Lead Article

Which Way is Infertility Treatment Heading? – A Review on the Latest Techniques in Assisted Reproductive Technology

Prathiba Govindaiah*, Jyothi, Devika Gunasheela

Abstract

Across the world, among millions of couples who fail to conceive, some seek medical help and undergo treatment, some resign to their fate, while others resort to adoption. The incidence of infertility in India is estimated to be between 10% and 15%. The birth of the world's first baby through the in vitro fertilization (IVF) technique opened up a new horizon in the management of infertility.

The purpose of this review article is to provide information on latest techniques and advances in assisted reproductive technology (ART). Preimplantation genetic screening and fertility preservation are the current burning issues in ART.

A systematic search of MEDLINE, Cochrane Library, PubMed, and reference list of articles on ART was conducted and is summarized in this article.

The techniques used in IVF continue to evolve as we strive to improve success rates while minimizing multiple pregnancies. However, we should all remain cognizant that currently, live birth rates generally do not exceed 50% per stimulated IVF cycle, even in young women with good prognosis, and in older women, success rates are dramatically lower.

*Correspondence
Dr Prathiba Govindaiah
Gunasheela Surgical and Maternity Hospital
No. 1, Dewan N Madhava Rao Road
Basavanagudi, Bangalore 560 004
Karnataka, India

E-mail: prathiba_g@hotmail.com

Introduction

Louise Brown is the world's first baby to be born through in vitro fertilization (IVF), in 1978, at Oldham in England. It was the determination and dedication of Patrick Steptoe and Robert Edwards, together with Jean Purdy, that led to the first IVF baby. This opened a new exciting horizon in the management of infertility and brought a ray of hope to millions of childless couples, worldwide.

In the early years, when Edwards was working at the National Institute for Medical Research, London, he conducted some of the earliest work on in vitro

maturation (IVM) of human oocytes using small slices of human ovaries, provided by a gynecologist in London. Steptoe presented papers on laparoscopic recovery of preovulatory human oocytes from follicles, under direct vision. During their initial years, none of their first 40 patients became pregnant, until, in 1976, they achieved the first pregnancy, which was, unfortunately, ectopic. Edwards and Steptoe had performed 102 embryo transfers, including the ectopic pregnancy, which had failed. Nevertheless, they succeeded in their 103rd embryo transfer, where Ms Leslie Brown, who was referred to Steptoe for infertility treatment, conceived. This pregnancy was achieved in a natural-cycle IVF, with one oocyte collected and fertilized, and the embryo transferred at the 8-cell stage. The birth of Louise Brown heralded "the baby of the century." In 1979, Edwards and Steptoe were successful with their work on the second IVF baby, Alastair Macdonald, who was the world's first boy conceived through IVF.[1]

Infertility, although not life threatening, causes intense mental agony and trauma that can only be best described by infertile couples themselves. There are no detailed figures on the prevalence of infertility in India; however, a multinational study performed by the WHO shows that the incidence of infertility in India is between 10% and 15%. In a population of 1000 million Indians, an estimated 25% (250 million individuals) may be conservatively estimated to be attempting parenthood at any given time. By extrapolating the WHO estimate, in the country, approximately 13 to 19 million couples are likely to be infertile at any given time.

Brief History of IVF in India

Across the world, among millions of couples who fail to conceive, some seek medical help and undergo years of treatment, some resign to their fate, while others adopt children. Couples go to any extent to fulfill the dream "my own child, in its full meaning, is my own child, my own blood." The world's second and India's first IVF baby, Kanupriya, alias Durga, was born on October 3, 1978, in Kolkata, through the efforts of Dr Subhas Mukherjee and his 2 colleagues.

India's first officially and scientifically documented IVF baby, Harsha, was born on August 6, 1986, in Mumbai, through the collaborative efforts of the Indian Council of Medical Research's (ICMR) Institute for Research in Reproduction and the King Edward Memorial (KEM) Hospital. This work was executed after being approved by the Scientific Advisory Committee of the ICMR's Institute for Research in Reproduction and the Ethics Committee for Human Experimentation of the KEM Hospital.

Dr Sulochana Gunasheela, a pioneer in South India in the field of Reproductive Medicine, was the second to deliver a female baby through IVF in 1978. She was an academician of the highest caliber; her sincere commitment to reproductive medicine was truly a benchmark in history. Her intelligence, vision, organization, and caring for others were exemplary.

Roadblocks That Led To Advancements in IVF

Fertilization with IVF was often unsuccessful, particularly when semen samples had a low sperm count and poor functional characteristics.

Attempts were first directed to increase the number of oocytes and ensure their nuclear maturity by individualizing superovulation protocols. Another approach was to promote the interaction between sperms and oocyte by increasing the number of sperm cells in the inseminating medium, sperm processing with density gradient, and removing the cumulus oophorus cells.

However, these efforts improved fertilization rates only in cases of mild impairment in the semen parameters. For severe sperm dysfunctions, more aggressive methods were used.

Advances in Follicle Stimulation

The concept of controlled ovarian hyperstimulation in the context of assisted reproductive technology (ART) was pioneered by Howard and Georgeanna Jones in the early 1980s.[2]

Gonadotropins

Gonadotropins compose one of the cornerstones of infertility management and have been used extensively over the last 3 decades.

Gonadotropins extracted from the urine of postmenopausal women have traditionally been used to stimulate folliculogenesis in the treatment of infertility and in ART. The products, such as human menopausal gonadotropin, comprise not only a mixture of follicle-stimulating hormone (FSH), luteinizing hormone (LH), and human chorionic gonadotropin (hCG) but also other biologically active contaminants such as growth factors, binding proteins, and prion proteins.

There are 3 generations of gonadotropin preparations:

a. First generation, which is derived from human urine
b. Second generation, which is purified urinary gonadotropins
c. Third generation, which is highly purified

The production of human gonadotropin preparations requires collection of a huge volume of postmenopausal urine. For this, urine is collected from postmenopausal women, leading to batch-to-batch inconsistency; there is also a theoretical risk of contamination with urinary proteins.[3-5] In the last few years, with recombinant DNA technology, it has been possible to produce human FSH in vitro without the need for extraction from human fluids.

The advantages of recombinant preparations are high purity, unlimited supply, batch-to-batch consistency, and no risk of contamination by urinary proteins.

