the Oldham general hospital, Manchester city, United Kingdom. He advised her to try a new experimental technique to bypass her tubal blockage. Accordingly, Lesley underwent a laparoscopic oocyte retrieval during a natural non- stimulated ovulatory cycle. Mr. Robert Geoffrey Edwards, a British physiologist, used her husband's sperm to fertilize the retrieved oocyte in the lab. A few days later, an 8-cell stage embryo was placed inside Lesley's uterine cavity. At 11.47 PM on July 25th 1978, Louise Brown was delivered by an elective caesarean section at Oldham hospital by the registrar John Webster at gestational age of 38 weeks and 5 days due to coincidence of maternal pre-eclampsia. Louise was healthy at birth and her weight was 5 pounds and 12 ounces (2700 grams). By the birth of Louise Brown, the world celebrated the start of a new era of assisted human reproductive technology.

Early attempts: The history of IVF dates back as early as the 1890's when Walter Heape, a professor at the University of Cambridge, UK, reported the first known case of embryo transplantation in rabbits. In 1932, Aldous Huxley described the technique of IVF in his science fiction novel "Brave New World". In 1934, Gregory Pincus mixed rabbit's eggs and sperm in the glass top of his watch and implanted the developing embryo in a surrogate rabbit. Fourteen years later, in 1948, Miriam Menken and John Rock retrieved more than 800 oocytes from women. However, it was not until 1959 that Min Chueh Chang, a young Chinese reproductive investigator, obtained indubitable evidence of *in vitro* fertilization by achieving live births for the first time from a white rabbit by using eggs and sperm from black ones.

The first human IVF pregnancy was in 1973 reported by professors Carl Wood and John Leeton in Melbourne, Australia. Unfortunately, it ended in an early embryo death (less than one week) (1). In 1976, Patrick Steptoe and Robert Edwards published a report on an ectopic pregnancy following a transfer of a human embryo at its late morula stage (2).

ART Progress: The birth of Louise Brown on July 25th 1978 (3) was followed by the birth of Courtney Cross on October 16th 1978 and Alastair MacDonald on January 14th 1979, the world's first three IVF babies. Since then, IVF has become a common procedure with a record of more than 5 million births worldwide. The years followed have brought rapid progress that allows more infertile couples to have their own genetic babies (4).

Back to the year 1978 (Figure 1), Alex Lopata, in Melboure, Australia described the first ovarian cycles stimulated by clomiphene citrate (5).

In 1979, Alastair MacDonald, the world's third IVF baby (also considered as the world's first IVF male baby) was born on January 14th. Pez and his colleagues in France started tracing the growth of the graffian follicles by using pelvic ultrasonography (6). Their sonographic findings were correlated with the laparoscopic observations. They further indicated that the diameter of the graffian follicles, as measured by ultrasound, is a better predictor of follicular maturation relative to the serum Oestradiol levels alone (on day 8th of the cycle E_2 level should be ≥300 *pg*).

In 1980, the Melbourne IVF team led by Alan Trounson succeeded to deliver the Australia's first IVF baby (the world's fourth IVF baby) a female called Candice Elizabeth Reed (7). In the same year, the first American IVF clinic was opened in Virginia, United States (6). WHO published the "Laboratory manual for the examination of human semen and semen-cervical mucus interaction" to standardize semen analysis (8). Alan Handyside,

