FOGSI Focus Recent Advances In Infertility

E D I T O R S

Dr Nandita Palshetkar
President FOGSI & Editor in Chief

Dr Pratik Tambe
Chairperson Endocrinology Committee

Dr Hrishikesh Pai

Dr Rishma Dhillon Pai

FOGSI Focus on Recent Advances in Infertility

Editors

Dr Nandita Palshetkar
MD FCPS FICOG FRCOG

President, FOGSI (2019)
Chairperson, MSR (2017-18)
Vice President, AMOGS (2016-18)
Past President, MOGS (2016-17)

Dr Hrishikesh Pai
MD FCPS FICOG MSc FRCOG

Past Secretary General, FOGSI
Past President, IAGE
Past President, ISAR
Past President, MOGS

Dr Pratik Tambe
MD FICOG

Chairperson, FOSGI Endocrinology Committee (2017-19)
Managing Council Member, MOGS
Managing Council Member, IAGE, MSR, AMC (2015-18)

Dr Rishma Dhillon Pai
MD FRCOG (UK) DNB DGO FCPS FICOG

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President's Message

It gives me great pleasure to know that the FOGSI Focus on Recent Advances in Infertility is ready for release. The aim of this FOGSI Focus is to highlight the latest evidence based guidelines, approaches to diagnosis and treatment and protocols for practice on this subject.

As busy practitioners, we rarely have time to read and update ourselves with current evidence. We hope this volume will help you to keep abreast of the latest developments in this field and give you some valuable tips and pointers which you can implement in day-to-day practice.

The Presidential theme for my FOGSI year 2019 is "We for Stree – Safer, Stronger, Smarter". During the year, we will attempt to focus on academic, social and community health initiatives aimed at improving the profile of women in our country. I urge every single one of you to unite and stand with us and contribute to a series of initiatives which will refocus our contributions not only toward the health of Indian women, but their social, financial and educational upliftment as well.

FOGSI is committed to delivering continuing medical education programmes and helping our members to perform to the best of their ability in the areas in which they practice. We also have a raft of initiatives to help women from poor socioeconomic areas receive appropriate care through our social and community healthcare initiatives eg. The FOGSI Saving Mothers initiative. The FOGSI Manyata project also aims to bring a certain minimum standard of care to the women of India via accreditation and training of private nursing homes and healthcare personnel.

I congratulate the editors Dr Hrishikesh Pai, Dr Pratik Tambe and Dr Rishma Dhillon Pai for their hard work and sincere efforts in helping to write, collate, edit and publish this FOGSI Focus on time.

Dr Nandita P. Palshetkar

Dr Nandita Palshetkar
MD FCPS FICOG FRCOG

President, FOGSI (2019)
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Past President, MOGS (2016-17)

FOGSI Focus on Recent Advances in Infertility

Preface

Respected colleagues and dear friends,

It is with immense pride that we bring to you this **FOGSI Focus on Recent Advances in Infertility.**

This volume includes the latest advances, clinical trials, reviews and meta-analyses on both the clinical front and regarding laboratory technology which is essential to providing our patients the best possible success rates during infertility treatment. The authors of the various chapters have decades of infertility practice experience between them and include some of the most senior, respected teachers and eminent practitioners in this field.

We hope that this compilation serves as a ready reckoner and a quick reference guide to common practical scenarios in day-to-day practice. If this publication helps you in your approach to patient management and impacts the lives of the women that we serve, our purpose in publishing this FOGSI Focus will have been well served.

We wish to thank our FOGSI President Dr Nandita Palshetkar and the Office Bearers, without whose blessings and encouragement, this book would not have materialised. We also wish to place on record our gratitude to our publishers Sharp Printers and the academic partners Sun Inca who will help bring this volume to FOGSIans across the length and breadth of India.

Yours sincerely,

Dr Nandita Palshetkar **Dr Hrishikesh Pai**
Dr Pratik Tambe **Dr Rishma Dhillon Pai**
(Editors)

Table Of Contents

New PCOS Guideline: An Overview

Dr Ameet Patki
MD, DNB, FCPS, FRCOG (UK)
Medical Director, Fertility Associates, Mumbai
Consultant Khar Hinduja and Surya Hospital, Mumbai

Dr Mrinmayi Dharmadhikari
MS, DNB
Clinical Fellow, Fertility Associates, Mumbai

Background
Polycystic ovary syndrome is one of the most common endocrinopathies prevalent in reproductive-aged women. Patient presentation is variable, ranging from being asymptomatic to having multiple gynaecologic, dermatologic, or metabolic manifestations.

Diagnosis of PCOS
In adolescents less than 20 years of age, after two years of onset of menarche, where both androgen excess and ovulatory dysfunction are present the most common diagnosis is PCOS. Ultrasound is not recommended for diagnosis in this age group. In adult women if any two of three of the following, viz.androgen excess, ovulatory dysfunction or polycystic ovarian morphology are present. Ultrasound is required where either androgen excess or ovulatory dysfunction is not present.

Table 1. Criteria for Diagnosis of PCOS[1]

CLINICAL FINDING	**NATIONAL INSTITUTES OF HEALTH CRITERIA, 1990** (must have both of the findings marked below)	**ROTTERDAM CRITERIA, 2003** (must have any two of the findings marked below)	**ANDROGEN EXCESS AND PCOS SOCIETY, 2009** (must have a plus either b or c)
Hyperandrogenism*	X	X	A
Oligomenorrhea	X	X	B
Polycystic ovaries		X	C

PCOS = polycystic ovary syndrome.
*—Clinical or biochemical evidence of excess androgen.

Ovulatory disturbance is a key feature of PCOS, leading to sub fertility. Modifiable lifestyle factors, especially excess weight, exacerbate infertility as well as response to infertility treatment and pregnancy outcome. Hence, lifestyle intervention for weight loss is recommended.

Lifestyle modification[3]
Customization of diet according to individual needs, allowing for a flexible and individual approach to reducing energy intake and avoiding unphysiologically restrictive and nutritionally unbalanced diets, are important.

To achieve weight loss in those with excess weight, an energy deficit of 30% or 500 - 750 kcal/day or a total intake of 1,200 to 1,500 kcal/daycould be prescribed for women, also considering individual energy requirements, body weight and physical activity levels.

Exercise should be encouraged in PCOS patients. Health professionals should encourage and advise the following for prevention of weight gain and maintenance of health.

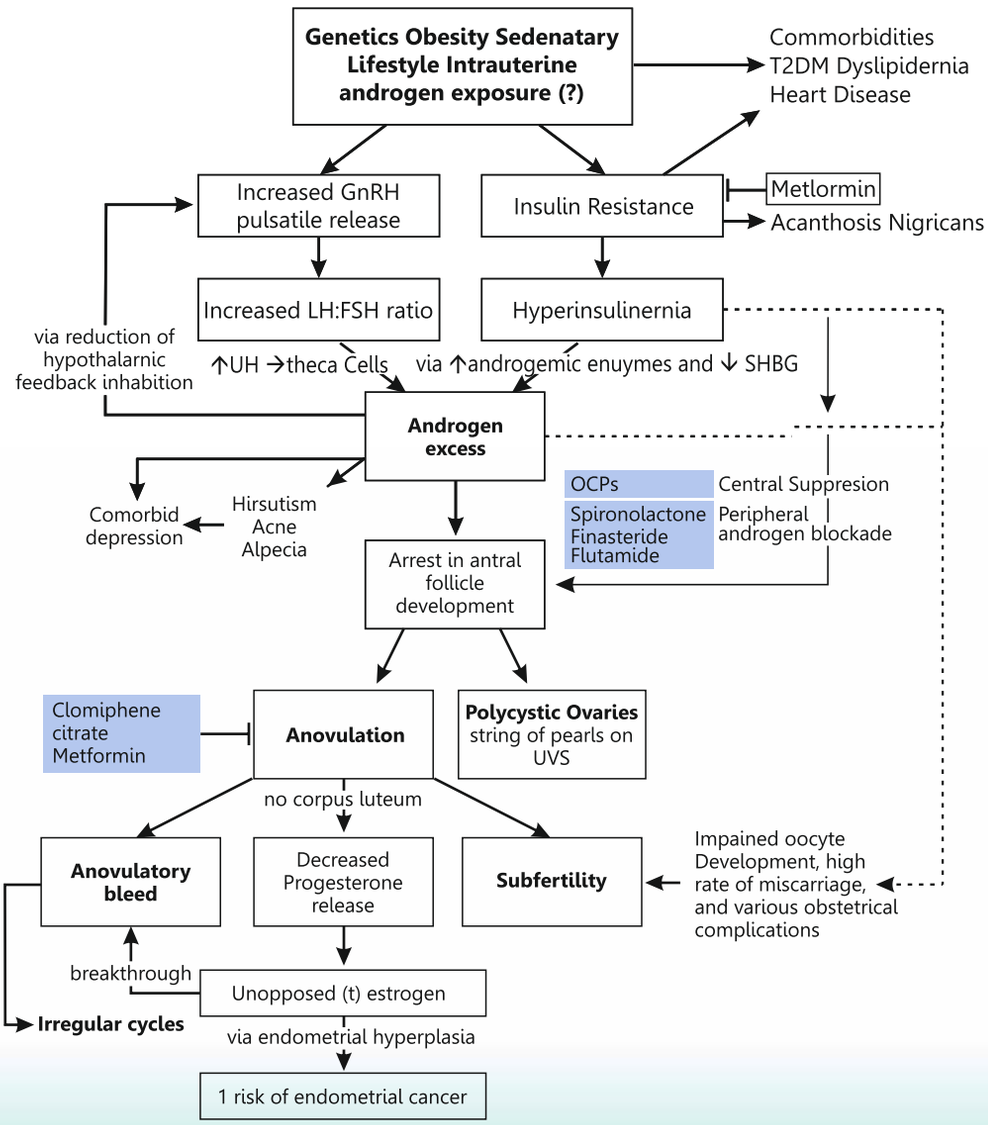
- in adults from 18 – 64 years, a minimum of 150 min/week of moderate intensity physical activity or 75 min/week of vigorous intensities or an equivalent combination of both, including muscle strengthening activities on 2 non-consecutive days/week.

- in adolescents, at least 60 minutes of moderate to vigorous intensity physical activity/day, including those that strengthen muscle and bone at least 3 times weekly.

- activity be performed in at least 10-minute bouts or around 1000 steps, aiming to achieve at least 30 minutes daily on most days.[3]

Pathophysiology of PCOS

Alex Rotstelin, Ragini Srinivasan, and Eric Wong



Pharmacotherapy
When prescribing pharmacological therapy in PCOS, benefits, adverse effects and contraindications in PCOS and general populations need to be considered and discussed before commencement.

COCP (combined oral contraceptive pills), insulin sensitisers and other pharmacological treatments are commonly used in the treatment of PCOS.

COCP alone should be recommended in adult women with PCOS for management of hyperandrogenism and/or irregular menstrual cycles.[3]

COCP alone should be considered in adolescents with a clear diagnosis of PCOS for management of clinical hyperandrogenism and/or irregular menstrual cycles.[3]

COCP could be considered in adolescents who are deemed ''at risk'' but not yet diagnosed with PCOS, for management of clinical hyperandrogenism and irregular menstrual cycles. 35 microgram ethinyloestradiol plus cyproterone acetate preparations should not be considered as first line in PCOS as per general population guidelines, due to adverse effects including venous thromboembolic risks. The lowest effective estrogen doses (such as 20-30 micrograms of ethinyloestradiol or equivalent), and natural estrogen preparations are preferred.[3]

Insulin sensitisers
In combination with the COCP, metformin should be considered in women with PCOS for management of metabolic features where COCP and lifestyle changes do not achieve desired goals, adolescents with PCOS and BMI >25 kg/m2 where COCP and lifestyle changes do not achieve desired goals, high metabolic risk groups including those with diabetes risk factors, impaired glucose tolerance, or high-risk ethnic groups. In combination with the COCP, anti-androgens should only be considered in PCOS to treat hirsutism, after six months or more of COCP and cosmetic therapy have failed to adequately improve symptoms.

Inositol (in any form) should currently be considered an experimental therapy in PCOS.[3]

Ovulation induction in PCOS
Letrozole
Aromatase inhibitors (AI) are effective as ovulation-inducing agents and include letrozole and anastrozole.Letrozoleis very widely used. These agents inhibit the aromatase-facilitated conversion of androgens to oestrogens and increase secretion of follicle stimulating hormone (FSH) in turn stimulating ovarian follicle development and maturation.[4]

Letrozole should be considered first line pharmacological treatment for ovulation induction in women with PCOS with anovulatory infertility and no other infertility factors to improve ovulation, pregnancy and live birth rates.[3]

Gonadotrophins
Gonadotropin therapy is used in anovulatory PCOS patients who have been treated with other first line ovulation induction agents and if they have failed to ovulate or if responses have reduced the chances of conception (e.g. persistent hypersecretion of luteinizing hormone (LH), or an anti-estrogenic endometrial effects).

To prevent overstimulation and multiple pregnancy, the traditional standard step-up regimens[5] were replaced by either low-dose step-up regimens[6,7] or step-down regimens[8] with gonadotropins used alone and different gonadotropin preparations appearing to work equally well.[9] It can be difficult to predict stimulation responses in PCOS and to achieve a single dominant follicle to reduce multiple pregnancy and OHSS and careful monitoring of follicular development by ultrasound is required with triggers used only with two or less follicles over 14mm.

Gonadotrophins can be used as second line pharmacological agents in women with PCOS who have failed first line oral ovulation induction agent therapy and are anovulatory and infertile, with no other infertility factors.[3]

Gonadotrophins could be considered as first line treatment, in the presence of ultrasound monitoring, following counselling on cost and potential risk of multiple pregnancy, in women with PCOS with anovulatory infertility and no other infertility factors.[3]

Gonadotropin therapy provides better per cycle and cumulative pregnancy rates as well as live birth rates compared to the use of oral anti-oestrogens and /or no therapy in anovulatory women with PCOS.

Laparoscopic ovarian surgery

LOS is an intervention that can lead to a singleton birth in women with PCOS. There is no convincing evidence however of inferiority over other common ovulation induction agents.

Laparoscopic ovarian surgery could be used as a second line therapy for women with PCOS, who are clomiphene citrate resistant, with anovulatory infertility and no other infertility factors.[3]

Laparoscopic ovarian surgery could potentially be offered as first line treatment if laparoscopy is indicated for another reason in women with PCOS with anovulatory infertility and no other infertility factors are identified. However, it is important to note that LOS is an invasive surgical intervention and that there is a small risk of reduced ovarian reserve or loss of ovarian function and adhesion formation.[3]

In vitro fertilisation

Ovulation induction therapies are first and second line treatment in management of infertility in women with PCOS, anovulation and no other fertility factors. Yet resistance to and failure of ovulation induction the rapies and the inability to overcome other concomitant causes of infertility means that Assisted Reproductive Technology (ART) therapies have a role in PCOS. A number of challenges exist across the vast array of protocols available for IVF and concerns in PCOS do exist including OHSS, high estradiol levels, accelerated endometrial maturation. In the absence of an absolute indication for IVF ± ICSI, women with PCOS and anovulatory infertility could be offered IVF as third line therapy where first or second line ovulation induction therapies have failed.[3]

A gonadotrophin releasing hormone antagonist protocol is preferred in women with PCOS undergoing an IVF ± ICSI cycle, over a gonadotrophin releasing hormone agonist protocol, to reduce the duration of stimulation, total gonadotrophin dose and incidence of ovarian hyperstimulation syndrome (OHSS). The duration of stimulation with a GnRH antagonist approach is around a day shorter than the standard 'down regulation' approach with a GnRH agonist. The rate of OHSS appears less with a GnRH antagonist approach in comparison to the standard 'long-down regulation' approach with a GnRH agonist.

Trigger

Triggering final oocyte maturation with a gonadotropin-releasing hormone (GnRH) agonist and freezing all the embryos could be considered as an option in women with PCOS having an IVF/ICSI cycle with a GnRH antagonist protocol and is especially useful in those at an increased risk of developing OHSS or where fresh embryo transfer is not planned. GnRH- agonist triggers are associated with lower pregnancy rates in fresh embryo transfers, which can be overcome in frozen cycles.

Exogenous LH in PCOS

The chronic low dose step-up protocol with exogenous FSH in securing single (fewer) dominant follicle selection is an alternative method to avoid multi-follicular development. During late follicular development, LH is essential to achieve adequate ovarian steroidogenesis and develop the subsequent capacity of the follicle to ovulate and luteinise.

Increased LH secretion or elevated LH/ FSH ratio in PCOS may influence fertility, with inhibition of oocyte maturation due to the premature rise in LH, deleterious effects on granulosa cell steroidogenesis as well as on endometrial receptivity and with potential increased early pregnancy loss.

The choice of gonadotropin depends on the availability, convenience, and cost. In standard IVF/ICSI protocols, hormones used for controlled ovarian stimulation (FSH alone or addition of LH as a supplement) have little impact on the fertility outcomes. Endogenous LH levels may fall to very low levels in older women (>35) during ovarian stimulation, especially with GnRH-antagonist use and LH supplementation has been proposed.

Exogenous recombinant luteinising hormone treatment should not be routinely used in combination with follicle stimulating hormone therapy in women with PCOS undergoing controlled ovarian hyperstimulation for IVF ± ICSI.[3]

Metformin

Metformin has been studied to restore ovulation and enhance pregnancy rates in PCOS through a range of mechanisms. These mechanisms provide a physiological rationale for management of insulin resistance in IVF in PCOS. It has also been suggested that metformin may reduce serum estradiol levels during ovarian stimulation and it has also been hypothesised that metformin may reduce the production of vascular endothelial growth factor, both of which are important factors involved in the pathophysiology of OHSS.There fore, it was deemed important to explore the effectiveness and safety of metformin as a co-treatment in achieving pregnancy or live birth and reducing OHSS in IVF in PCOS.

IVM

The term in vitro maturation (IVM) treatment cycle is applied to "the maturation in vitro of immature cumulus oocyte complexes collected from antral follicles" (encompassing both stimulated and unstimulated cycles, but without the use of a human gonadotrophin trigger).

In units with sufficient expertise, IVM could be offered without the risk of OHSS for women with PCOS, where an embryo is generated, then vitrified and thawed and transferred in a subsequent cycle.[3]

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Managing Male Factor - What's New?

Dr Narendra Malhotra
MD, FICMCH, FIAJAGO, FICOG, FICMU
Past President, FOGSI
Past President, ISAR

Dr Sunil Jindal
MS , DNB , MNAMS
Andrologist and Reproductive Medicine Specialist
Jindal Hospital , Meerut.

Background and aetiology

Infertility is a condition affecting an estimated 70 million people globally. The World Health Organization (WHO) estimates that 9% of couples worldwide struggle with fertility issues and that male factor contributes to approximately 50%. Many genetic and lifestyle factors have been implicated in male infertility; however, about 30% of cases are still thought to be idiopathic.

The prevalence varies throughout developed and underdeveloped countries, being higher in the latter in which limited resources for diagnosis and treatment exist.[1] A male factor is solely responsible for infertility in approximately 20% and contributory in another 30–40% of couples; as such, a male factor is implicated in more than 50% of couples attempting to conceive.[2]

The category 'unexplained male infertility' (UMI) is reserved for infertile men with infertility of unknown origin with normal semen and in which female infertility factors have been ruled out. The reported prevalence of UMI ranges from 6 to 27%. Men classified as having idiopathic male infertility have an unexplained reduction in semen quality with no history associated with fertility problems and have normal findings on physical examination and endocrine laboratory testing.

There is a need to identify the causes of so called idiopathic infertility before personalised treatment recommendations can be made. Researchers are exploring genomics, proteomics, transcriptomics, metabolomics, sperm DNA fragmentation, capacitation and nano technology are shading light in an effort to discover better male fertility biomarkers. Some have redirected their efforts to look at current lifestyle factors and their impact on fertility. Several studies have demonstrated an ominous decline in overall sperm quantity and quality in the last several decades. In this chapter, we discuss new updates and advances in male fertility.

Male infertility & overall health

Studies suggest that male infertility may be an early sign of poor overall health. Men with abnormal semen parameters may be at a higher risk of malignancy. Testicular cancer risk increases up to 20-fold in men with abnormal semen parameters.[3] This risk even translates to first-degree relatives of men with abnormal semen analyses. It is also suggested that male infertility has a correlation with increased risk of prostatic cancer. One study found a threefold increase in cancer in azoospermic men. Many studies have suggested that semen parameters correlate with male morbidityand mortality.[4]

Despite advances in understanding male infertility, idiopathic causes still account for 30%. Avariety of medical comorbid conditions have been found to affect semen parameters, viz., renal disease, liver failure, hemochromatosis, chronic obstructive pulmonary disease, cystic fibrosis, and multiple sclerosis. The impact of medical conditions on fertility includes effect on hormonal levels, impairment of sexual function (including ejaculatory function) and impairment of testicular function/spermatogenesis. By medically optimising a man's health, improvements in medical disease status can improve semen parameters, sexual function, and fertility potential.[5]

Lifestyle

Much of past research was performed on retrospective data sets with their own inherent bias and limitations. The Longitudinal Investigation of Fertility and the Environment (LIFE) study was designed to be one of the first prospective studies to analyse fertility factors in couples of unknown fertility status.[6]

In a 2015 report, the study authors detailed the negative effects of heavy occupational exertion (sperm concentration and total count), hypertension (strict morphology), and increasing total number of medications (sperm count). Their second publication focused largely on correlations between semen quality and measures of obesity, with 82% of the overall male cohort being overweight or obese (body mass index [BMI] ≥25) at baseline.

Findings included a linear decline in ejaculate volume associated with increasing BMI and waist circumference (WC). WC was also noted to have a negative relationship with total sperm count (TSC); no significant correlations with sperm concentration, motility, morphology, DNA integrity, or vitality were found. Overall, an increasing frequency of men with abnormal ejaculate volume, sperm concentration, and TSC were seen with increasing body size, though, demonstrating correlations in a population of men without known infertility.

Levine et al, in 2017, published a study demonstrating declining sperm concentrations in the US and worldwide.[7] The etiologies behind these findings of decreasing sperm counts are difficult to pinpoint but may be due in part to increasing rates of overweight and obese men of childbearing age. Additionally, pesticide exposure and illicit drug and tobacco use could be implicated as well, although no causal relationship between these behaviours and decreased sperm parameters currently exists.

Alcohol

Alcohol appears to interfere with the production of GnRH, FSH, LH, and testosterone, as well as impair the functions of Leydig and Sertoli cells. As a result, the production, morphological development and maturation of spermatozoa could be impaired. Partial or complete spermatogenic arrest and Sertoli cell-only syndrome were more commonly present amongst heavy drinkers compared to non-drinkers. Direct exposure of spermatozoa to alcohol (at concentrations corresponding to that of serum after moderate and heavy drinking) was found to be harmful to sperm motility and morphology in a dose-dependent manner. [8]

Smoking

Sperm concentration in male smokers was reported to be typically 13-17% lower than that of non-smokers. Moreover, cigarette smoking has been negatively associated with sperm count, motility, and morphology. The decline in semen quality was found to be more marked in heavy (>20 cigarettes/day) and moderate (10- 20 cigarettes/day) smokers compared to mild smokers (1-10 cigarettes/day). The effect size was higher in infertile males than in the general population. Smoking is also associated with increase in DNA damage, aneuploidies, and mutations in sperm.

Alcohol and smoking together appear to exert a synergistic and additive effect that could adversely alter sperm parameters.[9]

Biomarkers

Our main stay in evaluation of the male is a semen analysis. However, this is not a precise measure of male fertility potential as there are many variables and is not a reliable predictor of fecundity. The sperm apart from DNA also has RNA and other molecules, which are a complex profile of proteins. The investigation of these novel technologies to quantify and study each of these – omics areas is to discover new fertility biomarkers.

Seminal plasma contains concentrated levels of proteins derived from the male reproductive system and may prove the most fruitful. Batruch et al were able to identify over 2,300 individual proteins from semen samples of fertile and infertile men using mass spectrometry. Prostaglandin D synthase (PGDS) levels were found to positively correlate with sperm concentration, motility, and morphology. While other groups have started to compare seminal plasma proteomic profiles between small cohorts of fertile and infertile men, larger collaborative studies are needed before any results can be validated for possible clinical applications.

Discrimination between obstructive azoospermia (OA) and nonobstructive azoospermia (NOA) for proper patient counselling and management in select circumstances of undetermined azoospermia. Follicle-stimulating hormone (FSH) and testicular size are commonly used to predict between the two, though with limited sensitivity.[10]

To avoid a testicular biopsy, efforts are on to identify biomarkers such as PGDS, acrosomal vesicle protein 1 (ACRV1), lectin galactoside-binding soluble 3 binding protein (LGALS3BP), extracellular matrix protein 1 (ECM1), and testis expressed 101(TEX101).TEX101, a testicular protein, aids in differentiating hypospermatogenesis, maturation arrest and Sertoli cell-only patterns of NOA. A combined assay using ECM1 and TEX101 is currently under development.

Reactive oxygen species and sperm DNA fragmentation

Reactive Oxygen Species: Seminal oxidative stress (OS) and sperm DNA fragmentation (SDF) are two advanced sperm function tests that are increasingly used in the evaluation of infertile men.

Reactive oxygen species (ROS) play an important role in cell signalling and homoeostasis. They are produced by the sperm cell in small quantities providing beneficial functional effects including initiation of sperm capacitation, regulation of sperm maturation, and enhancement of cellular signalling pathways.[11] However, high levels of ROS may have paradoxical effects on sperm function, ultimately resulting in infertility.

Increased DNA damage and lipid peroxidation effects of exaggerated ROS levels in seminal plasma. Several exogenous (testicular hyperthermia, environmental and habitual exposures) and endogenous (immature spermatozoa, leucocytes, varicocele) conditions have been recognised as potential causes of increased ROS production. ROS are counterbalanced by antioxidants that help maintain the equilibrium in the redox potential desired for optimal sperm function.

Impact on fertility

Seminal fluid is rich in antioxidants that nourish andprotect the sperm. An accurate measure of seminal oxidative stress is the oxidation reduction potential (ORP). Elevated levels of ORP and sperm DNA fragmentation are reliable information to predict the low fertility potential in the semen sample.

Decreased rates of infertility have been found in men with seminal fluid containing high levels of reactive oxygen species (ROS).[12] These ROS are associated with sperm dysfunction, germ cell DNA damage with the possibility of impaired fertility, but the exact mechanism is not completely understood. These associations have led clinicians to treat infertile men with antioxidant supplements. A variety of clinical trials have suggested that the use of antioxidant supplements benefit patients by improving sperm function and DNA integrity. However, most of these studies are not randomised controlled trials.

Antioxidants

In a randomized controlled trial, the combination of vitamins A, C, E plus NAC and zinc increased sperm concentration with no impact on pregnancy rate. This group of patients also had varicocele correction surgery and the increase in sperm concentration can be confounded and not be associated with use of the antioxidant.

A systematic review of 17 randomised trials, including 1,665 infertile men, was conducted to evaluate the effects of oral antioxidants (vitamins C and E, zinc, selenium, folate, carnitine and carotenoids) on sperm quality and pregnancy rates in infertile men. Fourteen of the 17 (82%) trials showed an improvement in either sperm quality or pregnancy rate after antioxidant therapy. Ten trials examined pregnancy rate and six showed a significant improvement after antioxidant therapy.[13]

Managing elevated DNA fragmentation

Advances in the past several years has been the management of elevated DNA fragmentation index (DFI) and intracytoplasmic sperm injection (ICSI) which have led to a new era of male fertility management. Much of the DNA damage in ejaculated sperm occurs at the epididymal level & Greco et albegan exploring the use of surgically retrieved testicular sperm in couples with elevated DFI.[14] His study reported 18 couples who had previously failed ICSI with ejaculated sperm, where repeat ICSI was performed using testicular sperm. Sperm DFI rates proved to be much lower in the testicular samples and eight of 18 (44.4%) couples were able to achieve a pregnancy with testicular sperm.

The use of testicular sperm to optimise outcomes in couples with failed fertility attempts has gained popularity with several recent publications and research presentations. A report by Patel et aldetailed outcomes for 44 couples with elevated DFI (>24% on sperm chromatin structure assay) undergoing ICSI with sperm obtained via testicular sperm aspiration. Only 28 of the couples had failed prior ICSI or had a miscarriage. Overall pregnancy rate was reported at 38.6% and even slightly higher in the cohort with prior failed ICSI or miscarriage (42.9%). In contrast to prior studies, Patel et al reported higher fertilisation rates and better embryo quality with testicular versus ejaculated sperm.

A recent review of 147 couples undergoing IVF with elevated sperm DFI levels (>30% on sperm chromatin dispersion assay despite oral antioxidant therapy) revealed significant reductions in DFI using testicular sperm over ejaculated specimens (8.3% and 40.7%, respectively). Significant improvements in clinical pregnancy (51.9% versus 40.2%), miscarriage (10.0% versus 34.3%) and live birth (46.7% versus 26.4%) rates were also seen for the testicular-ICSI versus ejaculated- ICSI groups, respectively.

Many groups are now investigating the use of testicular sperm for couples with elevated sperm DFI with promising pregnancy, live birth, and miscarriage rates.[15] Clearly larger studies with longitudinal follow-up are needed to better characterise the role of testicular sperm retrieval for assisted reproductive techniques.

Surgery

Over the past decade, the indications for and techniques of surgery for male infertility have been significantly refined, resulting in substantially increased success in the management of male-factor infertility.

These advances include

1. refined microsurgical techniques for sperm retrieval combined with intracytoplasmic sperm injection (ICSI) for men with nonobstructive azoospermia;
2. improved techniques for microsurgical reconstruction for obstruction;
3. the use of varicocelectomy for enhancement of spermatogenesis in azoospermic or severely oligospermic men, for prevention of future infertility and androgen deficiency in young men, and for treatment of androgen deficiency in men of all ages;[16]
4. increasing use of genetic and molecular biologic markers to better select patients for surgical treatment. Even men with nonobstructive azoospermia caused by Klinefelter syndrome - where treatment was once regarded futile - can now father biologic offspring with assisted reproductive techniques.

