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Controversies in Infertility

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President's Address

Dr. Rishma Dhillon Pai
President FOGSI

Dear Colleagues,

Couples seeking help for subfertility has increased in the recent years due to delayed child bearing age and availability of Assisted reproductive techniques (ART). The field of ART is making rapid strides by improving techniques of gamete handling, laboratory cultures, cryopreservation and embryo transfer. Yet the pregnancy rates per started cycle of stimulation remain extremely low.

We are revisiting issues of optimal ovarian stimulation, sperm function tests, hysteroscopic optimization of the uterine cavity in this issue. Whether improved gamete handling in the laboratory with improved embryology techniques including the Time lapse microscope has lead to an increase in pregnancy rates needs to be assessed objectively.

I congratulate Dr Mala Arora, Dr Monika Gupta and her team of contributors for bringing forth this issue of ICOG campus newsletter on Infertility.

Chairperson's Address

Dr. Mala Arora
Chairperson ICOG
chairpersonicog@gmail.com

The advent of contraception in the 1960 s gave women the choice of delaying fertility. The birth of Louise Brown in 1980 opened up vista's for assisted reproductive techniques (ART). The turn of the century is now witnessing an increasing need/ demand for ART which is now a sub-speciality of Ob/Gyn.

This issue highlights many controversies in the field of ART like the best method of tubal testing, and the need for hysteroscopy in the infertile woman. Polycystic Ovarian disease has increased in epidemic proportions and contributes a major chunk to the infertile pool. Guidelines for its prevention, diagnosis and management are included. Cryopreservation by rapid vitrification has allowed us to freeze all embryos and replace them in subsequent cycles, thereby eliminating the dreaded complication of ovarian hyper-stimulation syndrome and improving overall pregnancy rates.

Pre implantation genetic diagnosis (PGD) of known genetic diseases is now possible even to the level of single gene deletion by the advent of complete genomic hybridization (CGH) microarray, single nucleotide polymorphism (SNP) and next generation sequencing (NGS). However, the need for pre implantation genetic screening (PGS) of all embryos to improve implantation rates and prevent pregnancy loss is still controversial. Improved laboratory culture techniques including time lapse have helped us to achieve a 50% blastocyst rate. Biopsy of trophectoderm cells is least traumatic to the embryo as it does not disturb the inner cell mass. PGS may now be indicated in women with recurrent implantation failure (RIF) recurrent pregnancy loss (RPL) abnormal gamete morphology and advanced maternal age. However the issue of mosaicism and its auto correction at the embryo level is still a limiting factor for PGS.

FIGO has developed a fertility tool box with seven tools i.e.

Tool 1 – Why should we care about infertility

Tool 2 -Overcome personal barriers

Tool 3 – Overcome Societal Barriers

Tool 4 – Diagnose Infertility

Tool 5– Treat Infertility

Tool 6 – Refer / Resolve Infertility

Tool 7 – Prevent infertility

This is to guide patients, health care providers and policy makers to make the right choices. It can be accessed free of cost at the website **www.fertilitytool.com**

Secretary's Message

Dr. S. Shantha Kumari
Secretary ICOG

Dear All !

"Men and women of full age, without any limitation due to race, nationality or religion, have the right to marry and to found a family"

-The UN Declaration of Human Rights, Article 16.1.

The 20th century has witnessed several major advances in reproductive medicine. In Vitro fertilisation (IVF) has become a routine and widely accepted treatment for infertility. However, IVF is only one of many procedures in the increasingly complex and sophisticated assisted reproduction.

With the newer advancements in infertility arena, many contentious and controversial issues have arisen like oocyte donation, cryopreservation of gametes and embryos and concept of 'designer babies' which need to be addressed . National guidelines and regulations need to be formulated and in place to prevent misuse of the infertility treatments and at the same time benefiting and not denying the necessary treatment protocols for the couple desirous of bearing children.

I congratulate Dr Mala Arora and Dr Monika Gupta for doing a fantastic job of bringing out this issue on controversies in infertility management which deals with intricate topics right from evaluation to much advanced inclusions in this field.

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From the Editor's Pen

EDITOR-IN-CHIEF

Dr. Monika Gupta

MD, DNB, FICOG, MAMS

Associate Professor, Obgyn

VMMC & Safdarjung Hospital, New Delhi

FOGSI-Kamini Rao Yuva Orator Awardee 2016

ICOG Travel Fellowship (ART) Awardee 2016

Joint Secty, AOGD 2015-16

drmonikagupta@hotmail.com

09312796171

www.icogonline.org

Greetings to All!

I thank once again, our FOGSI and ICOG fraternity for appreciating and giving feedbacks for previous issues of ICOG Campus. It gives us great pleasure to bring out this issue on 'Controversies in Infertility'

Although management of Infertility has become a specialized subject, all of us have to face the routine cases of Infertility in our day to day practice. A lot of advances have been occurring in the field of infertility over the last decade. There has also been a tremendous increase in the awareness amongst patients coming for infertility treatment. With the newer advancements and protocols, comes the problem of controversies due to comparisons with the hitherto existing technologies. Thus, it is absolutely necessary that all practicing gynecologists keep themselves upto date in trends and protocols related to infertility management.

Keeping this in mind, we have invited the stalwarts in this field to share their experience and knowledge to throw light on the existing myths and facts in the field of advanced infertility management. We have carefully chosen the important issues of PCOS and infertility, practicality in choosing the tubal patency test and hysteroscopy in management of infertility. We also have included the insights into recent advancements like Pre-implantation Genetic diagnosis (PGD), embryo vitrification and cryopreservation with targeted tips for our members practicing at different parts of country. There is also an appraisal on latest equipment embryoscope with a mind stimulating latest journal search and brainteasers.

I hope you will find this issue of immense benefit in your day to day practice.

I acknowledge the two guest editors for this issue, Dr. Duru Shah and Dr. Bharti Dhorepatil who are very senior and respectable figures in the field of infertility. I thank Dr. Bharti Dhorepatil to have contributed an article on PGD/PGS as well.

I wish you all a happy reading

eNEWSLETTER TEAM

Polycystic Ovarian Syndrome and Infertility

Dr. Sonia Malik

Director & HOD, Southend Fertility & IVF, Delhi & NCR.

Chairperson FOGSI Infertility Committee (2014-16)

INTRODUCTION

Polycystic ovarian syndrome (PCOS) is a common syndrome presenting in the Indian clinics but still remains a subject of controversies. It was first described by Stein and Leventhal in 1935 but its cause was not understood, and it has taken more than 80 years to understand this disorder to some extent. All controversies surrounding PCOS are not only due to its heterogeneity and complexity but also to its uncertain etiology.[1] It would be pertinent to address the controversies surrounding its etiology and diagnosis if one were to understand and solve the management controversies – specially pertaining to infertility.