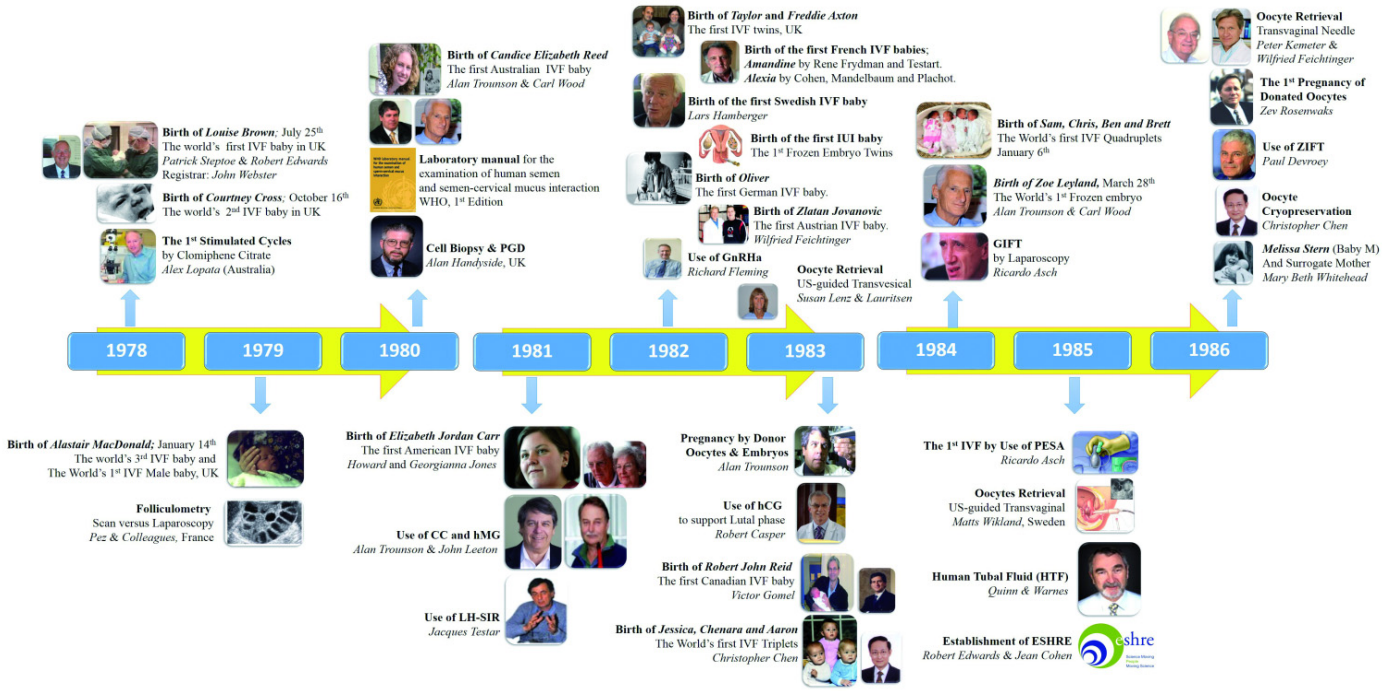

Figure 1. Timeline of major ART milestones (Year 1978–Year 1986)

in the United Kingdom, introduced Preimplantation Genetic Diagnosis (PGD) to identify genetically abnormal embryos by cell biopsy (8).

In 1981, Howard and Georgianna Jones announced the delivery of the first American IVF baby, Elizabeth Jordan Carr in Virginia after ovarian stimulation by human menopausal gonadotrophin (hMG), (6) while Samantha Steel was the first IVF baby born to American parents in England on the same year. Clomiphene Citrate (CC) and human menopausal gonadotrophin (hMG) were introduced in the IVF treatment protocols by Alan Trounson and John Leeton in Australia (9). In stimulated ovarian cycles, the number of mature oocytes retrieved increased and by the administration of human chorionic gonadotrophin (hCG) identification of the exact timing of ovulation (±36 hours later) and oocyte collection was attained. Moreover, Alan Trounson noticed that a delay between oocyte collection and insemination allows the immature oocytes collected to complete its meiotic maturation in the culture media (10, 11). The Clamart's IVF working group in France, led by Jacques Testar, developed a luteinizing hormone-Surge Initiating Rise (LH-SIR) assay that could detect the LH-surge at its initial rise (not at its peak) in human plasma for an accurate prediction of ovulation and best timing for oocytes retrieval (6, 12).

In 1982, the first IVF twins, Taylor and Freddie Axton, were born at Queen Elizabeth hospital in King's Lynn, United Kingdom. The first French IVF babies were born in the same year; Amandine, at Clamart in February followed by Alexia at Hôpital Sèvres in June (6). The first Swedish IVF baby was born in Gothenburg (13). The first frozen embryo twins were born in Australia in 1982 and the world's first delivery after intrauterine insemination (IUI) was done in the same year as well (6). On April 16th at the University Hospital in Erlangen, Oliver, the Germany's first test-tube baby was born by caesarean section and Zlatan Jovanovic was the first IVF baby born in Vienna's AKH hospital in Austria. Culture media for growing embryos started to be used (14). Richard Fleming was the first who demonstrated that gonadotrophin releasing hormone (GnRH) agonists could be used to eliminate premature luteinization of the graffian follicles and control the process of ovarian stimulation (15). Danish gynaecologists, Susan Lenz and Jörgen G Lauritsen, demonstrated how to use the ultrasound as a guide for trans abdominal trans-vesical oocyte aspiration (16).

In 1983, Alan Trounson's working group in Australia succeeded to achieve the first pregnancy in a woman with bilateral oophorectomy by using donor oocytes (17) and in an infertile woman with primary ovarian failure by using donor embryo (18). The Monash IVF team in Australia reported a successful pregnancy by the first frozen embryo (19). In the same year, in-vitro maturation (IVM) for fertilizing immature oocytes was introduced (20). Gleicher and his colleagues reported the early use of vaginal route oocytes retrieval via culdocentesis by the aid of transabdominal ultrasonography (21). Robert Casper and his co-workers were the first to describe the use of low dose human chorionic gonadotrophin (hCG) to support the luteal phase in assisted ovarian cycles (22). The first report on the Canadian IVF baby, Robert John Saunders Reid, was written by the working group of Victor Gomel at the University of British Columbia. In the same year, the birth of world's first IVF triplets was reported by Christopher Chen.