Varicocele

The only choice of surgery for infertility is microscopic inguinal & subinguinal approach.Typically, semen parameters improve 3 to 6 months after repair. Ameta-analysis from 2016 confirmed that repairing varicoceles prior to ART improves pregnancy and live birth rates in oligospermic and azoospermic men.

One recent study of menwith non-obstructive azoospermia (NOA) showed an increased return of sperm to the ejaculate following varicocele repair and higher rates of live births when compared with controls with NOA and no varicocele.[17] Similarly, a 2016 meta-analysis concluded that varicocelectomy in men with NOA and clinical varicocele improved surgical sperm recovery rates. In 2012, Mansour Ghanaie et al published a randomised control trial examining varicocele repair in couples with recurrent firsttrimester miscarriages. They showed that varicocelectomy significantly improved semen parameters but interestingly also increased pregnancy rates and decreased miscarriage rates significantly.[18]

Table 1 Technique of Varicocelectomy[19]

Technique	Artery Preserved	Hydrocele (%)	Failure (%)	Potential for serious morbidity
Retroperitoneal	No	7	15 -25	No
Conventional inguinal	No	3 - 30	5 - 15	No
Laparoscopic	Yes	12	3 - 15	Yes
Radiographic	Yes	0	15 -25	Yes
Microscopic inguinal or subinguinal	Yes	0	0.5 - 1.0	No

Sperm retrieval

PESA and TESA are highly effective methods for retrieving sperm in the group of men with OA. Successful sperm retrieval (SRR) was achieved in over 85% of the cases using PESA, but more than one aspiration was required in several cases. In cases of failed PESA, TESA was adequate to obtain sperm in practically all cases. Motile spermatozoa were obtained in approximately 73% of the cases after the first or second PESA aspirations, and TESA was carried out as a rescue procedure after failed PESA in about 14% of the individuals.

Successful sperm retrieval using percutaneous techniques appears to be independent of the cause of obstruction, since SRR rates did not differ among groups. In the group of men with NOA, SRR rates were in the range of 50–70% in most aetiology-specific causes of NOA. Testicular histopathology results were predictive of sperm collection using both TESA and micro-TESE.

According to our data involving 176 individuals, overall SRR rates by TESA were 64.4%, but only 20.7% and 33.3% in cases of Sertoli-cell only and maturation arrest, respectively. On the other hand, SRR by TESA was 100% and 82.3% in NOA men presenting with either hypospermatogenesis on testicular histology or a history of a previous successful TESA attempt. Using micro-TESE, overall SRR rates were 52.3%, but higher than TESA in cases of maturation arrest and Sertoli cell-only.

All reviews have found microdissection testicular sperm extraction (micro-TESE) to have the highest sperm retrieval rates. A systemic review by Deruyver, et al[20] reported SRRs of 16.7-45% by conventional TESE (cTESE), vs42.9% to 63% with micro-TESE. A meta-analysis by Bernie, et al,[21] compared SRR by testicular sperm aspiration (TESA), cTESE and micro-TESE. They found that cTESE was twice more likely to find sperm than TESA, and that micro-TESE was 1.5-times more likely to find sperm as compared to cTESE.

Table 2 Surgical Techniques for Sperm Retrieval[22]

	Advantages	Disadvantages
MESA (microsurgical epididymal sperm aspiration)	Microsurgical procedure allows lower complication rate. Epididymal sperm has better motility than testicular sperm. Large number of sperm can be harvested for cryopreservation of multiple vials in a single procedure.	Requires an aesthesia and microsurgical skills. Not indicated for nonobstructive azoospermia.
PESA (percutaneous epididymal sperm aspiration)	No microsurgical skill required. Local an aesthesia. Epididymal sperm has better motility than testicularsperm.	Complications include hematoma, pain, and vascular injury to testes and obstruction of the epididymis. Variable success in obtaining sperm. Smaller quantity of sperm obtained than with MESA. Not in dicated for nonobstructive azoospermia.
TESA (testicular sperm aspiration)	No microsurgical skill required. Local an aesthesia. Can be used for obstructive azoospermia.	Immature or immotile testicular sperm. Small quantity of sperm obtained. Poor results in nonobstructive azoospermia. Complications include hematoma, pain, and vascular injury to testes and epididymis.
TESE (testicular sperm extraction)	Higher retrieval rate than TESA T echnique for nonobstructive azoospermia.	Requires an aesthesia
Micro TESE	Preferred retrieval technique in NOA Lesser testicular tissue and less drop in post surgical testosterone	Requires an aesthesia and microsurgical skills.

Conclusion

Larger studies with longer follow-up are necessary to define our evaluation and treatment of men struggling to conceive. Currently, our ever-expanding research on infertility demonstrates the complexity of the human body and hopefully, with concerted collaborative efforts we will begin to better understand the challenges our patients face in trying to become parents. With current and future efforts examining the molecular and genetic factors responsible for spermatogenesis and fertilisation, we may be better able to understand aetiologies of male factor infertility and thus improve outcomes for our patients.

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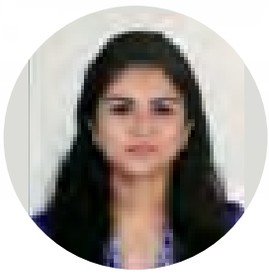
Anti-Mullerian Hormone in Reproductive Medicine

Dr S Shanthakumari
MD DNB FICOG FRCPI FRCOG
President Elect FOGSI (2021)
Chairperson ICOG (2018 -2019)
Vice President FOGSI (2013)

Dr Milind Shah
MD DGO DFP FICOG FIAOG
President of ISOPARB (2016-18)
Deputy Secretary General, FAOPS
(Asia Oceania Federation of Perinatal Societies)
Vice President of FOGSI (2011)



Dr Saloni Savaskar MS
Senior Resident at VMGMC, Solapur

Introduction

Serum anti-Mullerian hormone (AMH) is a unique biomarker that has a critical role in folliculogenesis as well as steroidogenesis within ovaries. AMH which was initially considered as a male hormone has emerged as an invaluable tool for assessment of ovarian function in childhood, adolescence, and adult women.

AMH is the best single serum test for ovarian response management with age dependent association with live birth rate and time to conception. In females, there is mild peak at puberty followed by highest levels of secretion between 23-25 years of age.[1] By knowing AMH levels we can improve menopause prediction, we can monitor ovarian damage and identify women at risk of several ovary related disorders like polycystic ovarian syndrome and premature or primary ovarian insufficiency. It can be useful also in males for certain conditions related to reproductive medicine.

Physiology

AMH is a dimeric glycoprotein and belongs to transforming growth factor (TGF-) family.[2] AMH, also called as Mullerian Inhibiting Substance (MIS), has been known for its function in male sexual differentiation – activated by SOX9 in Sertoli cells and inhibits development of the male reproductive tract.

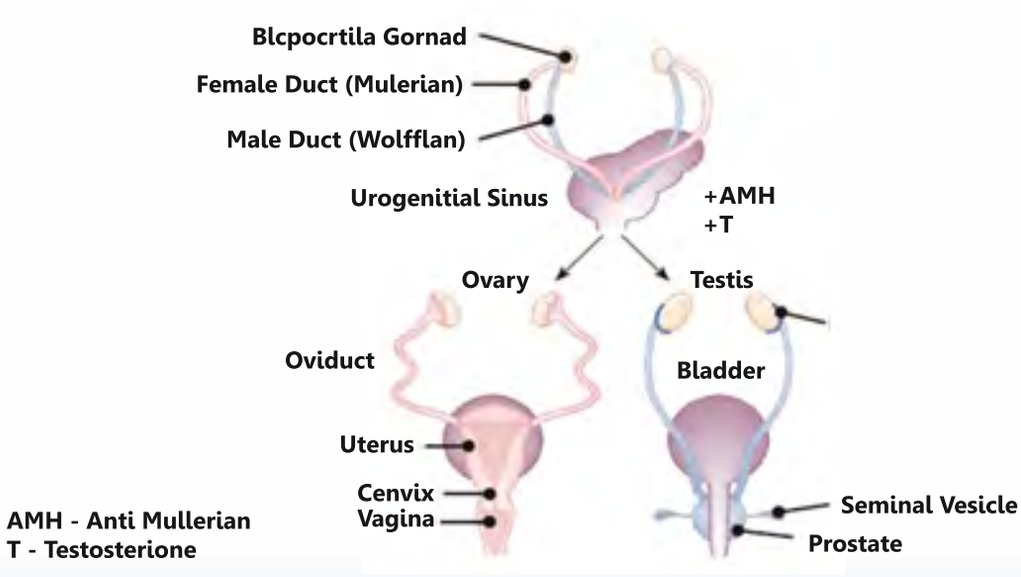


Fig. 1: Influence of MIS on development

Folliculogenesis

The functional unit of the ovary is the follicle. AMH is secreted by the granulosa cellswithin the follicles.The concentrations of AMH have been found to be proportional to the number of follicles.The levels cannot be detected in the resting primordial follicles but reach their maximum in the preantral and small antral phase and gradually disappear in the large antral and preovulatory phase.

AMH expression is on the higher side until a follicle grows up to diameter of ≤ 4mm. In the larger (4-8mm) antral follicles, the AMH expression gradually disappears.[3] Also the intra follicular concentrations of AMH shows a gradual decrease with increase in diameter and a steep fall is observed around 8mm.[4] This rapid decline corresponds with the selection of a dominant follicle. In recent times, sensitive assays have been developed to measure the level of AMH in serum.

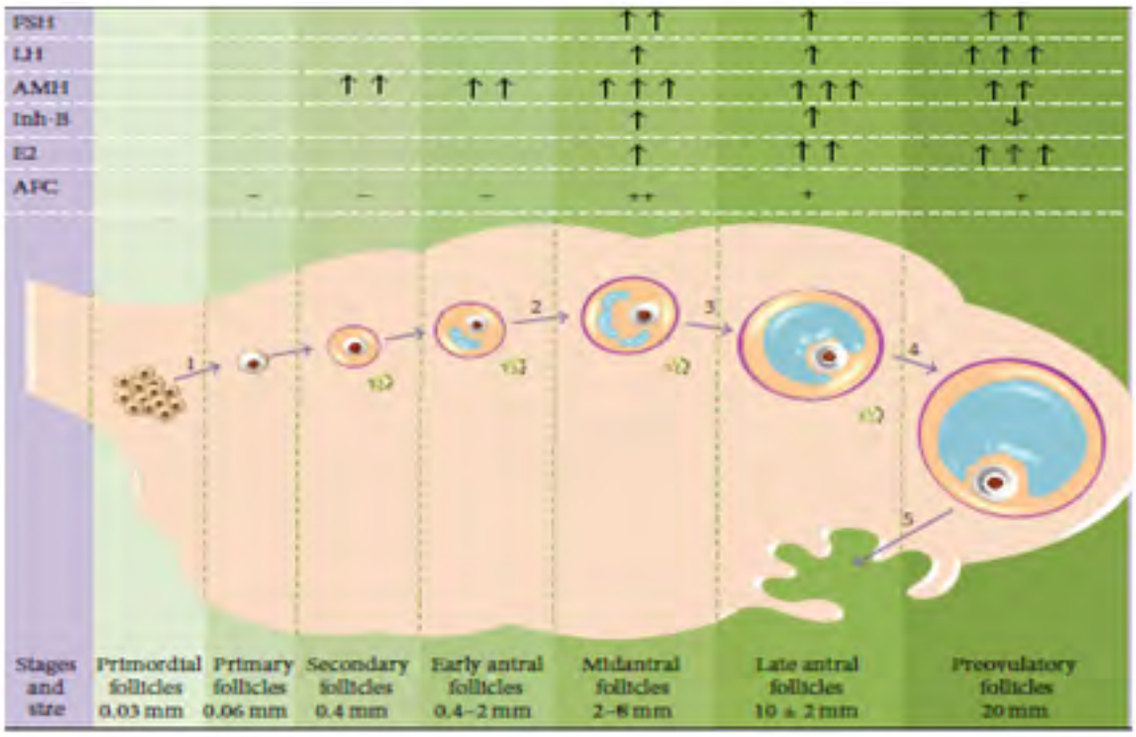


Fig 2 Schematic representation of hormonal patterns in the ovarian cycle

AMH is produced by primary and preantral follicles and has two mechanisms of action:
Pathway 1: inhibition of initial follicle recruitment;
Pathway2: inhibition of FSH dependent growth and selection of the primary and preantral follicles.

Assessment of s AMH levels

Measurement of serum AMH levels was first reported in the 1990s. It is assessed with the help of Enzyme Linked Immunosorbent Assay (ELISA). This uses monoclonal and polyclonal antibodies for detecting levels of AMH. One of the most sensitive assays is the IOT assay with a sensitivity of 0.1ng/mL. Levels of AMH are fairly constant and testing can be done on any day of the cycle. Similarly, pregnancy also does not influence AMH levels.

However, it is now recognised that serum AMH results can have variability due to some common biological fluctuations within some patients due to use of hormonal contraceptives or other medications, certain surgical procedures, specimen treatments, assay changes and laboratory calibration differences. It has been observed that smoking also reduces levels of AMH.

AMH levels raised in	AMH levels reduced in
Polycystic ovary syndrome	Premature menopause
Granulosa cell tumors	Cryptorchidism in boys
Sertoli - Leydig cell tumors	Increased age
Gonadotropin independent precocious puberty in boys	Administration of chemotherapy or radiation
Delayed puberty in boys	Oophorectomy
	Increased Body Mass Index
	Administration of gonadotrophins

Table 1 Variability in AMH levels

Clinical applications

Assessment of ovarian reserve

The basic advantage of using AMH for ovarian reserve is that it is not cycle dependent, that is, it can be measured on any day of the cycle and it does not show inter-cycle variability.

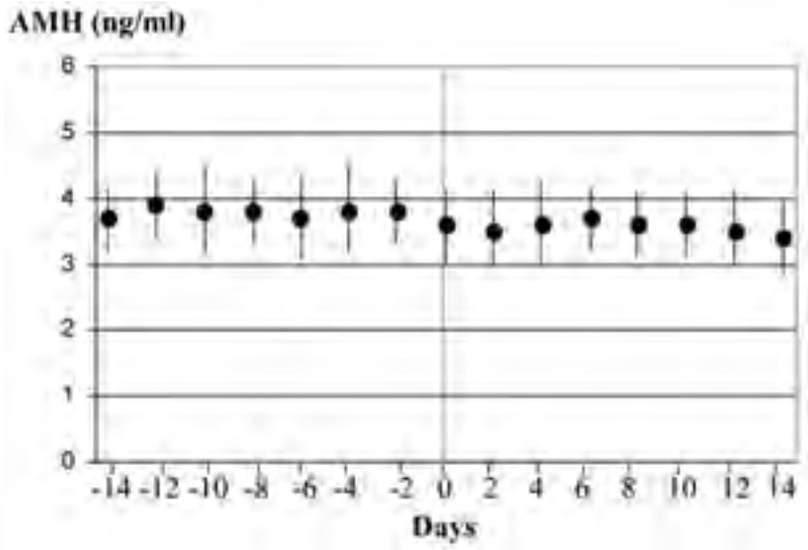


Fig 3Intra-cycle variability of AMH levels[5]

In ART practice

AMH predicts the ovarian response with accuracy[6] and this helps fertility physicians to choose the best possible ovarian stimulation protocol. In addition, it helps us guide patients with realistic expectations about their fertility and outcome of ovarian stimulation protocols. AMH guided ovarian stimulation can lead to individualisation of therapeutic strategies for infertility treatment.

It can predict hyper-response and help identify women who are at risk of ovarian hyperstimulationsyndrome (OHSS).[7] The clinical implication is that we can use the GnRH antagonist protocol for these patients and intervene early in the incipient stage to limit the progression of OHSS. However, AMH levels cannot be used as a marker to reliably predict pregnancy.

Levels less than 0.3 ng/mL predict poor ovarian response with sensitivity of 85% and specificity of 82.3%. However, future research on AMH levels within follicular fluid may pave the way to establish it as a marker of "quality" besides "quantity" of the growing follicles.

In PCOS

Polycystic ovarian syndrome is the most common endocrine disorder in women. Serum AMH is about 2-4 fold higher in women with PCOS than healthy women. This is because of the increased production of AMH by the preantral follicles which are in large number in PCOS.

When the levels in women with PCOS were compared it was found that AMH production was about 75 times higher per granulosa cell in anovulatory PCOS and about 20 times higher in ovulatory PCOS than healthy ovaries.[8] There has been a proposal that AMH levels could be included in the criteria for the diagnosis of polycystic ovarian morphology (PCOM).[9] Levels more than 6.79 ng/mL are associated with increased incidence of ovarian hyperstimulation syndrome.

Granulosa cell tumors of the ovary

Serum AMH levels have been found to increase in patients with adult type granulosa cell tumors. AMH levels are elevated in primary as well as recurrent tumors and thus it can be used for follow-up.[10]

Pubertal issues in males

Serum AMH determination is done in conjunction of serum testosterone and serum LH. In normal and precocious puberty there is a negative correlation between AMH and testosterone. Fall in serum AMH is marker for Sertoli cell pubertal development.[11]

Gonadotropindependent precocious puberty – declining AMH
Gonadotropin independent precocious puberty – abnormally high AMH production[12]

Ambiguous genitalia

AMH helps in differentiating gonadal and non-gonadal causes of mild virilisation in phenotypic prepubertal girls. Undetectable levels are found in 46XX prepubertal girls. Increased levels are found in androgen insensitivity syndrome, dysgenetic testes and ovotestes.[13]

Conclusions

Thus, AMH has helped us show different insights into the ovarian function, not only during reproductive years but also during childhood and adolescence. However, more research still needs to be done. As it has low predictive value, its use in ART should be aimed for effective designing of stimulation protocols and counselling, at the same time keeping in mind that patients should not be deprived of treatment on the ground of very low AMH.

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Augmenting the Ovarian Reserve

Dr Rishma Dhillon Pai
MD FRCOG (UK) DNB DGO FCPS FICOG
Consultant: Lilavati, Jaslok and Hinduja Hospitals, Mumbai
President, FOGSI 2017
President, ISAR (2018-19)
President, IAGE (2018-19)
Vice President, MOGS

Dr Unnati Mamtora
DNB DRM (Germany)
Fellowship in Reproductive Medicine & Endoscopy
Fertility Consultant, Bloom IVF

Background
The ovarian reserve of a woman denotes the egg-producing capacity of the woman in her reproductive career. It determines the fertility potential of the woman and is one of the limiting factors in the success of any fertility treatment.

Poor responder patients form the most challenging group in the practice of fertility clinician. On several occasions, their cycles are cancelled due to poor growth of follicles, lack of oocytes retrieved or failure to fertilise.[1-4] With advanced age, both the egg quality and quantity deteriorates. Hence as women age, they experience a parallel decline in fertility.

The incidence of poor responders among infertile women is reported in 9-24% IVF cycles and is associated with very low clinical pregnancy rates. With the advancement of age over the age of 37 years, there is a gradual decline in the follicular pool over time. The mechanism of this decline in ovarian insufficiency is not fully understood.

Definition
There is no proper definition to define the poor responder patients. The most recent definitions are the Bologna criteria and the Poseidon criteria.

"Poor ovarian responders" should be considered patients having at least two of the following three Bolognia criteria:

1. a previous episode of poor ovarian response (≤3 oocytes) with a standard dose of medication;
2. an abnormal ovarian reserve with AFC <5–7 follicles or AMH <0.5–1.1 ng/mL;
3. women above 40 years of age or presenting other risk factors for a poor response such as previous ovarian surgery, genetic defects, chemotherapy, radiotherapy and auto immune disorders.

Due to the diversity in the inclusion criteria, the Bologna criteria have been criticised.[8]

POSEIDON criteria
In 2016, poor ovarian reserve was redefined by reproductive clinicians.[9] As a result, the new POSEIDON (Patient-Oriented Strategies Encompassing Individualized Oocyte Number) classification was developed, providing a more detailed classification to reduce the heterogeneity of the Bologna criteria.

According to the POSEIDON classification patients are sub-divided into four sub-groups based on quantitative and qualitative parameters, namely:

(i) age
(ii) antral follicle count and/or AMH
(iii) ovarian response – if a previous stimulation was performed.

Hence, the expected poor responder patient according to the age of the patient is classified as either Poseidon Group 3 or 4.

POSEIDON GROUP 1

Your patients <35 years with adequate ovarian reserve parameters (AFC>5; AMH>1.2 ng/ml) and with an unexpected poor or suboptimal ovarian response.

- **Subgroup 1a: <4 oocytes***
- **Subgroup 1b: 4-9 oocytes retrieved***

***after standard ovarian stimulation**

POSEIDON GROUP 2

Older patients >35 years with adequate ovarian reserve parameters (AFC>5; AMH>1.2 ng/ml) and with an unexpexted poor or suboptimal ovarian response.

- **Subgroup 2a: <4 oocytes***
- **Subgroup 2b: 4-9 oocytes retrieved***

***after standard ovarian stimulation**

POSEIDON GROUP 3

Your patients (<35 years with) Poor ovarian reserve pre-stimulation parameters (AFC<5: AMH<1.2 ng/ml)

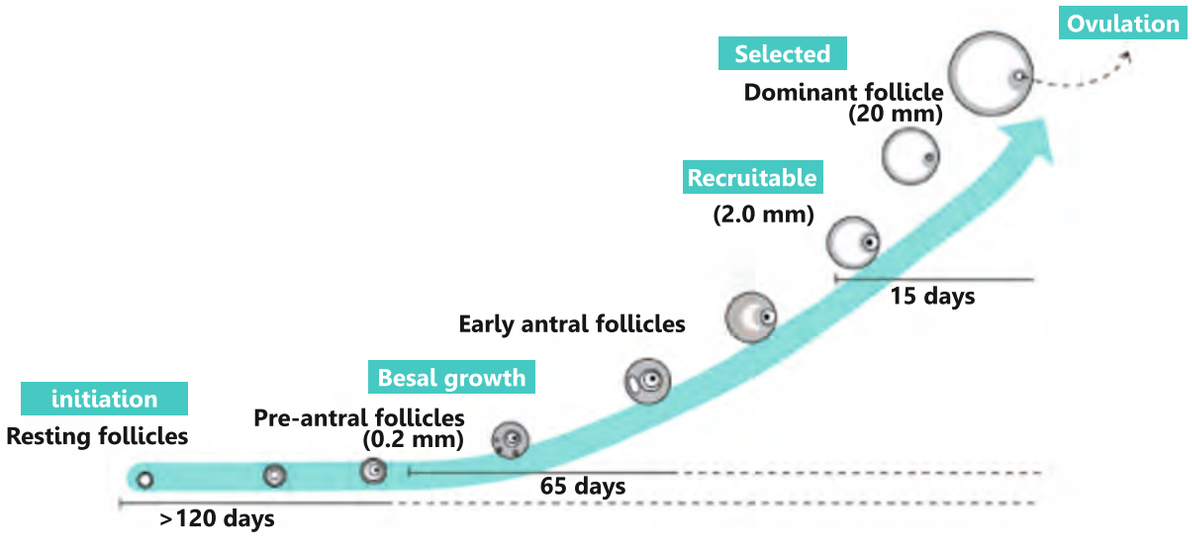
POSEIDON GROUP 4

Your patients (<35 years) with poor ovarian reserve pre-stimulation parameters (AFC<5: AMH< 1.2 ng/ml)

Fig 1 POSEIDON Classification

Aetiology

There is a gradual physiological decline in the follicular pool of a woman in her reproductive life after the age of 38 years. In poor responders, the mechanism of ovarian insufficiency is prematurely determined and not fully understood.



Small growing follicles (Shown with orange arrows) at the very early stages of oocyte maturation determine how many occytes will be availablw for retrieval in a subsequent IVF cycle.

Fig 2 Stages of Oocyte maturation

Some causes of the decrease in ovarian reserve have been identified:

- congenital
- ovarian surgery especially in case of endometrioma
- genital tuberculosis
- Chronic pelvic infections
- genetic defects
- chemotherapy, radiotherapy
- autoimmune disorders
- single ovary
- chronic smoking
- unexplained infertility
- diabetes mellitus Type I
- transfusion-dependent B-thalassemia
- uterine artery embolisation for the treatment of uterine leiomyoma

It is believed that 10% of all the women undergoing IVF treatment will show poor response to gonadotropin stimulation.[5-7] The reduction in the ovarian reserve may be due to many mechanisms and altered expression of certain genes including FSH receptor mutation.

Age is the most common cause of the decline in the ovarian reserve. Every woman is born with a finite number of germ cells which gradually declines throughout her reproductive life. The fertility peaks around the age of 30 years and then gradually declines. The decline is mainly due to follicular atresia.[10]

Premature ovarian insufficiency represents the extreme spectrum in which is characterised by secondary amenorrhoea, hypoestrogenism and high gonadotropin levels in the women below the age of 40 years. Spontaneous premature ovarian insufficiency occurs in 1% of the women above the age of 40 years, 0.1% in women younger than 30 years and 0.01% in women below the age of 20 years.[11,12] These patients form the poor prognosis patient group in ART.[13-16]

Diagnosis

Various tests have been used to predict the ovarian reserve and the ovarian response to the stimulation include the estimation of s anti-Mullerian hormone (AMH) and the antral follicle count (AFC). They are the most sensitive markers of the ovarian reserve.[17] AFC and ovarian volume have a very important role in predicting the response to ovarian stimulation.[18]

The ovarian antral follicle count (AFC) is assessed by transvaginal ultrasound. All the follicles between 2-12 mm are defined to be recruitable. Any follicle which is more than 2 mm shows response to stimulation. The correlation between the AMH levels and the AFC has been well known and established.[19-21]

Another endocrine marker that is used to assess the ovarian reserve is Inhibin B. Inhibins are the glycoproteins that are produced by the theca and the granulosa cells. They suppress the FSH production.[22,23] Inhibin B values decrease and FSH levels increase when the size of the follicle pool decreases. The value of Inhibin B varies with the menstrual cycle. It should be done during the beginning of a follicular phase of the menstrual cycle[24]

Although the association between diminished basal serum concentrations of inhibin-B, poor response to ovarian stimulation in ART is well established, several reports did not recommend the use of only inhibin B as a trustworthy marker of ovarian reserve.[25]

Management

Patients with low ovarian reserve are the ones who belong to a poor prognosis group. Many strategies are used in the management of these patients. Modification of the stimulation protocols and supplementation with adjuvants are used in managing these patients. Oocyte cryopreservation can be offered to young women with low reserve or POF patients.

IVF Stimulation Protocols

Gonadotropins

The most commonly used protocols include an intake of gonadotropins ranging from 300 to 450 IU per day with GnRH agonist with long, stop or microdose flare protocols or GnRH antagonist protocols.There was a previous understanding that increasing the gonadotropin dose would increase the response to the stimulation. However, newer studies have shown that increasing the dose of the gonadotropin does not prove beneficial.[26-28]

Also, the use of highly purified hMG gave better results than rFSH.[29] Addition of LH in younger patients proves to be beneficial, but this is not true for older patients.[30] Sankara et al reported that long agonist protocol had better results in terms of higher number of oocytes retrieved, higher pregnancy rates and lower cancellation rates with a better cohort of follicles responsive to stimulation. Also, microdose protocol uses the lower dose of GnRH agonist for two days in the follicular phase of the cycle in previously suppressed OCP cycle prior to COH.

Natural cycle IVF

The natural cycle should not be used as the first choice but should be used in patients with failure of response to classical stimulation protocol. Natural cycle IVF minimises the cost of the treatment. It results in natural oocyte selection, improved oocyte quality and a higher endometrial receptivity.[31,32] However, it has been reported that it is also associated with increased LH surge and a higher incidence of cancellation rates, low oocyte retrievable rates, low pregnancy rate per embryo transfer cycle.[33]

Minimal stimulation / modified natural cycle antagonist cycle IVF

Mild ovarian stimulation has the advantages of low-cost treatment with less discomfort to the patients. It is also called a patient-friendly protocol for the same reason. It decreases the incidence of ovarian hyperstimulation syndrome.[34]

Studies comparing the conventional IVF protocols with the mild stimulation protocol in women above the age of 37 years have concluded that the mild stimulation protocol had better pregnancy rates and lower cancellation rates as compared to the conventional protocols.[35]

Dual trigger (hCG and GnRH agonist) have been tried and have been reported to yield a better number of oocytes but there was no difference in the number of embryos formed, implantation rate and miscarriage rate was not significantly improved.[36]

Adjuvants

Dehydroepiandrosterone supplementation

It has been noticed that with the advancement in the age, the androgen levels in the ovarian microenvironment decrease. Dehydroepiandrosterone (DHEA) when supplemented, results in an increase in the testosterone levels which results in better oocyte quality, better embryo, and higher pregnancy rates.

The adrenal glands, the theca cells of the ovary, the central nervous system form the source for DHEA. In the ovarian follicle, this DHEA is converted into androstenedione and estrone, the source of testosterone and estrone according to the two cell two gonadotropin theory. Cason et al first described the advantages of DHEA supplementation.[37]

DHEA administration results in the improvement of both the follicular microenvironment and the oxygen levels in the follicular fluid. Recent trials have shown that DHEA supplementation in the patients with a poor ovarian reserve for at least three to four months results in improved oocyte quality, better fertilisation rates and improved pregnancy results.