Injectable corifollitropin alfa

Injectable corifollitropin alfa, a novel hybrid molecule with sustained follicle-stimulating activity, is an exciting development that may decrease the treatment burden in women undergoing IVF. Corifollitropin alfa is a recombinant molecule constructed by coupling the carboxy terminal peptide of the β-subunit of hCG to the β-subunit of FSH.[6] Its pharmacokinetic profile initiates and sustains multifollicular growth with a single injection, by maintaining FSH level above the threshold required for the first 7 days of controlled ovarian stimulation for IVF. This can replace 7 daily injections of recombinant FSH.[6,7]

Gonadotropin-releasing hormone analogues

There are 2 types of analogues: gonadotropin-releasing hormone (GnRH) agonist and GnRH antagonist, both of which are used to suppress untimely LH surge but have different modes of action.

GnRH agonist

Earlier, premature LH surges occurred in 20% to 50% of stimulated cycles, leading to high cycle cancellation rates or poor cycle outcomes.[8] The advent of GnRH agonists, in the early 1980s, to prevent LH surges was a breakthrough in assisted reproduction, as it allowed the clinician to take control of the stimulated cycles and more flexibility in scheduling oocyte retrieval.[9]

Agonists bind to and stimulate the GnRH receptors, but ultimately cause internalization and depletion of receptors (downregulation), hence suppressing the LH surge.

GnRH antagonists

The development of third-generation GnRH antagonists revolutionized the field of reproductive medicine. Antagonists block the GnRH receptor by competitive inhibition and prevent LH surge faster than the agonists do. GnRH antagonists have become the most popular GnRH analogues as they shorten the treatment cycle and agonist trigger for ovulation can be given while using GnRH antagonist to prevent ovarian hyperstimulation syndrome (OHSS).

Advances in Laboratory Techniques

Zona drilling

Zona drilling involved the creation of a circumscribed opening in the zona by application of an acidic solution through a fine-glass micropipette.[10] This often allowed more than one sperm to enter the perivitelline space,

but the acidic pH of the medium was demonstrated to be deleterious to the egg.

Partial zona dissection

Later, a new technique called partial zona dissection was used, which involved slicing the zona with a glass needle before insemination.[11] This procedure resulted in moderate increase in fertilization rates but increased polyspermy.

Subzonal sperm injection

Subzonal sperm injection, another approach developed at that time, involved the mechanical insertion of a sperm directly into the perivitelline space. It is more effective than the other manipulative techniques.[12]

Intracytoplasmic sperm injection

Intracytoplasmic sperm injection (ICSI) involves direct insertion of a single spermatozoon directly into the oocyte, bypassing all the preliminary penetration/fusion steps of fertilization. The first human pregnancies with ICSI occurred in 1992; since then, thousands of babies are born through ICSI.[13,14] ICSI can be adopted when the sperm sample is extremely poor or after failure of fertilization with IVF. Patients having nonobstructive azoospermia can be successfully treated with testicular sperm extraction (TESE)-ICSI if viable "mature" spermatozoa are obtained. ICSI is preferred in serodiscordant couples because it virtually avoids the interaction of oocytes with semen, thereby reducing the risk of viral exposure.[15] Reassuringly, no seroconversions have been reported after assisted reproduction in discordant couples.[16]

Sperm Selection Techniques

Intracytoplasmic morphologically selected sperm injection

Intracytoplasmic morphologically selected sperm injection (IMSI) was introduced in 2001. It is a more sophisticated form of ICSI, whereby, before injection, the spermatozoon is selected at higher magnification. The spermatozoon is evaluated for fine integrity of its nucleus, thus ensuring that a normal spermatozoon with a vacuole-free head is injected. Furthermore, Bartoov et al introduced motile-sperm organelle morphology examination.[17] At high magnification, the fine nuclear morphology of motile spermatozoa is examined in real time, using the inverted light microscope equipped with high-power differential interference contrast optics.

Earlier, the advantages of IMSI were shown in case–control studies, mainly among patients in whom conventional ICSI failed repeatedly,[18-22] according to a review by Nadalini et al.[23] No difference was found in oocyte fertilization rates between ICSI and IMSI. It was not clear if embryo development improved with IMSI.[18-22] However, significantly higher implantation and pregnancy rates and significantly lower abortion rates were reported after IMSI.

Randomized controlled trials are scarce,[24-27] are sometimes underpowered, and have been conducted only in cases of male factor infertility[24,25] or in unselected infertile populations.[26,27]

As IMSI does not seem to be effective in unselected patients undergoing ART, relevant indications for its use need to be defined. Evidence suggests a higher clinical pregnancy rate with IMSI in cases of severe male factor infertility.

Microdissection testicular sperm extraction

Microdissection TESE is a relatively new technique that improves the chance of retrieving sperm from an individual with nonobstructive azoospermia. This technique involves using an operating microscope to identify microscopic foci of spermatogenesis within the testicular parenchyma rather than using the traditional random biopsy approach.

Sperm retrieval rates of up to 63% have been reported with this new technique compared with about 45%

with conventional open or needle biopsy.[28] It has also been reported that sperm retrieval using microdissection TESE was successful in up to 69% of men with nonmosaic Klinefelter syndrome and in 32.4% of men with Sertoli cell–only syndrome on initial testicular biopsy.[29,30]

Despite a more prolonged operating time and extensive dissection, reported short-term and long-term complication rates are lower compared with conventional techniques. However, it is still recommended that these men undergo regular follow-up for their hormonal profile to detect subsequent hypogonadism.[29,31]

In the Laboratory

The live birth rate currently expected through IVF is possible because of the major advances in embryology.