In 1984, the world's first IVF quadruplets were born on January 6th at the Royal Women's Hospital, in Melbourne, Australia. On March 28th, the birth of the world's first frozen embryo, Zoe Leyland, was a breakthrough in ART history. This baby was delivered by caesarean section at the Queen Victoria Medical Centre in Melbourne, Australia by Alan Trounson and Carl Wood (6). During the year 1984, the first legislation to regulate the IVF and human embryo research in the Australia by Government of Victoria, the Infertility (Medical Procedures) Act 1984, was promulgated (6, 23). The first surrogacy embryo transfer baby was born in California, USA, (6) while the first reported pregnancy following trans laparoscopic gamete intrafallopian transfer (GIFT) was announced by the endocrinologist Ricardo Asch (24). The first pregnancy following IVF and oocyte donation in a woman with primary ovarian failure was successful (25). The early trans vaginal oocyte retrieval was introduced by Pierre Dellenbach and his colleagues in Strasbourg, France (26). The first two pregnancies following the transfer of intact frozen-thawed embryos were successful (27).

In 1985, the first pregnancy achieved by IVF using percutaneous epididymal sperm aspiration (PESA) succeeded (28). It was the year of the first human birth after replacement of hatching blastocyst cryopreserved at an expanded blastocyst stage (29). A Nordic group, led by the gynaecol-

ogist Matts Wikland in Gothenburg, Sweden, described the possibility of using transvaginal scanning for oocyte retrieval for the first time (30). By this technique, the ovaries' visualization was more effective than by the abdominal approach, and the smaller follicles were easily punctured. The procedure could be done under local anaesthesia and the patient could leave the IVF center after an hour. The first report on the use of abdominal ultrasonography as a guide for embryo transfer was published. [31] Dan Szollosi with Jacqueline Mandelbaum described the microstructures of the human oocyte, which became known as "Oocyte Dysmorphia" (32). Testart's group in France published impressive results about using propanediol and sucrose as cryoprotectants for embryo freezing, instead of using dimethyl sulphoxide (DMSO) (33). Quinn and Warnes published a formula entitled Human Tubal Fluid (HTF) that mimics *in vivo* environment to which the embryo is exposed (34). The European Society of Human Reproduction and Embryology (ESHRE) was established by the help of Robert Edwards (from UK) and Jean Cohen (from Paris) after its first meeting in Bonn in 1985.

In 1986, Lupron® (GnRH agonist) was used for the first time to prevent premature ovulation. Monash IVF team reported the world's first pregnancy achieved by surgical sperm retrieval from a patient with bilateral vas deferens ducts obstruction (6). Wilfried Feichtinger and Peter Kemeter used ultrasound-guided trans-vaginal needle aspiration of mature graffian follicles for oocytes retrieval (35). It was the year of the first successful pregnancy following IVF donated oocytes which was achieved by a woman with non-ovarian failure, Zev Rosenwaks (36). Daniel Navot and his co-workers reported the ability to induce endometrial cycles artificially and to establish pregnancy in the absence of functioning ovaries (37). Paul Devroey and his colleagues reported the first successful pregnancy following laparoscopic zygote intrafallopian transfer (ZIFT) (38). The introduction of the direct intraperitoneal insemination (DIPI) was in 1986 (6). Christopher Chen in Australia reported the world's first pregnancy resulting in the birth of twins by using a previously cryopreserved oocyte (slow freezing with dimethylsulfoxide-DMSO/rapid thawing technique) (39). The first Soviet IVF baby, Lena, conceived at Leonov's laboratory in Moscow was born in February. A few months later, another IVF child, Kirill, Lena's brother, was born in Saint Petersburg. Melissa Stern (Baby M) was born on March 27th in the United States. Her surrogate and biological mother, Mary Beth Whitehead (who conceived by artificial insemination) refused to yield custody of Melissa to the couple (William Stern and his wife, Elizabeth Stern) with whom she made the surrogacy contract. The court of New Jersey found it in the best interest of the infant to award custody of Melissa to her biological father William Stern and his wife Elizabeth Stern, rather than to her surrogate mother, Mary Beth Whitehead.

In 1987 (Figure 2), the first report on using ultrasound machine during embryo transfer (ET) was published. Laws-King and his co-workers reported a new technique called SUZI (sub-zonal injection) that would advance assisted reproductive technology and offers a hope for couples with recurrent failed cycles (40). The introduction of a new effective method, Ultra-rapid freezing, for cryopreservation of the human embryos was in 1987 (41). Norway was the first country in the world to pass a law on ART on June 12th, 1987 (Norwegian law on Assisted Reproduction and Genetics). In the Nordic law, treatment is limited to the married or cohabiting couples. Same sex couples, lesbians and single women were excluded from the IVF treatment and further surrogacy and embryo donation was not permitted.