A recent trial reported that DHEA promotes ovarian function, enhances pregnancy possibilities and by decreasing aneuploidy, decreases the percentage of miscarriage. DHEA supplementation seems objectively to enhance the ovarian reserve.[38] These effects were maximised with 3-4 months of administration of 75 mg of DHEA.[39]

Growth hormone
Growth hormone alters the activity of insulin-like growth factor which in turn modulates the activity of the FSH on the granulosa cells, facilitating the action of gonadotropins on the granulosa cells.[40] GH supplementation increases the clinical pregnancy rates and the live birth rates, especially in women with poor ovarian response in previous cycles and previous IVF failure patients.[41]

Estradiol
Estradiol administration in the luteal phase of the previous cycle has been shown to have some beneficial effect on the stimulation in the next cycle in terms of increase in more coordinated growth of a cohort of the follicles. Some authors have reported enhanced embryo quality and increased pregnancy rates with supplementation of estradiol in the previous cycle.[42,43]

However, recent studies have shown that routine supplementation of estradiol and progesterone as luteal support in long protocols in IVF/ICSI cycles is not recommended.[44,45]

Androgens and androgen modulating drugs
Fabregues et al reported that androgen increase the FSH receptor response to the gonadotropin stimulation resulting in better development of follicles in poor responder patients.[46]

Addition of oral antioxidants
It has been proposed that oral antioxidants rejuvenate the mitochondrial stores in the granulosa cells resulting in improved results in IVF/ICSI cycle. But a recent trial conducted by Bentov et al found no improvement in the fertilisation rate and the clinical pregnancy rates between the control group and those who received oral anti-oxidants. We require more randomised controlled trials to routinely recommend the use of oral anti-oxidants in poor responders.

Oocyte cryopreservation
In younger women who present with the low ovarian reserve but want to delay their conception due to social or medical reasons (before chemotherapy and radiotherapy), can be offered with oocyte cryopreservation. It is no more experimental and can be offered to all the women who present to the IVF clinician for medical or social reasons.[47]

Intraovarian platelet rich plasma
Evidence of enhanced ovarian function was noted in the women who received intra ovarian platelet rich plasma (PRP) instillation.[48]

The procedure involves instillation of 5ml PRP into the ovaries through a transvaginal ultrasound-guided procedure. Autologous PRP substrate isolated and activated with calcium gluconate is for this procedure. It has been reported to result in an increase in the AMH and a decrease in FSH. The number of oocytes reported to be obtained on oocyte retrieval is reported to be increased. After Intraovarian PRP instillation, the ovarian function was reported to be improved as early as two months.

Intraovarian rejuvenation using autologous stem cells
Autologous transfusion of the stem cells in the ovaries of the poor prognosis women group has been tried in the past. It was reported to improve the AFC and the AMH of the 81.3% of women who underwent the procedure. This technique optimized the growth of the existing follicles possible growth of fibroblast growth factor 2 and thrombospondin 1 within apheresis.[49]

Ovarian tissue cryopreservation
Women with premature ovarian insufficiency can be offered ovarian tissue cryopreservation with in vitro activation.

In a recent prospective study comprising 37 infertile women with POI and age more 40 years, ovarian tissue cryopreservation with in vitro activation was studied. This prospective clinical study included 37 infertile women with POI with a history of amenorrhea for more than >4 months, age <40 years and serum FSH levels of >35 mIU/mL.[50]

Laparoscopic ovariectomy was performed, ovarian cortices were dissected into strips and vitrified. These strips were warmed and smaller cubes obtained by fragmentation were later activated by culturing for two days with Akt stimulator.

The washed cubes were transplanted under serosa of the fallopian tube under laparoscopic guidance. This was followed by follicular monitoring and measurement of serum estrogen levels. After oocyte retrieval from mature follicles, IVF was performed. Out of the 37 patients who underwent this procedure, histology showed residual follicles in 54%. Amongst these, 9 patients showed growth of follicles on follicular monitoring, 6 patients underwent oocyte retrieval and 2 live births and 1 miscarriage was reported.

The augment technique- autologous germ cell mitochondrial energy transfer

In the augment technique, the egg precursor cells taken from the small biopsy of the ovary are then cultured and vitrified for storage. Mitochondria from the precursor cells are then injected with sperm into the maternal egg.

However, this technique has been controversial due to ethical issues. Mutations in the DNA could result in genetic diseases and its impact on the future generations is unknown. Ovarian stem cell activation has been reported to be successfully conducted in the mice and form the future potential in humans.

Oocyte donation and adoption

When all the rest fail, oocyte donation may be offered as a last resort in women where the above fail to achieve the result.[51]

Conclusion

The management of poor prognosis women with low ovarian reserve needs a holistic approach. They often form a challenge to treat in the practice of an IVF clinician. Modifying the stimulation protocol with highly individualised egg retrieval along with the addition of adjuvants may prove beneficial in the management of these women. Newer techniques are available and are being tried in the management of these women. However, more studies and trials are required to recommend the same. Lastly, oocyte donation and adoption may be offered to patients who fail to achieve the results despite enormous advances.

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Current Concepts in Ovulation Induction

Dr Pratik Tambe
MD FICOG
Chairperson, FOSGI Endocrinology Committee (2017-19)
Managing Council Member, MOGS
Managing Council Member, IAGE, MSR, AMC (2015-18)

Dr Rohan Palshetkar MS
Youth Coordinator FOGSI
Treasurer, Maharashtra Chapter IAGE
Youth Council Member MOGS

Dr Jiteeka Thakkar MS
Youth Council Member MOGS

Background
An ovulatory infertility is a common problem faced in infertility practice. The causes of an ovulation have been classified by the World Health Organisation into 3 categories based on the gonadotropin profile:

WHO type 1 (hypogonadotropic hypogonadism) (10%)
Caused by any lesion affecting the pituitary or hypothalamus and affecting gonadotropin production including idiopathic, weight-related amenorrhoea, Sheehan syndrome, extreme stress and strenuous exercise, Kallman's syndrome, craniopharyngiomas etc.

WHO type 2 (normogonadotropichypogonadism)
The commonest cause of an ovulation accounting for 85% of cases and is most commonly caused by polycystic ovarian syndrome. Hyperprolactinaemicamenorrhoea is another cause, where in addition to amenorrhoea and infertility, women may have galactorrhoea.

WHO type 3 (hypergonadotropichypogonadism) (5%)
This is usually an indication of ovarian failure.

Treatment strategies and goals
In an ovulatory women, the purpose of treatment in ovulation induction is the development of at least one follicle, whereas in other causes of infertility, ovarian stimulation is used to increase the number of follicles, known as super ovulation or controlled ovarian hyper stimulation. Induction of ovulation is possible in the first two types. However, in the third type, ovulation induction is usually unsuccessful due to follicular depletion and the only way to achieve a pregnancy may be through oocyte donation.

Clomiphene citrate
Clomiphene is the oldest, most widely used ovulation induction agent and is structurally similar to estrogen, allowing it to bind competitively to the estrogen receptor (ER).[1] While most modern fertility physicians have now shifted to aroma tase inhibitors as a first line agent for oral ovulation induction, the role of clomiphene citrate and the significant body of evidence on this molecule built up over several decades needs to be discussed.

As a selective ER modulator (SERM), CC has both estrogen agonist and antagonist properties; however, it is the agonist properties, which manifest in the setting of lowendogenous estrogen levels, that are relevant in the setting of ovulation induction.[2] When endogenous estrogen levels are low, CC competitively binds ERs. CC also binds nuclear ERs for longer periods than endogenous estrogen, thus, depleting ER availability and communicating a false low estrogenstate to the hypothalamus. This causes the hypothalamic-pituitary ovarian feedback axis to increase GnRH secretion, increasing pituitary gonadotropin release and hence follicular activity.[1]

Treatment regimen:
Dosing is started 2 to 5 days after the onset of a spontaneous or progestin-induced menses or arbitrarily in amenorrhoeic patients with a negative pregnancy test. Ovulation and pregnancy rates are similar regardless of whether CC is initiated on cycle day 2, 3, 4, or 5. Treatment with CC is associated with higher rate of pregnancy if started early (days 1 through 5 than 5 through 9) in the menstrual cycle.[3] Typically, CC 50 mg is given daily for 5 days; if ovulation occurs, it is expected 5 to 10 days after the last dose of CC. If the patient remains an ovulatory at 50 mg/ day, 50mg/d increments may be done with each cycle until ovulation is achieved with standard effective doses ranging from 50 to 150 mg/d.

In women who ovulate, 52% do so taking 50 mg, 22% taking 100 mg, and fewer with subsequent increases.[4] There are varying opinions about maximum CC dosing, and although doses greater than 100 mg/d are not approved by the FDA, the American College of Obstetricians and Gynecologists supports doses of up to 150 mg/d before considering alternatives and recognizes that some women, particularly those with higher body mass indexes, will require higher doses to achieve ovulation.[5]

Indications
An ovulatory infertility
Traditionally, the first-line treatment for an ovulatory and oligo - ovulatory women, though recent guidance has now advocated the use of aroma tase inhibitors as the first line of therapy, especially in PCOS as per the new ESHRE/ASRM joint PCOS Guideline.

Unexplained infertility
Clomiphene citrate is used empirically for the treatment of unexplained infertility, but there is currently no definitive evidence that it offers significant benefit over placebo alone in these patients. However, when CC is used in combination with IUI, CC has shown a therapeutic benefit over placebo.[6]

Clomiphene citrate is an efficient, inexpensive and well tolerated drug with a well-known safety profile when used correctly.[22] A recent review by Kathrine B P et al from 2014 supports the use of clomiphene citrate as first-line treatment for ovulation induction in PCOS. [23] The continuation of treatment for another six cycles of clomiphene citrate before switching to, for example, gonadotrophins may be cost-effective theoretically.[24]

Tamoxifen
This is another SERM that is similar to CC in structure, has also proven successful as an induction agent.[8] Traditionally, it has been used in the medical management of breast cancer. The lack of superiority data and side effects, including hot flashes, limit its clinical utility.

Aroma tase inhibitors
The aroma tase inhibitors used for ovulation induction include anastrozole and letrozole, that function as competitive, non steroidal inhibitors of aroma tase. Hence, androgens cannot be converted to estrogens, thereby creating a low estrogen state, releasing the hypothalamic-pituitary axis from the negative feedback of estrogen.This causes compensatory increase in the pulsatile GnRH secretion and thereby causes follicular growth.[9]

Specifically, after letrozole treatment is ceased, estrogen levels increase immediately, which leads to a more abrupt decrease in follicle-stimulating hormone (FSH). This decrease makes gonadotropin support of multiple follicles less likely and increase in estrogen allows for the production of cervical mucus and endometrial proliferation.

Therapy regimen and efficacy
Letrozole is typically given in doses of 2.5 to 7.5 mg daily and the dose can be adjusted in 2.5mg/d increments. Anastrozole is given as 1mg daily. Both medications are administered in a very similar regimen to that of CC. Prolonged treatment courses (extended regimen for 10 days) and single-dose regimens (20 mg once on cycle day 3), have been studied with some positive results.[14,15]

Letrozole with timed intercourse or IUI can be used and although it has been most studied in adjunct with hCG and USG, these additions are likely unnecessary in patients with ovulatory dysfunction who don't suffer from a secondary source of infertility. In an ovulatory women who do not respond to CC treatment, aroma tase inhibitors have been effective with a 60% ovulation rates and between a 12% and 40% pregnancy rates.[13,16]

Indications

Letrozole is indicated in CC-resistant women or in those who are unable to use CC because of side effects, such as vasomotor symptoms, visual changes, or headaches.[10] Aroma tase inhibitors should also be used in women who have a thin endometrium (<7 mm) when taking CC, as the proliferation of the endometrium is stimulated by estrogen, and letrozole does not have the same antiestrogen effects as CC.[11,12]

In a recent Cochrane review, letrozole appears to improve live birth and pregnancy rates in subfertile women with anovulatory polycystic ovary syndrome, compared to clomiphene citrate.[27] This differs from a previous review, which did not detect a difference.[28]

Adjuvant regimens

These have traditionally been described in textbooks of reproductive endocrinology (especially glucocorticoids and bromocriptine) and are mentioned here for completeness; their utility is restricted in day-to-day practice.

Clomiphene and glucocorticoids

With CCresistance in the presence of normal and elevated dehydroepiandrosterone(DHEA) levels, the addition of dexamethasone (0.5–2 mg) or prednisone (5 mg) during the follicular phase has shown an increase inovulation and pregnancy rates when compared with CC plus place bo.The mechanism of the glucocorticoid effect has not been fully elucidated, but it ishypothesised that androgen suppression has direct effects on the oocyte and indirect effects on cytokines and intra follicular growth factors.[16]

Clomiphene and human chorionic gonadotropin

Patients using CC for ovulation induction for unexplained infertility or coexisting male factor infertility requiring IUI may benefit from an injection of hCG, used as a surrogate luteinising hormone surge to trigger ovulation.

Clomiphene and metformin

The combination of metformin and CC deserves consideration in PCOS patients whoare CC resistant before proceeding with alternative therapies. If metformin is used, it is usually administered at 1500–2000 mg daily,which can be increased after a starting dose of 500 mg daily.

A meta-analysis has suggested that metformin may improve success in weight management.[25] Otherwise, the role of metformin in ovulation induction is controversial. Interestingly, metformin may have a role as pretreatment before standard assisted reproduction techniques. A recent RCT demonstrated improved pregnancy rates after 3–9 months of metformin before assisted reproduction techniques.[26]

Exogenous gonadotropins

Gonadotropins were first obtained by purifying urine; nowadays many commercially available preparations are from highly purified urinary source medications or are the product of recombinant technology. Advantages of the recombinant preparations include more consistent supply, less variation in biological activity, and the absence of antigenic urinary protein; however, based on available evidence, efficacy for ovulation induction may be similar regardless of preparation.[17,18]

Indications

Hypogonadotropic hypogonadism

Oral ovulation induction agents are typically not effective in WHO group I patients, who do not have intact hypothalamic-pituitary-ovarianaxes. Exogenous gonadotropinsrestore normal cyclic ovulation for these patients.[19] Historically, GnRH was delivered in a pulsatile fashion via a pump, and although GnRH pump efficacy for monofollicular development was quite good, they had several limitations and are no longer available commercially.

Clomiphene citrateresistant an ovulation
Exogenous gonadotropins are not considered first-line therapy for ovulation induction for most an ovulatory patients and should only be considered in WHO II patients after a failure to respond to oral therapy. [20]

Unexplained infertility
Super ovulation is often the goal of using gonadotropins in this population attempting to optimise cycle fecundity.

Therapy regimen and efficacy
As a prerequisite, extensive counseling is essential. The couple must understand the expected expenditure and the commitment required to monitor medication effects. Close monitoring of serum estradiol levels and follicular number and growth is mandatory to minimize risk of ovarian hyper stimulation syndrome (OHSS). The dose and duration of gonadotropin treatment required to induce ovulation varies and must be determined empirically.

Patients remaining an ovulatory (CC-resistant) and patients failing to conceive during CC treatment (CC failure) are generally treated with exogenous gonadotropins. Recently, it has become more accepted to treat clomiphene resistant patients with a combination of CC and an insulin sensitiser before treatment with exogenous gonadotropins is started.

Individual differences in the FSH response dose (amount of FSH required to induce ongoing follicle growth and ovulation) may be the main factor of hyper- responsiveness and severe complications during ovulation induction by FSH.

The **'step-up'** protocol aims at surpassing the FSH-threshold to reduce the chances of complications. However, this approach may cause the treatment period to be prolonged and late follicular phase FSH accumulation, causing increased risk of multi follicular growth.

The **'step-down'** protocol overcomes these problems by imitating the physiological FSH profile more closely. The starting dose of FSH is presumed to be the response dose; hence, dominant follicle growth is established more quickly. Hence, the dose of FSH can be slowly reduced, resulting in the development of a single dominant follicle.[24]

Frequent monitoring of response is important, especially during the step-down protocol, because the duration of FSH threshold being suppressed determines whether there will be mono- or multi follicular growth. Stimulation can be cancelled when multi follicular growth is apparent and more than three follicles >12mm in diameter are present. Starting with a very high initial dose in women with a low FSH threshold is the most prominent danger in step- down ovulation induction.

A low-dose, **'step-up regimen'** may be used during the first stimulation cycle to determine the response dose for an individual patient. Subsequent cycles can be performed according to a low-dose step-down regimen, starting 37.5 IU above the response dose in the preceding 'dose finding' cycle.

GnRH agonists and antagonists
Among the various GnRH agonist protocols, namely ultra short, short and long, the long GnRH agonist protocol has been used as the gold standard in IVF since its discovery in the 1980s. GnRH antagonists have recently offered an alternative approach in IVF treatment.

The long GnRH agonist protocol involves administration of 0.1 mg GnRH agonist (e.g., triptorelin / leuprolide) starting on preceding cycle-day 21 followed by administration of gonadotropin at 150-225 international units (IU) daily starting on cycle-day 2. The adjustment of dose is based on follicular development and administration of GnRH agonist and gonadotropin lasts until the hCG trigger injection, which is around 14 days post GnRH agonist regimen or when follicles reach 16 to 18 millimeters (mm) in size.

For the GnRH antagonist protocol, administration of gonadotropin is initiated after monitoring of patients' follicles sizes on cycle-day 2/3. Gonadotropin dosage varies according to the follicular response. Approximately after the 6th day of gonadotropin injection or when follicular size reaches more than or equal to 14 mm, subcutaneous administration of the GnRH antagonist(eg.Cetrorelix 0.25 mg/d) begins.

Myoinositol
It has been found in recent studies that insulin sensitisers like myo-inositol improved the ovulation and pregnancy rate in insulin-resistant patients with PCOS when given alone or in combination with clomiphene citrate.[29]

J Pundir et al conducted a systematic review and meta-analysis on inositol treatment in women with polycystic ovarian syndrome, published in the BJOG in 2017.[26] Ten trials and a total of 362 women were on inositol (257 on myo-inositol; 105 on di-chiro-inositol), 179 were on placebo and 60 were on metformin. Inositol was associated with significantly improved ovulation rate (RR 2.3; 95% CI 1.1–4.7; I2 = 75%) and increased frequency of menstrual cycles (RR 6.8; 95% CI 2.8–16.6; I2 = 0%) compared with placebo. One study reported on clinical pregnancy rate with inositol compared with placebo (RR 3.3; 95% CI 0.4–27.1), and one study compared with metformin (RR 1.5; 95% CI 0.7–3.1). No studies evaluated live birth and miscarriage rates.[30]

They concluded that inositol appears to regulate menstrual cycles, improve ovulation and induce metabolic changes in polycystic ovary syndrome; however, evidence is lacking for pregnancy, miscarriage or live birth. A further, well - designed multi centric trial to address this issue to provide robust evidence of benefit is warranted.

Cochrane meta-analysis
42 RCTs and 7,935 women were analysed in a Cochrane meta-analysis in 2018.[27] Live birth rates were higher with letrozole (with or without adjuncts) compared to clomiphene citrate (with or without adjuncts) followed by timed intercourse (OR 1.68, 95% CI 1.42 to 1.99; 2,954 participants; 13 studies; I2 = 0%; number needed to treat for an additional beneficial outcome (NNTB) = 10).

There is evidence for a higher pregnancy rate in favor of letrozole (OR 1.56, 95% CI 1.37 to 1.78; 4629 participants; 25 studies; I2 = 1%; NNTB = 10; moderate-quality evidence). There is little or no difference between treatment groups in the rate of miscarriage by pregnancy (20% with CC versus 19% with letrozole; OR 0.94, 95% CI 0.70 to 1.26; 1210 participants; 18 studies; I2 = 0%) and multiple pregnancy rate (1.7% with CC versus 1.3% with letrozole; OR 0.69, 95% CI 0.41 to 1.16; 3579 participants; 17 studies; I2 = 0%).

There is low-quality evidence that live birth rates are similar with letrozole or laparoscopic ovarian drilling (OR 1.38, 95% CI 0.95 to 2.02; 548 participants; 3 studies; I2 = 23%). There is low-quality evidence that pregnancy rates are similar (OR 1.28, 95% CI 0.94 to 1.74; 774 participants; 5 studies; I2 = 0%). There is insufficient evidence for a difference in miscarriage rate (OR 0.66, 95% CI 0.30 to 1.43; 240 participants; 5 studies; I2 = 0%), or multiple pregnancies (OR 3.00, 95% CI 0.12 to 74.90; 548 participants; 3 studies; I2 = 0%).

Additional comparisons were made for Letrozole versus placebo, Selective oestrogen receptor modulators (SERMS) followed by intrauterine insemination (IUI), follicle stimulating hormone (FSH), anastrozole, as well as dosage and administration protocols. There is insufficient evidence for a difference in either group of treatment due to a limited number of studies. Hence, the reviewers concluded that more research is necessary. [27]

Conclusions
Although clomiphene citrate as a treatment modality has existed for more than 50 years, an increased awareness of the effect of obesity and different PCOS phenotypes has emerged. Accordingly, ovulation induction in women suffering from oligo- and anovulation seeking fertility treatment has to be individualised according to weight, treatment efficacy and patient compliance, with the aim of achieving mono follicular growth, mono-ovulation and subsequently the birth of a singleton baby.

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New Consensus in Managing Endometriosis

Dr Prakash Trivedi MD
President, FOGSI 2015

Dr Sanjay Patel
MD, Diploma in Endoscopy (Germany)
Director, Mayflower Women's Hospital Ahmedabad

Introduction

Endometriosis is an estrogen-dependent disease and thus, usually affects reproductive-aged women. This condition has a prevalence rate of 20-50% in infertile women[1,2,3,4] but it can be as high as 71-87% in women with chronic pelvic pain.[1,5] The laparoscopic approach is the method of choice for treating endometriosis conservatively.[6,7]

Signs and symptoms

- Painful periods (dysmenorrhoea)
- Pain with intercourse
- Pain with bowel movements or urination
- Excessive bleeding
- Infertility
- Other symptoms: fatigue, diarrhoea, constipation, bloating or nausea, especially during menstrual periods.

Pathophysiology of infertility in endometriosis patients

- Increased prostaglandin level
- Sperm motility and binding
- Vascular endothelial growth factor (VEGF)
- Tumor necrosis factor alpha(TNF-□)
- Immunological abnormalities
- Abnormal follicular development
- Reduced embryo implantation

Some patients with minimal endometriosis and normal pelvic anatomy are also infertile.

Type of endometriosis

1. Pelvic endometriosis
- Ovarian endometrioma
- Superficial pelvic peritoneum involvement

2. Extrapelvic disease (DIE/ deep infiltrating endometriosis) refers to lesions that penetrate to a depth of 5 mm or more
- Uterosacralligaments
- Retro cervical space,
- Bowel,
- Ureter,
- Bladder
- Others

Complete resection requires appropriate surgical expertise that often includes a multidisciplinary surgical team (general surgeon, urologist).

Preoperative preparation

- Informed consent and preoperative counselling
- Thromboprophylaxis
- Antibiotic prophylaxis
- Bowel preparation

Surgical procedures

Aims

- Diagnosing the location and extent of endometrial lesions
- Treating the lesions with some form of destructive therapy

Objectives

- Excision or ablation of all endometriosis implants
- Cystectomy or resection of endometriomas
- Removal of endometrial growths, scar tissue, and adhesions
- Restoration of normal anatomy

Destruction of endometriotic lesions

- Modalities
- Ablation– eradication of lesions by laser vaporisation, electrosurgical fulguration, or ultrasonic cutting and coagulation
- Excision – removal of lesions
- Radical resection of endometriosis refers to removal of all visible implants at the time of surgery.

Ablation can be performed with laser or electro diathermy. Overall, the recurrence rate is 19% and is similar for all techniques.[8]

Exploration and diagnosis

- Inspect the pelvis
- Uterus, cervix, bilateral ovary and fallopian tubes, and ligaments (uterosacral ligaments, round ligaments)
- Peritoneal surfaces (anterior and posterior pelvic cul-de-sac, pelvic sidewalls)
- Sigmoid colon
- Bimanual pelvic examination under laparoscope for posterior cul-de-sac lesions
- Appendix (2 to 4 percent involvement)
- Deep infiltrating endometriosis by peritoneal mobility

Adhesiolysis

Resect all adhesions that may compromise fertility or that correspond to the location of the patient's pain. The term previously used for this was "frozen pelvis."

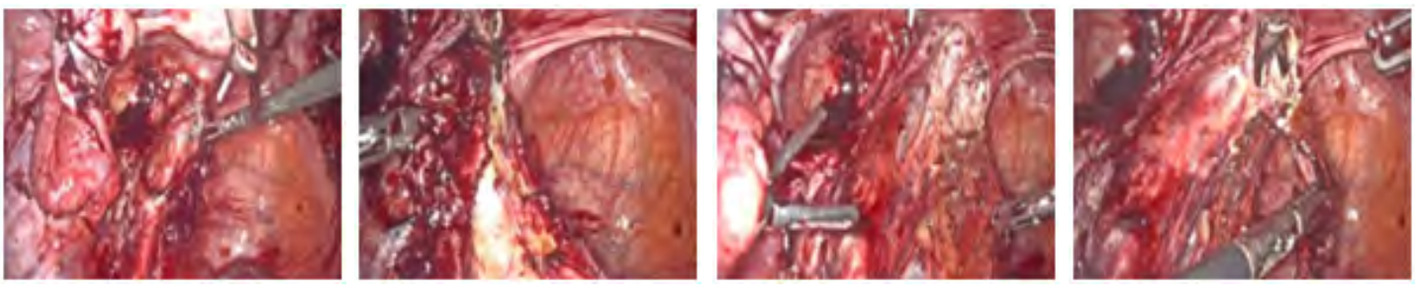
Techniques

Management of endometrioma

Laparoscopic cystectomy is the gold standard and preferred approach for the treatment of endometriosis and endometrioma. Laparoscopic cystectomy was found to yield better pain relief and pregnancy rates than drainage.[9,10]Medical therapy with gonadotropin-releasing hormone (GnRH) agonists reduces the size of the cyst but does not influence pain relief.[11]

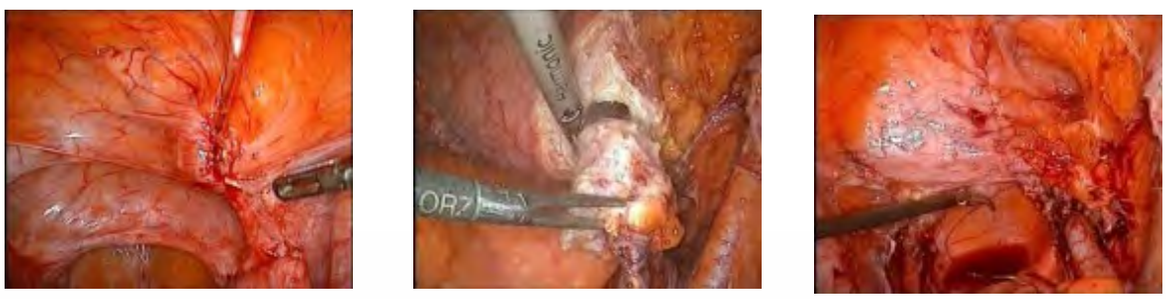
Excision of the entire cyst by laparoscopy appears to be the optimum treatment approach. Fenestration, ablation of the lining of an endometrioma or aspiration is less effective with increased recurrence rate.