In vitro maturation

IVM is the ability to mature oocytes in a laboratory environment; it is another advanced technology that is currently under focus. IVM is the practice of retrieving immature human oocytes, which then undergo the transition (maturation) from prophase I to metaphase II, including extrusion of the first polar body, in vitro.[32-34]

Since Pincus and Enzmann[35] described IVM of rabbit oocytes in 1935, it has been the primary method of breeding of rabbits and hamsters through IVF. Some of Robert Edwards' earliest work involved IVM of human oocytes from small slices of human ovaries, provided by a gynecologist in London. IVM of human oocytes was first reported in 1965, and a successful pregnancy and delivery was reported in 1989.[36]

Over the past decade or so, IVM protocols have evolved, including priming with hCG, FSH, and/or LH and making specific changes in the IVM oocyte culture media.[37-41]

IVM can be performed in the following cases: women at risk of OHSS, those with PCOS or polycystic ovary–like ovaries, those with estrogen-sensitive cancers, and those who require rapid fertility preservation before beginning potentially gonadotoxic treatments.[42,43] The procedure is cheaper than IVF as it does not involve expensive gonadotropin injections.[44,45]

However, despite these advantages, the maturation rate and the developmental potential of embryos derived from oocytes matured in vitro are significantly lower than those of oocytes matured in vivo. Although the pregnancy rate following IVM is slightly lower than that with conventional IVF, several recent reports regarding the pregnancy rates following improvements in clinical protocols and culture conditions are promising.

A 2-year study on IVM versus IVF in patients with PCOS was conducted at Gunasheela IVF Centre from June 2013 to June 2015. The reported fertilization rates were 68.7% versus 74.8% and clinical pregnancy rates per embryo transfer were 48% versus 67.4%, respectively.

Time-lapse imaging

Evaluation of embryos in vitro has improved greatly over the past 2 decades. Classical embryo assessment has been supplemented by the evaluation of several additional morphologic characteristics that allow prediction of the developmental potential of an embryo and the probability of achieving pregnancy among infertile couples. There is a well-documented close correlation between morphologic appearance and developmental stage of the embryo at given time points and developmental competence (ALPHA and ESHRE, 2011).[46]

Time-lapse imaging of the developing embryo has provided embryologists more morphologic observations for assessing embryo quality.[47-50] An automated time-lapse monitoring system with continuous embryo surveillance provides comprehensive data on embryo kinetics. The dynamic nature of cell cleavage and embryo development is well known, as demonstrated with fragmentation, evenness of blastomeres,

and appearance and disappearance of pronuclei and nuclei,[49] along with increase in the number of blastomeres over time due to cell divisions.

The inherent limitation in evaluating a dynamic process by a few snapshots at discrete time points is reflected in the recent observation that the result of embryo scoring can change markedly within few hours.[51] Frequent evaluation outside the incubator enables the assessment of timing of events; it also exposes the embryos to undesirable changes in temperature, humidity, and gas composition. Therefore, a conflict exists with the use of traditional incubators—between the need to obtain a detailed picture of embryo development and the risk of compromising stable culture conditions.

We can overcome this limitation by using time-lapse monitoring, with cameras incorporated in the incubation chamber. This offers the benefit of stable culture conditions during inspection, making it a promising clinical method of extending and refining morphologic evaluation to include dynamic parameters.

Embryo viability and implantation potential can be estimated with quantitative measurements of the timing of embryo development, that is, morphokinetic parameters.[49,52] These recent findings suggest that morphokinetic parameters may supplement current embryo selection methods for possibly increasing pregnancy rates with IVF.

Blastocyst culture

Extended embryo culture or "blastocyst culture" involves culturing embryos in the laboratory until day 5 or 6 after fertilization rather than the traditional day 2 or 3. The success of blastocyst culture is because of the recognition of vastly different nutrient requirements compared with the cleavage-staged embryo, but it highly depends on meticulous quality control within the laboratory. Without excellent laboratory techniques, the advantage of extended culture is lost because of poor embryo development. The major advantage of blastocyst culture is that it generally allows better selection of a single, best-quality embryo (single embryo transfer), based on morphologic assessment of the embryo, thus improving the implantation rate per embryo transferred.[53] One disadvantage of blastocyst culture is the increased rate of monozygotic twins (2%–4%) compared with that in case of cleavage-staged embryos (0.5%).[54]

Embryo cryopreservation

Cryopreservation of IVF embryos was achieved relatively early in 1983, when the first pregnancy resulting from a cryopreserved embryo was reported.[55]

Earlier, cryopreservation of excess embryos was by the "slow freezing" method, which took approximately 2 to 3 hours. This has now been replaced by a technique of rapid freezing called "vitrification."

Vitrification is a technique that has become widespread throughout IVF laboratories. It involves the use of concentrated cryoprotectant solutions with rapid cooling, allowing samples to reach low temperatures in a glassy state that has the molecular structure of a viscous liquid rather than crystalline, hence avoiding intracellular ice crystal formation.

Compared with the conventional "slow freezing" method, vitrification is faster, easier, and does not require sophisticated equipment. Vitrification has also been associated with improved cryosurvival of embryos (> 90%), particularly blastocysts; higher implantation rates; fewer miscarriages; and higher live birth rates.[56]

Oocyte cryopreservation

Although embryo cryopreservation is the most established method of fertility preservation,[57] oocyte cryopreservation now represents the most apt option for single reproductive-age women in need of delaying childbearing for any reason. Owing to challenges in the structure of the oocyte and the optimization of freezing methods, it took more than 2 decades for oocyte cryopreservation to evolve into a technique with acceptable clinical success rates. This transition was made possible by 3 important achievements: improvements in cryoprotectants, introduction of vitrification, and use of ICSI.[58-61]

In addition, oocyte cryopreservation is likely to become a useful adjunct to routine IVF in various clinical scenarios:

1. In cases of unavailability of sperm at the time of egg retrieval[62,63]
2. In the presence of ovarian hyperstimulation syndrome[64]
3. In poor responders[65,66]
4. In patients at risk of losing their fertility potential because of genetic abnormalities such as Turner syndrome[67]
5. In couples who do not wish to cryopreserve supernumerary embryos for ethical, legal, or religious concerns[68]
6. In women who are prescribed immunosuppressants for conditions such as systemic lupus erythematosus, certain hematologic diseases, and renal diseases
7. In the presence of endometriosis
8. In working women who defer child bearing for pursuing higher achievements in their career and thus prefer to freeze oocytes and use them when they are ready to become a mother

The most applicable indication for oocyte cryopreservation that has now become a reality[69,70] is the establishment of donor oocyte banks. However, in addition to all these indications, elective oocyte cryopreservation for deferring childbearing remains the most debatable and, surprisingly, the most common indication for oocyte cryopreservation.

