Editorial

Dilemmas in ART – Looking for solutions

Though the assisted reproductive technology (ART) has given hope to a large number of couples suffering from infertility, it has also hosted innumerable technical, ethical, legal, and social challenges for the future.

Advancements in the field have resulted in a landmark shift in the way physicians and the general population perceive infertility and related issues. The reproductive science is unswervingly challenging the society to re-evaluate the way in which human life, social justice, and claims to resulting genetic offspring are viewed. These issues will challenge the technology and legal organizations to modify existing laws to accommodate its unique situations.

We also need to devote resources and energies to identify and remove the environmental and physical causes of infertility. Prevention, education, and increased access to appropriate and cost-effective fertility care, including insurance coverage, are also imperative so that more families throughout the world are able to have children when they are ready safely.

HISTORY AND EVOLUTION OF *IN-VITRO* FERTILIZATION GLOBALLY

The beginning

The history of *in-vitro* fertilization (IVF) and embryo transfer (ET) dates back as early as the 1890s when Walter Heape, a professor, and physician at the University of Cambridge, England, who had been conducting research on reproduction in a number of animal species, reported the first known case of embryo transplantation in rabbits, long before the applications to human fertility were even suggested.

In 1934, Pincus and Enzmann,[1] from the Laboratory of General Physiology at Harvard University, published a paper in the Proceedings of the National Academy of Sciences of the USA, raising the possibility that mammalian eggs can undergo normal development *in vitro*. Fourteen years later, in 1948, Miriam Menken and John Rock[2] retrieved more than 800 oocytes from women during operations for various conditions. One hundred and thirty-eight of these oocytes were exposed to spermatozoa *in vitro*. In 1948, they published their experiences in the American Journal of Obstetrics and Gynaecology.

However, it was not until 1959 that the indisputable evidence of IVF was obtained by Chang[3] who was the first to achieve births in a mammal (a rabbit) by IVF. The newly ovulated eggs were fertilized, *in vitro* by incubation with capacitated sperm in a small Carrel flask for 4 h, thus opening the way to assisted procreation.

Professionals in the fields of microscopy, embryology, and anatomy laid the foundations for future achievements. The recent rapid growth of IVF–ET and related techniques worldwide are further supported by the social and scientific climate which favors their continuation.

Through the years, numerous modifications have been made in the development of IVF–ET in humans: refinement of fertilization and embryo culture media; earlier transfer of the embryo; improvements in equipment; use of a reduced number of spermatozoa in the fertilization dish, embryo biopsy among others.

The purpose of this introduction is to acknowledge those who initiated new steps in the development of the treatment protocols and techniques that we now use facilitating such simple and promising IVF–ET procedures.

Year	Discoveries
1961	Palmer from France described the first retrieval of oocytes by laparoscopy.[4]
1965	In 1965, Edwards *et al*. tried to fertilize human oocytes *in vitro* at Johns Hopkins Hospital in the USA.[5]Later, Monash research team reported the first IVF pregnancy in Melbourne, Australia[6] but resulted in early miscarriage.
1976	Menezo formulated the world's first B2 culture medium, known as "the French medium." This specific medium reflected the follicular, tubal, and uterine environments of the sheep, rabbits, and humans.[7]Steptoe and Edwards reported an ectopic pregnancy by transferring a late morulae/early blastocyst stage human embryo.[8]