Uterosacral cardinal endometrioma

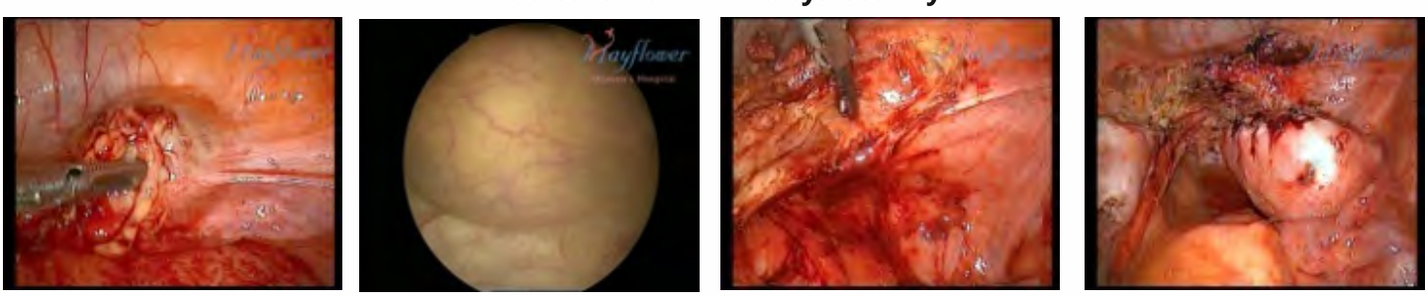


Bladder endometrioma

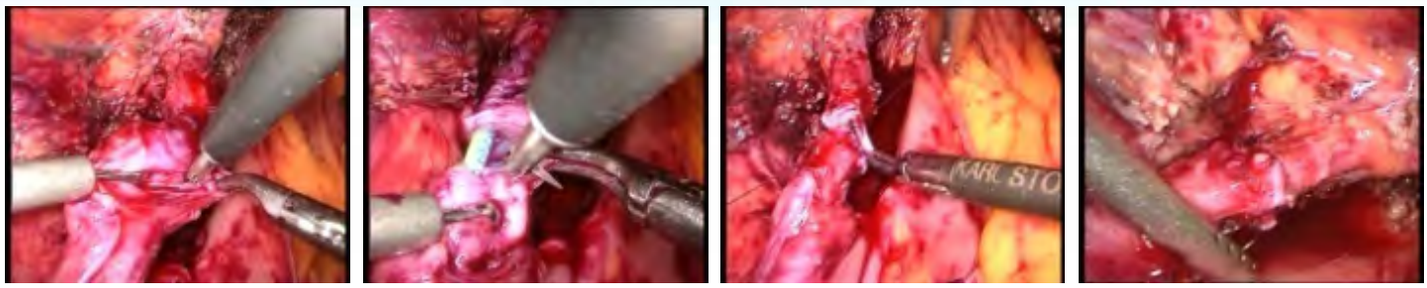
Intrinsic involvement: excision and suturing



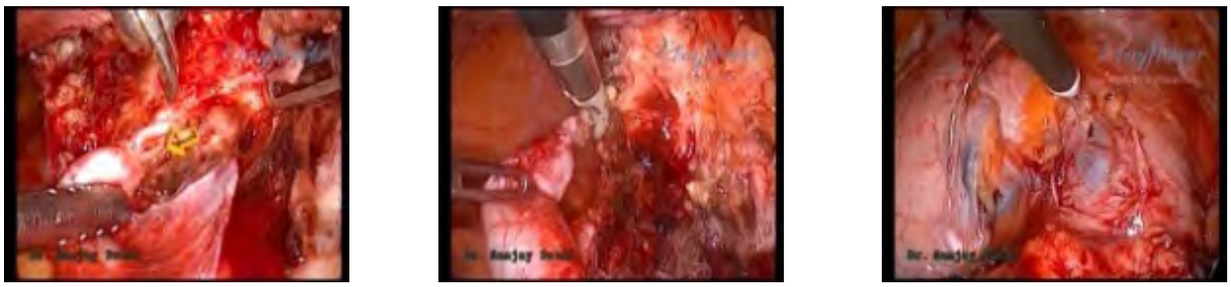
Extrinsic involvement: cystectomy

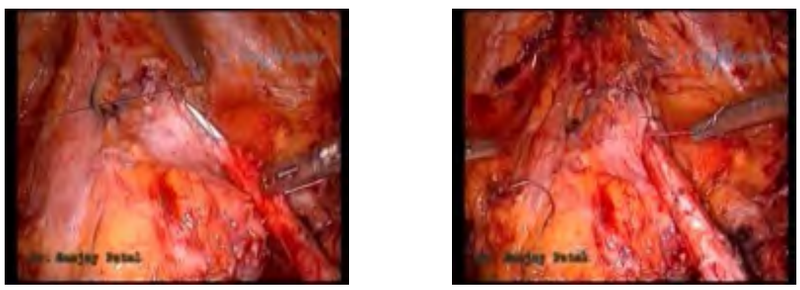


Bilateral ureteric endometriosis
Intrinsic involvement : Resection anastomosis

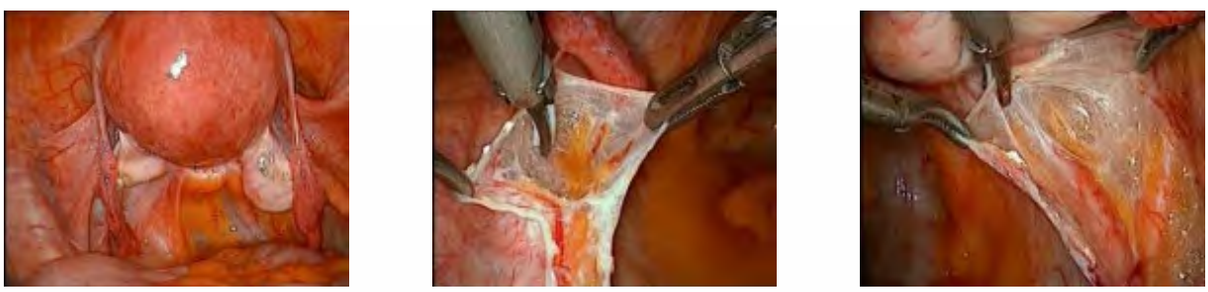


Implantation

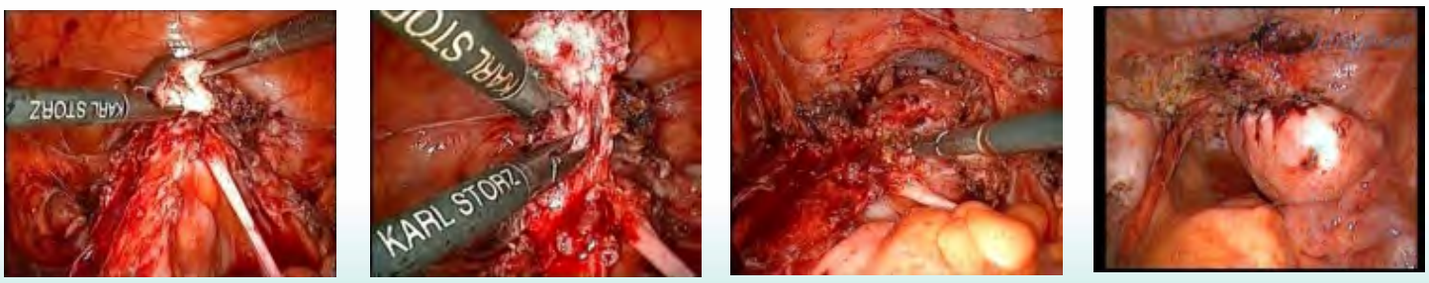




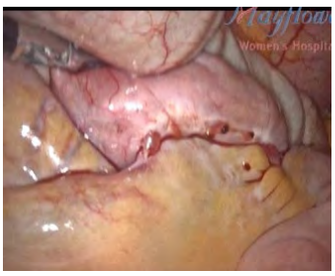
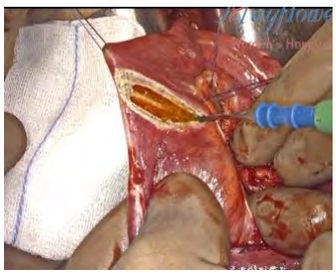
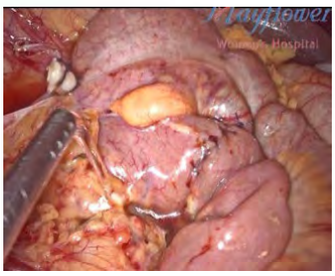
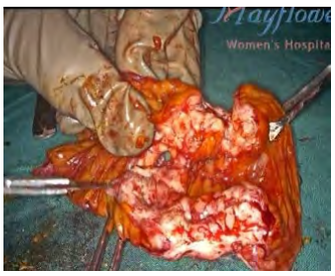
Extrinsic involvement: Ureterolysis



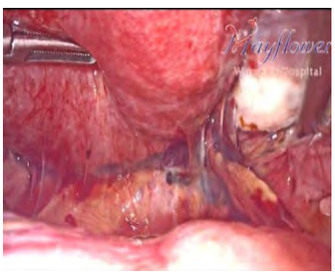
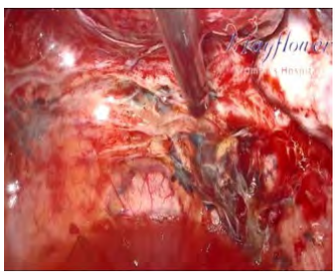
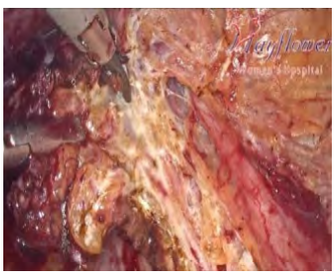
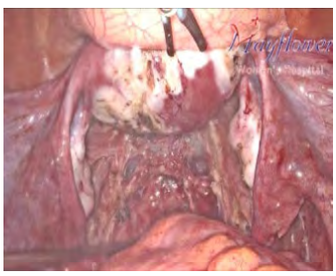
Colorectal and intestinal endometriotic nodule
Extrinsic involvement: Shaving



Intrinsic involvement: Resection anastomosis

Peritonectomy

Others: scar endometriosis, diaphragmatic and other organ involvement

Complications

Surgical injuries to adjacent structures (e.g., nerves, blood vessels, ureters) at the time of excision or ablation, wound infection and adhesion formation.

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Unexplained Infertility: Does it Exist?

Dr Bhaskar Pal
MD DGO DNB
Secretary General IAGE
Vice President FOGSI (2017)

Dr Kedar Ganla
MD DNB FCPS DGO DFP
Consultant Fertility Physician,
Ankoor fertility Clinic, Mumbai

Dr Rana Choudhary
DNB DGO DFP DCR FCPS FICMCH FICOG MRM MNAMS
Associate Consultant,
Ankoor fertility Clinic, Mumbai

Dr Priyanka Vora
DGO DFP FCPS BIMIE
Associate Consultant,
Ankoor fertility Clinic, Mumbai

Introduction

It has been observed over the past few decades that the overall incidence of infertility is constant. However, the success rates in terms of achieving pregnancy has markedly increased because of the advent and use of assisted reproductive technologies like intra uterine insemination (IUI), in vitro fertilitisation (IVF) and intra cytoplasmic sperm injection (ICSI).[1] In approximately 15% to 30% of couples no obvious cause can be found.[2] Unexplained Infertility (UI) remains a puzzle till date and is basically a diagnosis of exclusion. If the standard investigations like semen analysis, ovulation detection tests and tubal patency are normal, the couple is labelled as suffering from unexplained infertility.[3]

However,some authors have questioned the use of the term UI. They have argued that women having mild endometriosis, premature ovarian ageing and immunological infertility can be misdiagnosed as UI.It is interesting to note that with thecurrent management options, further investigations and more 'accurate' diagnosis is unlikely to changecourse of action in these couples.

Diagnosis of Unexplained Infertility: the basic infertility evaluation

Infertility is defined as the failure to conceive after 1 year of unprotected intercourse. The NICE guidelines have categorised infertility into the following types:[4]

- Male factor,
- Tubal disease,
- Anovulation,
- Endometriosis and
- Unexplained infertility (UI).

The American Society for Reproductive Medicine (ASRM) has also published guidelines for a standard evaluation in fertility seeking couples.[5]These investigations include:

1. Semen analysis,
2. Assessment of ovulation,
3. Tubal patency tests using a hysterosalpingogram

They have stated that further tests in the form of ovarian reserve testing and diagnostic laparoscopy may be added.

A study of literature shows that the conception rate is strongly influenced by female partner's age and infertility duration, and treatment independent cumulative live birth rates have been around 33-60% at the end of 3 years. It is interesting to note that pregnancy rate does not change neither does the management in this group of patients. As there is unavailability of precise diagnostic methods, many times UI is over diagnosed.

Aetiology

The following conditions may be the underlying cause in these couples with unexplained infertility:

Male factor

WHO has laid down guidelines stating parameters for a normal semen analysis. However, there is an overlap between 'normal' and 'abnormal' values. Use of intracytoplasmic sperm injection (ICSI) has revolutionised the treatment for male infertility which includes UI due to immunological origin. Sometimes there is an increase in sperm DNA fragmentation index which could be the cause of UI. This is more common in men with diabetes and can be improved by maintaining strict glycemic control and use of antioxidants.

Immunological factors

Upto 20% of couples having infertility may have underlying autoimmune disease. Various tests like antiphospholipid, antinuclear, antithyroid and antisperm antibodies have been used for a long time. Preliminary studies reported an association between early reproductive failure/ miscarriage and abnormal immune function.

However recent studies have failed to confirm a causal effect. Matsubayashi et al reported elevated peripheral NK activity in patients with UI in 2001. This has led to confusion in the therapy for these patients. Therapies like intravenous immunoglobulin have not shown consistent results inimprovement of live birth rates in couples with IUI and repeated unexplained IVF failures as per Stephenson and Fluker. Hence use of this therapy routinely is thus questionable.

Mild tubal disease

Assessment of the uterine contour and tubal patency is an integral part of basic infertility evaluation. This may be achieved by hysterosalpingography (HSG).Mol et al in 1999 reported a sensitivity of 0.81 and a specificity of 0.75 for HSG in identifying tubal occlusion as compared to laparoscopy However, patent fallopian tubes on HSG do not confirm ideal tubal functioning. For example, women with severe endometriosis may have adherent ovaries in the cul de sac with normal fallopian tubes.

Laparoscopy

Laparoscopic direct visual examination of the pelvic reproductive anatomy is the gold standard and test of choice to identify otherwise unrecognised peritoneal factors, specifically endometriosis and pelvic adhesions. Laparoscopy is more reliable in predicting pregnancy, (fecundity rate ratios of 0.38 and 0.19 when a one- and two-sided occlusion appeared, respectively) compared with HSG (expressed by a kappa statistic of 0.42). However, even this has some limitations in terms of accuracy in assessing tubal patency and function.

In women with patent tubes, it would be reasonable to adopt an expectant approach (taking into account age and duration of subfertility) before considering IVF. Thus, inability to exclude a diagnosis of mild tubal defects is unlikely to change the overall plan of management.

Endometriosis

In the absence of a detailed laparoscopic examination of the pelvis, endometriosis could often be misdiagnosed as UI. In a meta-analysis of women with endometriosis by Barnhart et al in 2002, pregnancy rates were found to be higher in women without endometriosis. The management of infertility in these women depends on the age and severity of endometriosis. The management strategies for minimal/mild endometriosis are similar to those used for UI, i.e. superovulation/IUI and IVF (NICE, 2004). This questions the justification for diagnosing minimal and mild endometriosis separate from UI.

Management of minimal / mild endometriosis

- Medical treatment is ineffective.
- Laparoscopic resection or ablation of lesions in minimal and mild endometriosis led to contradictory
- results. Also the cost of therapy increases without much increase in success rate of pregnancy.
- Expectant management OR superovulation/IUI and IVF is the accepted management.

Endometrium

Defect in the endometrial perfusion may also be the reason for UI. This can be diagnosed by endometrial Doppler evaluation in the follicular as well as luteal phase. Various therapies like ecospirin, nitric oxide donors, l-arginine, sildenafil etc have been used to improve the perfusion and pregnancy rate in these women.

Additional investigations in UI

Assessment of ovarian reserve

As age advances there is high rate of follicular atresia and poor follicular growth. This is known as 'poor ovarian response'. Thus, women with advanced age or history of prior ovarian surgery are at risk for diminished ovarian function or reserve and maybe the underlying cause of UI.

Hence, the evaluation of the ovarian reserve maybe offered to women with UI. The testing includes a cycle day 3 serum follicle-stimulating hormone (FSH) and ultrasonographic ovarian antral follicle count. The results are not absolute indicators of infertility but abnormal levels correlate with decreased response to ovulation induction medications and lowered live birth rates even after IVF.

In the absence of reliable and accurate markers for ovarian reserve, older women will be offered the same treatment options, such as controlled ovarian stimulation with IUI, IVF, and ultimately oocyte donation.

Management

Treatment for unexplained infertility is mostly empirical because it does not address a specific defect or functional impairment. It is possible that unexplained infertility represents the lower extreme of the normal distribution of fertility with no defect present. It is possible that routine infertility evaluation misses subtle defects because of imperfect or incomplete methods.

Studies of couples with unexplained infertility who are followed without any treatment report a broad variation in cumulative pregnancy rates. Although expectant management is associated with the lowest cost, it results in the lowest cycle fecundity rates, and is therefore inferior to the commonly available reproductive techniques outlined below. It may provide an option for a couple with unexplained infertility in whom the female partner is young with a good ovarian reserve.

The principal treatments for unexplained infertility include expectant observation with timed intercourse and lifestyle changes, oral ovulation induction, controlled ovarian hyperstimulation (COH) with IUI, and IVF.

Expectant management and lifestyle change

Epidemiological studies indicate cigarette smoking, abnormal body mass index (BMI), and excessive caffeine and alcohol consumption reduce fertility. Thus weight reduction, reducing caffeine intake (2 cups of coffee/day), and alcohol intake to no more than 4 standardised drinks per week may benefit in these women.[6]

Laparoscopy

Whether operative laparoscopy improves pregnancy outcomes in a subject with unexplained or minimal/mild endometriosis is of debate. A Cochrane review from 2002 found that laparoscopic surgery in the treatment for mild endometriosis and may improve pregnancy success rates, but further research is needed. However, current literature does not support performing a diagnostic laparoscopy in all patients.

IUI

Intrauterine insemination (IUI) can be performed in conjunction with natural ovulation timed with LH kit, ovulation induction using clomiphene citrate / letrozole, or injectable gonadotropins. It has been estimated that 37 cycles of IUI without additional ovarian stimulation would be needed to obtain an additional pregnancy compared with control cycles. A Cochrane review confirmed that IUI with ovulation induction increased the live birth rate compared with IUI alone.[8]

COH and IUI

Over the past decade, there has been a marked increase in the use of COH, with or without IUI, in the treatment of UI. Clomiphene citrate, letrozole and gonadotropins have been used for COH, with or without IUI. Subtle ovulatory defects missed by standard testing may be overcome by doing COH with IUI and we get an increased number of oocytes available for fertilization. Also, when washed sperms are introduced into the uterine cavity it increases the density of motile sperm available to ovulated oocytes and maximise the chance of fertilization.

Use of clomiphene citrate with timed intercourse in patients with unexplained infertility has been shown to have a small effect on pregnancy rates: combined analysis of the available evidence revealed that 40 cycles with empirical clomiphene citrate therapy were necessary to achieve 1 additional pregnancy. Gonadotropin therapy is superior to clomiphene citrate therapy, and both are most effective when combined with IUI.

Gonadotropins

A meta-analysis of 27 studies involving 2,939 cycles revealed that the pregnancy rate per cycle was 8% with gonadotropin treatment alone and 18% with gonadotropin treatment combined with IUI. The cumulative pregnancy rate rises with the number of attempted COH/IUI cycles; however, there is some evidence suggesting that the number of COH/IUI cycles prior to treatment with IVF should be limited to three. IVF can be offered if the couple fail to conceive after 3 trials of COH and IUI.

Role of Double IUI

There are several studies addressing the effect of IUI on 2 consecutive days over single IUI. Available trials on this issue are difficult to interpret because they are not restricted to patients with unexplained infertility, but also included subjects with other types of infertility. Although some studies suggested marginal benefits of double IUI over single, the most recent randomised trial concluded that among patients undergoing COH/IUI, results of single and double IUI do not differ statistically.

IVF/ICSI

IVF/ ICSI is the treatment of choice for unexplained infertility when other treatment modalities explained above have failed. Studies have shown a 40% live birth rate for women younger than 35 years of age and 38% for women 35 to 37 years of age with UI.

The optimal treatment strategy needs to be based on individual patient characteristics such as age, treatment efficacy, side-effect profile such as multiple pregnancy, and cost considerations.

Conclusions

1. Couples should undergo a semen analysis, ovulation testing, assessment of ovarian reserve, and imaging to assess for tubal and uterine factors before a diagnosis of unexplained infertility is made.

2. The principal treatments for unexplained infertility include expectant observation with timed intercourse and lifestyle changes, clomiphene citrate, letrozole and intrauterine insemination (IUI), controlled ovarian hyperstimulation with Gonadotropins with IUI, and in vitro fertilisation (IVF).

3. Although expectant management is associated with the lowest cost, it results in the lowest cycle fecundity rates. It may provide an option for a couple with unexplained infertility in whom the female partner is young and has good ovarian reserve.[7]

4. IVF/ ICSI is the treatment of choice for UI when the less expensive, but also less successful treatment modalities have failed.

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Immunology of Fertility

Prof Pratap Kumar MD
Kasturba Medical College
Manipal Academy of Higher Education

Introduction
Immunological responses could be the cause in many cases of infertility and miscarriage. Several auto antibodies, produced by the immune system directed against one or more of the individual's own proteins have been investigated as possible influences on reproductive success and failure. Auto antibodies may persist for many years in the circulation as a marker of a prior autoimmune attack, but their presence does not necessarily indicate a current disease process. Hence there is a lot of unclear data on the subject which seem a dilemma in every one's mind.

What are the various immune problems?

Antiphospholipid antibodies
Some of the researched areas amongst the immunological reasons that contribute to infertility are the presence of anti-phospholipid antibodies and antinuclear antibodies.[1] The anti-phospholipid antibodies such as lupus anticoagulant, anti-cardiolipin and anti- ß2 glycoprotein are associated with recurrent miscarriage (RM) or as possible factors involved in infertility.

Anti-thyroid antibodies
Thyroid antibodies to thyroid antigens, such as anti-thyroglobulin and anti-thyroid peroxidise, antibodies to nuclear antigens.

Antinuclear antibodies (ANAs)
These are a specific class of auto antibodies that have the capability of binding and destroying certain structures within the nucleus of the cells. Presently the ANAs have been categorised in two main groups; the first includes auto antibodies to DNA and histones and the second one consists of auto antibodies to extractable nuclear antigens that are including auto antibodies to Smith antigen, ribo nucleo proteins, SSA/Ro, SSB/La, Scl-70, Jo-1. These are called as ANA profile.[2]

There are three distinct groups which need to be dealt with: recurrent pregnancy loss, other severe placenta-mediated pregnancy complications, such as severe pre-eclampsia, fetal growth restriction, abruption, stillbirth, and placental histopathological lesions. Out of the above, this chapter deals with recurrent pregnancy loss and infertility and immune problems.

Recurrent pregnancy loss
In clinical practice we find that early pregnancy loss is very common; at least 15% of clinically recognised pregnancies end in miscarriage. Recurrent pregnancy loss – defined as two or three more consecutive losses – affects up to 5% or 1% of couples, respectively[3]

A Cochrane review [4] determining whether anticoagulant treatment improves the chance of a livebirth in women with a history of at least two unexplained miscarriages with or without inherited thrombophilia concluded that neither low dose aspirin alone nor LMWH / low dose aspirin have a demonstrable benefit with respect to live-birth: lowdose aspirin versus placebo.

In addition, a 2016 meta-analysis[5] of randomised controlled trials comparing LMWH with no LMWH in women with inherited thrombophilia and previous late or recurrent early pregnancy loss suggested no benefit from additional treatment with LMWH.

Data review and Consensus

The EULAR (European League Against Rheumatism) has set out recommendations for the management of antiphospholipid syndrome in adults.[6]

Treatment recommendations according to EULAR are as follows:

Women with a high-risk APL serum profile (ACA >20 units) but, no history of thrombosis or pregnancy complications (with or without SLE) Low Dose Aspirin (100-150 mg daily) during pregnancy should be considered.

In women with a history of obstetric APS only (no prior thrombotic events), with or without SLE: With a history of pregnancy loss ≥ 3 recurrent spontaneous miscarriages < 10th week of gestation fetal loss (≥ 10th week of gestation), combination treatment with low dose aspirin (LDA) and LMWH at prophylactic dosage (1mg/kg of heparin daily) during pregnancy is recommended (Evidence level 2b).

Women with a high-risk APL serum profile (ACA >20 units) but, no history of thrombosis or pregnancy complications (with or without SLE) Low Dose Aspirin (100-150 mg daily) during pregnancy should be considered. In women with a history of obstetric APS only (no prior thrombotic events), with or without SLE: With a history of pregnancy loss ≥ 3 recurrent spontaneous miscarriages <10th week of gestation fetal loss (≥ 10th week of gestation), combination treatment with low dose aspirin (LDA) and LMWH at prophylactic dosage (1mg/kg of heparin daily) during pregnancy is recommended (Evidence level 2b).

In women with a history of clinical 'non-criteria' obstetric APS such as: The presence of two recurrent spontaneous miscarriages <10th week of gestation, or delivery ≥ weeks of gestation due to severe pre-eclampsia or eclampsia, treatment with LDA alone, or in combination with heparin might be considered based on the individual's risk profile (Evidence level 4).

What to do with the treatment failures?

When the initial treatment does not work inspite of heparin and aspirin, probable inflammatory pathology is thought of. Treatment options include aspirin and therapeutic heparin 1mg/kg twice daily; + hydroxychloroquine 6mg/kg/day shown to decrease aPL titres with improved outcomes.[7]

Other therapies

Statins – Pravastatin- Decreases inflammation and prolongs pregnancy in the face of severe preeclampsia
B cell modulators – Belimumab, in excess microthrombus formation very low and intractable thrombocytopaenia
Alteration of complement – Eculizumab, Steroids, IVIgG are all areas of ongoing research.

Thyroid auto immunity

Thyroid auto immunity (TAI), due to anti-thyroperoxidase (TPO) and anti-thyroglobulin antibodies, was often considered to have a negative impact on reproductive outcome. The assumed effect extended from infertility to miscarriage to live birth rates. But many recent studies have found this to be not true. The meta-analysis by van den Boogaard, with 330 TAI patients and 1,430 controls, showed no difference in clinical pregnancy rate after IVF.[8]

Autoantibodies inpremature ovarian failure
These women are at risk of autoimmune hypothyroidism (18.5%). Testing should therefore include thyroid stimulating hormone (TSH), free thyroxine (T4), and anti-TPO antibodies and anti-thyroglobulin antibodies.

Natural killer (NK) cells
The results of a review demonstrate no significant difference in the percentage of peripheral or uNK cells in infertile women compared with fertile controls. There was no difference in the percentage of uNK cells in women with RM compared with fertile controls.

However, meta-analysis of studies comparing the numbers of peripheral NK cells in infertile women and women with RM versus fertile controls showed significantly higher levels of peripheral NK cell numbers in infertile women and women with RM.[9]

Assisted Reproduction
Routine steroid use in IVF is faulty but in indicated cases is beneficial. Corticosteroids alone or in combination with low-dose aspirin are reported to improve pregnancy rate after IVF in women with anti-nuclear antibodies, anti-cardiolipin antibodies, anti-thyroid antibodies or lupus anticoagulant.[10]

ANA positive status associated with adverse IVF / ICSI outcomes irrespective of high (>1.320)/low (<1:320) titre. Prednisone (10 mg daily) plus low-dose aspirin for three months prior to IVF in previous IVF/ET failure showed improved pregnancy outcomes. Pregnancy rate (12.5% vs. 57.1%),implantation rate (6.06% vs. 27.9%), improved fertilisation and embryo formation rates were observed.[11]

Glucocorticoid supplementation during ovarian stimulation for IVF or ICSI were analysed in four RCTs were included in a review (416 women) by Cochrane in 2017 with various dose regimens and type of steroids - 1mg of dexamethasone received daily until the day of oocyte aspiration,10mg of prednisolone administered daily until the day of hCG administration versus placebo,0.5 mg of dexamethasone versus placebo. Glucocorticoids possibly increase the clinical pregnancy rate, there may be little or no impact on live birth rate. Though the data is unclear regarding adverse effects, most studies show no harm.

Conclusion
At present, within a heterogeneous set of underlying causes, inherited thrombophilias appear to be, at best, a weak contributor to adverse pregnancy outcomes. This is not the case for heritable thrombophilias. More understanding as to when to start only aspirin or aspirin and heparin needs a proper understanding of the clinical scenario. It is important to present this information to patients in an empathetic manner and remind them and ourselves, of the importance of primum non nocere – first doing no harm.

The balance of immunologic tolerance between the mother and the embryo has been given increasing importance during the process of embryo implantation and fetal development. It is increasingly clear that the immunological background and environment during fertilisation are very crucial and decisive factors for impregnation.

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Non-Invasive Embryo Assessment

Mrs Rushika Mistry
MSc Life Sciences (Applied Medicine)
Embryologist, Bloom IVF Lilavati Hospital, Mumbai

Dr Sarita Sukhija MD
Consultant, Reproductive Medicine

Introduction

With recent advances in IVF, the main aim of the IVF specialists is reducing multiple pregnancies while maintaining good clinical results. SET (Single embryo transfer) is progressively becoming a reality. Traditional embryo assessments are based on time-point evaluations. On the other hand, time lapse monitoring systems allow complete observation of embryo development. Introduction of imaging systems allows us to assess embryos in a different way through their morphokinetics.

Morphological embryo assessment is the most time tested method available for embryo selection worldwide. In morphological assessment, the embryo selection is done on the basis of embryo morphology and the development rate as assessed by light microscopy. The embryos are assessed at a particular time interval on particular days. The Istanbul consensus on embryo grading can be easily followed in modern IVF labs.

Importance of embryo scoring

A precise embryo evaluation is of paramount importance to sustain a successful IVF programme. In most IVF clinics around the world,this quality assessment relies mainly on the morphological evaluation of cleavage stage embryos. The purpose of every IVF lab today is to illustrate morphological aspects as well as the latest evaluation techniques to help the embryologist select the best suitable embryo to maintain high pregnancy rates.

A series of dynamic and complex events are triggered following sperm-oocyte interaction that sequentially leads to fertilisation, zygote formation, embryo development and a blastocyst. The contemporary goal of IVF is to reduce the number of embryos transferred to the patient, maintain high pregnancy rates and select the best embryo for higher implantation.

Timing of observation and scoring of oocytes and fertilised embryos

A standardised timing of observations should be followed relative to the timing of insemination. This allows assessment, appropriate grading and identification of the progress of growth and stages that the fertilised zygote goes through over a period of time.

Type of observation	Timing(hours post insemination)	Expected stage of development
Fertilization check	**17 ± 1**	Pronuclear stage
Syngamy check	**23 ± 1**	50%atsyngamy (20% may be at 2 cell stage)
Early cleavage check	**2628 ± 1**	2 cell stage
Day 2 embryo assessment	**44 ± 1**	4 cell stage
Day 3 embryo assessment	**68 ± 1**	8 cell stage
Day 4 embryo assessment	**92 ± 2**	Morula
Day 5 embryo assessment	**116 ± 2**	Blastocyst

Oocyte scoring system

Oocyte morphological assessment in the lab is first based on the cumulus corona cells. Following removal of the cumulus corona cells, one can assess the maturity of oocytes.