The first live birth with oocyte cryopreservation was reported in 1986, with slow freezing,[71] but because of its very low success rates, there were only 5 live births reported over a decade. Over the recent years, oocyte cryopreservation, especially with vitrification, has proven to be an efficient technique, resulting in pregnancy outcomes similar to that of IVF with fresh oocytes.

Technologies Evaluating Embryos

Determining the optimal embryos in a given IVF cycle for uterine transfer remains a significant challenge in ART. Traditionally, embryo morphology has been the vastly used method for determining embryo quality. Numerous grading systems have been developed to grade embryo morphology.[72,73] However, embryo morphology alone has been shown to be a suboptimal indicator to determine embryos having normal chromosomal status (euploidy) or optimal implantation potential.[72,73] Hence, a variety of modalities have been developed to evaluate embryo quality both directly and indirectly.

Preimplantation genetic diagnosis

Preimplantation genetic diagnosis (PGD) and preimplantation genetic screening (PGS) involve obtaining one or more cells from the developing embryo and evaluating the genetic composition of the cells for a specific genetic defect known to exist in the parents (PGD) or to screen for the presence of embryo aneuploidy (PGS).[74,75] The results then guide the decision for selecting appropriate embryos for the transfer.[74] In 1990, Handyside et al reported the first pregnancies using this procedure in 2 couples known to be at risk for transmitting adrenoleukodystrophy and X-linked mental retardation.[76] PGD has helped to successfully detect the presence of numerous genetic disorders, such as sickle cell anemia and retinoblastoma.[77,78]

Advancements in genetic medicine show that medical conditions are linked to specific genetic markers.[79] The expansion of genetic medicine in the future will certainly broaden the uses of medically indicated PGD. It is also used for human leukocyte antigen (HLA) typing.[80] This technology is generally used in cases when a child is affected by a particular disorder that could benefit from some type of human tissue transplantation, for example, a child with leukemia who requires bone marrow transplantation. In these cases, PGD has been used as a modality to ensure that the next child that the couple conceives will be HLA compatible with their existing child having the particular illness. This practice is relatively uncommon, but has generated considerable debate regarding the ethics of HLA typing with PGD.[81] In couples who had recurrent pregnancy loss (RPL) and a documented balanced reciprocal/Robertsonian translocation or chromosomal inversion in one or both of them, PGD coupled with IVF has been shown to have some benefit in improving pregnancy and live birth rates.[82-84]

Traditionally, florescence in situ hybridization (FISH) has been used in PGD of translocations. FISH identifies both balanced and unbalanced chromosomal translocations. In recent years, microarrays are being increasingly used. Microarrays evaluate all 23 pairs of chromosomes, including the chromosomes involved in structural aberration, for aneuploidy and other chromosomal imbalances.[83-85]

Preimplantation genetic screening

Chromosomal aneuploidy is believed to be the single greatest causal factor for pregnancy failure.[86] PGS is the practice of evaluating cells from a developing embryo to identify aneuploidy. PGS was introduced as a technology that could greatly improve pregnancy efficiency in women at risk for miscarriage who are undergoing IVF, including couples who had RPL or women of advanced maternal age.

PGS for aneuploidy was first performed with the FISH technique for approximately 5 chromosomes using a cell taken from the cleavage-staged embryo. FISH then progressed to the routine evaluation of 9 to 14 chromosomes.[74] This PGS methodology resulted in suboptimal results, leading to criticism.[68] There are several reasons why PGS using FISH at the cleavage stage did not produce optimal results. Primarily, FISH only evaluates a portion of possible aneuploidies (9–14 of 23 pairs of chromosomes[56]); therefore, it does not detect many chromosomal aneuploidies. Furthermore, high rates of aneuploidy/euploid mosaicism are known to exist in cleavage-staged embryos.[87,88]

The most common methods of performing 23-chromosome PGS is with microarray technology, using single nucleotide polymorphism or comparative genomic hybridization (CGH) platform[75] and, more recently, next-generation sequencing. Other forms of 23-chromosome PGS evaluation include CGH on metaphase chromosomes and real-time polymerase chain reaction.[89,90] PGS evaluating 23 chromosomes and day 5 biopsy has resulted in excellent pregnancy rates in specific patient populations such as couples who had RPL.[91-93]

The recent technological advances in preimplantation genetic testing suggest that there will be a wider implementation of PGD/PGS in the future. However, PGD and PGS require close collaboration between obstetricians, fertility specialists, IVF laboratory staff, and geneticists.

Fertility Preservation

The survival rates for young patients with cancer are increasing with the advent of new treatment modalities. Counseling a young patient with cancer regarding fertility treatment has become the norm.

The American Society of Clinical Oncology panel says that oncologists should address the possibility of infertility and options for fertility preservation in all patients who are to be treated in their reproductive years, and thereby refer them to the reproductive endocrinologists during treatment planning.[94]

Fertility can be impaired following surgery, chemotherapy, or radiotherapy treatment for cancer. The damage caused by chemotherapy agents is drug and dose dependent and is related to age at the time of treatment (Table).[95]

Table. Risk Levels of Infertility With Various Chemotherapeutic Agents

Risk Level	Chemotherapeutic Agents
High	Alkylating agents: cyclophosphamide, ifosfamide, chlormethine, busulfan, melphalan, chlorambucil, carmustine, lomustine, mechlorethamine, and procarbazine
Medium	Cisplatin, carboplatin, and doxorubicin
Low (< 20%)	Vincristine, methotrexate, dactinomycin, bleomycin, mercaptopurine, vinca alkaloids (vinblastine), and fluorouracil
Unknown Level of Risk	Nitrosources and antimetabolites: cytosine arabinoside
Unknown Risk	Taxanes, oxaliplatin, monoclonal antibodies (trastuzumab, bevacizumab, and cetuximab), tyrosine kinase inhibitors (erlotinib and imatinib)

Adapted from Feigin E, et al.[96]

Fertility preservation in female adolescents with malignancies

Total body, abdominal, or pelvic irradiation may cause ovarian and uterine damage, depending on radiation dose, fractionation schedule, and age at time of treatment.[97] Techniques for freezing testicular and ovarian tissue are still experimental.