DIAGNOSIS

Much after the first description of the syndrome by Stein Leventhal, the first classification was attempted by the National Institute of Child Health and Human Development in1990. This was a consensus based on the opinion of specialists without much evidence.[2] These criteria included: i) chronic anovulation with ii) clinical and/ or biochemical hyperandrogenism with exclusion of other etiologies of androgen excess and anovulatory infertility but without any reference about the polycystic ovaries found on ultrasound.[2]

However, in 2003 ESHRE/ASRM consensus established the Rotterdam criteria. Therefore, in order to diagnose PCOS two of the following three criteria need to be met: i) oligo- or chronic anovulation, ii) clinical and/ or biochemical signs of hyperandrogenism and/or iii) polycystic ovaries with the same specification as earlier regarding the exclusion of other androgen excess and anovulatory infertility etiologies[1]

While gynecologists primarily follow the 2003 Rotterdam criteria, the endocrinologists disagree to label a person as having PCOS in the absence of hyperandrogenimea. Hence, Androgen Excess Society (AES) in 2006 put forth that PCOS should be, first of all, considered a disorder of androgen excess or hyperandrogenism, a minority however considered the possibility that there may be forms of PCOS without any evidence of hyperandrogenism[3] To summarize, the 2006 AES guidelines state that in order to diagnose PCOS the following two criteria are necessary: i) hirsutism and/ or hyperandrogenemia, and ii) oligo-anovulation and/or polycystic ovaries after the exclusion of other etiologies of anovulatory infertility and androgen excess[3]

PCOS PHENOTYPES

The heterogeneity of the syndrome is the result of a mix of the three cardinal symptoms depending on the ethinicity, diet and environment (Figure 1). In addition to the four classical phenotypes[4] many more can be present (Figure 2). These are as follows:

- **Phenotype A** (i.e. classic PCOS) including polycystic ovaries, hyperandrogenism and oligo-anovulation,
- **Phenotype B** (i.e. hyperandrogenic anovulation) including hyperandrogenism with oligo-anovulation
- **Phenotype C** (i.e. ovulatory PCOS) including polycystic ovaries (e.g. without ovulatory dysfunction) and hyper-androgenism,
- **Phenotype D** (i.e. non-hyperandrogenic PCOS) including polycystic ovaries and oligoanovulation[4]

All phenotypes are found in the Indian population.

BIOCHEMICAL TESTS AND CLASSIFICATION SYSTEMS

It is however paradoxical that although the disorder is recognized as an error of insulin metabolism, none of the guidelines or texts advise testing for insulin resistance. Clinical hyperandrogenimia is an expression of insulin resistance. Not only that, the presence of hyperandrogenemia itself is debatable, but also the biochemical criteria needed to make the diagnosis. First, there is no unanimous consensus on the androgen levels in women which should be measured to diagnose PCOS[5] High serum levels of testosterone (i.e. total or free), androstenedione, and dehydroepiandrosterone (DHEA) are classically used in order to define hyperandrogenemia, yet some studies showed that decreased sex hormone-binding globulin (SHBG) levels, and increased free testosterone (i.e. not total testosterone) concentration and DHEA concentrations, are most suggestive for PCOS hyperandrogenemia.[6,7] Moreover, controversies regarding which methods should be used when assessing androgens in women still exist.[6]

There are also no clear guidelines for the cut offs of clinical hyperandrogenemia. Various classifications have been put forth to score hirsutism and acne. The popular one used is the modified Ferriman Gallaway scale and the Acne Score by the Indian Dermatologists Association[8] Nigra Albicans and areas of pigmentation indicate severe insulin resistance and maybe an onset of metabolic syndrome. Oligomenorrhea, frequently a consequence of anovulation, is defined as

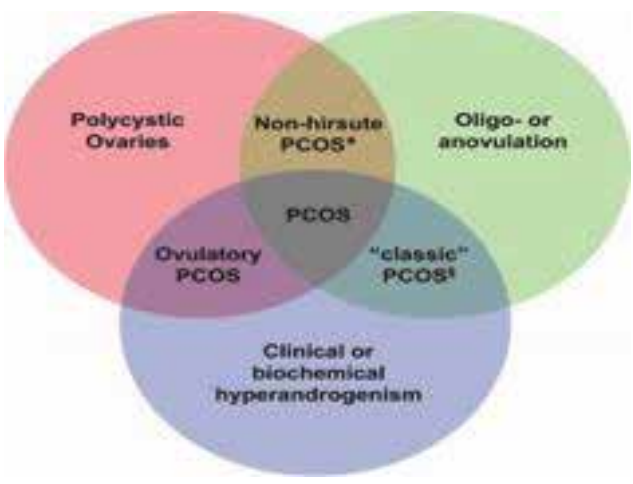

Fig. 1: Heterogenity of PCOS

Features	Phenotypes									
	A	B	C	D	E	F	G	H	I	J
Hyper Androgenemia	+	+	+	+	-	-	+	-	+	-
Hirsutism	+	+	-	-	+	+	+	+	-	-
Oligo Anovulation	+	+	+	+	+	+	–	–	–	+
Polycystic Ovaries On USG	+	–	+	–	+	–	+	+	+	+
NIH 1990 Criteria	√	√	√	√	√	√				
Rotterdam 2003 Criteria	√	√	√	√	√	√	√	√	√	√
AES 2006 Criteria	√	√	√	√	√	√	√	√	√	

Fig. 2: Characteristics of Various Phenotypes in PCOS

"menstrual cycles at more than 35 days interval".[6] We need to specify the fact that ovulatory dysfunction, an important criterion for diagnosing the PCOS, is not necessarily associated with menstrual irregularity.[9]

Polycystic ovaries are usually defined by Rotterdam criteria based on total follicle number (e.g. the presence of 12 or more follicles throughout the ovary measuring from 2 to 9 mm in diameter) or on increased ovarian volume (i.e. more than 10 cm3).[1] Until present, studies showed that more than 50% of healthy women have more than 12 follicles per ovary,[10] more so because of the availability of better ultrasound machines and the use of the transvaginal route. A recommendation therefore is to consider a polycystic ovary when the number of follicles exceeds 20 per ovary.[11]

Endocrinal derangement like high LH or an augmented FSH: LH ratio, which is the result of an aberration in insulin and leptin metabolism is no longer required to be tested in order to diagnose PCOS. However, AMH is now being considered as a diagnostic test for adolescent PCOS where TVS is not possible. AMH is also emerging as a useful marker for ovarian response and helps to prognosticate response to treatment. A high AMH may indicate hyper response but can also be an indication for resistance to stimulation.[12]

ETIOLOGY

A number of theories have been put forth over the years regarding the etiology of PCOS. A series of theories related to the origin of PCOS have been proposed in the last three to four decades, all seemingly plausible, but none qualified as the main cause of PCOS. Some of them are as follows:

1. **Intrauterine theory**: Exposure to androgens during intrauterine life or neonatal period can alter fetal ovaries[13] or can lead to congenital masculinization of hypothalamus[14] thus explaining PCOS hyperandrogenemia while reduced pancreas growth *in utero.*[15]
2. **Insulin-Like Growth Factor 1 (IGF1):** the levels of IGF1are increased in infancy after periods of protein excess, influences ovarian steroidogenesis.[16]
3. PCOS **hyperandrogenemia** is the result of increased adrenal production during puberty.[17]
4. **Enlarged ovaries** found in PCOS women may be related to excessive androgen production.[18] Studies have shown that ovarian secretion of IGFs is the possible cause of increased insulin resistance and adrenal androgen secretion.[19,20]
5. **The genetic theory:** The theory concluded that PCOS was transmitted in an X-linked dominant fashion,[21] a hypothesis inferred years ago without much evidence but which opened new vistas for studies confirming the role of heritability in PCOS. Some studies showed that up to 80.5% of women sibling of PCOS women are affected[22] attesting the genetic origin of PCOS and infirming in the same time both autosomal dominant or X-linked dominant modes of inheritance.
6. **Environment and diet:** With the understanding of long term consequences of PCOS like diabetes and metabolic syndrome, the latest theory talks of the altered gut microbiome as the cause of PCOS.[23] Disturbances in bowel bacterial flora ("Dysbiosis of Gut Microbiota") brought about by a poor diet creates an increase in gut mucosal permeability, with a resultant increase in the passage of lipopolysaccaride (LPS) from gram negative colonic bacteria into the systemic circulation. The resultant activation of the immune system interferes with insulin receptor function, driving up serum insulin levels, which in turn increases the ovarian production of androgens and interferes with normal follicle development. The Dysbiosis of Gut Microbiota (DOGMA) theory of PCOS accounts for all three components of the syndrome-anovulation/menstrual irregularity, hyper-androgenism (acne, hirsutism) and the development of multiple small ovarian cysts. However, it needs more studies to justify it as a cause.