In 1988, Patrick Christopher Steptoe died on March 21st. In the same year, the world's first baby after home monitoring of fertility treatment was born (6). Leeanda Wilton and Alan Trounson introduced the early embryo biopsy technique for genetic study (42). The first report about two babies born after microsurgical epididymal sperm aspiration (MESA) in men with congenital bilateral absence of vas deferens (CBAVD) was published in 1988 (43). In May, Linda Kirkman gave birth to Alice, who was conceived from her mother Maggie's egg, fertilized by sperm from a donor since her husband, Sev, had no sperm. The world's first IVF surrogate birth occurred in Australia (6). It was the year of the successful pregnancy achieved through sub-zonal sperm injection (SUZI), (44) and after oocyte zona pellucida drilling and mechanical partial zona dissection that facilitated sperm penetration (45).

In 1989, the first report on biopsy taking from pre-implanted human embryos and sex detection by DNA amplification was published (46). It was the same year when laser was used for the first time in the field of assisted reproduction (47). Yael Gonen and her co-workers in Toronto, Can-

Figure 2. Timeline of major ART milestones (Year 1987 – Year 1995)

ada, pioneered the use of ultrasound for assessment of endometrial quality instead of IVF procedures (48).

In 1990, successful deliveries following human embryo vitrification, (49) and biopsy of preimplantation embryos sexed by Y-specific DNA amplification were reported (50). A Dutch embryologist, Jacques Cohen, published the first report on assisted hatching in human embryos (51). The use of first polar body biopsy for genetic diagnosis was done in the same year (52). Yael Gonen and her co-workers proposed the use of GnRH agonist instead of hCG to trigger the endogenous LH-surge for IVF cycles (53). The use of combined oral contraceptive pills for follicular synchronization and cycle scheduling in IVF program has been suggested (54). The British Human Fertilization and Embryology Act set out a framework for ART practice and research under the license from the Human Fertilization and Embryology Authority (HFEA) (6).

In 1991, *in vitro* maturation (IVM) of donor oocytes in a non-stimulated cycle resulted in a successful pregnancy (55). GnRH antagonist was introduced to prevent premature LH-surge in a controlled ovarian hyperstimulation program (6). Daniel Palanker used Excimer laser for zona pellucida drilling (56).

In 1992, the first successful pregnancy after intracytoplasmic sperm injection (ICSI) by Gianpiero Palermo and Andre Van-Steirteghem occurred in Brussels, Belgium (57). A further successful in-vitro fertilization and embryo transfer (IVF-ET) was achieved after treatment with recombinant human FSH (rh-FSH) (58). It was the year of delivery of the first British SUZI treated baby. Two births were recorded by Pasquale Patrizio from frozen embryos produced by epididymal aspirated sperm (59). A published scientific report stated that men with congenital bilateral absence of vas deferens (CBAVD) have a mild form of cystic fibrosis (CF) (60). Administration of luteinizing hormone releasing hormone (LHRH) agonist induced ovulation by triggering endogenous LH-surge (61).

In 1993, Sherman Silber and his co-workers reported, for the first time, that infertile men with non-obstructive azoospermia become able to father their own babies by the use of Testicular Sperm Extraction (TESE) and ICSI procedures (62). The genetic cause of congenital bilateral absence of vas deferens (CBAVD) among infertile men (cystic fibrosis mutations) could be transmitted to their offspring (63). The first live birth was reported following treatment with rh-FSH (64).

In 1994, a successful in-vitro oocyte maturation (IVM) and fertilization in non-ovulating women with polycystic ovary (PCO) syndrome was achieved by Alan Trounson and his co-workers in Australia (65). It was the year of birth of the first British triplets after maternal surrogacy (6). The first IVF unit in the world, Midland Fertility Ser-

vice in United Kingdom was accredited with ISO 9002:1994. The first highly purified FSH preparation was developed (66) and the first pregnancy after the use of r-hLH was recorded (67).

In 1995, a successful human birth was reported by Frank Barnes after *in vitro* primary oocyte maturation (IVM), ICSI, and assisted hatching (68). The first report of aneuploidy testing was published by Jacques Cohen, (69) while the first report of spermatids to achieve pregnancy was published by Simon Fishel and Green (70). Dmitri Dozortsev and his co-workers discovered oocyte activation during ICSI procedure triggered by a water-soluble, heat-sensitive, non-species, specific cytosolic sperm factor (71).

In 1996 (Figure 3), Manuel Gil-Salom and his co-workers reported successful pregnancies employing ICSI after cryopreserved testicular sperm (72). The males with severe oligo-astheno-zoospermia were discovered to have deletion in their Y-chromosomes (6). Andrea Jurisicova, a Canadian embryologist, was the first who recognized that preimplantation embryo fragmentation leads to a programmed cell death (73). Robert Casper and his colleagues at the University of Toronto, Canada used hypo-osmotic swelling test for selection of viable immotile sperm for ICSI procedure in men with complete astheno-zoospermia (74). Although the successful fertilization of a human oocyte by a late stage spermatid using ICSI was first reported by Vanderzwalmen in 1995, it was not before the year 1996 when Jan Tesarik and Simon Fishel announced the delivery of the world's first two successful testicular spermatid babies (6). This technique introduced the concept of using immature sperm (spermatids) to overcome the problem of males' non-obstructive azoospermia (75). On July 5th, Ian Wilmut and Keith Campbell, succeeded to clone a sheep (Dolly) at Roslin Institute in Scotland by using cells from the mammary glands of an adult sheep and enucleated egg cell.