Oocyte grading	1	2	3
Cumulus amount	Scant to none	Moderate	Abundant
Cumulus appearance	Dense,dark,clumpy,opaque, slightly elastic	Slightly translucent,moderately elastic	Translucent,mucus like,elastic
Cumulus expansion	Slight to none,compact	Moderate	Fully expanded
Corona expansion	Slight to none	Moderate	Radiating
Oocyte shape	Irregular	Slightly irregular	Regular
Ooplasm	Dark colour,central clumping of organelles	Mainly uniform,pale, honey colour,dark centre	Uniform appearance ,pale honey colour

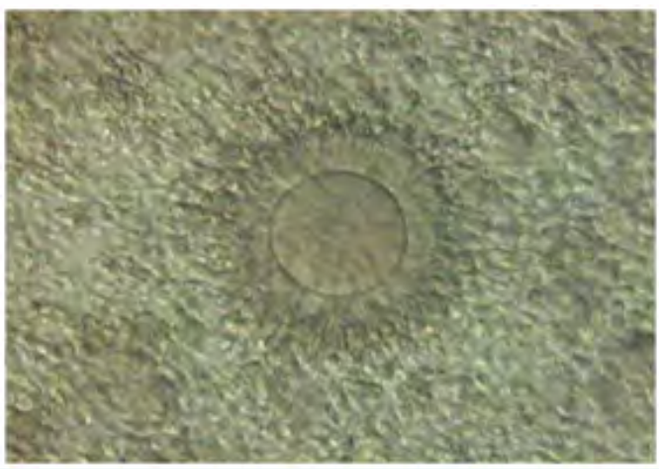


Fig 1 Normal oocyte cumulus complex

Assessing oocytes

The recent introduction of polarized light microscope enables a noninvasive visualization of MS enhancing a better assessment of oocyte meiotic stage (Wang et al.2001). The developmental competence of human embryos is directly influenced by the normality of nuclear and cytoplasmic maturation of the oocytes.

Intracytoplasmic anomalies are incorporations, refractile bodies, dense central granulations, vacuoles, sER discs.Extracytoplasmic anomalies are first polar body morphology, perivitelline shape (PVS) size and granularity, discolouration, zona pellucida (ZP) defects, shape anomalies.

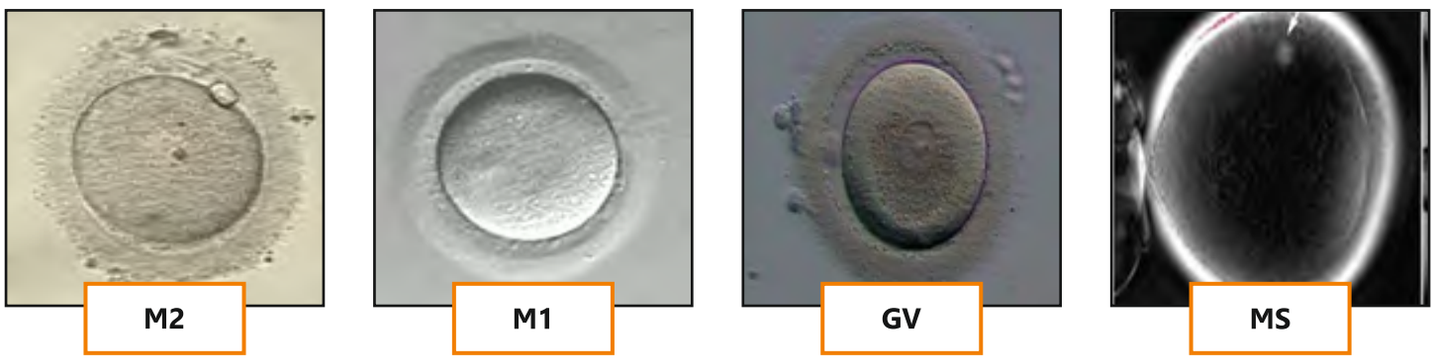


Fig 2 Stages of oocyte maturation

Pronuclei scoring system

Assessment of PN check is usually done 17+1 hours post insemination. The most popular zygote grading system involves checking of pronuclear size and symmetry.Size, number, equality and distribution of nucleolar precursor bodies (NPBs) along with the appearance of cytoplasm also have to be observed.

Category	Rating	Description
1	Symmetrical	Equivalent to Z1 and Z2
2	Non symmetrical	Other arrangements, including peripherally sited pronuclei
3	Abnormal	Pronuclei with 0 or 1 nucleolar precursor bodies (NPBs)

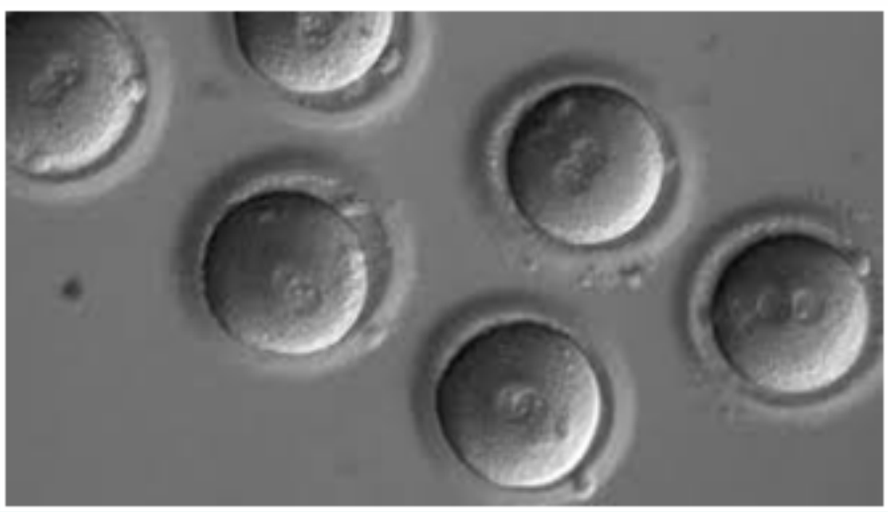


Fig 3 Pronuclear stage (PN)

Embryo scoring System

The next step in embryo evaluation is made at 24-28 hours after insemination or ICSI, where the presence of first cleavage, blastomere symmetry and the extent of fragmentation are examined.

On an average, embryos that have cleaved more slowly than the expected rate have lower implantation potential and that embryos that have cleaved faster than the expected rate are likely to be abnormal and have a reduced implantation potential too. Cleavage directly to three or more cells has been shown to be associated with chromosomal abnormality (Hardarson et al.2006), which can be seen in a time lapse video clearly.

Incidence of chromosome abnormalities increases from 50-60% in non-fragmented embryos to 70-90% in embryos with >35% fragmentation. The relative degrees of fragmentation are defined as:

Mild: <10% Moderate: 10-25% Severe: >25%

Multinucleation Assessment

Multinucleation assessment should also be considered as an important tool in selection of an embryo, as multinucleated embryos are associated with an increased level of chromosome abnormality and as a consequence increased risk of spontaneous miscarriage.

On Day 2 i.e.44+1hr,one should expect even size blastomeres at 2, 4 and 8 cell stages. For all other stages one would expect a difference in the cells as the cleavage phase has not been completed. Other morphological parameters such as cytoplasmic granularity, membrane appearance and the presence of vacuoles can also be scored.

Grade	Rating	Description
1	Good	<10% fragmentation Stage specific cell size No multinucleation
2	Fair	10 - 25% fragmentation Stage specific cell size for majority of cells No evidence of multinucleation
3	Poor	Severe fragmentation (>25%) Cell size not stage specific Evidence of multinucleation

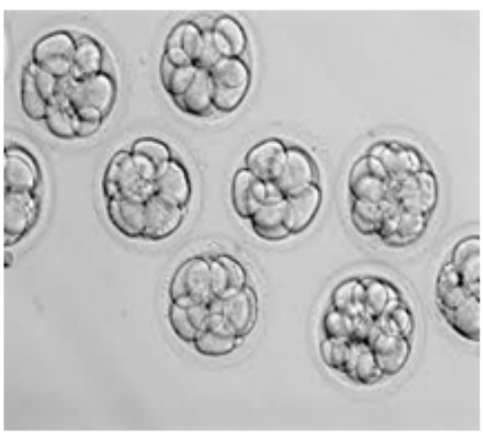


Fig 4 Day 2 embryos (4 cell stage) Fig 5 Day 3 embryos (8 cell stage)

Morula stage scoring system

Entering into 4th round of cleavage at 92+1 hours would be compacting and virtually will be occupying all the embryo volume. Disproportionate compaction that involves less than half of the embryo, with two or three cells remaining as discrete blastomeres is associated with a poor prognosis.

Grade	Rating	Description
1	Good	Entered into a fourth round of cleavage Evidence of compaction which involves virtually all the embryo volume.
2	Fair	Entered into a fourth round of cleavage Compaction involves the majority of the volume of the embryo.
3	Poor	Disproportionate compaction involving less than half of the embryo, with two or three cells remaining as discrete blastomeres.

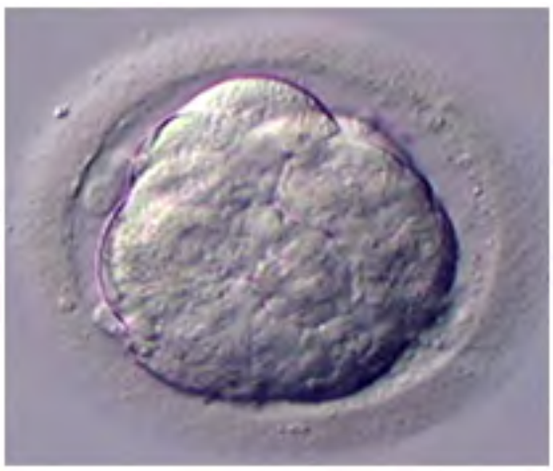


Fig 6 Morula

Blastocyst stage scoring system

Finally an optimal embryo at 116 + 2 hrs will be a fully expanded blastocyst,with an intermediate cell mass (ICM) that is prominent,easily discernible and consisting of many cells,with the cells compacted and tightly adhered together and a trophectoderm (TE) that comprises many cells forming a cohesive epithelium. While the ICM has a high prognostic value for implantation and fetal development, a functional TE is also essential.

	Grading	Rating	Description
Stage of development	1 2 3 4		Early Blastocyst Expanded Hatched/hatching
Inner cell mass	1	Good	Prominent, easily discernible, with many cells that are compacted and tightly adhered together.
	2	Fair	Easily discernible, with many cells that are loosely grouped together.
	3	Poor	Difficult to discern, with few cells.
Trophectoderm	1	Good	Many cells forming a cohesive epithelium.
	2	Fair	Few cells forming a loose epithelium.
	3	Poor	Very few cells .

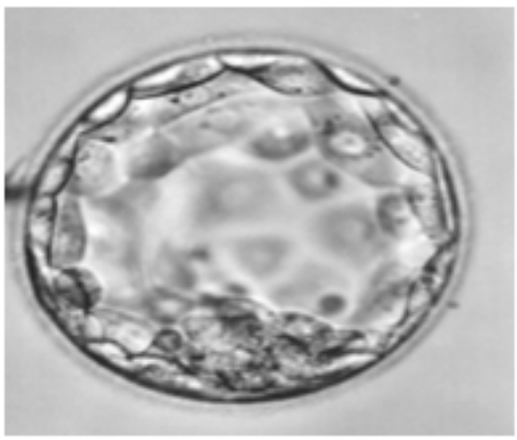
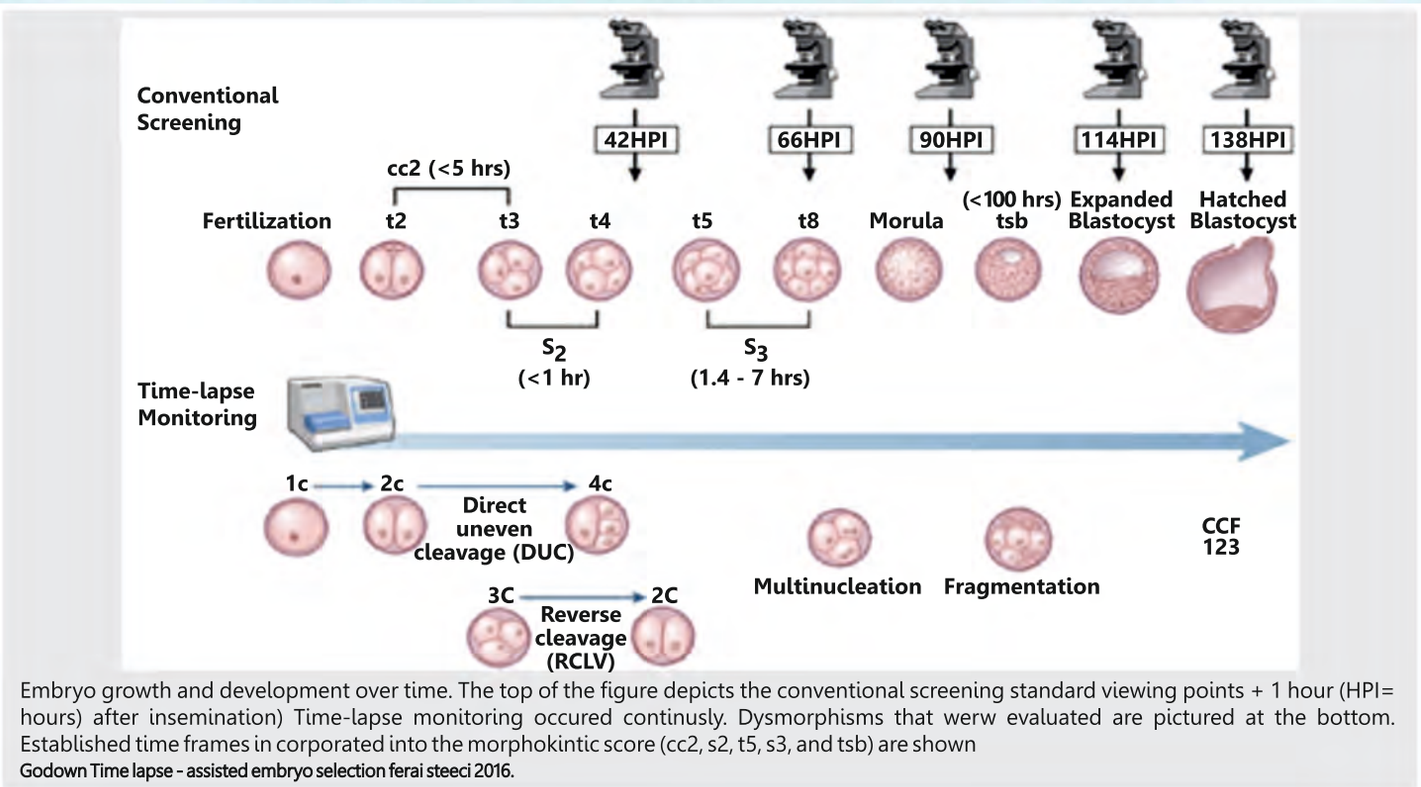


Fig 7 Blastocyst

Time lapse imaging

Embryo assessment is one of the most critical procedures that play a role in the success of ART. Traditional embryo assessment is challenged by different factors such as subjectivity, low efficiency and external factors.New "Non-Invasive" techniques may provide valuable additional information to optimize embryo assessment and maximise the chances of IVF success. Time-lapse allows the embryologist to review and analyse the full course of embryo development at their convenience. The increased number of critical quantitative parameters allows embryologist to select the best viable embryo.



Embryo growth and development over time. The top of the figure depicts the conventional screening standard viewing points + 1 hour (HPI= hours) after insemination) Time-lapse monitoring occured continusly. Dysmorphisms that werw evaluated are pictured at the bottom. Established time frames in corporated into the morphokintic score (cc2, s2, t5, s3, and tsb) are shown

Godown Time lapse - assisted embryo selection ferai steeci 2016.

Fig 8 Difference between conventional screening and time lapse monitoring

Alternate future options for selecting embryo for implantation

The noninvasive methods like metabolomics, where from the proton NMR spectrum, alanine, pyruvate and glucose levels were found to be reduced in the culture media of the embryos that resulted in pregnancy whereas, glutamate levels were found to be higher in embryos that did not result in pregnancy.

Protein markers like sHLAG (soluble human leukocyte antigenG) when present in the culture media show no correlation to embryo morphology, whereas lack of it has a negative predictive value. Several genes expressed in the cumulus cells have been correlated with predicting pregnancy(COX2,STAR, PTX3).

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Preimplantation Genetic Testing (PGT)

Dr Hrishikesh Pai
FCPS FICOG MSc (USA) FRCOG
Consultant Gynaecologist and Infertility Specialist

Mrs Rushika Mistry
MSc Life Sciences (Applied Medicine)
Embryologist, Bloom IVF Lilavati Hospital, Mumbai

Background
In vitro fertilisation (IVF) has revolutionised the treatment of infertility and is estimated to have led to more than 6 million births all over the world. It is now possible to test embryos formed by IVF for genetic diseases by utilising Preimplantation Genetic Testing (PGT). The primary goal of PGT is to identify genetic defects in embryos created through IVF before transferring them to the uterus, thus decreasing abortions and births with genetic abnormalities.

Definitions
The terms Preimplantation Genetic Screening (PGS) and Preimplantation Genetic Diagnosis(PGD) are now replaced by new terminology in the international glossary of infertility and fertility care.

PGT-A: Preimplantation Genetic Testing for Aneuploidy
This is routine screening of embryos to identify euploid embryos for transfer and screen and exclude those embryos with sporadic chromosome abnormality. It is therefore used to select embryos that are most likely to result in a successful pregnancy.

PGT-M: Preimplantation Genetic testing for Monogenic/single gene disorders
This is typically used when parents are known carriers of a single gene mutation. It is used to help reduce the risk to have a child with a known inherited disorder caused by a single gene.

PGT-SR: Preimplantation Genetic testing for Structural Rearrangement
Commonly used when one of the parents is a known carrier of a balanced or Robertsonian translocation (Structural Chromosomal Rearrangement). The resulting embryos may carry imbalance in chromosome number indicative of translocation. It reduces the risk of having a pregnancy or a child with an unbalanced structural abnormality.

Indications of PGT

- Advanced maternal age
- Recurrent pregnancy loss
- Multiple IVF failures
- Male factor infertility
- Patients with a family history of X linked disorders with 25% risk of having an affected embryo
- Carriers of autosomal recessive disease
- Carriers of autosomal dominant diseases
- HLA matching

Genetic abnormalities that can be detected using PGT

Aneuploidy

This is the presence of an abnormal number of chromosomes in a cell, instead of the usual 46 chromosomes. It does not include a difference of one or more complete sets of chromosomes.

Trisomy

A type of polysomy in which there are three instances of a particular chromosome, instead of the normal two. A trisomy is a type of aneuploidy.

Tetrasomy

A form of aneuploidy with the presence of four copies, instead of the normal two, of a particular chromosome.

Monosomy

A condition of having a diploid chromosome complement in which one chromosome lacks its homologous partner.

Nullisomy

A genome mutation where a pair of homologous chromosomes that would normally be present is missing.

Single gene disorder

Also known as monogenic disease, is when a single mutation in a specific gene leads to ahereditary disease which can occur early during childhood or have a late onset.

Mosaicism

The presence of two or more different cells lines with different chromosomal number or structure in one embryo resulted by errors in chromosomal segregation during mitosis. Mosaicism could be present only in the intermediate cell mass (ICM) and not in the trophectoderm (TE). It may be misdiagnosed as a euploid embryo and could result in an unfavourable outcome. It is advisable to perform amniocentesis/ CVS for confirmation of test results.

Mito Score: Mitochondria play an important role in energy production and have their own DNA that is known as mitochondrial DNA or mtDNA, which is responsible for predicting implantation potential of embryo. The mitochondrial score"MitoScore" represents the total mtDNA content in euploid embryos.

Genetic Analysis Techniques available for testing:

There are three main techniques available for testing and are as follows:

FISH (Fluorescence in situ hybridization)

It is a molecular cytogenetic technique that uses fluorescent probes that bind to only those parts of a nucleic acid sequence with a high degree of complementarity of sequence. Fluorescence microscopy can be used to find out where the fluorescent probe is bound to the chromosome.

It analyses a limited number of chromosomes at a time (13, 16, 18, 21, 22, X and Y). It was the initial method used for analysis because of its accuracy in results, but it has its technical limitations like the number of probes required to get reliable reports and the requirement of specific parent karyotyping prior to testing.

Array CGH (Comparative genomic hybridization)

It is a molecular cytogenetic technique for the detection of chromosomal copy number changes on a genome with a high resolution scale. It is a significant advance in technology that allows detection of chromosome imbalances that are too small to be detected by microscope. It allows locus by locus measure of CNV (copy number variation) with increased resolution as low as 100 kilobases. With recent technologies, new methods for comprehensive chromosome screening (CCS) like a CGH, qPCR and SNP array can detect both euploid and aneuploidy embryos but are unable to detect mosaicism.

NGS (Next generation sequencing)
DNA sequencing is the process of determining the sequence of nucleotides in a section of DNA. The first commercialised method of DNA sequencing was Sanger sequencing. Next-generation sequencing (NGS), also known as high-throughput sequencing, is the term used to describe a number of different modern sequencing technologies. These technologies allow for sequencing of DNA much more quickly and cheaply than the previously used Sanger sequencing and revolutionised the study of genomics and molecular biology.

These technologies include:
Illumina (Solexa) sequencing
Illumina sequencing works by simultaneously identifying DNA bases, as each base emits a unique fluorescent signal, and adding them to a nucleic acid chain.

Roche 454 sequencing
This method is based on pyrosequencing, a technique which detects pyrophosphate release, again using fluorescence, after nucleotides are incorporated by polymerase to a new strand of DNA.

Ion Torrent: Proton/ PGM sequencing
This method of sequencing measures the direct release of H+ (protons) from the incorporation of individual bases by DNA polymerase and therefore differs from the previous two methods as it does not measure light.

Process of PGT
The process of PGT includes the following steps:

Embryo biopsy
The embryo biopsy procedure consists of opening the zona pellucida and removal of the cellular material. Zona opening can be performed in three ways:

- Mechanical - Direct Puncture
  - Partial Zona Dissection
- Chemical (Acid Tyrode's pH=2.3)
- Photothermolysis(Laser)

The developmental stages at which biopsy cab be performed are as follows:

Polar bodies from oocytes (Day 0/ Day 1)
23hours after the ovum pick up, 1st polar body is observed. A small hole of 1825µm (not <15µm) should be made in the zona pellucida with laser or mechanical opening and not to use Ac. Tyrode here as it can be harmful and could compromise the viability of the oocyte. Pipettes for pb biopsy can be bevelled or not and the inner diameter should be 1215µm.

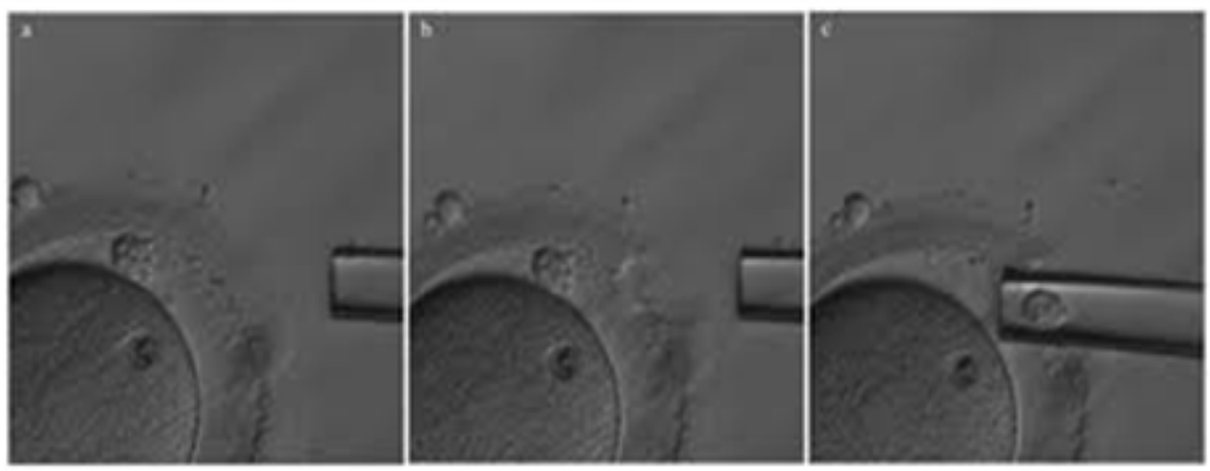


Fig 1 Polar body biopsy

Blastomeres from early cleavage stage embryos (Day 3)

For blastomere biopsy at day 3 of cleavage stage the blastomere removal is performed in three ways:

a) Blastomere removal by Ac. Tyrode Aspiration

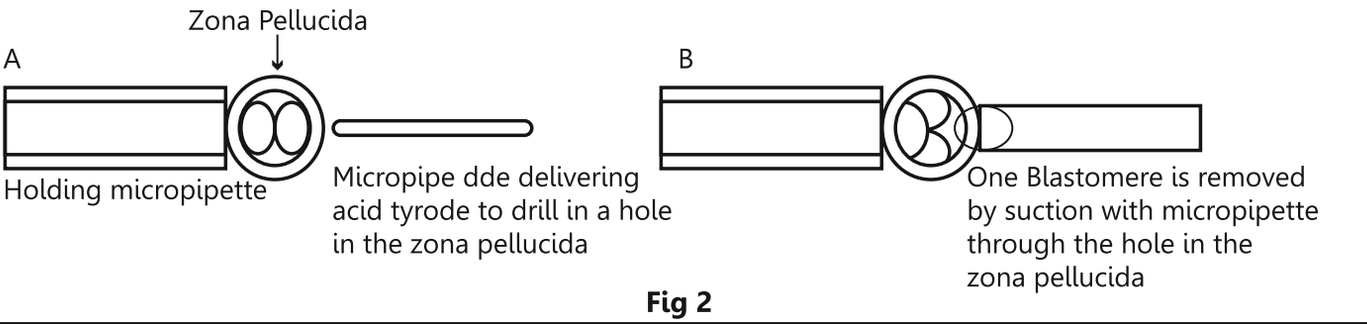


Fig 2

b) Blastomere removal by Extrusion

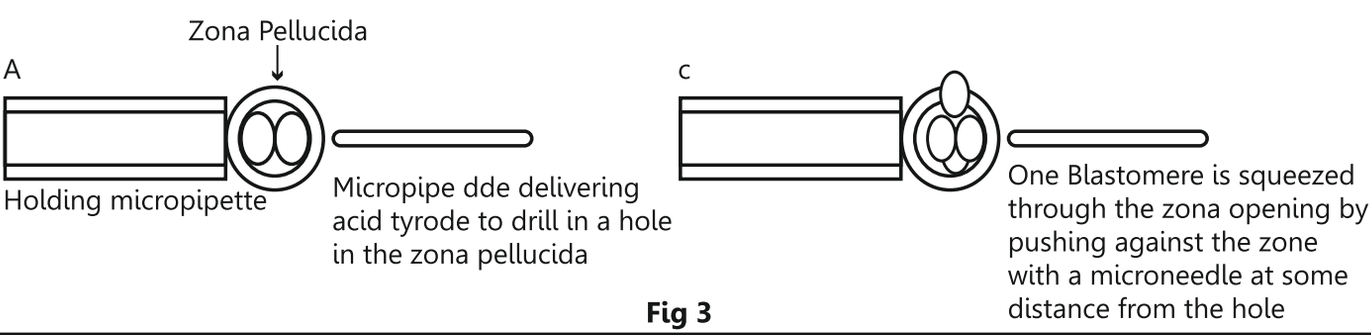
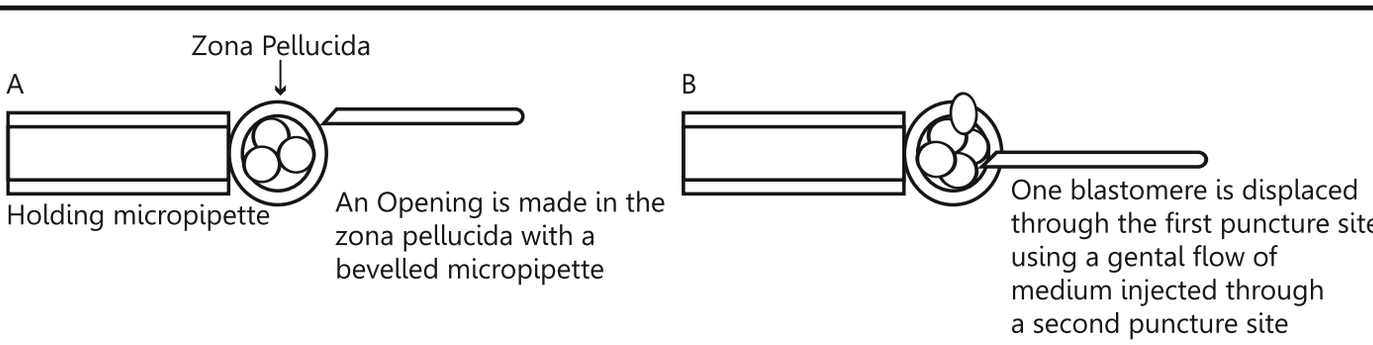


Fig 3

c) Blastomere removal by Displacement



It allows the detection of maternal, paternal and early post-fertilisation defects and gives enough time for the genetic diagnosis if it is performed on day 3 and transfer on day 5. Ca2+ Mg2+ free culture medium facilitates embryo biopsy with no detrimental effect on embryo development and pregnancy rates (Veiga et al 1994; Santaló et al, 1996; Dumoulin et al, 1998). Limit exposure time to maximum 10 mins and after biopsy, gently flush the embryo repeatedly.

Trophectoderm cells from blastocysts (Day 5)

Blastocyst biopsy is an emerging technique as it provides more cells to analyse the defects if present in the embryo. It is interesting in monogenic diseases as more DNA is available. A lower degree of mosaicism is observed at this stage and ICM remains fully intact. However, it requires a high blastocyst formation rate, an optimised culture system and specific laboratory expertise. Genetic results are be obtained in <24 hours in order to avoid cryopreservation.

Fig 5 Trophectoderm biopsy

Transportation of biopsied material to the genetic lab

The first step in transportation of the sample is proper documentation and form filling with witnessing of the procedure. There should be one blank PCR tube for control. The biopsied cells are placed in the PCR tubes with minimum volume of washing media under the stereozoom microscope. The PCR tubes are then placed in the cooler rack, covered with parafilm and kept inside the shipping box with ice packs. Take care that cooler does not move inside the box during transportation.