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Year	Discoveries
1978	The first IVF birth occurred in Oldham, England on July 25, 1978.[9]On October 3, 1978, the birth of the world's second test-tube baby was announced by Dr. Subhas Mukerji in Calcutta. The news was widely reported in the media in India and to some extent abroad.[10]Lopata in Melbourne described the first cycle stimulated with Clomiphene Citrate.[11]
1979	Pez *et al*. started using ultrasound to track the growth of follicles. They showed an appreciable relationship between the echographic and laparoscopic observations.[12]
1980	Culture medium was introduced.[13]
1981	The delivery of first IVF baby, using hMG was announced by Howard and Georgianna Seegar Jones in the United States. Later, Wood *et al*. introduced a foot-controlled fixed aspiration pressure control.[14]Use of Clomiphene Citrate and hMG in the IVF treatment protocol was introduced.[15]LH assay (LH-SIR0) was developed by the Clamart group in France to detect the initial LH rise in plasma allowing accurate prediction of the ideal time for the retrieval of oocytes.[16]
1982	The first IVF birth in Sweden[17] and "test tube baby" (Twin pregnancy) in Austria were reported.[18]The first demonstration on the use of GnRH agonists to eliminate premature luteinization and control ovarian stimulation was given by Fleming *et al*.[19]The need for a delay between oocyte collection and insemination was reported to allow oocytes to complete maturation.[20]Lenz and Lauritsen demonstrated trans abdominal transvesical oocyte aspiration using an ultrasound-guided needle.[21]
1983	The Monash IVF team achieved the first pregnancy in a woman without ovaries by using donor eggs creating artificial menstrual cycles and a special hormonal formula for the first 10 weeks of pregnancy.[22]Monash IVF team reported on the birth of the first frozen embryo baby.[23]Maturation and fertilization of morphologically immature human oocytes in an IVF was reported by Veeck *et al*.[24]First successful delivery following egg donation.[25]Casper *et al*. were the first to describe the use of low-dose hCG to support the luteal phase in ART cycles.[26]World's first IVF triplets reported by Christopher Chen.The first report on human pregnancy following cryopreservation, thawing, and transfer of an eight-cell embryo.[27]
1984	First surrogacy ET baby born in California.First report on pregnancy following trans-laparoscopic Gamete intrafallopian Transfer procedure.[28]The first report on pregnancy following IVF and egg donation in a woman with primary ovarian failure.[29]An unusual report of the possibility that abnormal spermatozoa could be enriched and give rise to healthy babies.[30]The first publication demonstrating human chorionic gonadotropin secretion by the human embryo was published.[31]The world's first IVF quadruplets were born on January 6, 1984, in Melbourne.
1985	Human pregnancy by IVF using sperm aspirated from the epididymis.[32]First report of the use of abdominal ultrasound guidance for ET.[33]The first reported birth after replacement of hatching blastocyst cryopreserved at the expanded blastocyst stage.[34]In 1985, Quinn and Warnes published a formula entitled human tubal fluid that mimics the *in-vivo* environment to which the embryo is exposed.[35]Transabdominal ultrasound-guided ET.[36]
1986	First report on pregnancy after trans-laparoscopic zygote intrafallopian transfer.[37]Szollosi *et al*. described the microstructures of the human oocyte, which became known as "oocyte dysmorphia."[38]
1987	Fertilization of human oocytes by microinjection of a single sperm under the zona pellucida.[39]
1988	First two babies born after epididymal sperm aspiration for men with congenital absence of the vas deferens and naming of the technique micro epididmyl sperm aspiration were reported.[40]Pregnancy was obtained from micromanipulation using

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Year	Discoveries
	zona drilling or mechanical partial zona dissection.[41]The first preclinical evaluation of pronuclear formation by microinjection of human spermatozoa into human oocytes.[42]
1989	First report on the use of laser techniques in the field of assisted reproduction for application in gametes or embryos.[43]Gonen *et al*. in Toronto pioneered the use of ultrasound for endometrial quality (thickness and pattern) related to IVF pregnancy.[44]Embryo biopsy technique was developed in mice by Wilton and Trounson.[45]
1990	The first successful human cleavage-stage embryo vitrification followed by a successful delivery.[46]First report of assisted hatching in human embryos.[47]The first report on polar body biopsy, transfer of the embryo, and achieving pregnancy.[48]
1991	*In-vitro* maturation (IVM) in an unstimulated cycle resulted in pregnancy in a donor oocyte program.[49]Navot *et al*. confirmed that the age-related decline in female fertility is attributable to oocyte quality.[50]ET catheter is visualized by vaginal ultrasound.[51]
1992	Assisted zona hatching was introduced in IVF programs to breach the zona pellucida and promote the natural process of hatching.[52]Report of the first pregnancy after intracytoplasmic sperm injection (ICSI) in Brussels.[53]First two births from the replacement of frozen embryos produced with epididymal sperm.[54]Report on using Erbium YAG laser for the micromanipulation of oocytes and spermatozoa.[55]
1993	The second-term pregnancy after ICSI reported by a group in Sweden.[56]Confirmation that men with congenital absence of the vas deferens have cystic fibrosis mutations which can be transmitted to the offspring.[57]First report on the use of testicular sperm extraction (TESE) and ICSI.[58]
1995	Pregnancies after TESE and ICSI in non-obstructive azoospermia.[59]Birth after blastocyst development from IVM oocyte plus ICSI plus Assisted Hatching.[60]
1996	The Valencia group reported on the first pregnancy employing cryopreserved testicular sperm following IVF–ICSI.[61]Discovery that some men with severe oligoasthenospermia have deletions in the Y-chromosome.[62]Casper *et al*. were the first to demonstrate and introduce the use of the hypo-osmotic swelling test for the selection of immotile sperm for ICSI.[63]
1997	Sun *et al*. described the use of terminal deoxynucleotidyl transferase nick-end labelling for the detection of DNA fragmentation in sperm and correlation with IVF outcome. They showed the almost uniform presence of deoxy ribonucleic acid (DNA) fragmentation in round spermatids as the explanation for the failure to achieve pregnancy with these immature gametes.[64]First births of babies from frozen oocytes following the use of ICSI: Porcu *et al*., birth of a healthy female after ICSI of cryopreserved human oocytes.[65]
1998	Gardner introduced sequential media and blastocyst transfer which now greatly assists in the move to single ET.[66]Births after intracytoplasmic injection of sperm obtained by testicular extraction from men with non-mosaic Klinefelter's syndrome.[67]
1999	First unaffected pregnancy using preimplantation genetic diagnosis (PGD) for sickle cell anemia.[68]Birth following vitrification of human oocyte.[69]Chian *et al*. demonstrated that hCG priming prior to immature oocyte retrieval in women with polycystic ovaries increases the maturation rate and produces high pregnancy rates of 40% per IVM started cycle.[70]
2000	Oktay and Karlikaya were the first to report on ovarian tissue transplants after frozen storage.[71]The development of a completely chemically defined protein-free embryo culture medium and the births of the first batch of babies generated from the fertilization of eggs collected and inseminated in the said protein-free medium using spermatozoa also prepared in the same protein-free medium in both conventional IVF and ICSI.[72]