In 1997, Sun, Jurisicova and Robert Casper described the use of terminal deoxynucleotidyl transferase-mediated dUTP-biotin end labeling (TUNEL) for detection of DNA fragmentation in sperm and its correlation to IVF outcome (76). They found that sperm with fragmented DNA were less likely to fertilize the oocytes. The first successful birth after the transfer of enucleated donor oocyte cytoplasm into a recipient oocyte (Ooplasm Donation) was in 1997 (77). The Australia's first twins were born after open testicular biopsy. Gamete intra-fallopian transfer (GIFT) was accomplished by Porcu and Dal Prato through trans-cervical falloposcopy (78).

In 1998, Hannatu Kupchi, the first successful IVF baby, was born in Nigeria. The first case report on a successful pregnancy after controlled ovarian hyperstimulation induced by recombinant FSH and GnRH antagonist (Ganirelix®) was published by Joseph Itskovitz-Eldor (79). David Gardner at Colorado Center for Reproductive Medicine in the USA introduced a serum-free medium for blastocyst transfer and culture (80). Gianpiero Palermo and his assistants succeeded to get sperm

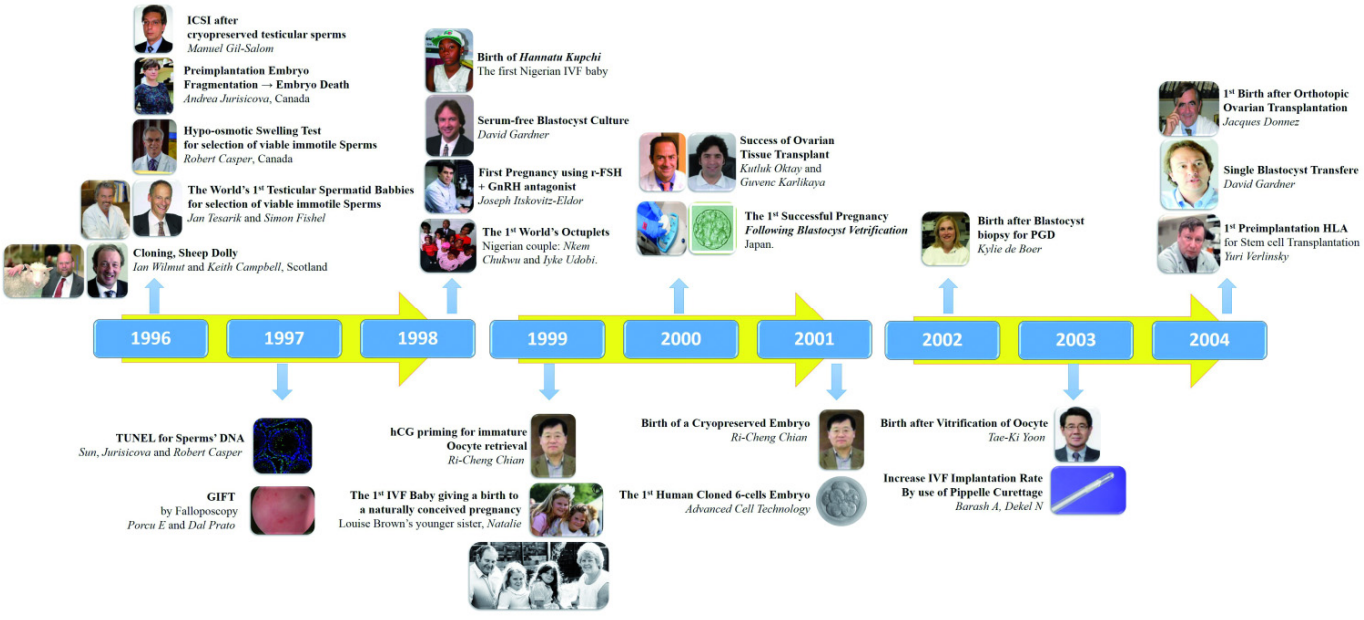

Figure 3. Timeline of major ART milestones (Year 1996 – Year 2004)

from men with non-mosaic Klinefelter's syndrome by testicular extraction and achieve pregnancy by using ICSI procedure (81). A live birth following cryopreservation of immature oocytes, thawing, IVM and then fertilization through ICSI procedure took place (6). The DNA sequence of the human chromosomes (Human Genome Project) was broadcasted. On December 1998, the first set of octuplets in the world (8 babies; 6 girls and 2 boys) were born at St. Luke's Episcopal Hospital in Houston, Texas, USA, after the use of ovulation induction therapy to a Nigerian-born American couple, Nkem Chukwu and Iyke Louis Udobi. Monash IVF-team in Australia announced the birth of twins by using Cell Robotics Laser Assisted Hatching technique (6).