The increasing incidence of the disorder world over including India, is due to the changing life style and diet and an ever-increasing incidence of obesity.[24]

CONTROVERSIES REGARDING TREATMENT

Since the exact cause is not known, treatment is largely need based and symptomatic hence controversial.

LIFESTYLE MODIFICATION AND BARIATRIC SURGERY

Some studies showed that lifestyle changes are primary therapy in PCOS overweight and obese women for the treatment of metabolic complications.[25] These include reducing body mass index and preventing further weight gain. The main goal is a 5-10% initial weight loss, followed by long term weight loss of 10 to 20% and achieving a waist circumference of less than 88 cm.[26] Few Indian studies also talk of the benefits of yoga for weight loss and reversal of PCOS.[27] Therefore, lifestyle changes are the most effective form of treatment for reducing weight, improving insulin sensitivity and decreasing the incidence of metabolic syndrome and type II diabetes, and indirectly improving risk factors for cardiovascular disease.[28,29] Studies also showed that weight loss has some fertility benefits.[30] Initial studies on pharmacological treatment showed good results concerning the weight loss, maintenance of weight loss and reduction of cardiovascular risks but eventually, some of these drugs were proven to increase the risk for cardiovascular events and were removed from the market.[31,32] Studies now indicate that bariatric surgery is associated with improvement or complete resolution of type II diabetes, hypertension, hyperlipidemia and obstructive sleep apnea.[34] Complete resolution of all features of PCOS, even hirsutism, hyperandrogenism, anovulation or menstrual irregularity have also been reported by some recent studies.[35,36]

PHARMACOLOGIC TREATMENT

When fertility is desired, ovulation (in up to 80% cases of obese women) needs to be restored. First line therapy for this again is weight loss.[36] Insulin sensitizing drugs, improve menstrual regularity along with reduction of body mass index and androgen levels.[37] A meta-analysis in 2009, concluded that metformin indeed leads to significant weight loss compared to placebo but when given to the patients on a diet or on a program of life changes does not contribute majorly.[38] Studies showed that metformin also effectively induces ovulation in PCOS women.[39]

When drugs need to be used, Clomiphene citrate is the first line drug used to induce ovulation. Research shows that it induces ovulation in 57% of the cases with pregnancy rates higher than 23%.[40] While clomiphene citrate and metformin are frequently given together, most studies conclude that the association is irrelevant, and the pregnancy rates after clomiphene citrate plus metformin compared to pregnancy rates after clomiphene citrate alone do not differ.[41] Metformin, an insulin sensitizing drug, is frequently added when a pregnancy is not achieved after weight loss and clomiphene.

Gonadotrophins are often used as second-line treatment, in low doses due to the increased risk of ovarian hyperstimulation syndrome.[42] Studies showed that in case of PCOS the low-dose protocols are safer, therefore the "step-up" and "step-down" protocols which use 37.5- 75IU/day are to be taken into consideration if the patient is unresponsive to the first-line treatment. The step-down protocol is the protocol of choice for mono-follicular development.[43,44]

Ovulation induction in PCOS can be tricky. On one hand, if the threshold is not reached, there may be anovulation and if it is overstepped, it may result in ovarian hyperstimulation. Hence, careful titration and monitoring is required. Another problem maybe a premature rise of LH due to an excessive rise in estradiol levels. In order to effectively counter these problems, the concept of an OHSS Free Clinic has emerged. This entails the use of antagonist protocol and a GnRh agonist trigger, freezing the embryos formed, and a deferred embryo transfer.[45]

As regards anovulation associated with PCOS, when there is failure of first-line treatment and second-line gonadotrophin therapy is considered too risky, in vitro

fertilization (IVF) is to be taken into consideration.[46]

Other indicated patients for IVF are the patients of PCOS who have associated pathologies such as tubal damage, endometriosis or male infertility.[47]

SURGICAL OPTIONS

Another second-line therapy is laparoscopic ovarian drilling (LOD). It is recommended in cases resistant to clomiphene citrate lean PCOS, high LH, recurrent miscarriage in PCOS.[47] LOD may have similar or better pregnancy rates (60%) in comparison to gonadotrophin therapy when properly performed in properly selected cases.[48] While four monopolar or laser punctures have been shown to be effective, majority uses between four and ten punctures and this might lead to premature ovarian failure.[47,49] Neither gonadotrophin therapy nor LOD are risk free: while the first one needs a close monitoring and associates the risk of ovarian hyperstimulation syndrome, LOD associates intraoperative and postoperative risks, especially in overweight women.[47]

NEWER DRUGS

A new drug is inositol, which although were discovered in 1850's, it found its use in the treatment of PCOS as late as 1993. Their efficacy in ovarian stimulation and their beneficial effect on insulin sensitivity has been clearly showed in women suffering from PCOS both obese and normal weighted along with their capacity of lowering androgen levels.[50] Studies showed that inositol also improves the metabolic profile by lowering total cholesterol level, triglyceride level, glucose and insulin levels.[50]

TREATMENT OF HIRSUTISM

Hirsutism and acne, are the most disturbing features in the life of women with PCOS and deserve attention. Besides the local treatment and mechanical ways for hair removal, in case of mild hyperandrogenic symptoms usually the oral contraceptive pill is effective. Both severe hirsutism and acne respond to antiandrogens either androgen receptor blockers or 5-alpha-reductase inhibitors (finasteride) often incorporated with oral contraceptives. These could be used as monotherapy or dual therapy along with oral contraceptive pill.[51]

In some cases, adding metformin to monotherapy or to dual therapy has benefic effects on hyperandrogenisc symptoms due to its effectiveness on lowering testosterone levels and increasing SHGB levels.[51]

CONCLUSIONS

The heterogeneity and complexity this syndrome is has led to controversies in diagnosis etiology and treatment especially with regards to infertility issues. Every PCOS guideline is a consensus rather than a nondisputable fact. There is a persistent need for more and greater studies researching new ideas, new genes, and new therapies.

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CORRIGENDUM FOR JULY 2017 ISSUE

The first author's name for the article "Newer Drugs in Medical Management of Uterine Myomas" was wrongly printed as 'Dr Taruna Bansal' instead of 'Dr Taruna Sharma

Tubal Patency Tests: Which One to Do?

Dr. Divya Pandey
Assistant Professor,
VMMC & Safdarjung Hospital, New Delhi

Dr. Monika Gupta
Associate professor,
VMMC & Safdarjung Hospital, New Delhi

INTRODUCTION

Any optimal screening infertility investigation protocol should be designed taking its diagnostic accuracy, cost-effectiveness, reliability and minimal invasive nature into consideration. The currently practiced screening tests of tubal patency evaluation during an initial infertility work up, which are regarded as accurate are not free from disadvantages.

The purpose of this article is to thoroughly review associated risks, potential advantages and finally the weighted efficacy of the two most widely performed tests of tubal patency: Laparoscopy with chromopertubation and Hysterosalpingography(HSG).