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Year	Discoveries
2001	Birth of an infant from cryopreserved embryos (zygotes) produced by IVM oocytes derived from an unstimulated patient with polycystic ovarian syndrome.[73]
2002	First, live birth following blastocyst biopsy and PGD analysis.[74]First clinical application of comparative genomic hybridization and polar body testing for PGD of aneuploidy.[75]
2003	First, live birth after ovarian stimulation using a chimeric long-acting human recombinant follicle-stimulating hormone agonist (recFSH-CTP) for IVF.[76]Implantation of the human embryo is the limiting factor in the success of IVF. Barash *et al*. showed an increasing implantation rate following endometrial injury, performed by Pipelle curettage as a simple outpatient procedure.[77]Normal birth after microsurgical enucleation of tripronuclear human zygotes.[78]
2004	Donnez reporting on the first Live birth after orthotopic transplantation of cryopreserved ovarian tissue.[79]Gardner *et al*. performed the world's first prospective single blastocyst trial, which showed the feasibility of single blastocyst transfer and in keeping high pregnancy rates.[80]The first preimplantation human leukocyte antigen matching for stem-cell transplantation to an affected sibling.[81]First report on oocyte cryopreservation to save fertility in patients with cancer.[82]
2006	First successful pregnancy after PGD for aneuploidy screening in embryos generated from natural-cycle IVF combined with IVM, achieved at the McGill Reproductive Centre.[83]Cryopreservation of intact human ovary with its vascular pedicle.[84]
2007	A novel multigradient freezing technique for the cryopreservation of the whole ovary, thawing the ovary resulted in normal ovarian architecture and no damage to the vascular wall.[85]The first report of DNA fingerprinting to identify the blastocyst of origin for live births and that gene expression profiles of biopsied trophectoderm can discriminate between viable and nonviable blastocysts.[86]Cryopreserved oocytes in patients with cancer: first ever birth of healthy twins after oocyte cryopreservation and bilateral ovariectomy.[87]
2009	Fishel *et al*. from CARE Fertility, Nottingham, reported about a live birth after polar body array comparative genomic hybridization.[88]Prof. Laufer at the Hadassah Medical Centre in Jerusalem reported on a viable pregnancy achieved in a woman who carries the defective BRCA2 genes after IVF embryos were tested and implanted.[89]
2014	First report on pregnancy and live birth from frozen-thawed embryos obtained from fresh oocytes, harvested from surgically removed ovary, after IVM and ICSI in a patient with advanced ovarian cancer.[90]

Evolving assisted reproductive technology in modern era

In 1978, the world witnessed the birth of the first "test tube baby."[9] Since then, there have been explosive advances in ARTs. Current optimizations surrounding the delivery of IVF including the utilization of minimal stimulation protocols and gonadotropin-releasing hormone (GnRH) agonist cycle triggers are being increasingly utilized to maximize patient safety. Modifications, such as IVM and cryopreservation seen in the embryology laboratory, continue to improve pregnancy rates. Concurrent with these advancements in IVF has been the emergence of related technologies, such as embryonic genetic diagnostic and screening and oocyte freezing, which potentially has broad applications for both fertile and infertile couples. Another technology such as time-lapse imaging may be a powerful tool to select embryos best suited for uterine transfer in IVF cycles. As these relevant applications of ART become increasingly utilized, it is incumbent on the society to ensure that these resources are made available in a morally responsible and equitable manner.