In 1999, the first unaffected pregnancy was reported after using preimplantation genetic diagnosis (PGD) for sickle cell anemia (82). Lilia Kuleshova and her colleagues announced a successful birth following vitrification of a human oocyte (83). During this year, the world's first baby was born to a man after multiple ejaculate resuspension centrifugation technique. Ri-Cheng Chian and his colleagues demonstrated that hCG priming prior to immature oocyte retrieval in women with PCO syndrome increases oocyte maturation and pregnancy rate (84). The world's first baby for a patient with cystic fibrosis was born. It was the world's first successful IVF-ICSI pregnancies after airplane transport of oocytes as reported by McGill Reproductive Centre, Toronto, Canada (85). Denmark reported the highest number of IVF cycles (1826 cycles) per million inhabitants followed by Finland (1440 cycles) and then Sweden, Iceland and Norway (around 1000 cycles). These exceptionally high numbers of IVF cycles are probably due to a combination of relatively high practice levels in the Nordic countries and a high public recognition of the technique (86). In spite of that, Louise Brown's younger sister, Natalie, in the United Kingdom who was also conceived through IVF in 1982 (the world's 40th IVF baby) was the first IVF baby in the world to give birth herself, naturally, to a daughter named Casey on May 1999.

In 2000, Kutluk Oktay and Guvenc Karlikaya were the first to report on the success of human ovarian tissue transplant after frozen storage (87). The first successful pregnancy in Japan was achieved through blastocyst vitrification. A completely defined new protein-free embryo culture medium was introduced (6).

In 2001, the implantation rate on the running IVF programs was improved by using transvaginal ultrasound guided embryo transfer catheter (88). Ri-Cheng Chian and his co-workers reported a successful birth of an infant developed from cryopreserved embryo which was produced by IVM oocytes and was delivered by a non-stimulated woman with PCO syndrome (89). Mc Gill group in Canada reported the first ongoing twin pregnancy after ICSI of PESA retrieved spermatozoa into *in vitro* matured oocytes (90). A baby was born after sperm retrieval from a moribund man (91). The first British license was awarded for PGD and HLA-tissue typing (6). Australian scientists succeeded in fertilizing mice eggs without using sperm. This step opened the door for future single-sex procreation. The first human cloned 6-cell embryos were achieved by a private American company, Advanced Cell Technology for the purpose of stem cell research (6).

In 2002, Kylie de Boer and her assistants reported the first live birth after blastocyst biopsy for PGD (92). A comparative genomic hybridization and polar body testing for PGD of chromosomal aneuploidy were applied for the first time (93).

In 2003, Tae-Ki Yoon and his working group reported a live birth after vitrification in a stimulated IVF-ET program (94). The first IVF birth after ovarian stimulation by a long-acting human recombinant follicle stimulating hormone (rFSH) agonist was reported (95). Dr. Barash and Prof. Dekel demonstrated increased IVF implantation rate following endometrial curettage by Pipelle curette as a simple outpatient procedure (96).

In 2004, Jacques Donnez reported the first live birth baby after orthotopic transplantation of cryopreserved ovarian tissue (97). Fertility preservation programs for women undergoing cancer treatment using IVM and oocyte vitrification were provided (98). The first report on the natural ovulatory cycle in IVF combined with IVM as a potential approach to infertility treatment was published (99). David Gardner and his colleagues performed the world's first single blastocyst transfer trial to improve pregnancy rate and to reduce the risk of multiple gestation (100). The British National Health System (NHS) funded PGD program and preimplantation HLA-tissue typing (101). A Korean group claimed to produce the first cloned human blastocyst. Furthermore, a live baby birth following pre-implantation genetic diagnosis for Retinoblastoma took place (102).

In 2005 (Figure 4), Adriana Iliescu was reported as the world's oldest mother to give birth at the age of 66 years and 320 days at Giulesti Maternity Hospital, Bucharest, Romania. She had an IVF using donated eggs and sperm. Sherman Silber, in USA, announced the first case of successful ovary transplantation between two identical twin sisters discordant for ovarian function. The first birth from thawed ovarian cortex transplants in a woman with post-chemotherapy ovarian failure was reported (103). The first baby was born alive after trophectoderm biopsy and preimplantation genetic testing of human blastocysts for beta Thalassaemia (104).