HYSTEROSALPINGOGRAPHY

HSG is the radiological evaluation of uterus and tubes in addition to assessment and detection of congenital abnormalities, leiomyoma, synechiae, polyps, tubal occlusion, Salpingitis Isthmic Nodosa(SIN), hydrosalpinx and peritubal adhesions (Fig 1).[1] Performing the procedure between day 6 to day 11 helps to ensure the absence of pregnancy and facilitates uterine cavity visualization to the maximum in presence of thin proliferative phase endometrium. Radiation exposure of patients during HSG using standard techniques is considered to be within margin of safety.

Post HSG procedure conception rates according to Cochrane meta-analysis varied from 24-38% (with oil contrast) to 17-23% (after water soluble contrast) in comparison to 8-21% without HSG procedure.[2]

Stumpf et al had reviewed five major risk factors for development of a post-HSG infection[3]

- History of infertility
- Previous Pelvic Inflammatory Diseases
- previous pelvic surgery for an infection
- adnexal mass
- adnexal tenderness at the time of procedure.

Patients with three or more risk factors have 40 times more risk of developing post-HSG infection. In these patients, investigators concluded that HSG should be avoided and laparoscopy should be considered. Besides these patient, where peritoneal or endometrial factor needs to be ruled out, laparoscopy along with same-sitting hysteroscopy will be the preferred option.

ACOG guidelines recommend empiric treatment in patients with a history of previous pelvic infection or if hydrosalpinx is noted, Doxycycline 100 mg twice daily for 5 days is commonly prescribed antibiotic.

LAPAROSCOPY WITH CHROMOPERTUBATION

This investigation is widely accepted as the ''gold standard'' method for tubal patency evaluation. Its advantages include simultaneous evaluation of the abdominal cavity and other pelvic structures for an enhanced diagnostic evaluation of other infertility etiologies. The procedure also allows for therapeutic excision of endometriotic lesions and restoration of abnormal pelvic findings. However, it incurs operative risks, costs, and a period of postoperative recovery.

A prospective, 12-month, Netherlands nationwide study was designed to discern the rate and characteristics of surgical complications in gynecologic laparoscopy. A total of 25,764 laparoscopic surgeries were performed in 72 Dutch hospitals with a reported complication rate of about 5.7 per every 1,000 laparoscopies.[4] The most common of these observed complications were hemorrhage from epigastric vessels and intestinal injury. Intuitively, the diagnostic procedures yielded less frequent complications (2.7/1,000) than the more involved operative laparoscopies (17.9/1,000). Similarly, a retrospective review of worldwide gynecologic laparoscopic studies performed in more than 1.5 million women revealed an overall procedure-related complication rate of 0.2%–10.3%; of which 20%–25% were unrecognized at the time of surgery.[5]

Cardiac abnormalities, most commonly arrhythmias, were reported in 27% of all laparoscopies. Brachial plexus injury was reported to occur in 0.16% of cases due to improper patient positioning. The overall hospital readmission rate was 0.5% and conversion to laparotomy 2.1%.[5] When there are no significant operative findings, laparoscopy may lead to an unnecessary delay in initiation of fertility therapy.

Historically, laparoscopy may have been more readily performed as a first-line screening evaluation for subfertility. As an invasive and expensive procedure, however,

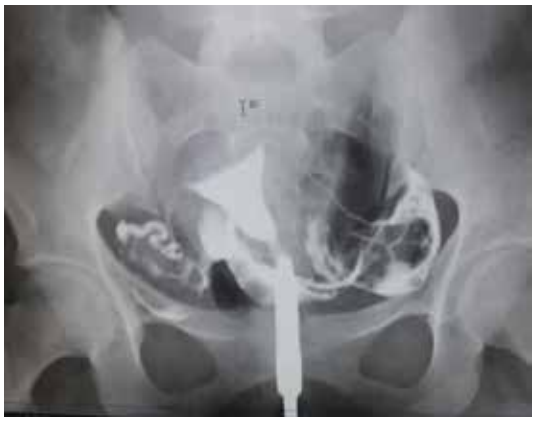

Fig. 1: Hysterosalpingogram showing normal uterrine cavity and clearly outlining both tubes with free spill on right side and loculated spill on left side

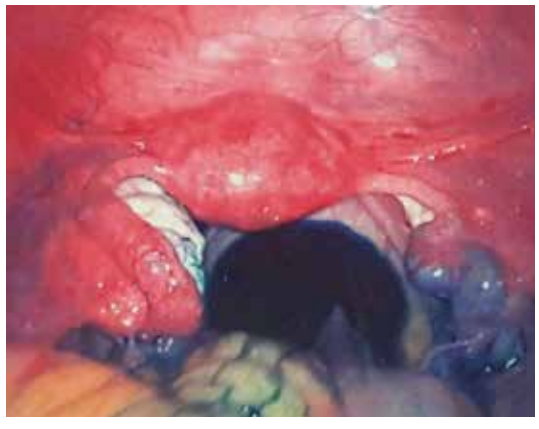

Fig. 2: Laparoscopic picture after chromopertubation showing bilateral free spill of dye

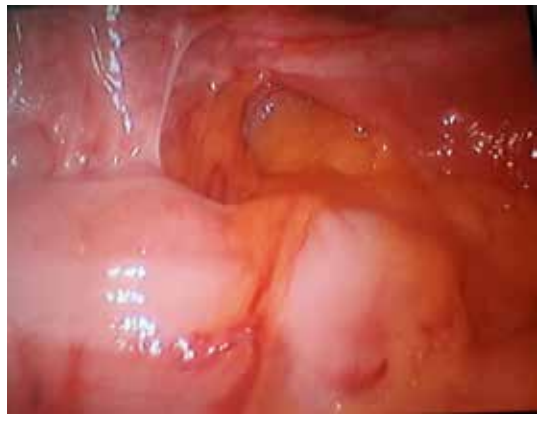

Fig. 3: Laparoscopic picture showing periuterine adhesions

it is not an ideal first-line, screening test for subfertility when suitable alternative office procedures are available (Table 1).

HYSTEROSALPINGO-CONTRAST-SONOGRAPHY

Another office procedure of tubal patency, Hysterosalpingo-Contrast-Sonography (HyCoSy) is based on the fact that normal fallopian tube is a poor sonic reflector, devoid of defined interfaces that produce clear organ outlines. HyCoSy is used to assess tubal patency by visual intra-tubal flow of echogenic contrast using B-mode (real time) ultrasound. When performed subsequent to Saline Infusion Sonography (SIS), HyCoSy can help in comprehensive evaluation of adnexal architecture, uterine cavity and myometrial assessment and tubal patency. Thus, like HSG, it is a relatively quick and non-invasive procedure especially amendable to the outpatient setting. Researchers have been concluded that HyCoSy procedure is comparable to traditional HSG for tubal evaluation and suggested that it can be used as time-efficient, simple to perform, well-tolerated, screening tool in the initial infertility evaluation.[6]

Table 1: Comparison of HSG, HyCoSy and Laparoscopy with Chromopertubation

Tubal Patency tests	Advantages	Disadvantages
Hysterosalpingography (HSG)	• Visualization of entire length of fallopian tube • Ability to diagnose tubal pathologies like SIN, Hydrosalpinx etc. • Therapeutic lavage with documented improvement in pregnancy rate.	• Radiation exposure • Contrast reaction • Trained staff and appropriate equipment and facilities • Visualization of pelvic adhesions and ovaries not possible
Hysterosalpingo-Contrast-Sonography (HyCoSy)	• Visualization of ovaries, uterus and fallopian tubes in same sitting	• Trained staff and appropriate equipment and facilities • Therapeutic lavage or improved pregnancy rate not proven.
Laparoscopic Chromoper-tubation	• Visualization of pelvic pathology(adhesions and endometriosis) • Possible concomitant therapeutic surgical correction or removal of pelvic pathology	• Invasive procedure with increased morbidity and mortality. • Risk of general anesthesia • Longer post-procedure pain and recovery • Higher medical costs

Adopted from Saunders et al. Tubal patency assessment. Fertil Steril 2011

Table 2: NICE,ESHRE and ASRM recommendations on tubal factor infertility.