The testes are highly susceptible to the toxic effects of radiation and chemotherapy at all stages of life. Radiation exposure of the testes to > 4 Gy causes irreversible azoospermia. Radiation exposure of 1.5 Gy causes sterility; however, this is reversible, and such recovery commonly takes 18 to 24 months. In men, cryopreservation of sperm before treatment, for later use, is the most common strategy to preserve fertility. Cryopreservation of testicular tissue from prepubescent males is still in experimental stages.[98]

Preservation of fertility in women is more complicated. A 2-Gy radiation dose destroys 50% of primordial follicles. Alkylating agents such as cyclophosphamide, ifosfamide, and busulfan are known to cause increased risk of sterility in both men and women.

Conservative fertility-sparing treatment such as radical trachelectomy of cervical cancer, hormonal treatment of early endometrial cancer, and conservative surgical management of early-stage epithelial ovarian cancer may be possible in certain women with early invasive disease.[99] Reducing the radiation dose to the ovary by shielding or surgically removing the ovaries from the field of radiation (ie, oophoropexy) may preserve ovarian function.[100]

If the cancer treatment can be delayed, it is possible to undergo ovarian stimulation and retrieve and freeze eggs (both mature and immature) or produce embryos that can be frozen for later transfer to the individual or a gestational carrier. Freezing ovarian tissue for later retransplantation or IVM of oocytes may be offered. Ovarian tissue cryopreservation is still experimental, and there have been only 17 live births worldwide from frozen ovarian cortical tissue transplantation in humans.[101]

What is in the Pipeline?

Maternal spindle transfer and pronuclear transfer to avoid mitochondrial disease

Mitochondrial disease can be due to mutation in mitochondrial DNA or mutations in nuclear DNA involved in mitochondrial function, and they usually affect the brain, muscle, heart, etc.

Cytoplasmic transfer was first proposed as a treatment for patients with infertility. It involves the transfer of a small portion of ooplasm, and hence mitochondrial DNA, from one oocyte to another. In 1997, Cohen et al reported the first cytoplasmic transfer that resulted in pregnancies in humans.[102]

Two other promising approaches have emerged more recently: maternal spindle transfer and pronuclear transfer. Both methods would result in the offspring inheriting nuclear genetic material from both the parents, while the mitochondrial DNA would be derived largely or perhaps exclusively from the oocyte obtained from a healthy donor. These methods could avoid mitochondrial disease not just in the resulting offspring, but also in subsequent generations. This procedure is under research, and lot more evidence is needed before it is widely accepted.

In vitro activation method for infertility treatment in patients with premature ovarian insufficiency

Kawamura et al[103] demonstrated that Hippo and Akt signaling pathways regulate follicle growth. Using an in vitro activation approach, they first removed ovaries from infertile patients, followed by fragmentation to disrupt Hippo signaling and drug treatment to stimulate Akt signaling. After grafting ovarian tissues back to patients, they found rapid follicle growth in some patients and successfully retrieved mature eggs. A live birth was also reported after IVF and embryo transfer.

Uterus transplantation

Uterine-related infertility is one of the main unresolved causes of infertility, affecting approximately 3% to 5% of the population.[104-110] It might be congenital (agenesis or malformation) or acquired (Asherman syndrome, myoma uteri, adenomyosis, or hysterectomy).

Currently, patients with uterine factor infertility can conceive through gestational surrogacy.[111] The first attempt in human uterus transplantation was by Fageeh et al in 2000.[112] The graft had to be removed

on the 99th day because of thromboses in the anastomosed site.

Brännström et al[113] performed 9 uterus transplantation surgeries from live donors. They have recently reported the first live birth after the procedure.[114] The outcomes of their 7 cases will provide very important information for future uterus transplantations.

Conclusion

The techniques used in IVF continue to evolve as we strive to improve success rates while minimizing multiple pregnancies. Clinicians are often under pressure to modify treatment regimens in the face of unsuccessful outcomes. However, we should all remain cognizant of the fact that currently live birth rates generally do not exceed 50% per stimulated IVF cycle, even in very young women with an excellent prognosis; in older women or those with comorbidities, rates are dramatically lower. It is important that all advances in techniques undergo adequate scientific scrutiny and unproven therapies be reserved for appropriate clinical trials.

References

1. Brinsden PR, Brinsden PR. Thirty years of IVF: the legacy of Patrick Steptoe and Robert Edwards. *Hum Fertil*. 2009;12(3):137–143.
2. Zarek SM, Muasher SJ. Mild/minimal stimulation for in vitro fertilization: an old idea that needs to be revisited. *Fertil Steril*. 2011;95(8):2449–2455.
3. Matorras R, Rodriguez-Escudero FJ. The use of urinary gonadotrophins should be discouraged. *Hum Reprod*. 2002;17(7):1675.
4. Crosignani PG. Risk of infection is not the main problem. *Hum Reprod*. 2002;17(7):1676.
5. Balen A. Is there a risk of prion disease after the administration of urinary derived gonadotrophins? *Hum Reprod*. 2002;17(7):1676–1680.
6. Fauser BC, et al. Advances in recombinant DNA technology: corifollitropin alfa, a hybrid molecule with sustained follicle-stimulating activity and reduced injection frequency. *Hum Reprod Update*. 2009;15(3):309–321.
7. Fauser BC, et al. Pharmacokinetics and follicular dynamics of corifollitropin alfa versus recombinant FSH during ovarian stimulation for IVF. *Reprod Biomed Online*. 2010;21(5):593–601.
8. Janssens RM, et al. Dose finding study of triptorelin acetate for prevention of a premature LH surge in IVF: a prospective, randomised, double-blind, placebo controlled study. *Hum Reprod*. 2000;15(11):2333–2340.
9. Meldrum DR. Ovulation induction protocols. *Arch Pathol Lab Med*. 1992;116(4):406–409.
10. Gordon JW, Talansky BE. Assisted fertilization by zona drilling: a mouse model for correction of oligospermia. *J Exp Zool*. 1986;239(3):347–354.
11. Cohen J, et al. Implantation of embryos after partial opening of oocyte zona pellucida to facilitate sperm penetration. *Lancet*. 1988;2(8603):162.
12. Laws-King A, et al. Fertilization of human oocytes by microinjection of a single spermatozoon under the zona pellucida. *Fertil Steril*. 1987;48(4):637–642.
13. Palermo G, et al. Pregnancies after intracytoplasmic injection of single spermatozoon into an oocyte. *Lancet*. 1992;340(8810):17–18.
14. Palermo GD, Cohen J, Rosenwaks Z. Intracytoplasmic sperm injection: a powerful tool to overcome fertilization failure. *Fertil Steril*. 1996;65(5):899–908.
15. Sauer MV, Chang PL. Establishing a clinical program for human immunodeficiency virus 1-seropositive men to father seronegative children by means of in vitro fertilization with intracytoplasmic sperm injection. *Am J Obstet Gynecol*. 2002;186(4):627–633.
16. Van Leeuwen E, et al. Reproduction and fertility in human immunodeficiency virus type 1 infection. *Hum Reprod Update*. 2007;13:197–206.
17. Bartoov B, Berkovitz A, Eltes F. Selection of spermatozoa with normal nuclei to improve the pregnancy rate with intracytoplasmic sperm injection. *N Engl J Med*. 2001;345(14):1067–1068.
18. Bartoov B, et al. Pregnancy rates are higher with intracytoplasmic morphologically selected sperm injection than with conventional intracytoplasmic injection. *Fertil Steril*. 2003;80(6):1413–1419.
19. Berkovitz A, et al. The morphological normalcy of the sperm nucleus and pregnancy rate of intracytoplasmic injection with morphologically selected sperm. *Hum Reprod*. 2005;20(1):185–190.
20. Berkovitz A, et al. How to improve IVF-ICSI outcome by sperm selection. *Reprod Biomed Online*. 2006;12(5):634–638.
21. Hazout A, et al. High-magnification ICSI overcomes paternal effect resistant to conventional ICSI. *Reprod Biomed Online*. 2006;12(1):19–25.
22. Berkovitz A, et al. Does the presence of nuclear vacuoles in human sperm selected for ICSI affect pregnancy outcome? *Hum Reprod*. 2006;21(7):1787–1790.