Many aspects of clinical practice in ART raise challenging issues, controversies, and dilemmas for service providers:

(1) *Surrogacy*: Central concern surrounding the use of surrogates and gestational carriers is the possibility that financial pressures could lead to the exploitation and commercialization of the service.[91] Additionally, the rights of the surrogate or gestational carrier to not relinquish the infant after delivery are not well described.[92]

At present, issues surrounding the individual rights, exploitation, and citizenship of the resulting offspring of international gestational carriers are largely unresolved internationally.[93] The proposed Indian Surrogacy (Regulation) Bill, 2016, inter alia, provides) to allow ethical altruistic surrogacy to the intending infertile Indian married couple only between the age of 23–50 years and 26–55 years for female and male respectively; (c) the intending couples should be legally married for at least five years and should be Indian citizens to undertake surrogacy or surrogacy procedures and prohibits surrogacy for international patients and commercial surrogacy.[94]

One need to debate keeping the individual need of infertile couple who do not have an option other than surrogacy and do not have one in family, whether banning the commercial surrogacy is justified or it requires a regulation.

(2) *Donor conception*: It would be a challenging issue in coming decades. With growing information and technology, the clientele may not agree for donor cycles. Stimulation protocols and stem cells would play an important role.

(3) *PGD*: In the near future, with refinements in microarray technology and the definition of genetic sequences associated with certain physical characteristics, it is conceivable that specific physical or mental characteristics may be evaluated to guide the decision as to which embryos to transfer.[95]

(4) *Cryofrozen embryos*: Embryo freezing is a robust and routine part of the IVF process, and approximately 60% of patients end up with some embryos in storage. This process provides patients with a "back-up" in case the initial fresh ET does not result in a pregnancy and if patients come back after few years to have a second child. Cryofrozen embryos of patients with single child norms would flood our cryobiology laboratories with challenges in disposing them.

However, the legal issues of whether an unborn is entitled to any rights, and if so what they are, have appeared in several different areas of law and need to be resolved.[96]

(5) *Fertility preservation*: Fertility preservation for patients with cancer using IVM, oocyte Vitrification, and the freezing of intact human ovaries with their vascular pedicles have also been reported.[97] However, this technology at present, in many countries, is only available to those with financial means. This poses ethical and social issues that will certainly see more attention in the future.

(6) *Deteriorating male factor*: Deteriorating male factor is the reduction of normal sperm count, motility, and morphology, and this increase in the deterioration of male factor and fertility fecundity has been a concern for ages. Various lifestyle factors such as tobacco smoking, chewing, and alcohol use as well as exposure to toxic agents might be attributed to the risk of declining semen quality and increase in oxidative stress and sperm DNA damage.[98] To achieve an acceptable pregnancy rate in such cases, ISCI is the only option. This could be a big challenge in the countries which do not believe in sperm donation. Stem cell biology would play a major role in such cases.

(7) *Posthumous use of gametes*: Posthumous use of gametes occurs when the surviving partner of a person dying on a battlefield or due to other reasons wishes to obtain and use their gametes (sperm) to conceive a child to continue his lineage. It is not permitted in most of the countries. It is important to refer to the law in each state/territory, and/or National Health and Medical Research Council, to determine whether the removal and or use of gametes after a person dies is permissible, and if so the circumstances in which this may occur because posthumous use of gametes can pose legal issues such as legitimacy of child born, inheritance rights of a child, and lifelong psychosocial implications.[99]

(8) *Legal issues*: Because of the rapidly evolving nature of the ART, legislation is often unable to keep pace and address all of the ethical and legal issues that are constantly emerging in the field. It is therefore incumbent upon physicians to continually monitor these issues and ensure that ART technologies are offered and delivered in a manner that balances patient care with social and moral responsibility.

CONCLUSION

ART as is a field that is dynamic and ever-changing. As technologies continue to proliferate, ethical and social challenges multiply, with complex questions of justice, rights, and conflicting principles continually rising. As an evolving society, we are long overdue to discuss these issues and to guard against leaving them solely in the province of researchers and reproductive medicine specialist.

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