NICE	ESHRE	ASRM
• Women who are not known to have comorbidities(such a PID,previous ectopic pregnancy or endometriosis) should be offered HSG to screen for tubal occlusion because this is a reliable test for ruling out tubal occlusion and it is less invasive and makes more efficient use of resources than laparoscopy. • Where appropriate expertise is available, screening for tubal occlusion using HyCoSy should be considered because it is an effective alternative to HSG for women who are not known to have comorbidities. • Women who are thought to have comorbidities should be offered laparoscopy and dye so that tubal and other pelvic pathology can be assessed at the same time.	• Women thought to have comorbidities should be offered laparoscopy so that any tubal and other pelvic pathology can be investigated and treated at the same time.	• The methods for evaluating tubal patency are complementary and not mutually exclusive. • Accurate diagnosis and effective treatment of tubal obstruction often requires more than one techniques

WHAT DOES THE EVIDENCE SAY?

The recommendations for tubal patency evaluation given by NICE, ESHRE and ASRM are elaborated in table 2.[7,8,9]

CONCLUSION

The most advantageous screening infertility protocol should necessitate methods that are diagnostically accurate, timely, cost-effective, reliable, and minimally invasive. Laparoscopy may disclose a definitive diagnosis and offer a treatment option in the same sitting in cases where clinical history, laboratory or other office procedures suggest tubal pathology. But it is definitely not the ideal first line screening test of tubal patency in all cases. The methods of tubal patency are complementary and not mutually exclusive. To reach an accurate diagnosis and thus effective management, more than one technique might be necessary.

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Hysteroscopy in Infertility: Myths and Facts

Dr. Fessy Louis T.
Assistant Professor,
AMRITA Institute of Medical Sciences
AMRITA Fertility Centre, Kochi, Kerala

Dr. Parvathy T
MCh Resident
Dept. Reproductive Medicine and Surgery
AMRITA Institue of Medical Sciences, Kochi, Kerala

INTRODUCTION

Hysteroscopy, the word derives from Greek words '*Skopeo' – to view* and '*Hystera' – Uterus*. It is the process of viewing and operating in the endometrial cavity from a transcervical approach. It allows for the diagnosis and treatment of various uterine conditions. Some of which, could lead to fertility problems.

A hysteroscope is a fiberoptic telescope (Figure 1). Some hysteroscopes are "rigid" and straight while others are semi-flexible. The hysteroscope contains several channels, all with a specific purpose. In addition to the "optic" channel that allows the doctor to see inside the uterus, one channel carries a fiber optic light in order to see inside the ordinarily dark uterus. One channel allows the introduction of fluid or gas to hold open the uterine walls and another channel is to allow the fluid back out again. Some hysteroscopes have an additional "operative" channel that allows the doctor to introduce instruments to do various tasks inside the uterus.

One of the basic steps of an infertility workup is to evaluate the shape and regularity of the uterine cavity. Acquired uterine lesions, such as uterine fibroids, endometrial polyps, intrauterine adhesions, or all of these, may cause infertility by interfering with proper embryo implantation and growth. Congenital uterine malformations are also thought to play a role in delaying natural conception.

Hysteroscopy has been proved to be the definite method for evaluation of the uterine cavity and diagnosis of associated abnormalities (Figures 2-4). Several studies have demonstrated that once the uterine cavity has to be investigated as part of the infertility workup, hysteroscopy is much more accurate than other diagnostic methods, mainly HSG.[1]

Abnormalities in the uterine cavity, such as endometrial polyps, submucous fibroids, uterine septum, and intrauterine adhesions, may disrupt the process of implantation of a fertilized egg into the inner layer of the cavity of the uterus. In subfertile women with a uterine cavity abnormality, removal of these abnormalities using hysteroscopy may be recommended to help increase the odds of pregnancy.

Some studies advocated and recommended the use of office hysteroscopy as a routine procedure in the infertility work-up.[2-5] It has become easy to perform in an outpatient setting without anesthesia. Moreover, it offers direct visualization and enables clinicians to diagnose and treat intrauterine pathology during the same session.

UTERINE SEPTUM (SEPTATE UTERUS)

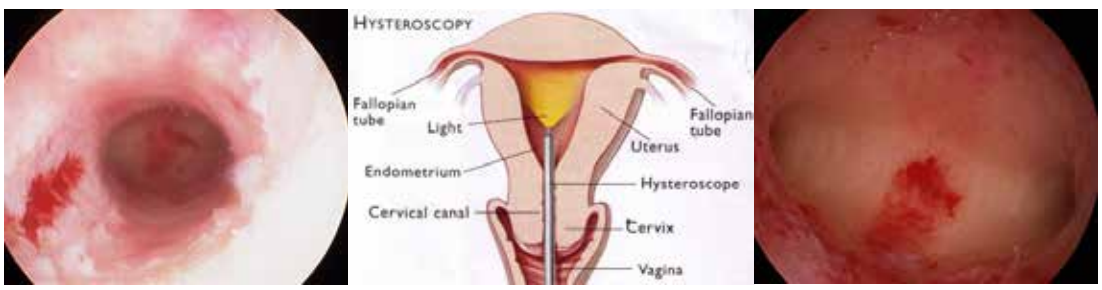
Uterine septum is not only associated with infertility but also is associated with increased rates of pregnancy loss as high as 90%.[6] The American Fertility Association (AFA), now known as the American Society of Reproductive Medicine (ASRM), explained these septum related pregnancy wastages is by structural alterations in the endometrium of the septum (Figures 5-7), which affects implantation.[7]

Mollo et al studied 2 groups with unexplained fertility, a group of women with septate uteri who underwent hysteroscopic metroplasty and a control group without septate uteri.[8]

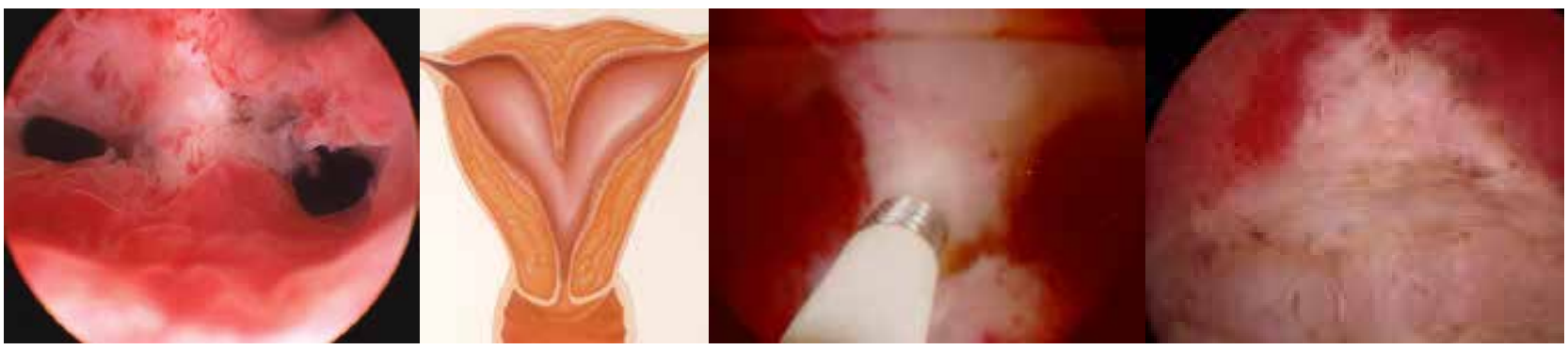
The 2 groups were similar in terms of age, duration of infertility and body mass index (BMI). The pregnancy rate and live birth rate were significantly higher in the hysteroscopic metroplasty group compared with the control group (38.6% vs 20.4%; p =0.016 and 34.1% vs 18.9%; p <0.05, respectively).