In 2006, Mohamed Bedaiwy and his group reported a successful cryopreservation of intact human ovary with its vascular pedicle (105). The first successful pregnancy after PGD for aneuploidy screening in embryos was generated from a natural ovulatory cycle reported by McGill Reproductive Centre, Toronto, Canada (106). Maria del Carmen Bousada became the world's oldest mother to give birth to twins at the age of 66 years and 358 days in Spain. She conceived by the aid of IVF using donated eggs and sperm. On December 20th, Louise Brown (the world's first IVF baby) who married to a nightclub security officer Wesley Mullinder gave birth herself to a naturally conceived son Cameron, at St Michael's Hospital in Bristol, United Kingdom.

In 2007, the first baby was born alive from an egg that had been matured *in vitro*, frozen, thawed and then fertilized at McGill Reproductive Center, Canada (6). A report was published from the Mc Gill Reproductive Center in Canada and the Maria Infertility Hospital in Korea on successful births after transfer of blastocysts that derived from matured oocytes by IVM (107). The first European baby was born after screening by using comparative genomic hybridisation (CGH) (6). The concept of mild treatment strategy that substantially reduces the risk of multiple gestation and overall costs was widely held (108). Pasquale Patrizio introduced a novel multi-gradient freezing technique for cryopreservation of the whole ovary that resulted in preservation of the normal ovarian architecture (109).

In 2008, a viable pregnancy was achieved for the first time in a 38 year old woman who carried a defective BRCA2 gene. Son Weon-Young and his colleagues recommended 38 *hr* interval between hCG injection and oocyte retrieval to increase *in vivo* and *in vitro* oocyte maturation rates (110). On December 2008, the Midland Fertility Services in the UK launched vitrification flash-freezing process. The first report, at Monash immunology and stem cell laboratories (MISCL), on DNA fingerprinting to identify the blastocyst of the origin for live births and that of gene expression profiles of biopsied trophectoderm could discriminate between viable and non-viable blastocysts (111). The first healthy twins were delivered after oocyte cryopreservation and bilateral ovariectomy for ovarian cancer (112). Rajo Devi Lohan and Omkari Panwar became the world's oldest mothers to give birth at the age of 70 years in India.

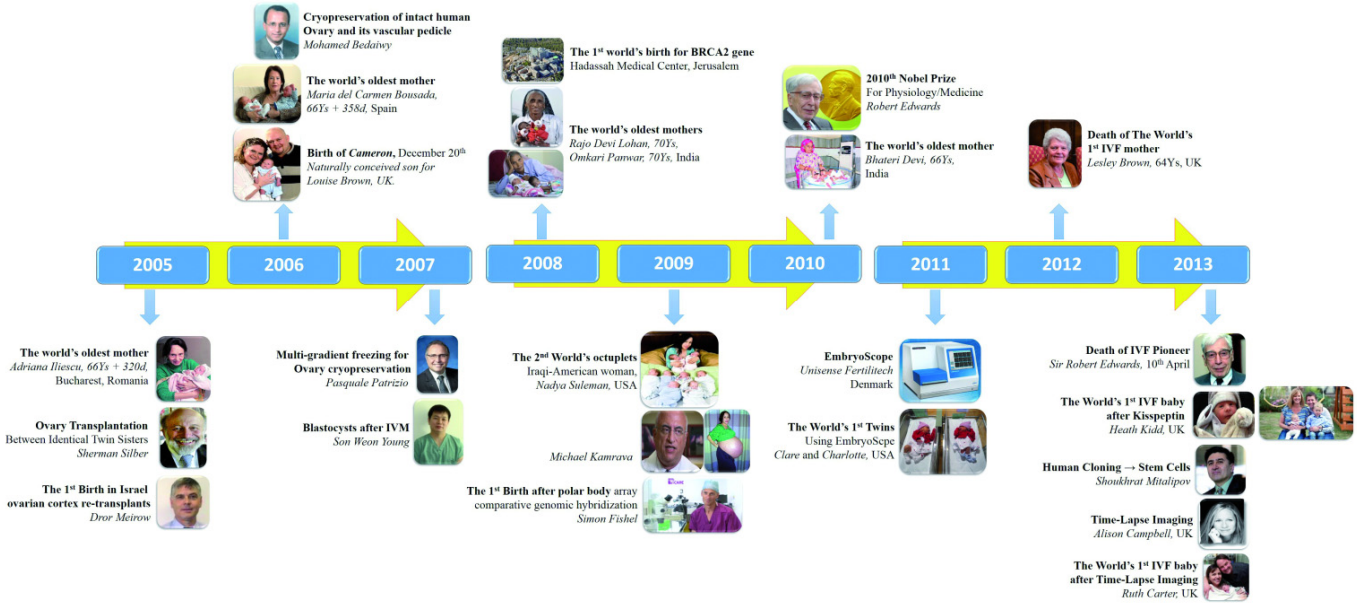

Figure 4. Timeline of major ART milestones (Year 2005 – Year 2013)

In 2009, octuplets (8 babies; 6 boys and 2 girls) were born to Nadya Suleman, a 33 year old Iraqi-American woman in California, USA (The second octuplets in the world). Her treating doctor Michael Kamrava had transferred twelve frozen embryos that left from her previous stimulated IVF cycles, which the medical board of California found it to be a "life-threatening practice" and withdrew his medical license. The first baby boy was born from vitrified oocytes in Australia (6). Cetrorelix acetate (LHRH antagonist) was approved by FDA for clinical use in IVF programs. Simon Fishel and his working group from CARE Fertility, Nottingham, reported a live birth after polar body array comparative genomic hybridization (113).