Amplification of DNA and library preparation

Prepare extraction master mix to extract gDNA and add preamp reagents and incubate for 75 mins (16 samples), 90 mins (24 samples) and 150 mins (96 samples) hands-on time, depending on the panels used. Add barcodes and amp master mix, incubate, pool, purify and quantitate the Library.

Template preparation

Pipette the library into ion chef cartridge (in case of Ion Torrent) for templating and chip loading. Load the cartridge onto ion chef system. This process takes 15 mins hands-on time.

Sequencing

Load reagents onto Ion GeneStudio™ S5 systems. Transfer chip ontoIon GeneStudio™ S5 systems for sequencing. This process takes 15 mins hands-on time.

Analysis

The results are analysed and interpreted using Torrent Suite™ Software or Ion Reporter™ Software.

Fig 6 Ion S5 System Thermo Fisher

Fig 7 Ion Chef Instrument Thermo Fisher

PGT Regulations in India:

In India, Ministry of Family Health and Welfare, regulates the concept under the Pre-Conception and Prenatal Diagnostic Techniques (Prohibition of Sex Selection) (PCPNDT) Act, 1994.This is an Act to provide for the prohibition of sex selection, before or after conception, and for regulation of pre-nataldiagnostic techniques for the purposes of detecting abnormalities or metabolic disorders or chromosomalabnormalities or certain congenital malformations or sex-linked disorders and for the prevention of their misusefor sex determination leading to female feticide.

Records to be maintained

Detailed record of the patients that have undergone counselling and tests is to be maintained in the register.

Form D : Form For Maintenance Of Records By The Genetic Counselling Centre.
Form E : Maintenance Of Records By Genetic Laboratory.
Form F : Form For Maintenance Of Record In Case Of Prenatal Diagnostic Test / Procedure By Genetic Clinic/ultrasound Clinic/ Imaging Centre.
Form G : Form Of Consent (for invasive techniques).

This is in addition to all the consent forms related to IVF procedure as per clinic protocols.

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Fertility Preservation

Dr Nandita Palshetkar MD FCPS FICOG FRCOG
President, FOGSI (2019)
Chairperson, MSR (2017-18)
Vice President, AMOGS (2016-18)
Past President, MOGS (2016-17)

Dr Pratik Tambe MD FICOG
Chairperson, FOSGI Endocrinology Committee (2017-19)
Managing Council Member, MOGS
Managing Council Member, IAGE, MSR, AMC
(2015-18)

Background

Modern women lead a different life when compared to women of even two or three generations ago. They have similar education, compete for places like their male counterparts and go on to have successful professional careers. They are confident, mature, in touch with current practices, self-aware and financially independent.

However, prioritising their career over motherhood is a difficult choice that women have always had to make. Often, by the time they are settled financially and professionally and have risen to a position of strength in their chosen field, they find that they may not have a suitable partner or ovarian aging has set in which limits their chances of motherhood.

Thanks to rapid advances over the past few decades in the field of assisted reproduction, women can now literally choose to have their cake and eat it too. Fertility preservation techniques have opened new doors and allowed women to cryopreserve their eggs or embryos and experience motherhood at a time of their choosing.

Scientific basis

Interestingly, the first live birth as a result of oocyte cryopreservation treatment was reported three decades ago. The technique required refinement and reproducibility at the hands of a wide variety of researchers. Important advancements and improvements in the clinical application of oocyte cryopreservation have taken place only over the last decade.

Randomised trials have now revealed important evidence on the safety of non donor oocyte cryopreservation, and confirmed that the clinical success of vitrification is comparable to that of IVF with fresh oocytes. Appropriate counselling of women for oocyte cryopreservation should incorporate statistics regarding age-specific success rates with cryopreserved oocytes for different indications.

Embryo freezing

Embryo cryopreservation is commonly practiced at most IVF clinics throughout the world.[1] It forms part of the strategy of segmentation to perform freeze thaw embryo transfers in a different cycle from the stimulation and oocyte retrieval cycle. This is especially helpful in patients with PCOS who are hyper-responders and are prone to the life-threatening complication of ovarian hyper stimulation syndrome (OHSS).[2]

Evidence is mounting that success rates with donor oocyte vitrification are similar to that of IVF with fresh donor oocytes. With wider media coverage and celebrity endorsements, attitudes toward oocyte cryopreservation among the lay populace have shown that elective use for the postponement of fertility is currently the most common indication for oocyte vitrification.

Oocyte vitrification

Oocyte cryopreservation now offers a new option for single women in the reproductive age group who need of delaying childbearing for any reason. Due to practical problems related to the structure of the oocyte and optimisation of freezing methods, it has taken more than 20 years for oocyte cryopreservation to evolve into a technique with acceptable clinical pregnancy rates.

This transition was made possible by three important achievements: utilisation of intracytoplasmic sperm injection (ICSI), improvements in cryoprotectants, and introduction of vitrification.[3-5] The improvements in the technique and the removal of the 'experimental' label on oocyte cryopreservation by the American Society of Reproductive Medicine (ASRM) Practice Guideline Committee have ushered in a new era.[6]

Clinical applications

Oocyte cryopreservation has taken the lead in fertility preservation. It is also useful in clinical scenarios such as the unavailability of sperm at the time of egg retrieval,[7] in cases of ovarian hyper-stimulation syndrome,[8] in poor responders,[9] accumulation of oocytes,[10] in patients at risk of losing their fertility potential due to genetic abnormalities such as BRCA mutation carrier status,[11] Turner syndrome,[12] fragile X syndrome, and deletions of the Xchromosome and for couples who do not wish to cryopreserve supernumerary embryos for ethical, legal, or religious concerns.

Donor oocyte vitrification has paved the way for the establishment of donor oocyte banks.[13,14] If this is practiced worldwide, IVF cycles using frozen-thawed donor oocytes may even outnumber those using fresh donor oocytes. Electiveoocyte cryopreservation (EOC) for deferring childbearing remains the most controversial indication, but is the most common indication for oocyte cryopreservation today. Most centres currently performing oocyte vitrification in the United States do so for elective indications.[15]

Efficacy and success rates

There are many factors that affect the success rates with oocyte cryopreservation such as factors related to the host (age, donor/nondonor oocyte, aetiology of infertility), stimulation protocols, cryopreservation methods (slow-freezing vs vitrification),protocols and devices (cryotop, cryoleaf, cryotip) used. Hence, it is difficult to compare success rates across various studies conducted over a period of time. A variety of trials on different techniques have reported variable success rates.[15-19]

Evolution

The first live birth with oocyte cryopreservation was reported in 1986 with slow freezing,[20] but due to low success rates, there were only five live births reported.[21] After intra cytoplasmic sperm injection (ICSI) was discovered in 1997,[22] further optimisation of oocyte cryopreservation required another decade. In 1999,the first live birth with oocyte cryopreservation after vitrification was reported.[23]

Slow freezing vs vitrification techniques

An early meta-analysis concluded that success rates with oocyte cryopreservation using slow freezing were lower than that of IVF with fresh oocytes. Comparisons of vitrification with either slow freezing or fresh oocyte cycles could not be performed because of the limited number of reports with vitrification at that time.

Following the first RCT comparing slow freezing and vitrification, which showed that vitrification was more successful in terms of both embryological and clinical outcomes,15more researchers reported improved clinical outcomes using vitrification. [24-27]

The success rates remain lower for slow freezing compared with vitrification. Over the past 10 years, oocyte cryopreservation using the vitrification technique, has resulted in pregnancy outcomes similar to that of IVF with fresh oocytes.[28]

Since 2006, implantation and live birth rates have increased from 2 to14% and 2 to 27% for slow freezing, while they ranged from 13 to 20% and 23 to 35%, respectively, for the vitrification method.

Scientific evidence: safety and efficacy

This falls into two main categories: 1. studies assessing donor oocyte freeze that cycles and [2]. Studies assessing infertile women undergoing IVF who have supernumerary oocytes for cryopreservation.

Autologous oocyte cryopreservation

Most reports on cryopreservation of non donor oocytes are observational studies in infertile women undergoing IVF who prefer cryo preservation of their surplus oocytes. These women have usually declined embryo cryopreservation due to ethical or legal concerns.

Published RCTs on non donor oocyte cryopreservation report the out comes of IVF using vitrified/warmed non donor oocytes from infertile patients.[18-21] Studies comparing slow freezing and vitrification have concluded that vitrification is superior to slow-freezing in terms of oocyte survival, fertilization, implantation, and clinical pregnancy rates.[18]

Two RCTs were conducted in infertile couples with supernumerary oocytes available to vitrify and warm only if pregnancy was not achieved in the fresh cycle.[16,17] Fresh sibling oocytes were transferred in the first cycle. If pregnancy failed to occur, then the cryo preserved sibling oocytes were thawed, fertilised and transferred to the same patient in a subsequent cycle. Using this design, the authors were able to compare the fertilization and embryo developmental rates of vitrified and fresh sibling oocytes. Both studies concluded that similar fertilisation and embryo development rates were achieved with fresh and vitrified oocytes.

A more recent RCT adopted a unique design, which allowed the comparison of clinical outcomes with non donor vitrified and fresh oocytes. In this study, the authors divided retrieved oocytes from infertile patients less than 35 years of age. One group of oocytes underwent temporary vitrification while their others remained in culture. Later, vitrified oocytes were thawed; vitrified and non vitrified oocytes were fertilised with ICSI, and resulting embryos were cultured to the blastocyst stage.[18]

Embryos of sufficient quality to transfer or cryo preserve under went trophectoderm biopsy for genotyping and a karyo type was assigned to each embryo. Blastocysts obtained from vitrified and fresh oocytes were then transferred in pairs and embryonic an euploidy was assessed in each one. To determine the identity of the implanted embryos, DNA fingerprinting was performed on cell-free fetal DNA enriched from maternal serum specimens drawn at 9 weeks of gestation or on newborn DNA taken from a buccalswab.

The authors detected no differences between the two groups regarding an euploidy. In addition, the ongoing pregnancy rate per transferred embryo was similar for vitrified and fresh oocytes. Hence, oocyte vitrification does not seem to increase the rate of an euploidy or diminish the implantation potential of viable blastocysts. The authors demonstrated that clinical success rates with non donor vitrified oocytes from young infertile women are similar to their sibling fresh oocytes.

Donor oocyte cryopreservation

The largest RCT including 600 recipients of donor oocytes demonstrated similar ongoing pregnancy rates with vitrified donor oocytes when compared with fresh donor oocytes. This study reported implantation and clinical pregnancy per embryo transfer rates of 39.9 vs 40.9% and 55.4 vs 55.6% for vitrified donor and fresh donor oocytes, respectively.[14] Oocyte donors are typically young women under the age of 35; therefore, the results of these studies may be extrapolated to young patients seeking fertility preservation. These studies also pave the way for donor oocyte cryo banking.

Pooling of oocytes

In poor responders, accumulating cryo preserved oocytes in consecutive cycles followed by thaw, ICSI, and embryo transfer is reported to yield comparable success rates to those observed in normal responders.[9,10] When this strategy was applied to poor responders over 40, live-birth/patient success rates were higher (15.8%) for the vitrified oocyte group compared with the fresh oocyte group (7.1%). A recent study has reported a similar approach for patients undergoing EOC. 132 patients underwent multiple cycles of EOC with an average age of 38.4 at first and 39 at subsequent cycles. When more than one cycle was applied, subsequent cycles resulted in greater oocyte yield, albeit with the implementation of a higher dose.[35]

Elective oocyte cryopreservation

A study analysing 491 women in EOC programs reported that the mean age of the patients undergoing EOC was 38, similarto other studies. More than 80% of women undergoing EOC were over 35 years old (range: 36–41).This shows that EOC is primarily utilised by older reproductive age women, although to achieve higher success rates with IVF, both the age of inquiry and application of EOC should be at an age younger than 35 years.[36-39]

Patients suffering from cancer

Although oocyte cryopreservation is proposed for preserving fertility incancer patients, the data on clinical success of oocyte cryopreservation in such patients is limited. For the purposes of counselling, success rates can beextrapolated from other healthy populations. Some studies suggest comparable results with nondonorpatients,[30,31] whereas others show diminished oocyte yield.[32,33]

The British Fertility Society has recently issued updated guidelines for fertility preservation for medical reasons. [40] Their recommendations are summarised as follows:

Women should be informed that risk of congenital anomalies or genetic disease is not increased after cancer treatment. (Evidence level C)
Women who received radiotherapy to a field that included the uterus should be informed of the obstetric risks. (Evidence level C)
Women should be informed that there is no evidence of increased risk of cancer recurrence as a result of pregnancy, with most cancers. (Evidence level C)

The risk of infertility diminished ovarian reserve and premature ovarian insufficiency should be assessed based on age, type and dose of chemotherapy. (Evidence level C)
Patients undergoing pelvic, abdomino-pelvic or cranio-spinal irradiation should be informed of the risk of infertility, depending on the field of direct and scatter exposure. (Evidence level C)
The effect of delay in attempting conception due to prolonged endocrine therapy after breast cancer should be borne in mind when advising women about fertility preservation, even if they do not require gonadotoxic therapy. (GPP)

Women in the reproductive age range should be offered fertility preservation if:
There is a material risk of infertility as a result of the intended treatment. (Evidence level B)
The treatment for the disease has curative intent or there is good prospect for long-term survival. (GPP)
The woman is fit for ovarian stimulation and oocyte collection. (GPP)
The time required for ovarian stimulation and oocyte collection does not jeopardize prognosis. (GPP)
Women/couples should be advised that embryo cryopreservation is an established technique, with success rates for the transfer of frozen–thawed embryos comparable to those for the transfer of fresh embryos. (Evidence level D)
Women should be advised that oocyte cryopreservation is an effective technique, which may have a similar success rate to those using fresh oocytes. (Evidence level C)
Antagonist protocols should usually be employed as they shorten the duration of treatment and reduce the risk of OHSS. (Evidence level A)
An agonist trigger in an antagonist cycle should be considered as this minimizes the risk of OHSS, unless contraindicated. (Evidence level A)
Consider using an anti-oestrogen [letrozole, clomifene or tamoxifen] during ovarian stimulation in women with oestrogen-sensitive tumours. (Evidence level D)

Women/couples can be advised that:
Pregnancy outcome using cryopreserved oocytes/embryos reveals no increase in congenital anomalies. (Evidence level D)
Current data do not suggest that pregnancy outcomes from oocyte/embryos from oncology patients differ from other patients. (GPP)
OTC can be considered for post-pubertal patients, particularly where there is insufficient time for ovarianstimulation and oocyte cryopreservation. (Evidence level C)
Increasing numbers of live births from transplantation of frozen ovarian tissue suggests that OTC should be considered for pre-pubertal patients. (GPP)
These procedures should only be performed at centres with relevant expertise, facilities and HTA licensing. (GPP)
IVM should be offered only in specialized units with relevant expertise, facilities and HFEA licensing. (GPP)
In premenopausal women with early breast cancer consider temporary ovarian suppression with GnRHa started immediately before and continued during chemotherapy as this may partially preserve ovarian function. (Evidence level A)
Women should be advised that it is possible there is a benefit of using GnRHa when other cancers are treated with gonadotoxic chemotherapy. (GPP)
Consider ovarian transposition to move ovaries away from the field of irradiation. (Evidence level D)
Fallopian tubes should not be transected during ovarian transposition in order to retain the possibility of natural conception. (GPP)
Ovarian shielding is of limited use to prevent damage from irradiation and generally should not be offered. (Evidence level D)
Where feasible, fertility-preserving surgery should be considered in selected women with gynaecological malignancies. (Evidence level B)
Alternatives to fertility preservation should be discussed. (GPP)
Discuss fertility preservation options with girls and women at risk of premature ovarian insufficiency from non-malignant conditions. (GPP)
If appropriate, fertility preservation should be performed as early as possible in the treatment pathway. (GPP)
Offer counselling, both prior to fertility preservationand prior to use of stored material. (GPP)
Discuss fertility preservation as early as possible inthe cancer treatment pathway. (Evidence level C)
Decision making aids should be provided to supportwomen's fertility preservation decision-making,ideally at cancer diagnosis/treatment planning. (GPP)

Fertility preservation pathways in women with cancer

Present data suggest that young patients with cancer should be referred for fertility preservation counselling quickly to help with their coping process. Although the clinical application of novel developments, including oocyte vitrification and oocyte maturation in vitro, has resulted in reasonable success rates in assisted reproduction programmes, experience with these techniques in the setting of fertility preservation is in its in fancy.

It is hoped that these and other approaches, some of which are still regarded as experimental (eg, ovarian tissue cryopreservation, pharmacological protection against gonadotoxic agents, in-vitro follicle growth, and follicle transplantation) will be optimised and become established within the next decade. Unravelling the complex mechanisms of activation and suppression of follicle growth will not only expand the care of thousands of women diagnosed with cancer, but also inform the care of millions of women confronted with reduced reproductive fitness because of ageing.[41]

Oocyte in vitro maturation, ovarian cortex transplantation, cryopreservation of ovarian tissue, primordial and preantral follicle culture, derivation of germ cells from stem cells are all treatment pathways which are the subject of research in patients suffering from cancer.

Practical decision making

A recent paper discussed the options available to adolescents and young adults in Japan as regards fertility preservation. They concluded that GnRH agonist therapy combined with anti cancer agents may be useful for ovarian protection.

Vitrification is more popular as Japan is the birthplace of the protocol and a new closed technique has been developed for ovarian tissue cryopreservation.[42]

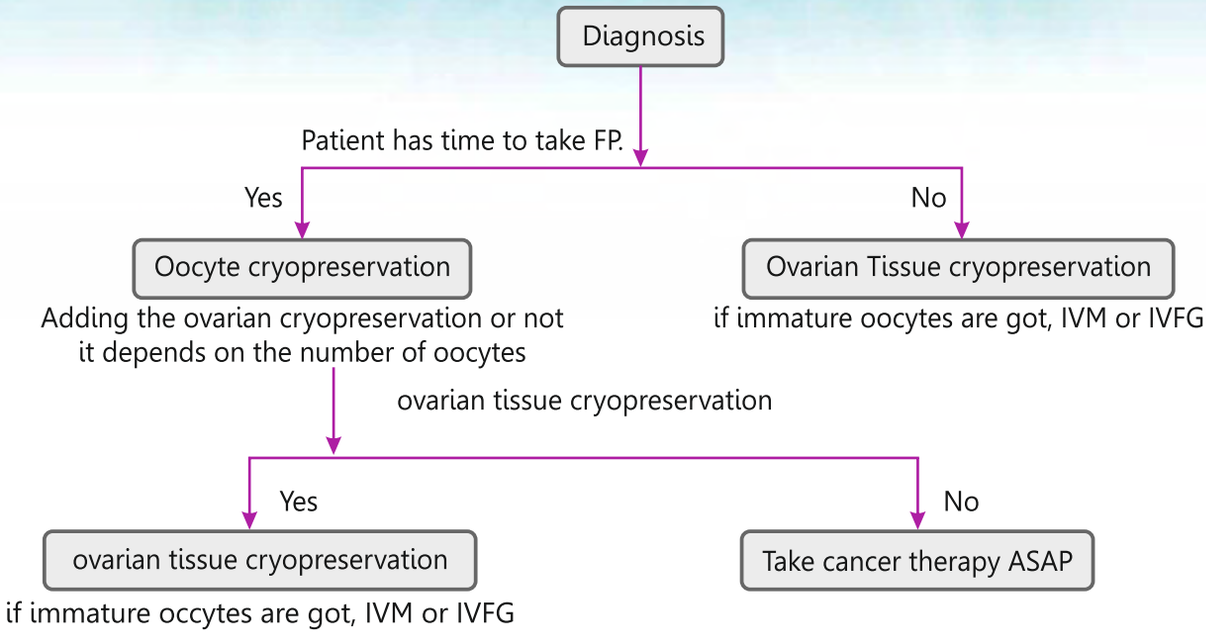

Fig 1. Female fertility preservation on young cancer patients in adolescent and young adult generation. FP. fertility preservation. IVM. in vitro maturation; IVFG, in vitro follicle growth; ASAP, as soon as possible

Ovarian tissue cryopreservation (OTC)

Ovarian tissue cryopreservation and transplantation is gaining ground as a successful method of preserving fertility in young women with cancer. However, OTC preserves more than just the reproductive potential; it restores the ovarian endocrine function and thus the entire female reproductive cycle with natural levels of essential hormones. A Korean group has reported 90 live births with a conception rate of about 30% after OTC and transplantation. Endocrine function recovery was observed in 93% between 3.5 and 6.5 months after transplantation.[43]

In a female population with an increased prevalence in the loss of ovarian function due to primary ovarian in sufficiency (POI) and aging, there is a need to develop new treatments and provide new opportunities to utilise the surplus of follicles that most women are born with and overcome major health issues associated with the lack of ovarian hormones. New applications for the potential utilisation of OTC include cell/tissue based HRT, social freezing, culture of immature oocytes, and a modern ovarian resection for women with polycystic ovaries.[44]

Conclusions

Following the first live birth with cryo preserved oocytes in 1986, clinical outcomes using cryo preserved oocytes have made great strides during the past decade. Recent RCTs show that fertilisation, embryo development, and pregnancy rates with vitrified non donor and donor oocytes are similar to fresh oocytes. Vitrification has become the protocol of choice.

These improvements in the cryopreservation technique and clinical outcomes may result in an increased utilisation of oocyte vitrification in clinical practice eg. patients with PCOS, social egg freezing and patients suffering from cancer. In order to provide appropriate counselling to women considering oocyte cryopreservation for fertility preservation or as an elective procedure for deferring child bearing, it is necessary to arrive at age-specific and indication-specific success rates so that we may better counsel and inform our patients.

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Application of Stem Cells in Infertility

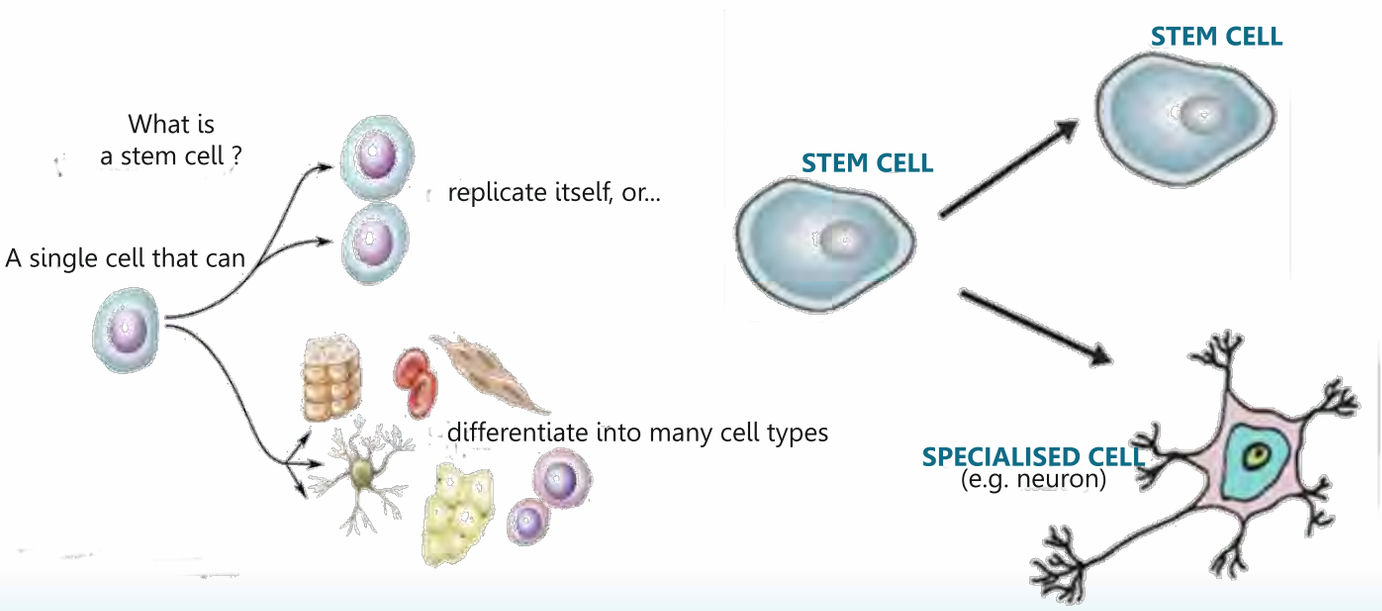
Dr Sunita Tandulwadkar **MD FCPS FICOG**
President, IAGE (2019-2020)
Chairperson, MSR (2018-2020)
Founder Honorary Secretary, Maharashtra Chapter ISAR
Vice President FOGSI (2017)

What are Stem Cells?

The body is made up of about 200 different kinds of specialised cells such as muscle cells, nerve cells, fat cells and skin cells. All cells in the body come from stem cells. Stem cells are undifferentiated "blank" cells that do not yet have begun to develop into specialised tissue and organs. Additionally, stem cells are self sustaining and can replicate themselves for long periods of time. The process of specialisation is called differentiation.Once the differentiation pathway of a stem cell has been decided, it can no longer become another type of cell on its own.

Unspecialized cells capable of renewing themselves through cell division, sometimes after long periods of inactivity.

Under certain physiologic or experimental conditions, they can be induced to become tissue--
or organ-specific cells with special functions.



History and Evolution of stem cells

All over the world, for centuries researches are going on in various fields. It is a known fact that certain species in animal kingdom like reptiles like lizard, earthworm etc can regenerate their certain body parts. Human share some of such abilities as our body is constantly regenerating skin, blood etc tissues.

Stem cells have an interesting. In the mid 1800s it was discovered that cells were basically the building blocks of life and that some cells had the ability to produce other cells.

Attempts were made to fertilise mammalian eggs outside of the human body and in the early 1900s, it was discovered that some cells had the ability to generate blood cells.

In 1968: the first bone marrow transplant was performed to successfully treat two siblings with severe combined immunodeficiency. Other key events in stem cell research include:

1978: Stem cells were discovered in human cord blood
1981: First in vitro stem cell line developed from mice
1988: Embryonic stem cell lines created from a hamster
1995: First embryonic stem cell line derived from a primate
1997: Cloned lamb from stem cells
1997: Leukaemia origin found as haematopoietic stem cell, indicating possible proof of cancer stem

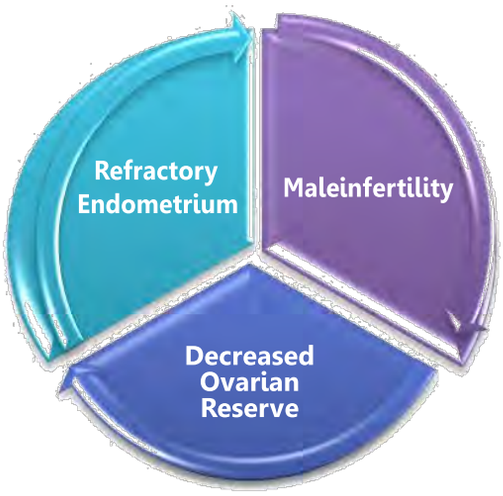
Why are stem cells special?

There are times in human history when new discoveries are made and those are recognized as milestones. In the world of medicines such achieved milestones have always helped in betterdiagnosis and treatment of the patients. Stem cells represent such a land mark because of their potential to regenerate and repair damaged tissues, making They are special because of their characteristic features.

- Self--renewal: stem cells can renew themselves almost indefinitely. This is also known as proliferation.
- Differentiation: stem cells have the special ability to differentiate into cells with specialised characteristics and functions.
- Unspecialised: stem cells themselves are largely unspecialised cells which then give rise to specialised cells.

Stem Cell Harvest and Infusion (Our BM method)

Stem cells can be isolated from several human organs like bone marrow, umbilical cord, adipose tissue etc. But, bone marrow is a rich source of stem cells. These stem cells are called Autologous Hematopoietic Stem Cells and they are considered to be adult stem cells. These cells can be easily harvested from the Bone marrow of hip bone of the patient undergoing the stem cell infusion. With the latest non-- touch, digital technology, Stem Cells can be isolated from the Bone Marrow of an individual within 30 minutes in the most sterile conditions. Since there is no hospitalization, no cut and no surgery, immediate mobilization is possible and it becomes patient friendly method of procedure.



Role of stem cells in Infertility

As can be seen it is clear that infertility is complex outplay of factors mentioned in Decreasing semen parameters and premature ovarian failure have become a global concern. For both male and female partners experiencing infertility, current treatment options rely solely on the premise that both partners roduce functional haploid gametes. For those couples where one partner is unable to produce a functional gamete, no treatment options are available other than the use of donor gametes. Stem cells are coming up as a miraculous therapy in various conditions, including fertility.

Azoospermia

Approximately 50% of human infertility is attributable to male defects, 70–90% of which arises from impaired spermatogenesis with the clinical presentation of abnormal sperm production, such as oligo- or azoospermia [1,2] In humans, azoospermia affects about 1% of the male population [3] and may be seen in up to 20% of male infertility situations.[4]

It is defined as the complete absence of any sperm in a man's semen. It is found in 20% of men who seek infertility treatment. Normally, man's testicles produce sperms which join with seminal vesicle secretions to become semen.

Types of azoospermia

a) Obstructive (sperm is blocked from exiting the testicle)
b) Non obstructive (sperm is not produced at all)

The testis has both exocrine and endocrine functions. The exocrine function involves the continuous production of spermatozoa, which are released from the testis, transported through the excurrent ducts, and eventually ejaculated. The endocrine activity consists of the secretion of testosterone, which is necessary to maintain secondary sexual characteristics, accessory sex organs, and spermatogenesis.

Pilot study of intra-testicular injection of autologous BMMNCs at our centre

4 Men with non-obstructive
azoospermia. Inclusion criteria

- Trial TESA failed to retrieve sperms.
- Genetically normal.