An older prospective study identified a reduction in pregnancy wastage from 87.5

Fig. 1: Hysteroscope



Figs. 2, 3, 4: Hysteroscopic view of cervical canal and Uterine cavity



Figs. 5, 6, 7: Hysteroscopic view of uterine septum and septal resection

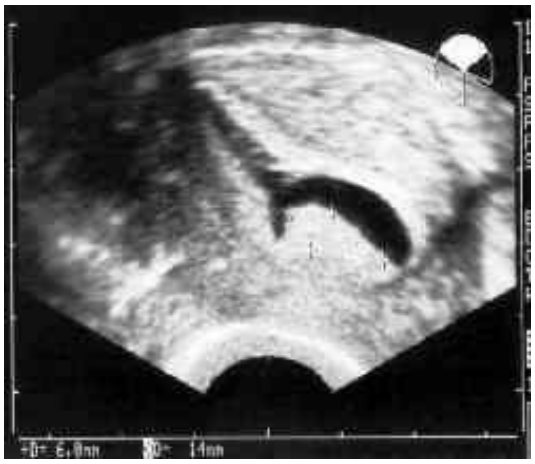


Fig. 8: Sonosalpingogram showing endometrial polyp

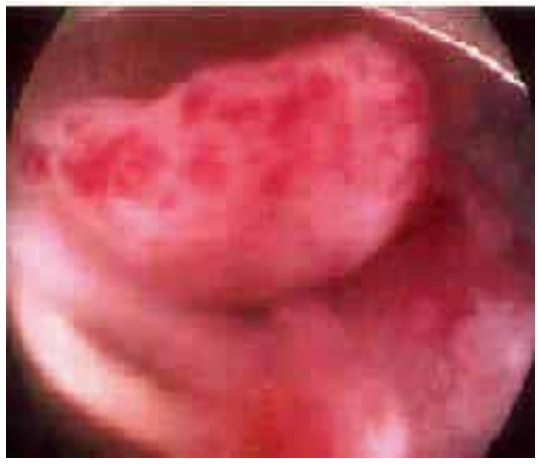


Fig. 9: Hysteroscopic view of same polyp

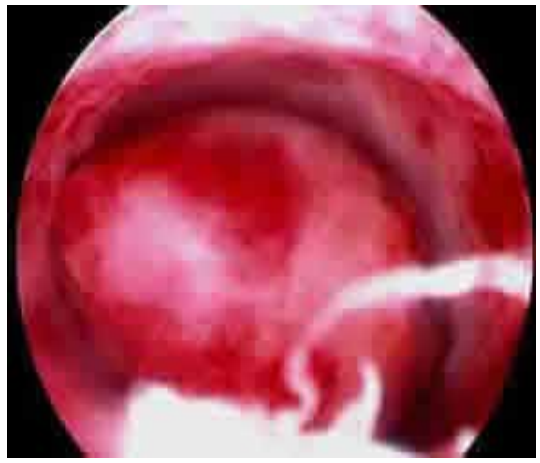


Fig. 10: A large uterine polyp

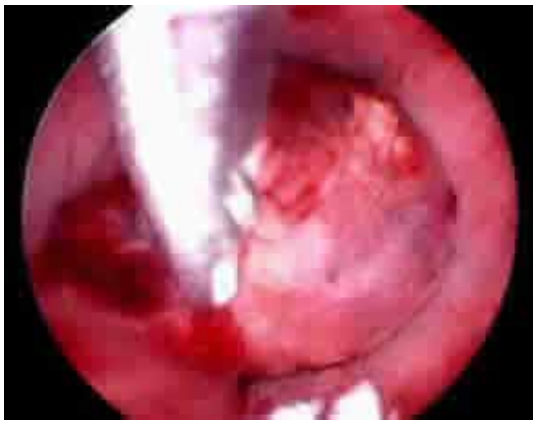


Fig. 11: Hysteroscopic resection of same polyp

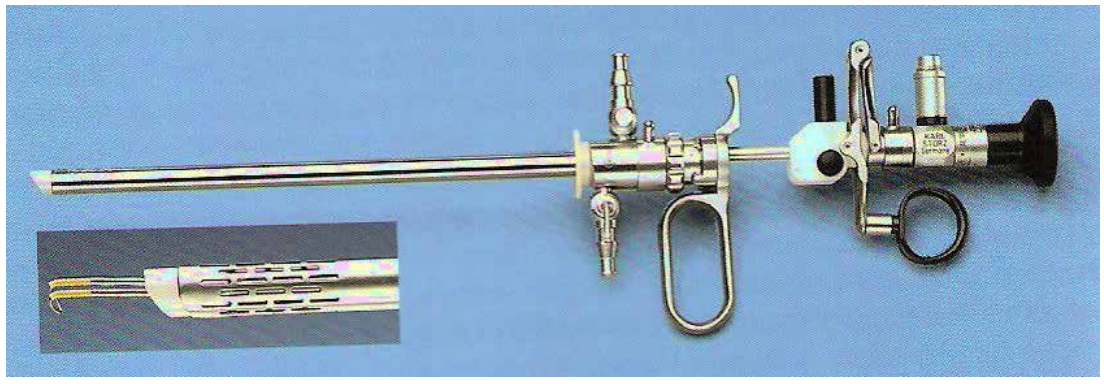
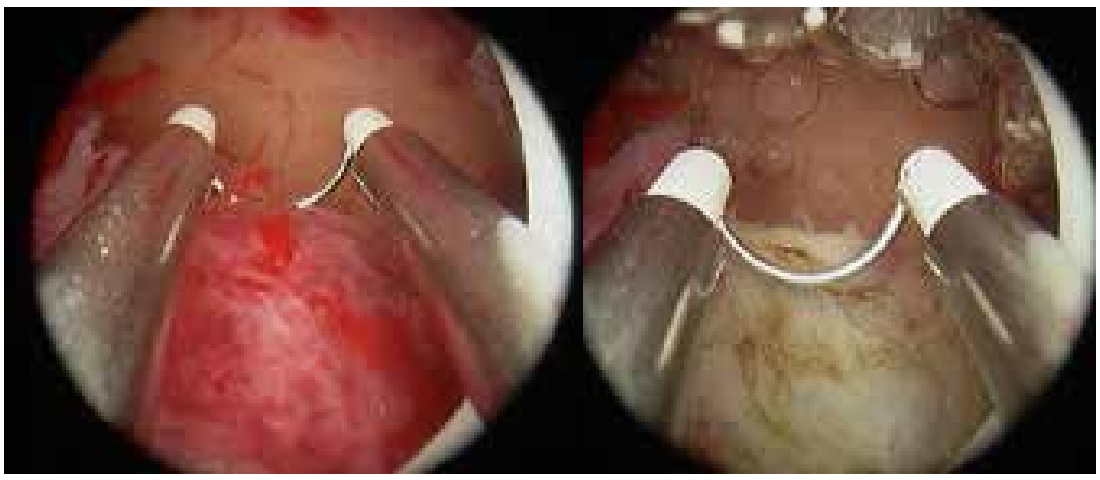


Fig. 12: Resectoscope



Figs. 13, 14: Myoma Resection using Resectoscope

to 44.4% and recommended hysteroscopic metroplasty as the treatment of choice in patients experiencing recurrent abortions.[9] Although, hysteroscopic metroplasty appears to improve fertility, the role of surgical correction in patients with primary infertility remains under debate.