In 2010, the Midland Fertility Services in the UK confirmed the first successful pregnancy from vitrified blastocysts. Several minor changes to the 8th HFEA Code of Practice were incorporated into the print version of the Code on April 2010. The Nobel Assembly at Karolinska Institute, Sweden, offered the 2010 Nobel Prize in Physiology or Medicine to the British physiologist, Robert Edwards for his remarkable work in the field of in vitro fertilization. An Indian woman became the oldest mother in the world to have triplets at the age of 66 years. After being childless for 44 years of marriage, Bhateri Devi gave birth to two boys and a girl after IVF.

In 2011, the novel monitoring system for continuous observation of early embryo development around the hour (EmbryoScope®) was introduced by Unisense Fertilitech, USA. In December, Clare and Charlotte were the world's first twins born to Ed and Caroline Marks by use of the new Embryo Scpe® at Cleveland Reproductive Center, Ohio, USA.

In 2012, the family of Lesley Brown, the first world's IVF mother, confirmed her death at Bristol Royal Infirmary. In the same year, the world's five-millionth IVF baby was born.

In 2013, Professor Sir Robert Edwards, scientist and co-pioneer of IVF, passed away peacefully in his sleep on the morning of April 10th after a long illness. In April, the first baby was born, Heath at Hammersmith hospital, UK to Suzannah and Calum Kidd, from Hitchen, by using a new lower risk IVF treatment, Kisspeptin for induction of ovulation without the risk of ovarian hyper stimulation syndrome (a clinical trial NCT01667406 led by Professor Waljit Dhillo at Imperial College London). In May, a group of scientists led by Shoukhrat Mitalipov, a reproductive biology specialist at Oregon Health Sciences Universities (OHSU) published a report on a successful human cloning. The approach involved nuclear transfer from human fibroblasts to enucleated oocytes and resulted in viable embryos developing to the blastocyst stage. The researchers planned to obtain embryonic stem cell from these developed blastocysts for the purpose of therapeutic cloning. Alison Campbell, a senior British clinical embryologist in Manchester, introduced the novel Time-lapse imaging for early developing preimplantation embryos for clinical selection of healthy-looking embryos without the need for biopsy and preimplantation genetic screening (PGS) in cases with recurrent IVF failure (114).

Bottom of Form: In June, Ruth Carter, 42-year-old clinical psychologist, became the world's first mother to give birth to a girl at Liverpool's Women Hospital, UK after using the new system Early Embryo Viability Assessment (Eeva) implementing the Time-lapse imaging technique.

ART Expectations: The prospects hold promise for rapid evolution in ART. Advances in molecular medicine will help in mapping the Y chromosome. Males with testicular failure will be able soon to father their own genetic children. Future researches with oocyte maturation, culture media and endometrial receptiveness may allow immature oocyte retrieval with *in vitro* maturation to replace the conventional *in vitro* fertilization. Cryopreservation of human oocytes will provide an alternative to embryo cryopreservation with its ethical repercussions. Advances in Time-lapse imaging technique will increase IVF success rate and avoid the need for invasive and expensive preimplantation genetic diagnosis (PGD) to screen out abnormal embryos. New drugs such as GnRH antagonists hold promise of leading to a better ovarian stimulation, egg quality and implantation rates. The transfer of cytoplasm from younger donor oocytes into older oocytes may improve the viability of developing zygotes and give a better chance for older women to carry their own biological babies.

Conclusion

Advances in assisted reproductive technology for infertile couples were among the great medical successes of the last century. ART has made huge strides and fast progress towards finding suitable treatment options for each infertile couple. Costs and complexity of treatment have been reduced to

alleviate the stress and social troubles. Problems related to the risk of multiple pregnancy and the use of stimulated cycles are nearly solved and new techniques for management of severe male factor infertility and the detection of genetic anomalies in the embryo prior to transfer are being introduced. Further refinements of the techniques and modification of treatments will probably occur with ongoing use and practice.

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Conflict of Interest

I hereby declare that this work is carried out in accordance with the requirements of the University of Bristol Regulations and Code of Ethics for Research Programs. Furthermore, it is approved by the Research Review Board. Except where indicated by specific reference in the text, this work is my own and there was no contribution of any other authors. Any views expressed in the study are those of the author. The work was self-funded. I did not receive any financial funding or support from any person or institution. In addition, I state that I have no competing interests.

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