Autologous BMMNCs infused intra-testicular under short propofol. Results of this study will help in changing the perspective of fertility potential in azoospermia patients.

Erectile dysfunction

Erectile dysfunction (ED) is defined as the consistent inability to obtain or maintain an erection for satisfactory sexual intercourse [5]. The process is most often initiated as a result of sexual arousal, when signals are transmitted from the brain to nerves in the penis. The most important organic causes of impotence are cardiovascular disease and diabetes, neurological problems (for example, trauma from prostatectomy surgery), hormonal insufficiencies (hypogonadism) and drug side effects.

Notwithstanding variations in definitions and methods, various large-scale studies (both cross-sectional and longitudinal) confirm the global presence of this disease, with an estimated overall prevalence rate of 10–20% worldwide [6]. There is a strong correlation between age and ED, with the prevalence increasing steadily from 6.5% in men aged 20–39 years to 77.5% in those aged 75 years. As Evaluated by Lin, in 35 published studies, mostly associated with cavernous nerve injury or diabetes. Adipose derived stem cells used in 18 studies and bone marrow derived in 9. Intracavernous injection done. All studies reported improved erectile function, as well as improved muscle, nerve or endothelium in the erectile tissue.[7]

Refractory Endometrium

For successful conception, apart from healthy embryo we need Excellent Endometrium so as to have healthy dialogue between embryo and endometrium.

The cavity of the uterus is lined by the endometrium. The human endometrium is a dynamic remodelling tissue undergoing more than 400 cycles of regeneration, differentiation and shedding during woman's reproductive years. Endometrial thickness is the key factor in the implantation of the embryo and in the achievement of pregnancy. Under normal conditions in response to naturally produced estrogen in the body uterine lining grows about 1-2mm every other day. Ideally, at the time of ovulation, the endometrium would be about 8mm or more in thickness. There is no officially accepted definition of a thin endometrium, which results in a lower rate of full-term pregnancy. The commonly accepted cut off is less than 7mm on the day of LH surge or HCG administration.[8] The prevalence of this pathology is 0.5% of infertile women undergoing assisted reproductive treatments. (Senturk and Erel 2008).

Asherman's syndrome is an uncommon gynaecological disorder cause d by the destruction of the endometrium due to repeated or aggressive curettages and/or endometritis (Yu et all 2008). As a result there is loss of functional endometrium in many areas. The uterine caity is obliterated by intra uterine adhesions, leading to amennorhoea, infertility, recurrent pregnancy loss,etc. It causes adhesion and fibrosis of the endometrium such that there is very little healthy endometrium for implantation. Refractory endometrium too, offers very limited treatment options.

A normal uterine cavity and endometrial lining are necessary in order to conceive and maintain a pregnancy. The only therapeutic modality available for such women is surrogacy.

Bone marrow stem cells contribute for endometrium regeneration. On the basis of these facts, bone marrow stem cells can be used for regeneration of damaged endometrium. Endometrium is dynamic, cyclically regenerating tissue, a unique model of physiological angiogenesis in adult.

Angiogenesis result either sprouting a new vessel through recruitment of local endothelial cells from neighbouring blood vessel and or by endothelial progenitor cells circulating in blood after release from bone marrow.

Woman with severe Asherman's syndrome treated with autologous bone marrow derived stem cells from endometrial regeneration led to a successful implantation[2,3]

Bone marrow stem cells exert their influence by the secretion of massive amounts of growth factors and cytokines to result in a therapeutic outcome called 'trophic' activity. It is known that stem cells secrete bioactive molecules that

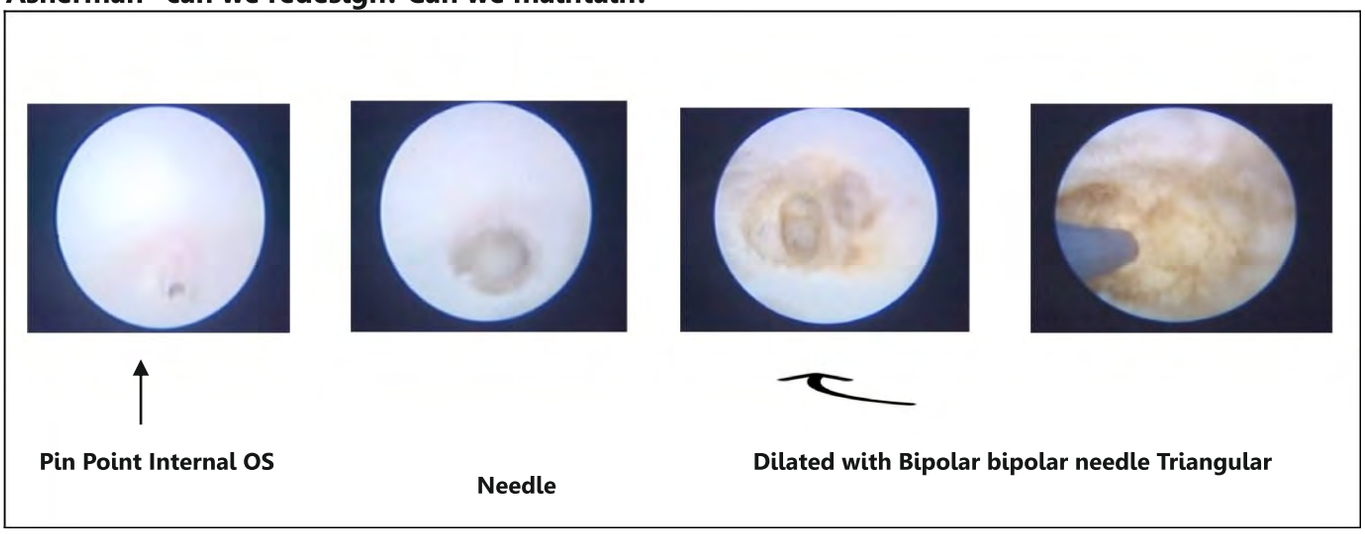
1) inhibit apoptosis and limit the field of damage or injury

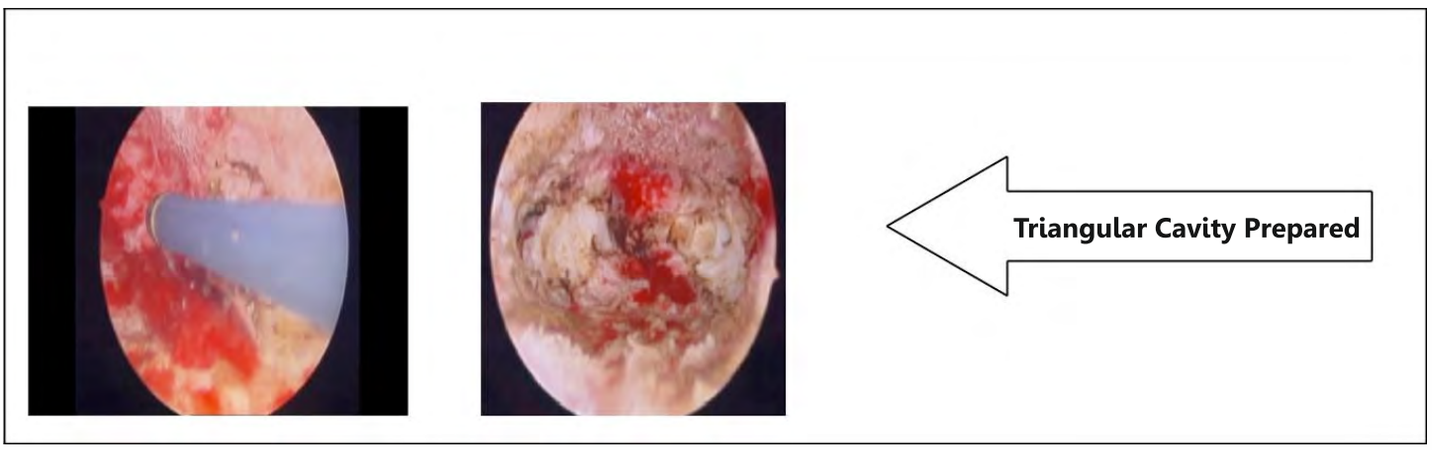
2) inhibit scarring or fibrosis at the site of injury

3) stimulate angiogenesis

4) stimulate the mitosis of tissue--specific and tissue--intrinsic progenitors [4,5]

Asherman –can we redesign? Can we maintain?





Premature Ovarian Failure

During the lifetime a numerically fixed pool of oocytes are committed for the fertility. This original pool would account in woman for approximately 106 oocytes in puberty, but the number declines with aging until exhaustion at menopause.

Premature ovarian failure (POF) is the loss of function of the ovaries before age 40. It is associated with sex steroid deficiency, amenorrhoea, infertility and elevated serum gonadotropins[6]. Majority of the cases underlying cause is not identified [7].10% of women undergoing IVF show diminished ovarian reserve, though the incidence is increasing by the day. However, pregnancy rate remains low despite a plethora of interventions and is associated with high pregnancy loss.

Known causes include

1) Genetic aberration which could involve the X chromosome and autosomes.
2) Autoimmune ovarian damage - Antiovarian antibodies are reported in POF by several studies
3) Iatrogenic following surgical radiotherapeutic or chemotherapeutic interventions as in malignancies
4) Environmental factors like viral infection and toxins for which no clear mechanism is known [16]

M. Edessy had injected bone marrow derived mesenchymal stem cells into the ovaries, via laparoscopically in 10 patients.Endometrial fractional biopsy was histopathologicaly (HP) and Immunohistochemically (IH) stained and evaluated according to Edessy stem cells score (ESS).

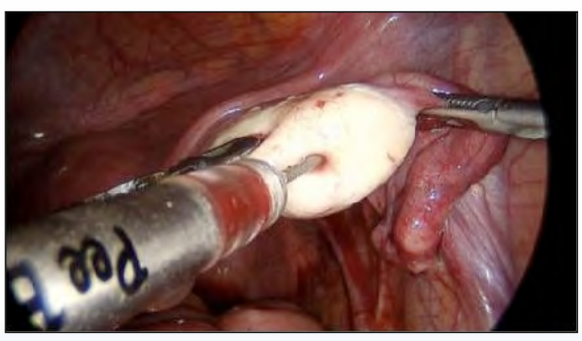
After transplantation two cases (20%) (ESS = 5 and 6) resumed menstruation after 3 months, one of them (10%) (Case no 5) (ESS = 6) got pregnancy after 11 months and delivered a healthy full term baby The 2 menstruating cases showed focal secretory changes after being atrophic endometrium in case 5 and distorted proliferative endometrium in case 10.[17]

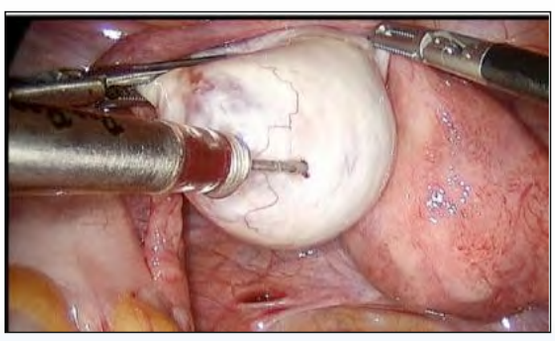
Several researchers in Eunice Kennedy Shriver National institute of child health and human development, US (january 2017) and Hospital university La Fe (September 2014) have confirmed the presence of ovarian stem cells, as well as bone-- marrow derived stem cells that have been able to colonise the ovaries, and have folliculogenesis.

The first baby of autologous stem cell therapy in POF is a reality and hope. The first stem cell baby "Zein Rajani", mature living female, 38 wks, 3.3 kg[18]

We at Ruby Hall IVF Endoscopy Centre have done 6 cases till now with encouraging results. One of 45 years lady whose AMH was 0.5 , shown improvement with 0.95 in 8 weeks. We could retrieve 3 eggs and one excellent day 3 Frozen Embryo Transfer gave successful pregnancy, 16 weeks of gestation now .

(NIPT showing Normal karyotyping)





Instillation of BMMSC in both ovaries

Ethical concerns

Indian Council of Medical Research has approved following guidelines

- National Guidelines for Stem Cell Research revised by ICMR in November 2017
- Stem cell use in infertility in India is for investigational purposes at present.
- Approval for clinical trials to be sought from Institutional Ethics Committee (IEC) and National Apex Committee for Stem Cell Research and Therapy (NAC- SCRT).

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Managing Viral Marker Positive Patients

Dr Asha Baxi
MS FRCOG FICOG
Chairperson,
FOGSI Infertility Committee

Dr Laxmi Shrikhande
MD FICOG FICMU
Consultant Gynaecologist
& Infertility Specialist
Vice President FOGSI (2012)

Dr Bhushan Shrikhande MBBS
Associate Consultant
Shrikhande Hospital &
Research Centre, Nagpur

Introduction

It has been estimated that globally, 37 million people were living with Human Immunodeficiency Virus (HIV) infection at the end of 2014. However, with the advent of highly active antiretroviral therapy (HAART), the natural history of the disease has transformed from one with a uniformly fatal outcome to a chronic infection with a near-normal life expectancy. In the backdrop of this transition, gynaecologists have seen rising footfalls from HIV positive patients who are predominantly in the reproductive age group and are seeking advice on fertility and reproductive issues.The provision of fertility care to seropositive couples poses myriadand novel ethical dilemmas to the treating gynaecologist. On one hand, the clinician might want to decline fertility care fearing neonatal transmission of HIV and on the other, he/she may be in a fixregarding the treatment option to be offered to a serodiscordant couple.

Principles of management

For HIV serodiscordant couples, any decision about fertility management should be the result of discussions between the couple, a fertility specialist and an HIV specialist. Advise couples where the man is HIV positive that the risk of HIV transmission to the female partner is negligible through unprotected sexual intercourse when all of the following criteria are met:

- the man is compliant with highly active antiretroviral therapy (HAART)
- the man has had a plasma viral load of less than 50 copies/ml for more than 6 months
- there are no other infections present
- unprotected intercourse is limited to the time of ovulation.

Advise couples that if all the above criteria are met, sperm washing may not further reduce the risk of infection and may even reduce the likelihood of pregnancy.

If couples who meet all the criteria in the recommendations perceive an unacceptable risk of HIV transmission after discussionwith their HIV specialist, consider sperm washing.Inform couples that there is insufficient evidence to recommend that HIV negative women use pre-exposure prophylaxis if the male partner satisfies the previous stated criteria. Given below is a flowchart outlining the management of fertility in serodiscordant couples.

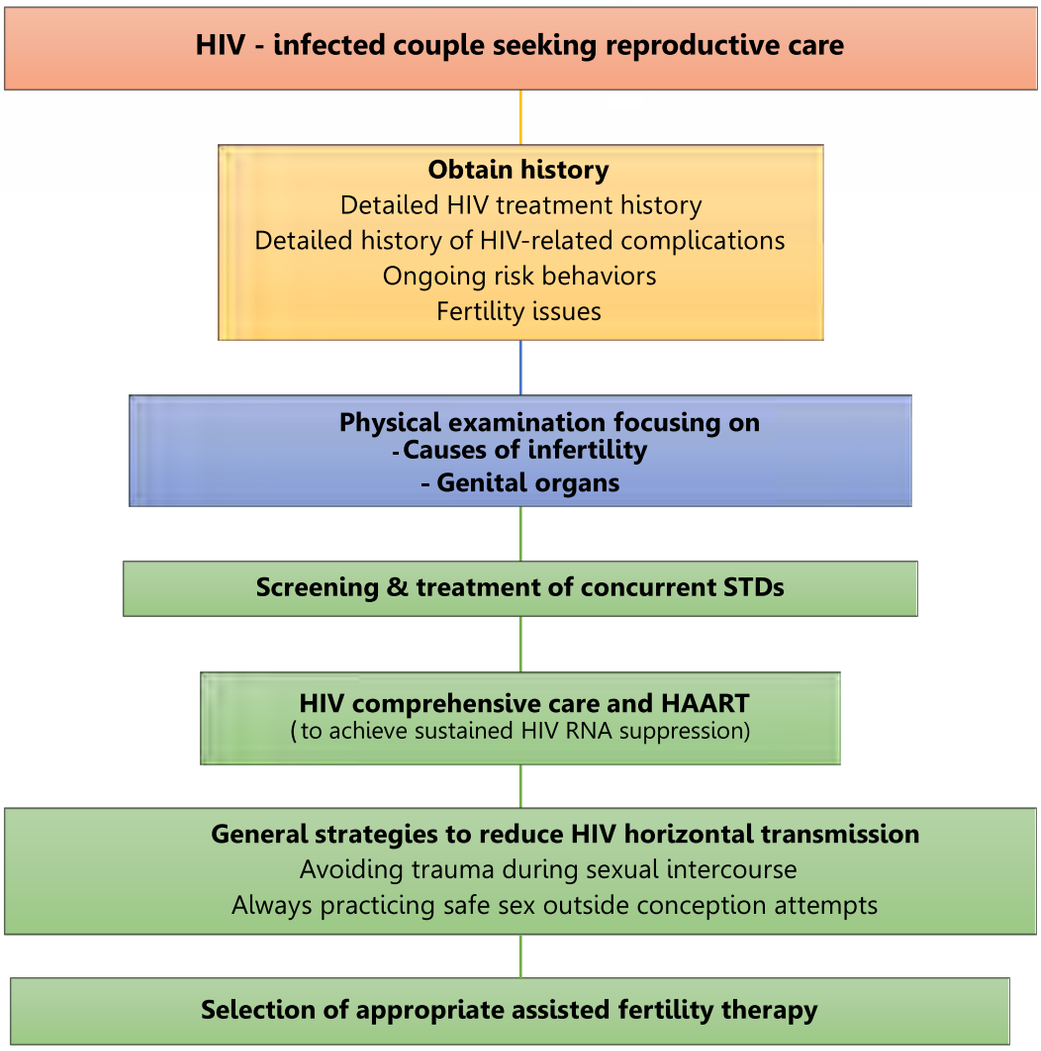

Fig 1 Approach to fertility management in HIV serodiscordant couples

Male partner positive, female partner HIV negative

HIV infection as well as HAART may be associated with semen abnormalities such as low sperm count, low motility, and low volume. Thus, the presence of HIV may affect the reproductive potential of a seropositive personand HIV should be well controlled as confirmed by an undetectable viral load both in serum and semen and a stable CD4 count. Only those patients who strictly adhere to HAART are appropriate candidates for fertility treatment.

The goal of treatment is to prevent transmission to the partner as well as the foetus. Keeping this in mind, the couple may be offered IUI by partner semen, IUI by donor semen, IVF and ICSI.In those cases where the partner's semen is used, it should be handled with all universal precautions.The processing lab must be informed of his HIV status and the semen should be as free as possible from HIV. For couples where the man is HIV positive and either he is not compliant with HAART or his plasma viral load is 50

copies/ml or greater, offer sperm washing. Inform couples that sperm washing reduces, but does not eliminate, the risk of HIV transmission.

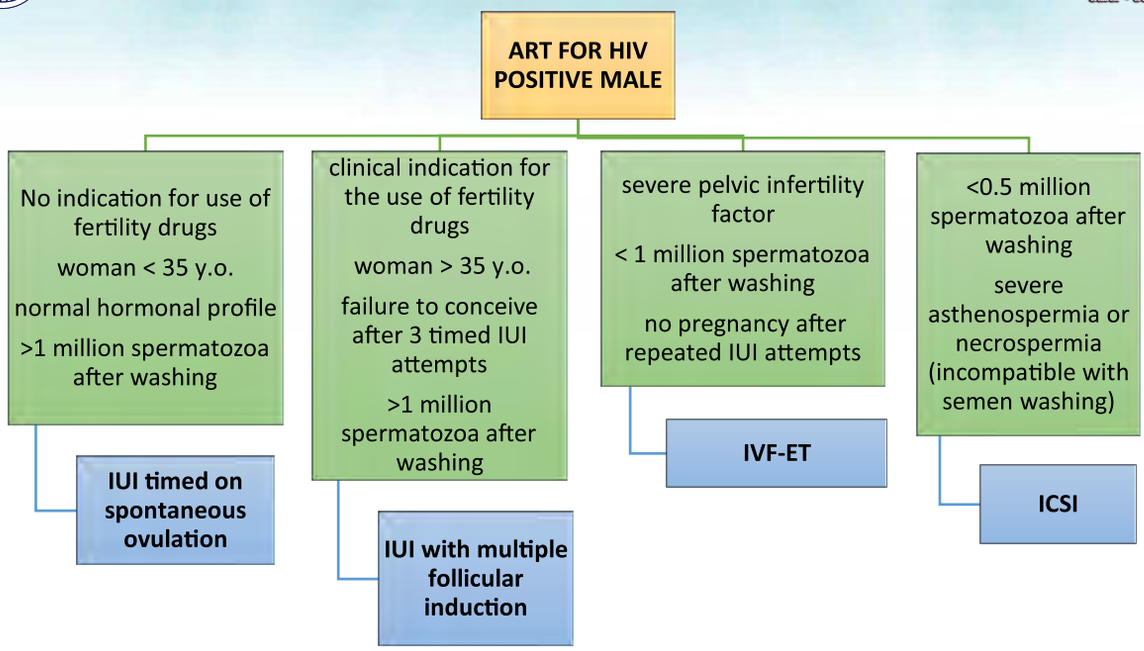

Fig 2 Approach to infertility in male seropositive partner

Female partner positive, male partner HIV negative

The woman must be fit enough to undergo pregnancy and as stated previously, only those with well controlled HIV are appropriate candidates for fertility services. Transmission of infection to the male partner can be avoided by using homologous insemination with the partner's sperm. If this option is not available to the couple, or for other reasons not desired, the risk of transmission can be minimised by using timed intercourse, assuring that the woman's viral load is suppressed to undetectable levels on antiretroviral therapy and/or that the uninfected male is taking antiretroviral therapy as pre-exposure prophylaxis. While clinicians need to emphasise that this option is not as safe as homologous insemination, it does represent an alternative option for select couples.

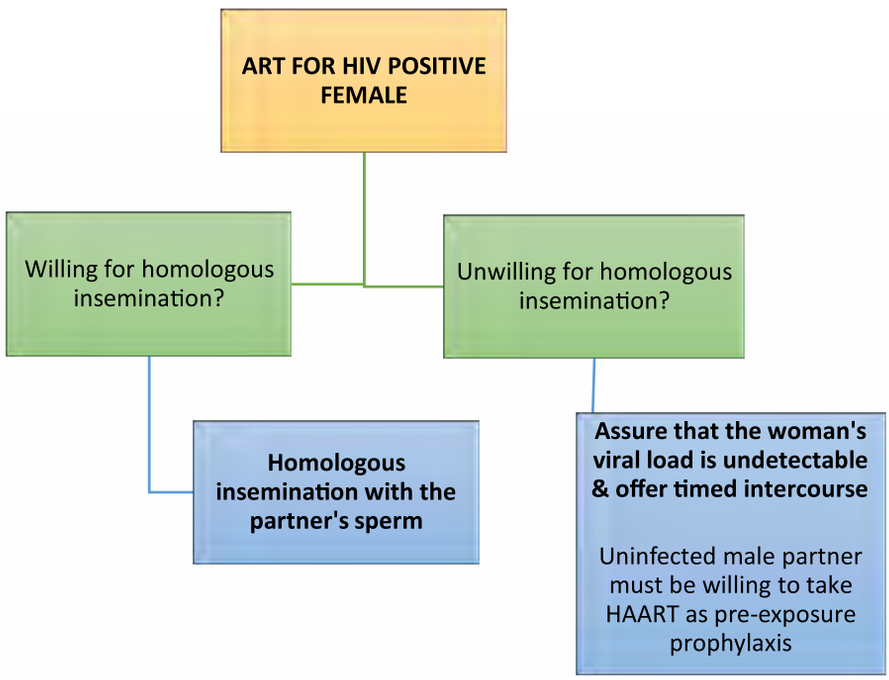

Fig 3 Approach to infertility infemale seropositive partner

Both partners are HIV positive

As with any couple presenting for evaluation and treatment, both members of an HIV-infected couple may have normal fertility potential or one or both may have impaired fertility. There is a real risk of one or both partners suffering from the long term consequences of HIV, leaving the child vulnerable. The risk of vertical HIV transmission looms as well.

Although such couples do not have the same concerns of HIV transmission as serodiscordant couples, the possibility of HIV superinfection must be raised in this scenario. There is atleast a theoretical risk that one HIV-infected partner can transmit their unique strain of HIV to another infected partner.

The best way of minimising these potential risks while optimising outcomes for the couple and their offspring is to encourage them to be compliant with HAART. As far as fertility is concerned, the couple should be thoroughly counselled about the pros and cons of fertility treatment. They should also be introduced to the idea of adoption or child-free living.

Can treatment be denied to a serodiscordant couple?

A clinician cannot refuse fertility treatment to a serodiscordant couple solely on ethical grounds. Rather, he/she is supposed to educate, counsel and empower the couple to make the best decision regarding their care.The physician should performall relevant tests to assess whether the couple is suitable for fertility treatment. It is also the duty of the physician to communicate that under fully suppressive treatment, HIV transmission risk is negligible. Before treating the couple, the physician must safeguard himself first by meticulously adhering to universal precautions at all stages of treatment.

Conclusions

Human immunodeficiency virus infection is a chronic disease which is treatable, but not yet curable. Significant advances in HIV treatment have delayed the onset of AIDS and its consequences in manyinfected persons. The potential for HIV-infected persons to live long and healthy lives, have uninfected children and not transmit the virus to their partners has resulted in increasing numbers of individuals to seek out optimal means for creating biologic families. Health-care providers and HIV-infected persons together share responsibility for the safety of the uninfected partner and potential offspring.

When an affected couple requests assistance to have their own child, they are best advised to seek care at institutions with the personnel and facilities that can provide the most effective evaluation, treatment, and follow-up. ART clinics with the necessary resources to provide care should offer services to HIV-infected individuals and couples who are willing to use recommended risk-reducing therapies. Clinics without sufficient resources to offer care should assist in referral to providers equipped to manage such patients.

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Future Trends in Lab Technology

Dr Sudesh Kamat
Chairperson Embryologist ISAR
Senior Consultant
Embryologist, Mumbai

Mrs Rushika Mistry
MSc Life Sciences (Applied Medicine)
Embryologist, Bloom IVF Lilavati Hospital, Mumbai

Introduction

In today's modern society, many couples delay childbearing until they are in their thirties. Infertility affects 2% of couples in their early twenties, 25% of couples in their mid-thirties, and more than 40% of couples in their late thirties and early forties. IVF has literally transformed the field of infertility since its inception in 1978 with the birth of first IVF baby, Louise Brown.

Probably the single most significant factor in the dramatic improvement in IVF pregnancy rates over the past 10-15 years has been modifications of technology in the embryology laboratory. Of these modifications, embryo culture media perfection has likely been the most significant. With this, pregnancy rates increased dramatically since the introduction of Human Tubal Fluid culture media formula which was developed in 1985.

One such modification that is the subject of much research today is the optimal oxygen (O2) concentration in embryo incubators. Other on-going research areas are evaluating the optimal pH of embryology media. In addition to media, various other embryology techniques have also been introduced that have optimised the results.

In this chapter we will discuss the importance and efficiency of the latest products available in the market to improve pregnancy outcomes and come closer to the aim of achieving pregnancy with a single embryo/blastocyst transfer.

Laboratory advances for sperm selection

The factor of outmost importance in the substantial improvement of IVF is the advancement of the laboratory practices. Routine semen analysis is the first step in evaluation of the infertile male, but is not a test of fertility. It provides no insights into the functional potential of the spermatozoon to fertilize an egg.

WHO laboratory manuals have been the primary reference handbooks for semen analysis for many years. The latest WHO (fifth edition, 2010) laboratory manual lists statistically derived fifth centile lower reference limits from several prospective semen analysis studies around the world. These are not cut-off values for diagnosis of sub-fertility, but reflect probabilities based on results from a fertile population.

Abnormal semen values suggest possible male factor infertility requiring further clinical and/or laboratory evaluation of the patient. These reference values for semen parameters are not the minimum values to define infertility, as men with semen variables outside these reference ranges are possibly fertile. To ensure accurate results, the laboratory should have a quality control program for semen analysis. Below are listed some of latest methods to evaluate the best sperms for fertilisation of an egg.

MACS

Magnetic- Activated Cell Sorting (MACS) system is an efficient method to select functionally active sperm and improve pregnancy rates. MACS Annexin V System is designed to selectively remove defective although morphologically indistinguishable cells from sperm preparations. The procedure magnetically labels unwanted spermatozoa, which are then passed through a separation column where they are selectively retained. Intact living spermatozoa without DNA fragmentation pass through the column and are collected for later use.

In most viable eukaryotic cells, the negatively charged phospholipid phosphatidylserine (PS) is located in the cytosolic leaflet of the plasma membrane lipid bilayer. PS redistribution from the inner to outer leaflet is an early and widespread event during apoptosis. Annexin V has high affinity to PS in the presence of physiological concentrations of calcium and has been used to isolate cells with exposed PS using MACS MicroBeads. PS-exposing cells are attracted by magnetic enrichment using Annexin V MicroBeads.

The sperms are magnetically labelled with Annexin V MicroBeads and passed through a MACS column which is placed in the magnetic field of a MACS Separator. The magnetically labelled PS- exposing sperms are retained in the column while the unlabelled cells run through. After removal of the column from the magnetic field, the magnetically retained PS-exposing sperms can then be eluted as a positively selected fraction.