Looking further at septum length, Shokeir et al studied women with septum length of ≥2.5 cm and compared them with women with a septum length of <2.5 cm.[10] All of the 42 women

(47.7%) who achieved pregnancy were age <40 years with <3 years of infertility; 8% of these pregnancies were spontaneous. The pregnancy rate was 66.7% in those with a septum length of ≥2.5 cm and 42.8% in those with a septum length of <2.5 cm The overall live birth rate was 40.1%.

Grimbizis et al reviewed 6 studies published before 2001 that reported a live birth rate of 6.1% in women with intact septums compared with 82% in those women who underwent hysteroscopic metroplasty.[11] Nouri et al performed a more recent literature search that revealed live birth rates ranging from 26% to 73%, with a cumulative rate of 45% after hysteroscopic metroplasty.[12] Both of these reviews evaluated studies in women with a septate uterus in both unexplained primary infertility and recurrent abortions.

POLYPS

These are uterine growths a few millimeters to centimeters in size. Polyps arise from the uterine lining (endometrium). A polyp may be attached to the uterine wall directly (Figures 8,9) or by a thin "stalk". Patients often have no symptoms from polyps but will occasionally notice irregular vaginal bleeding. This bleeding may occur in between periods or cause the period to be longer in duration or heavier than normal.

Polyps are also associated with an increased risk for miscarriage. Large polyps, which occupy the majority of the uterine cavity, are also probably responsible for infertility (Figure 10). Small polyps can be most easily vaporized in place. Polyps which are attached by a stalk can sometimes be removed by cutting through the stalk and removing the entire polyp through the cervix. Larger polyps may have to be removed by shaving small strips one at a time until the polyp is completely gone (Figure 11).[13]

Uterine polyps can cause infertility by many mechanisms which include irregular endometrial bleeding, inflammatory endometrial response, obstructive inhibition of sperm transport, physical obstruction of exposure of the embryo to the endometrium, interference with normal patterns of endocrine function, and inhibition of sperm binding to the zona pellucida.

The hysteroscopic removal of endometrial polyps suspected on ultrasound in women prior to IUI might increase the clinical pregnancy rate as per the Cochrane Database Syst Rev. 2013.[14]

FIBROIDS

These benign tumors arise from the muscle layers of the uterus. Often they will stay in the muscle layer but on occasion, fibroids can grow into the uterine cavity. Like polyps, fibroids can cause bleeding, infertility, and miscarriage.[15]

Hysteroscopic removal of submucous fibroids (Figures 12-14) improves the chance of

clinical pregnancy in women with otherwise unexplained subfertility as per the Cochrane Database Syst Rev. 2015. Intramural fibroids also negatively affect fertility, especially fibroids larger than 4cm, even without cavity distortion. Fibroids impair fertility by many mechanisms involving alteration of local anatomical location, inducing functional changes of the myometrium and endometrium, and finally endocrine and paracrine molecular mechanisms which could alone or in combination cause reduced reproductive potential, impaired gamete transport, diminished implantation, and creation of a hostile environment.[16]

Hysteroscopic excision of submucosal myomas seems to restore fertility with pregnancy rates after surgery similar to normal controls. Even open excision of intramural myomas seems to be associated with higher pregnancy rates when compared to non-operated controls, although evidence is still not sufficient. The results of endoscopic and open myomectomy are similar; thus, endoscopic treatment is the recommended approach due to its advantages in patient's post-operative course.

Casini et al analyzed whether the removal of fibroids before conception improves pregnancy rates and outcomes compared with no surgery.[17] In that study 92 patients underwent myomectomy, via either hysteroscopy or laparotomy, and 89 patients did not undergo surgery. All patients were followed-up for 12 months to determine the rate of clinical pregnancy. Higher pregnancy rates were observed in the patients who underwent myomectomy with submucous fibroids (43.35% vs 27.2% in the non-surgical group) or submucous and intramural fibroids (36.4% vs 15% in the non-surgical group) ($p < 0.05$). There was no statistically significant increase in pregnancy rate in the patients with only intramural or intramural and subserosal fibroids ($p > 0.05$).

Pritts et al in a meta-analysis of 23 studies evaluating women with fibroids and infertility found that large difference between infertile women with submucous fibroids and those without submucous fibroids as regard pregnancy rate, implantation, and ongoing pregnancy/live birth rates, as well as the spontaneous abortion rate.[18] They also found that women who underwent a hysteroscopic myomectomy had greater clinical pregnancy rate compared with those with fibroids left in situ.

Cochrane database found that in a subset of women with a submucous fibroid (n=94), there was a statistically insignificant increased odds of clinical pregnancy (odds ratio, 2.4; 95% confidence interval, 0.97-6.2; p =0.06). Shokeir et al found similar results in their randomized controlled study.[19]

ASHERMAN'S SYNDROME AND ART

Intrauterine adhesions are not life threatening, and may be asymptomatic in many patients. The main symptoms of Asherman's syndrome include pain, infertility, and abnormal menstrual patterns especially amenorrhea and scanty menstruation.[20] Hysteroscopy has been the method of choice in the investigation and treatment of the condition. Management of moderate to severe disease may be a challenge, and repeated surgery may be necessary in some cases and may not always produce the desired outcome.[21]

A prospective study evaluated 24 women with infertility (12 of whom had previously delivered) and 12 women with a history of recurrent abortions. Of these 24 women, 48% conceived after hysteroscopic adhesiolysis.[22] Among the 12 women with recurrent abortions, pregnancy wastage was reduced from 86.5 to 42.8% post-operatively.

A more recent study enrolled 357 patients with mild, moderate, and severe Asherman's syndrome who underwent hysteroscopic adhesiolysis between January 2012 and December 2015.[23] They found that the reproductive outcomes of 332 women (93%) were followed for an average duration of 27±9 months, and the overall conception rate after hysteroscopic adhesiolysis was 48.2%, which decreased with increased intrauterine adhesions (IUA) severity (mild, 60.7%; moderate, 53.4%; severe, 25%). The mean time to conception following hysteroscopic adhesiolysis was 9.7±3.7 months. The miscarriage rate was 9.4%, and the live birth rate was no lower than 85.6%. Eleven patients (7.9%) had postpartum hemorrhage, including 6 (4.3%) due to adherent placenta and 3 (2.1%) due to placenta accreta.