23. Nadalini M, et al. Impact of intracytoplasmic morphologically selected sperm injection on assisted reproduction outcome: a review. *Reprod Biomed Online*. 2009;19(Suppl 3):45–55.
24. Antinori M, et al. Intracytoplasmic morphologically selected sperm injection: a prospective randomized trial. *Reprod Biomed Online*. 2008;16(6):835–841.
25. Hammoud I, et al. Selection of normal spermatozoa with a vacuole-free head (x6300) improves selection of spermatozoa with intact DNA in patients with high sperm DNA fragmentation rates. *Andrologia*. 2013;45(3):163–170.
26. Balaban B, et al. Clinical outcome of intracytoplasmic injection of spermatozoa morphologically selected under high magnification: a prospective randomized study. *Reprod Biomed Online*. 2011;22(5):472–476.
27. Wilding M, et al. Intracytoplasmic injection of morphologically selected spermatozoa (IMSI) improves outcome after assisted reproduction by deselecting physiologically poor quality spermatozoa. *J Assist Reprod Genet*. 2011;28(3):253–262.
28. Schlegel PN. Testicular sperm extraction: microdissection improves sperm yield with minimal tissue excision. *Hum Reprod*. 1999;14(1):131–135.
29. Ishikawa T, et al. Learning curves of microdissection testicular sperm extraction for nonobstructive azoospermia. *Fertil Steril*. 2010;94(3):1008–1011.
30. Schiff JD, et al. Success of testicular sperm extraction [corrected] and intracytoplasmic sperm injection in men with Klinefelter syndrome. *J Clin Endocrinol Metab*. 2005;90(11):6263–6267.
31. Carpi A, Sabanegh R, Mechanick J. Controversies in the management of non obstructive azoospermia. *Fertil Steril*. 2009;91:963–970.
32. Loutradis D, et al. Oocyte maturation in assisted reproductive techniques. *Ann N Y Acad Sci*. 2006;1092:235–246.
33. Filali M, et al. Retrospective comparison of two media for in-vitro maturation of oocytes. *Reprod Biomed Online*. 2008;16(2):250–256.
34. Picton HM, et al. The in vitro growth and maturation of follicles. *Reproduction*. 2008;136(6):703–715.
35. Pincus G, Enzmann EV. The comparative behavior of mammalian eggs in vivo and in vitro: I. the activation of ovarian eggs. *J Exp Med*. 1935;62(5):665–675.
36. Cha KY, et al. Pregnancy after in vitro fertilization of human follicular oocytes collected from nonstimulated cycles, their culture in vitro and their transfer in a donor oocyte program. *Fertil Steril*. 1991;55(1):109–113.
37. Fadini R, et al. Effect of different gonadotrophin priming on IVM of oocytes from women with normal ovaries: a prospective randomized study. *Reprod Biomed Online*. 2009;19(3):343–351.
38. Chian RC, et al. Prospective randomized study of human chorionic gonadotrophin priming before immature oocyte retrieval from unstimulated women with polycystic ovarian syndrome. *Hum Reprod*. 2000;15(1):165–170.
39. Mattioli M, et al. Effects of LH and FSH on the maturation of pig oocytes in vitro. *Theriogenology*. 1991;36(1):95–105.
40. Roberts R, Franks S, Hardy K. Culture environment modulates maturation and metabolism of human oocytes. *Hum Reprod*. 2002;17(11):2950–2956.
41. Abir R, et al. Mechanical isolation and in vitro growth of preantral and small antral human follicles. *Fertil Steril*. 1997;68(4):682–688.
42. Oktay K, et al. In vitro maturation improves oocyte or embryo cryopreservation outcome in breast cancer patients undergoing ovarian stimulation for fertility preservation. *Reprod Biomed Online*. 2010;20(5):634–638.
43. Fadini R, et al. Embryo transfer following in vitro maturation and cryopreservation of oocytes recovered from antral follicles during conservative surgery for ovarian cancer. *J Assist Reprod Genet*. 2012;29(8):779–781.
44. Cha KY, Chian RC. Maturation in vitro of immature human oocytes for clinical use. *Hum Reprod Update*. 1998;4(2):103–120.
45. Trounson A, Wood C, Kausche A. In vitro maturation and the fertilization and developmental competence of oocytes recovered from untreated polycystic ovarian patients. *Fertil Steril*. 1994;62(2):353–362.
46. Alpha Scientists in Reproductive Medicine, ESHRE Special Interest Group of Embryology. The Istanbul consensus workshop on embryo assessment: proceedings of an expert meeting. *Hum Reprod*. 2011;26(6):1270–1283.
47. Payne D, et al. Preliminary observations on polar body extrusion and pronuclear formation in human oocytes using time-lapse video cinematography. *Human Reprod*. 1997;12(3):532–541.
48. Mio Y, Maeda K. Time lapse cinematography of dynamic changes occurring during in vitro development of human embryos. *Am J Obstet Gynecol*. 2008;199(6):660.e1–600.e5.
49. Lemmen JG, Agerholm I, Zeibe S. Kinetic markers of human embryo quality using time-lapse recordings of IVF/ICSI fertilised oocytes. *Reprod Biomed Online*. 2008;17(3):385–391.
50. Pribenszky C, et al. Pregnancy achieved by transfer of a single blastocyst selected by time lapse monitoring. *Reprod Biomed Online*. 2010;21(4):533–536.
51. Montag M, Liebenthron J, Koster M. Which morphological scoring system is relevant in human embryo development? *Placenta*. 2011;32(Suppl 3):S252–S256.
52. Arav A, et al. Prediction of embryonic developmental competence by time lapse observation and 'shortest half' analysis. *Reprod Biomed Online*. 2008;17(5):669–675.