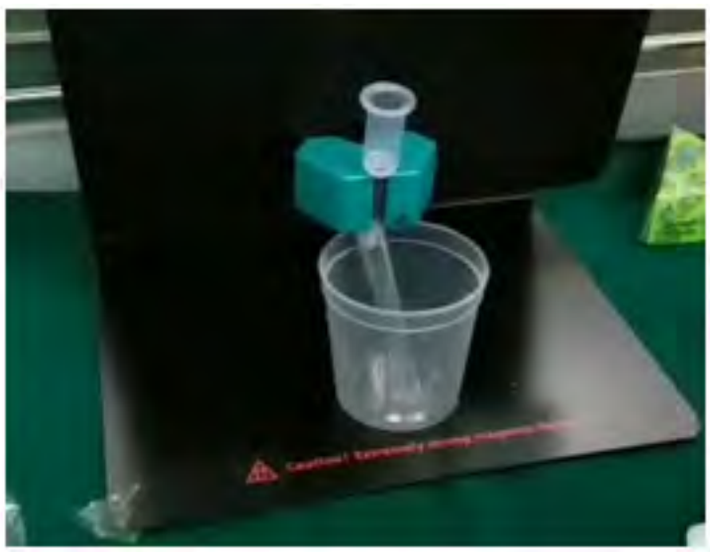

Fig 1 MACS setup

IMSI

In conventional IVF, the zona pellucida functions as biological barrier against abnormal sperm, so that in most cases only "normal" sperm is able to fertilise an oocyte.Since ICSI bypasses the natural sperm selection process, there is an increased risk of genetic abnormalities being transmitted to the offspring.

Hence, several sperm selection methods have been developed to select the most normal spermatozoa, prominent among which is the IMSI technique.ICSI is normally performed at a 200–400x magnification which enables the observation of major sperm morphological defects, such as head abnormalities (round, tapering etc), midpiece defects (bent neck, thick mid piece), tail defects (short stubby, double) whereas minor morphological defects, such as vacuoles in the sperm head, which seem to be related to the ICSI outcome are not identified.

In 2002, Bartoov et al, developed a new method of unstained, real time, high magnification motile sperm organelle morphology examination called MSOME. High magnification is made possible by the use of an inverted light microscope equipped with high power Normaski differential interference contrast optics enhanced by digital imaging to achieve a magnification of 6,600X. Inclusion of MSOME, together with a micromanipulation system enables the selection of a single motile spermatozoon with strictly defined morphologically normal nucleus to be injected into the retrieved oocytes. This modified IVF (in vitrofertilisation) procedure was then named IMSI (Intracytoplasmic morphologically selected sperm injection) by Bartoov et al.

The calculation of the "Total reached magnification" may vary depending upon the system components, but usually it is as follows:

1. Microscope Magnification (100x objective magnification x 1.5x magnification selector)
2. Video Coupler Magnification (1x)
3. Video Magnification (CCD x monitor diagonal dimension) (44.45x)

Total magnification = 100x1.5x1x44.45 = 6,600x

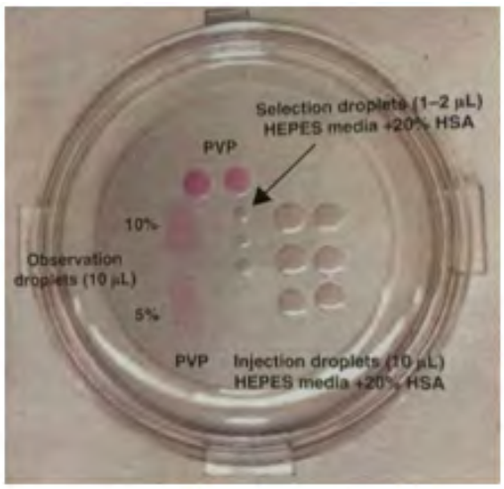

Fig 2 IMSI dish

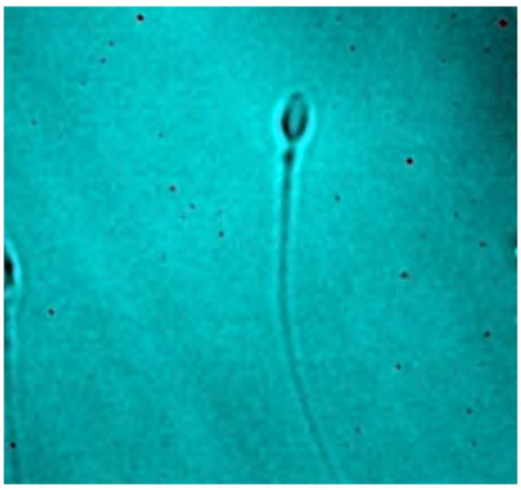

Fig 3 6600x magnified sperm

PICSI

PICSI is a variation of the ICSI technique where the 'P' stands for 'Physiological'. In the traditional ICSI the selection of the spermatozoon is made by the embryologist who observes the semen sample and selects one spermatozoon per egg, with good motility to carry out the ICSI procedure. With PICSI, it is intended to find a less subjective system in the sperm select system. This variation of ICSI is carried out using a similar molecule ie. hyaluronic-acid, that surrounds the oocyte naturally.

Sperm in vivo encounters hyaluronan in the cervical mucus and in the cumulus surrounding the oocyte. Penetration of the cervical mucus and cumulus by the sperm in vivo are critical elements in successful fertilisation and subsequent embryo implantation; hyaluronan is vital in this interaction. PICSI takes advantage of this naturally occurring encounter. A special dish with small dots of hyaluronan on the bottom of the dish is used with the standard ICSI injection. A drop of prepared sperm is added to the hyaluronan and the embryologist selects a hyaluronan bound sperm for injection. In this way more mature sperms are selected for injection. Many studies have shown that sperm bound to hyaluronan are more likely to have less DNA damage and a normal chromosome complement.

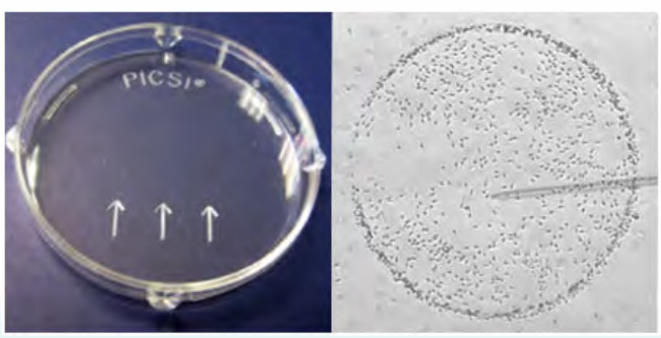

Fig 4 PICSI dish and sperms bound to hyaluronan drop

Microfluidics

Microfluidic Sperm Sorting Chip provides an alternative to procedures requiring extensive centrifugation and vortex mixing that cause irreversible damage to sperm. The sorted sperms exhibit more better morphology and motility. There is less DNA damage and lower levels of reactive oxygen species (ROS). The principle of the device is based on the fact that only motile sperm can traverse the border that separates the parallel streams of diluted semen and fresh medium. Thus, the laminar flow properties exhibited by media in micro channels allows motile sperm to swim away from non- motile sperm, debris, and seminal plasma and collect in a separate outlet reservoir.

Follow-up experiments demonstrated this microfluidic device design was not only biocompatible with human sperm, but that it could isolate motile, morphologically normal sperm. This novel approach, appears to offer a feasible alternative to, isolate sperm from oligozoospermic patients for use in intra-cytoplasmic sperm injection (ICSI) only. Microfluidic Sperm Sorting Chip is a flow-free, dual chambered, single use device. The lower chamber has a sample inlet and fluid channel separated from the upper collection chamber by a microporous membrane. A liquefied semen sample is injected via the inlet. The sorted sperms are collected from the upper chamber. Sperms are sorted by the separation of healthy motile sperms from the many compromised poorly motile sperms present in the semen sample.

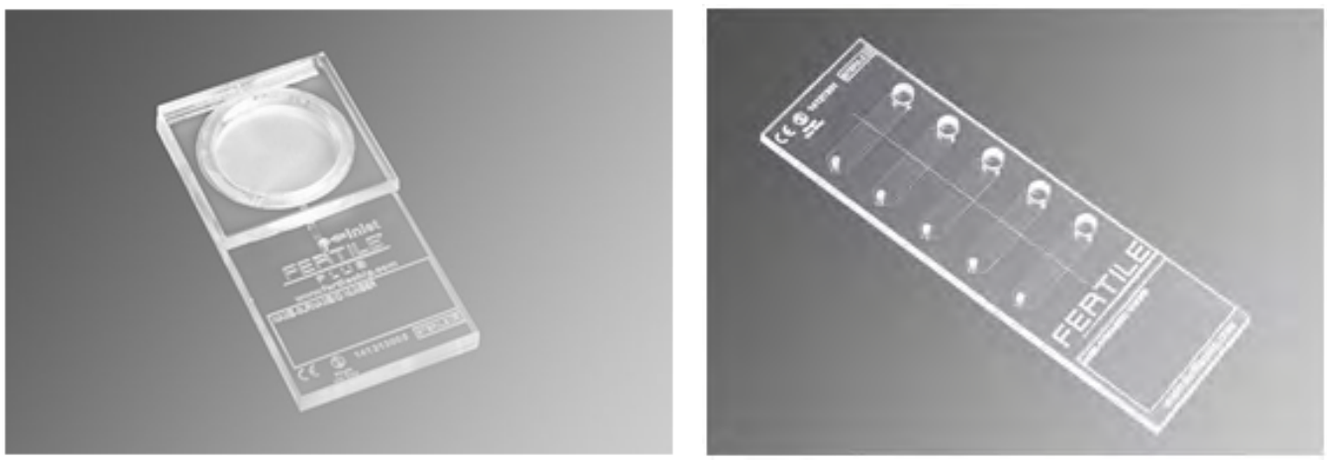

Fig 5 Microfluidics sperm sorting chip

Laboratory advances for improving IVF results

Spindle View PolScope

Oocytes after ovulation are at metaphase II, stage in which meiotic spindle is formed. The meiotic spindle plays an important role in the oocyte during chromosome alignment and separation at meiosis. It has been reported that spindle abnormalities in oocytes during meiosis contribute to the aneuploidy in embryos after fertilization, poor embryo development, spontaneous abortion and inherited diseases. The spindle is a very fragile mechanism and it must be avoided during sperm injection. Studies have shown that the damage of metaphase spindle reduces fertilisation and embryo development.

The instrument used to detect the spindle in the egg is called Spindle View PolScope system. The introduction of an orientation–independent polarised light microscopy system coupled with the image processing software, allows the visualisation of meiotic spindle in living eggs. The PolSscope functions by making use of optical deviation of light waves to allow it to pass through the egg under the microscope. After evaluating the deviation on a computer, the embryologist locates the spindle. Thus, the machine allows a thorough screening of the egg to understand its genetic qualities and if it is suitable for fertilisation.

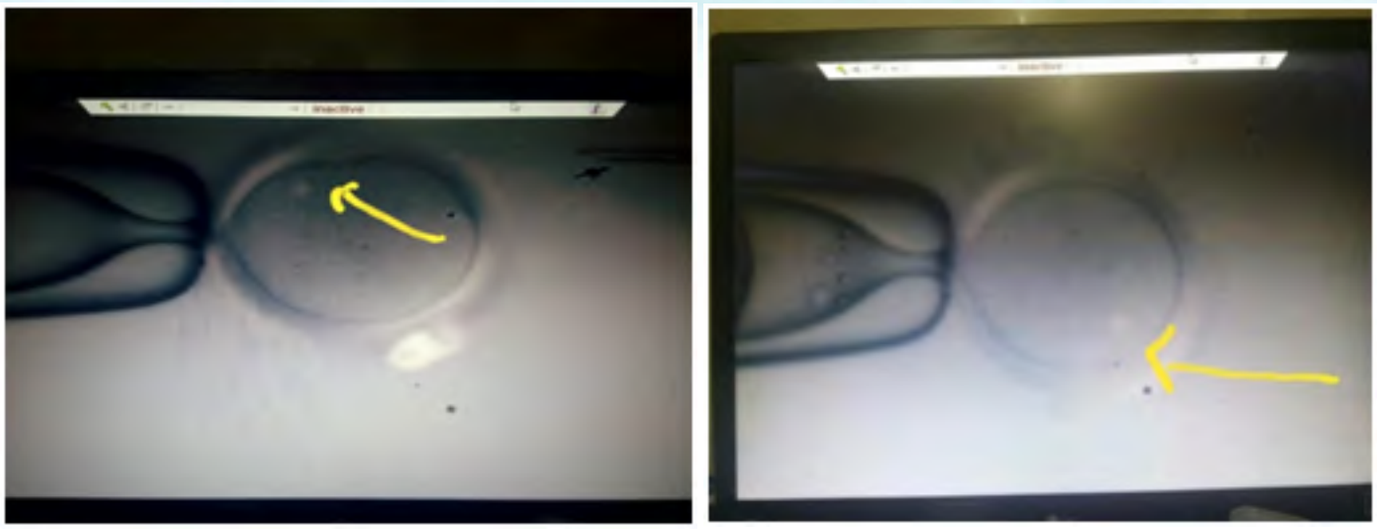

Fig 6 Meiotic spindle at 11o'clock of the polar body

Time lapse monitoring systems

The Embryoscope is a highly sophisticated instrument containing an incubator, a microscope and a computer.It is a tri-gas embryo incubator, which acquires a series of unattended measurements on individual embryos during their development.The measurements include time-lapse microscopy at multiple focal planes and logging of incubation conditions. Separate processing units control the incubation environment and the data acquisition to ensure safe and reliable operation.

The EmbryoViewer workstation is an accessory to Embryoscope.Out of the various time-lapse systems currently used, two of the most frequently used are the Primo Vision and Embryoscope systems, which use bright field technology. The EEVA (Early Embryonic Viability Assessment) system uses dark field technology. They differ by whether the embryos are put into the special incubator (the embryoscope) and monitored there or whether the equipment is fitted to the laboratory's own incubators (primovision). All the systems consist of a digital inverted microscope that takes a picture of the embryos at the interval of every 15 to 20 minutes. This is a simple way to document and save all the stages of embryo development. There is a minimal light exposure without disturbing the microenvironment of the embryos.

This system is user friendly and allows 6 units to be connected to one computer, thus all the embryos of six patients can be monitored in one incubator.The exact onset and duration of the first cell division can be accurately evaluated.The cleavage pattern, morphologic changes and embryo development dynamics helps in identifying and selecting the embryos with a higher implantation potential.Also, it allows detection of abnormal divisions, abnormal cleavages which may lead to chromosomal aneuploidies and repeated abortions. There is insufficient evidence to choose between TLS, with or without embryo selection software, and conventional incubation. Other models available in the market are Geri® and Miri® TL.

Fig 7 Time-lapse monitoring system (Embryoscope)

PGS and PGD
These are discussed in a separate chapter devoted to preimplantation genetic testing.

AUGMENT (Autologous Germline Mitochondrial Energy Transfer)
Ovarian aging is involved with decrease in both the total number and quality of the eggs. The decline in the quality is associated with mitochondrial dysfunction. Mitochondria isolated from egg precursor cells are of high quality and can serve as an autologous source of mitochondria for women. The mitochondria present in oogonial stem cells (OGC) are the same as those found in oocytes. In the AUGMENT technique, a biopsy of ovarian tissue is performed and harvested to isolate the egg precursor cells to obtain their mitochondria. Ovulation induction is done and the oocytes are collected.

The patient's own OSC-derived mitochondria along with a sperm are injected by ICSI. In this technique, there is no drawback of having a foreign mitochondrion present in the resultant embryo and offspring. The use of AUGMENT showed an improvement in clinical pregnancy and live birth rate as compared with no AUGMENT (presented at the 31st Annual Meeting of the European Society of Human Reproduction and Embryology (ESHRE).

CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats)
The CRISPR is a gene editing technique used to alter genes in human embryos. A simple version of the CRISPR/Cas system, CRISPR/Cas9, has been modified to edit genomes. By delivering the Cas9 nuclease complexes with a synthetic guide RNA (gRNA) into a cell, the cell's genome can be cut at a desired location thus allowing existing genes to be removed and/or new ones added. The alterations can be in the form of single nucleotide editing to the modification of multiple genome-wide genomic sites.

With this technology, it is easy to delete genes in cells or to create genetically modified karyotype. The technology can be used to correct genomic mutations or create new creatures by changing the inherited phenotypes. CRISPR opens the door to an unbeaten level of control over human genes.The CRISPR-Cas9 technology has limitations with targeting ability. Several off-target mutations have been detected by genome-sequencing due to its high specificity. This limits its use in correcting disease-associated mutations. It also creates an ethical controversy because it may be used to manipulate human germ cells. Thus, this technique should be used cautiously before modifying human inheritance.

Mitochondrial transfer
Mitochondrial DNA (mtDNA) mutations are a common cause of genetic disease with pathogenic mtDNA mutations. Mitochondria are the energy-producing powerhouses of the cell. Their function is dependent on proteins transcribed from nuclear and mitochondrial DNA (mtDNA). Mitochondrial DNA is circular in structure and contains 37 genes. mtDNA diseases are inherited by all offspring irrespective of gender, as all mitochondria in the embryo originate from oocyte cytoplasm. Mitochondrial transfer techniques have the potential to provide patients with novel reproductive options for the prevention of mtDNA disease and infertile women with advanced age, enhancement of fertility in women of advanced maternal age or those suffering from diabetes.

The mitochondrial transfer techniques consist of pronuclear transfer, spindle transfer, ooplasmic transfer and blastomere transfer. Cytoplasmic transfer was the first proposed as a treatment for patients with infertility. Cytoplasmic transfer involves the transfer of a small portion of ooplasm, and hence mtDNA, from one oocyte to another.In 1997, the first cytoplasmic transfer in humans was done, resulting in pregnancies. Pronuclear transfer involves removal of both pronuclei from a zygote containing mtDNA mutations and transfer to the perivitelline space of a donated enucleated zygote. Pronuclear transfer involves the movement of two pronuclei from the affected zygotes (also in the form of a karyoplast), into the enucleated healthy zygotes. The resulting zygotes contain nuclear DNA from each of the intending parents and a donor's mtDNA.

Spindle transfer aims to achieve the same results as pronuclear transfer – inheritance of parental nuclear DNA and donor mtDNA in the offspring. In this method, the transfer of parental nuclear DNA occurs before fertilisation. This technique involves excision of the metaphase II spindle from the donor oocyte, which will provide the cytoplasmic constituent (including mitochondria) to the embryo. Again, contained within a karyoplast, the donor nuclear DNA will be discarded. The chromosome-spindle complex will then be removed from the oocyte of the intending mother, and transferred to the enucleated healthy donor oocyte. Blastomere transfer involves transplantation of a blastomere from an affected embryo into an enucleated healthy donor oocyte.

With the imminent emergence of mitochondrial replacement therapy, there is increasing concern over the possible incompatibility that this could create in the communication network that exists between the nucleus and mitochondria, which could have deleterious effects on the metabolomics of a cell. These techniques are associated with some degree of mtDNA carry over during spindle or pronuclear transfer, and thus possible persistence of mutated mtDNA. The long-term safety and efficacy of these techniques in humans is unknown, and further clinical research is required.

Fertility preservation

This is discussed separately in a dedicated chapter.

Robotic ICSI

Since its invention about 20 years ago, ICSI has been conducted manually by a handful of highly skilled embryologists; however, success rates vary significantly among clinics due to poor reproducibility and inconsistency across operators. The aim of robotic ICSI is to standardise how clinical ICSI is performed.

Some of the technical aspects of robotic ICSI system include a cell holding device, motion control and computer vision algorithms. The system performs visual tracking of a single sperm, robotic immobilisation of sperm, aspiration of sperm with picoliter volume and insertion of sperm into an oocyte with a high degree of reproducibility. The system requires minimal human involvement (requiring only a few computer mouse clicks) and is human operator skill independent. Using the hamster oocyte-human sperm model in preliminary trials, the robotic system demonstrated a high success rate of 90.0% and survival rate of 90.7% (n = 120).

Conclusions

The scope of future lab advances in the field of assisted reproductive technologies is staggering. The technologies that are coming now are visible on the horizon and have the potential to expand the utilisation of ART to large portions of society with and without an infertility diagnosis. For infertile couples, these advances promise to further improve the effectiveness, convenience and availability of infertility treatment while bringing down costs and increasing the efficiencies. For individuals without a diagnosis of infertility, innovations such as oocyte cryopreservation and preimplantation genetic testing offer applications that may be applied socially on a big scale.

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Endometrial Scratch Therapy: Current Opinion

Dr Pratik Tambe MD FICOG
Chairperson, FOSGI Endocrinology Committee (2017-19)
Managing Council Member, MOGS
Managing Council Member,
IAGE, MSR, AMC
(2015-18)

Dr Ameya Purandare
MD, DNB, FCPS, DGO, DFP, MNAMS, FICMCH, FICOG,
Fellowship in Gyn Endoscopy(Germany)
Joint Secretary, FOGSI 2019
Chairperson, Food Drugs Medical Surgical Equipment
Committee, FOGSI 2015-17
Member, Managing Committee and Mentor, Youth Council, MOGS
Hon Secretary, AMOGS 2014-2015 and Zonal Co-Ordinator 2015-2019

Background

Even though it has been several decades since the birth of Louise Brown and the subsequent refinement of IVF/ET protocols, embryo implantation remains the final frontier. Until we have decoded the puzzle of blastocyst implantation, success rates in IVF programs will remain at a static plateau of between 30 and 50%. Embryo implantation depends on the aetiology of infertility, the quality of the embryos and endometrial receptivity.[1] The last of these plays an overwhelmingly important role in the establishment of a healthy pregnancy during assisted reproduction.

The implantation window is a critical period during which the endometrium is optimally receptive.[2] This occurs on average at Day 6-10 post ovulation. We have been made aware in recent times that the stimulation protocols, gonadotropin therapy and levels of estradiol consequent to these influence the endometrial thickness and appearance – which are identifiable and measurable – and the receptivity of the decidua – which is difficult to assess[3,4]

Manipulating, improving and creating a more receptive endometrium remains the Holy Grail of assisted reproductive therapy and as our understanding of the immunology, embryo-decidua crosstalk and receptivity in the peri-implantation period improves, our techniques are continually being refined.[5] A special subset of the infertile population are those couples who present with recurrent implantation failures and poor endometrial receptivity eg. subsequent to tuberculosis, intrauterine synechiae and Asherman syndrome.

In these patients, many strategies including a variety of adjuvants have been employed in the hopes of delivering better success rates. One such approach is the so called "endometrial scratch therapy". This chapter discusses the current medical evidence as it stands today on this unique approach to enhancing success rates in a population of patients who have precious few alternatives.

Procedure

The concept of endometrial scratch (ES) therapy is rather a simple one. A Pipelle biopsy cannula or similar endometrial aspiration cannula is used to negotiate the cervix and enter the uterine cavity under aseptic precautions. The cannula is moved in a cephalocaudal direction gently several times to injure/stimulate the endometrium. This may be performed as an office procedure without anaesthesia.

The optimum time during the menstrual cycle when this may be performed is a subject for discussion but most authorities recommend doing it during the luteal phase of the preceding cycle of the index or treatment cycle.

Postulated mechanisms of action

Mechanical irritation of the endometrium during the luteal phase leads to decidualisation of the endometrium. ES improves impaired endometrial receptivity by partially normalising the expression of estrogen and progesterone receptors (ERs, PRs) and pinopodes.[6] There is also conjecture that modulation of gene expression of factors such as laminin alpha 4, integrin alpha 6, matrix metallopreoteinase 1and glycodelin A takes place.[7] There is an increased secretion of cytokines and growth factors including interleukins, which have been demonstrated to increase embryo implantation.

Review of literature

A meta-analysis of 7 RCTs with 2,062 patients suffering from recurrent implantation failure concluded that the ES group has 70% greater incidence of clinical pregnancies when compared to the control group.[8]

The effect of endometrial scratching in women with unexplained infertility has also been investigated. A 105 women who were subjected to ES showed a significantly better clinical pregnancy rate (25.9% vs 9.8%)compared to the control group.[9]

According to a Cochrane meta-analysis in 2015, endometrial injury performed between day 7 of the previous cycle and day 7 of the embryo transfer (ET) cycle increased the rates of clinical pregnancy and live birth.[10] To improve the efficacy of endometrial injury, additional studies are needed to determine the extent of the endometrial injury, the number of endometrial biopsies, and the optimal time to perform biopsies, concluded another article.[11]

Updated evidence (2019)

According to a new study by Tumanyan et al published this year, the controversies surrounding the effect of ES on pregnancy outcome in women with RIF are mostly due to the poorly defined target population. They evaluated the effect of ES on clinical outcomes in women with strict criteria of RIF before IVF/ICSI and measured the expression of markers of endometrial receptivity.

Women with failed implantation after transfer of seven or more top quality day 3 embryos or three blastocysts underwent the scratch procedure on exact days of the cycle prior to IVF/ICSI. Results were compared to no scratch control group. Using histo pathology, immuno histochemistry, and scanning electron microscopy, they also examined the effect of injury on the endometrial receptivity in a separate series of observations with double ES.[6]

The cumulative pregnancy rate was significantly higher in the study group as compared to control (54.8% vs. 29.0%; $p<.05$). The effect of ES on the clinical outcome was seen during fresh ET, but not on the next FET cycles. They concluded that in a well-defined sub population of infertile women with RIF, ES significantly enhances pregnancy rates. ES has a specific impact on endometrial receptivity normalising the expression of some markers.[6]

ES for a first embryo transfer

An RCT from New Zealand published 2019 in the New England Journal involved 1,364 women randomised to ES by Pipelle biopsy between Day 3 of the preceding cycle and Day 3 of the ET cycle in a 1:1 ratio.[12]

The primary outcome was live birth. The frequency of live birth was 180 of 690 women (26.1%) in the endometrial-scratch group and 176 of 674 women (26.1%) in the control group (adjusted odds ratio, 1.00; 95% confidence interval, 0.78 to 1.27). There were no significant between-group differences in the rates of ongoing pregnancy, clinical pregnancy, multiple pregnancy, ectopic pregnancy, or miscarriage. The median score for pain from endometrial scratching (on a scale of 0 to 10, with higher scores indicating worse pain) was 3.5 (interquartile range, 1.9 to 6.0).

The trial concluded that endometrial scratching did not result in a higher rate of live birth than no intervention among women undergoing IVF.[12]

In April 2019, a systematic review and meta-analysis of published and unpublished data from randomised trials regarding ES for women undergoing a first embryo transfer was published.[13]

Seven RCTs including 1,354 participants were assessed. There was a nonsignificant difference between groups in terms of OPR/LBR, CPR, MR, MPR, and EPR. Subgroup analysis found that endometrial scratch injury (ESI) on the day of oocyte retrieval (achieved by a Novak curette) reduced OPR/LBR (RR 0.31, 95% CI 0.14-0.69) and CPR (RR 0.36, 95% CI 0.18-0.71), whereas ESI during the cycle preceding ET (performed through soft devices) had no effect on OPR/LBR and CPR. No difference in the impact of ESI was observed between fresh and frozen embryo transfer.

The reviewers concluded that the current evidence does not support performing ESI with the purpose of improving the success of a first ET attempt either during the index cycle or the preceding cycle.[13]

Systematic review and meta-analysis (2019)

In an article published in Jan 2019 in Human Reproduction Open, the reviewers analysed 14 RCTs involving 2,537 participants. The effect of scratching was assessed for three different patient groups: patients with no prior IVF/ICSI treatment (Group 0), patients with one failed full IVF/ICSI cycle, including cryo-thaw cycles (Group 1) and patients with two or more failed full IVF/ICSI cycles (Group 2).[14]

Most RCTs contained a high or unclear risk of bias on one or more items. Substantial clinical and statistical heterogeneity was present; therefore meta-analysis for LBR and CPR could only be performed on Group 1. For this group, no differences between scratch and control were found for both LBR (risk ratio (RR) 1.01 [95%CI 0.68–1.51]) and CPR (RR 1.04 [95%CI 0.74–1.45]).

Miscarriage and multiple pregnancy rates were evaluated for the three groups (0, 1 and 2) together. Both outcomes were not significantly different between scratch and control (miscarriage rate RR 0.82 [95%CI 0.57–1.17] and multiple pregnancy rate RR 1.06 [95%CI 0.84–1.35]).

Results of pooled analysis for the subgroups of Group 0 and 2 showed no significant difference for LBR, but CPR was significantly improved after endometrial scratching (Group 0 RR 1.28 [95%CI 1.02–1.62] and Group 2 RR 2.03 [95%CI 1.20–3.43]). Subgroup analysis of the overall groups showed no significant difference for miscarriage and multiple pregnancy rate.

They concluded that it remains unclear if endometrial scratching improves the chance of pregnancy for women undergoing ART and, if so, for whom. This means that endometrial scratching should not be offered in daily practice until results from large and well-designed RCTs and an individual patient data analysis become available.[14]

Conclusions

Endometrial scratching is a simple procedure which can be performed on an outpatient basis without causing too much discomfort or pain. While its routine use universally prior to embryo transfer has not yielded good results in most modern trials, the select population with previous IVF/ET failure, thin endometrium and recurrent implantation failure form the best candidates to whom this may be offered.

Although the treatment of patients with a history of RIF can be discouraging, techniques and methodologies striving to optimise IVF success in these patients continue to evolve. As our understanding of the complex embryo-decidual crosstalk, immunological mechanisms behind implantation and the role of cytokines and interleukins gets elucidated, we may have better targeted therapies to improve IVF success rates beyond the traditional barrier of 30-50%.

Until then, along with select adjuvants which help in increasing endometrial thickness and suppressing embryo/blastocyst rejection, judicious use of ES in the preceding cycle may help to improve success rates in patients with clinically proven previous and recurrent implantation failure. Owing to heterogeneity and various biases in most trials, a definitive conclusion may be difficult to reach regarding the utility of this simple procedure and it is the duty of the fertility clinician to counsel the couple appropriately so that they may make an informed choice regarding their treatment.

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