53. Thomas MR, et al. Clinical predictors of human blastocyst formation and pregnancy after extended embryo culture and transfer. *Fertil Steril*. 2010;94(2):543–548.

54. Chang HJ, et al. Impact of blastocyst transfer on offspring sex ratio and the monozygotic twinning rate: a systematic review and meta-analysis. *Fertil Steril*. 2009;91(6):2381–2390.

55. Trounson A, Mohr L. Human pregnancy following cryopreservation, thawing and transfer of an eight-cell embryo. *Nature*.1983;305(5936):707–709.

56. Kolibianakis EM, Venetis CA, Tarlatzis BC. Cryopreservation of human embryos by vitrification or slow freezing: which one is better? *Curr Opin Obstet Gynecol*. 2009;21(3):270–274.

57. Roberts JE, Oktay K. Fertility preservation: a comprehensive approach to the young woman with cancer. *J Natl Cancer Inst Monogr*. 2005;(34):57–59.

58. Gook DA. History of oocyte cryopreservation. *Reprod Biomed Online*. 2011;23(3):281–289.

59. Varghese AC, Nagy ZP, Agarwal A. Current trends, biological foundations and future prospects of oocyte and embryo cryopreservation. *Reprod Biomed Online*. 2009;19(1):126–140.

60. Boldt J. Current results with slow freezing and vitrification of the human oocyte. *Reprod Biomed Online*. 2011;23(3):314–322.

61. Rodriguez-Wallberg KA, Oktay K. Recent advances in oocyte and ovarian tissue cryopreservation and transplantation. *Best Pract Res Clin Obstet Gynaecol*. 2012;26(3):391–405.

62. Song WY, et al. Clinical outcome of emergency egg vitrification for women when sperm extraction from the testicular tissues of the male partner is not successful. *Syst Biol Reprod Med*. 2011;57(4):210–213.

63. Virant-Klun I, et al. Slow oocyte freezing and thawing in couples with no sperm or an insufficient number of sperm on the day of in vitro fertilization. *Reprod Biol Endocrinol*. 2011;9:19.

64. Lucena E, et al. Successful ongoing pregnancies after vitrification of oocytes. *Fertil Steril*. 2006;85(1):108–111.

65. Chung JT, et al. Normal birth following PGD for reciprocal translocation after serial vitrification of oocytes from a poor responder: a case report. *Reprod Biomed Online*. 2012;25(5):521–526.

66. Cobo A, et al. Accumulation of oocytes: a new strategy for managing low responder patients. *Reprod Biomed Online*. 2012;24(4):424–432.

67. Rodriguez-Wallberg KA, Oktay K. Fertility preservation and pregnancy in women with and without BRCA mutation-positive breast cancer. *Oncologist*. 2012;17(11):1409–1417.

68. Cobo A, et al. Vitrification: an effective new approach to oocyte banking and preserving fertility in cancer patients. *Clin Transl Oncol*. 2008;10(5):268–273.

69. Cobo A, et al. Use of cryo-banked oocytes in an ovum donation programme: a prospective, randomized, controlled clinical trial. *Hum Reprod*. 2010;25(9):2239–2246.

70. Nagy ZP, et al. Clinical evaluation of the efficiency of an oocyte donation program using egg cryo-banking. *Fertil Steril*. 2009;92(2):520–526.

71. Chen C. Pregnancy after human oocyte cryopreservation. *Lancet*. 1986;1(8486):884–886.

72. Borini A, et al. Predictive factors for embryo implantation potential. *Reprod Biomed Online*. 2005;10(5):653–668.

73. Ebner T, et al. Selection based on morphological assessment of oocytes and embryos at different stages of preimplantation development: a review. *Hum Reprod Update*. 2003;9(3):251–262.

74. Brezina PR, et al. Single-gene testing combined with single nucleotide polymorphism microarray preimplantation genetic diagnosis for aneuploidy: a novel approach in optimizing pregnancy outcome. *Fertil Steril*. 2011;95(5):1786.e5–8.

75. Brezina PR, Kearns WG. Preimplantation genetic screening in the age of 23-chromosome evaluation; why FISH is no longer an acceptable technology. *J Fertiliz In Vitro*. 2011;1:e103.

76. Handyside AH, et al. Pregnancies from biopsied human preimplantation embryos sexed by Y-specific DNA amplification. *Nature*. 1990;344(6268):768–770.

77. Xu K, et al. First unaffected pregnancy using preimplantation genetic diagnosis for sickle cell anemia. *JAMA*. 1999;281(18):1701–1706.

78. Xu K, et al. Preimplantation genetic diagnosis for retinoblastoma: the first reported liveborn. *Am J Ophthalmol*. 2004;137(1):18–23.

79. Brezina PR, Haberl E, Wallach E. At home testing: optimizing management for the infertility physician. *Fertil Steril*. 2011;95(6):1867–1878.

80. Kuliev A, et al. Preimplantation diagnosis and HLA typing for haemoglobin disorders. Reprod Biomed Online. 2005;11(3):362–370.

81. Brezina PR, Zhao Y. The ethical, legal, and social issues impacted by modern assisted reproductive technologies. *Obstet Gynecol Int*. 2012;2012:686253.

82. Kuliev A, Verlinsky Y. Current features of preimplantation genetic diagnosis. *Reprod Biomed Online*. 2002;5(3):294–299.

83. Treff NR, et al. Single nucleotide polymorphism microarray-based concurrent screening of 24-chromosome aneuploidy and unbalanced translocations in preimplantation human embryos. *Fertil Steril*. 2011;95(5):1606–1612.e1–2.

84. Otani T, et al. Preimplantation genetic diagnosis significantly improves the pregnancy outcome of translocation carriers with a history of recurrent miscarriage and unsuccessful pregnancies. *Reprod Biomed Online*. 2006;13(6):869–874.

85. Du L, et al. The rate of de novo and inherited aneuploidy as determined by 23-chromosome single nucleotide polymorphism microarray (SNP) in embryos generated from parents with known chromosomal translocations. *Fertil Steril*. 2011;96(3):S221.

86. Hassold T, et al. A cytogenetic study of 1000 spontaneous abortions. *Ann Hum Genet*. 1980;44(Pt 2):151–178.

87. Mastenbroek S, et al. In vitro fertilization with preimplantation genetic screening. *N Engl J Med*. 2007;357:9–17.

88. Munné S, et al. Chromosome mosaicism in human embryos. *Biol Reprod*. 1994;51(3):373–379.

89. Wells D, Alfarawati S, Fragouli E. Use of comprehensive chromosomal screening for embryo assessment: microarrays and CGH. *Mol Hum Reprod*. 2008;14(12):703–710.

90. Fiorentino F, et al. PCR-based detection of chromosomal imbalances on embryos: the evolution of PGD for chromosomal translocations. *Fertil Steril*. 2010;94(6):2001–2011.e6.

91. Scott R, et al. Noninvasive metabolomic profiling of human embryo culture media using Raman spectroscopy predicts embryonic reproductive potential: a prospective blinded pilot study. *Fertil Steril*. 2008;90(1):77–83.

92. Treff NR, et al. First pregnancies after blastocyst biopsy and rapid 24 chromosome aneuploidy screening allowing a fresh transfer within 4 h of biopsy. *Fertil Steril*. 2010;94(4):S126–S127.

93. Benner AT, et al. 23-chromosome single nucleotide polymorphism (SNP) microarray preimplantation genetic screening (PGS) on blastocysts, versus day-3 embryos, results in significantly higher clinical pregnancy rates. *Fertil Steril*. 2011;96(3):S221.

94. Lee SJ, et al. American Society of Clinical Oncology recommendations on fertility preservation in cancer patients. *J Clin Oncol*. 2006;24(18):2917–2931.

95. Janson PO. Possibilities of fertility preservation in children and young adults undergoing treatment for malignancy. *Acta Obstetricia et Gynecologica Scandinavica*. 2000;79(4):240–243.

96. Feigin E, et al. Fertility preservation in female adolescents with malignancies. In: Moorland MT, ed. *Cancer in Female Adolescents*. New York, NY: Nova Science Publishers; 2008:103–138.

97. Petersen PM, et al. Endocrine function in patients treated for carcinoma in situ in the testis with irradiation. *APMIS*. 2003;111(1):93–99.

98. Ajala T, et al. Fertility preservation for cancer patients: a review. *Obstet Gynecol Int*. 2010;2010:1–9.

99. Wallace WH, et al. Ovarian failure following abdominal irradiation in childhood: the radiosensitivity of the human oocyte. *Br J Radiol*. 1989;62(743):995–998.

100. Lushbaugh CC, Casarett GW. The effects of gonadal irradiation in clinical radiation therapy: a review. *Cancer*. 1976;37:1111–1125.

101. Sonmezer M, Shamonki MI, Oktay K. Ovarian tissue cryopreservation: benefits and risks. *Cell Tissue Res*. 2005;322(1):125–132.

102. Cohen J, et al. Birth of infant after transfer of anucleate donor oocyte cytoplasm into recipient eggs. *Lancet*. 1997;350(9072):186–187

103. Kawamura K, et al. Hippo signaling disruption and Akt stimulation of ovarian follicles for infertility treatment. *Proc Natl Acad Sci U S A*. 2013;110(43):17474–17479.

104. Milliez J. Uterine transplantation FIGO Committee for the ethical aspects of human reproduction and women' s health. *Int J Gynaecol Obstet*. 2009;106(3):270.

105. Saravelos SH, Cocksedge KA, Li TC. Prevalence and diagnosis of congenital uterine anomalies in women with reproductive failure: a critical appraisal. *Hum Reprod Update*. 2008;14(5):415–429.

106. Kwee A, et al. Emergency peripartum hysterectomy: a prospective study in The Netherlands. *Eur J Obstet Gynecol Reprod Biol*. 2006;124(2):187–192.

107. Erman Akar M, et al. Emergency peripartum hysterectomy. *Eur J Obstet Gynecol Reprod Biol*. 2004;113(2):178–181.

108. Farquhar CM, Steiner CA. Hysterectomy rates in the United States 1990–1997. *Obstet Gynecol*. 2002;99(2):229–234.

109. Quinn MA, et al. Carcinoma of the cervix uteri. FIGO 26th annual report on the results of treatment in gynecological cancer. *Int J Gynaecol Obstet*. 2006;95:S43–S103.

110. Del Priore G, et al. Uterine transplantation—a real possibility? The Indianapolis consensus. *Hum Reprod*. 2013;28(2):288–291.

111. Parkinson J, et al. Perinatal outcome after in-vitro fertilization-surrogacy. *Hum Reprod*. 1999;14(3):671–676.

112. Fageeh W, et al. Transplantation of the human uterus. *Int J Gynaecol Obstet*. 2002;76(3):245–251.

113. Brännström M, et al. First clinical uterus transplantation trial: a six-month report. *Fertil Steril*. 2014;101(5):1228–1236.

114. Brännström M, et al. Live birth after uterus transplantation. *Lancet*. 2015;385(9968):607–616.