HYSTEROSCOPY IN IN-VITRO FERTILIZATION

Before starting the treatment, a baseline TVS (Trans Vaginal Scan) is a must. And in suspected cases only hysteroscopy is advised. In failed IVF, the question arises whether to go for hysteroscopy before repeat IVF. The recommendation is to go for hysteroscopy if there is no clinical pregnancy even with good quality embryos.[24]

HYSTEREOSCOPY AFTER REPEATED IVF FAILURES

Hysteroscopy increases pregnancy rates even in the absence of intrauterine pathology in women with recurrent IVF failure. This could be explained by the cervical dilatation and/or direct hysteroscopic visualisation of the uterine cavity or alternatively by an immunological mechanism triggered by the hysteroscopic manipulation or by the effect of the distension medium on the endometrium.[25,26]

COMPLICATIONS

The commonest complications are perforation, bleeding and fluid overload. Most of the studies quote these complications, when done in proper settings, as less than 1%.[25] The complications depend not only on the inexperience of the surgeon, lack of proper instrumentation, but also from the complexity of the procedure and deviation from the standard techniques.

CONCLUSION

Hysteroscopy when done by proper technique and incorporating latest technologies, is a simple, valuable and precise, diagnostic as well as therapeutic tool in the hands of Gynecologists in treating Infertility.

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Live Work stations	All faculty & delegates

1. Oxygen therapy, Antishock garment, Fluid Resuscitation & vascular access, CPR in pregnant patient, CPAP & mechanical ventilation, ABG interpretation
2. Antishock garment to stabilize blood pressure
3. Fluid resuscitation & Vascular Access
4. CPR in pregnant patient
5. CPAP & Mechanical ventilation
6. ABG Interpretation"

Coup d'oeil into the Embryo - PGD / PGS Controversies

Dr. Bharati Dhorepatil
Founder Director
Dip. Endoscopy (Germany)
Ssmile IVF Center, Shree Hospital, Pune

Dr. Parul Sharma-Saoji
Post Graduate Fellow
Reproductive Medicine
Ssmile IVF Center, Shree Hospital, Pune

INTRODUCTION

Preimplantation genetic diagnosis was first described in 1990.[1] It is the testing of pre-implantation stage embryos or oocytes for genetic defects and was formulated for those whose potential offspring are at risk of severe Mendelian disorders, structural chromosome abnormalities or mitochondrial disorders. It was evolved to offer diagnosis of genetic disorders preconceptionally.[2] Prenatally such disorders can be diagnosed at amniocentesis or chorionic villus sampling. Pre-implantation embryo diagnosis requires in vitro fertilization, embryo biopsy and either using fluorescent in situ hybridization or polymerase chain reaction at the single cell level.[3] Genetic tests are done on the third day i.e. 8 cell stage after in vitro fertilization (IVF), & those without specific genetic traits are transferred a day or two later.

Genetic testing offered to a couple with an inherited genetic disorder or carriers of a structural chromosomal abnormality is termed Preimplantation Genetic Diagnostics or PGD. Genetic testing offered to infertile couples with increased risk of generating embryos with de novo chromosome abnormalities, and coined as preimplantation genetic screening, or PGS.[4]

RECOMMENDATIONS OF PGS/PGD-EMBRYO SELECTION

1. Women over the age of 39 years and those who, regardless of age have significant diminished ovarian reserve or, are running out of eggs and time, and need to "make hay while the sun shines"
2. Unexplained IVF failure / Recurrent Implantation failure
3. Certain cases of recurrent pregnancy loss (RPL).
4. Women who have alloimmune implantation dysfunction (IID) with activation of uterine natural killer cells (NKa)
5. Where karyotyping reveals one or other partner to have a balanced chromosomal translocation
6. Known or anticipated specific genetic abnormalities
7. Abnormal gamete cell morphology

CONTROVERSIES OF ANTENATAL TESTING

- Considering the fact that during the biopsy embryos could be traumatized especially the day 3 embryos [5]
- Being comparatively a new technique and technology, there is a "learning curve" in mastering the technique. The result variability exists and there are large differences- inter-center and even intra-technicians [6]
- An embryo is a mosaic if there are 2 (or more) different chromosomal patterns in the cells of that embryo. Being a single cell analysis tool mosaic embryo with normal or abnormal cells may be misdiagnosed, limiting its results.
- As of now there are no associated fetal malformations but the possibility cannot be negated later in life. Possible procedural risks can be there. Time-lapse imaging comparing the development of mouse embryos with and without blastomeres demonstrated key differences in the speed of growth, frequency of contraction and expansion, diameter of contraction and expansion, and hatching of the blastocyst from the zona pellucida. Mouse embryos that underwent blastomere biopsy had a slower growth pattern. Hatching occurred at the site where the blastomere was removed and did result in a hernia-like appearance.[7] Also, expansion and contraction occurred more frequently in the smaller embryos. It is not known if similar events occur in human embryos and if so, what effects it will have on the developing embryo and offspring.
- Another controversy is whether to consider treatable diseases for testing?
- Many of adult onset diseases are being considered in these screening. There is a controversy whether they should they be considered for screening.
- Misdiagnosis is a possibility , so confirmation with CVS/amniocentesis is recommended which in turn have their own associated risks to the mother and the fetus.
- It is likely when embyos are tested and found abnormal, other members of the family may exhibit similar genetic risks – whether to inform them or not ?
- Other dimesions for use of PGD like non medical indication for sex selection is not vaildated.

CONTROVERSIES SPECIFIC TO PREIMPLANTATION GENETIC TESTING

PGD has been used not only to diagnose genetic disorders but also to select for certain other characteristics, and this use of the technique is much more controversial.

- One of the unfortunate fact of current scenario is that embryo selection has by far become a beauty contest where people want optimisation of characteristics which they consider best for their progeny, which is best suited to survive having the best possible life.
- PGD/PGS are definitely for the individuals carrying risk of transmitted genetic disease rather than post gravid antenatal screening techniques and the need for termination of pregnancy[8]
- PGS/PGD being different from other antenatal testing techniques and applicable for IVF patients where the embryos are created in excess, the debatable question is what to do with these excess embryos?.[9,10,11]
- Ethical considerations pertaining to PGS include the moral status of the embryo, embryo freezing, embryo loss during cryopreservation, disposal or donation of unused embryos, abortion rates and parental interest and decisions. The moral status of the embryo is most important because of the legal, social, and other ethical implications involved.
- IVF in itself is expensive and PGS/PGD adds onto the cost employed in a single cycle. Also the number of embryos finally available for transfer are reduced.[12,13]
- PGS/PGD shouldn't be adopted as "a program of eugenics, to try and wipe out genetic disease", but rather to prevent disease meaning that embryos that are merely carriers of disease should be used for transfer. The conditions like Down's Syndrome or Turner's Syndrome, haemoglobin disorders like alpha and beta thallesemia impart definite morbidity to the child. However, PGD has since expanded to filter embryos with genes that merely confer risk (as

opposed to absolute causation) such as BRCA (predisposes to breast cancer) and adult onset diseases such as Huntington's disease. [14,15]

- A more controversial use of PGD is its role in producing a child with perfectly matched HLA-types to their siblings suffering from thalassemia. This 'saviour child' is conceived healthy and acts as a bone marrow donor to save the affected sibling.[16]
- PGD has also been used in non-medical sex selection – sex selection with no medical justification and solely for the purpose of 'family balancing'. [17,18,19]

CONCLUSION

While few would dispute using PGD to avoid life-threatening genetic diseases, to parents who are both carriers of genes for debilitating conditions, PGD offers a miracle by ensuring their child is unaffected. The ethical lines became blurry for some other scenarios.

Does PGD skirt the borders of eugenics? Does accepting the logic of PGD lead to a slippery slope where our moral stand posts eventually disappear. The notion of "the perfect child," which is associated with that of the designer baby, is new reproductive programming. [20,21,22] So controversies remain and whether PGD/PGS is a evasive slope or happy ending is yet to be decided with test of time .

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Fresh Embryo Transfer Vs. Freeze All - What is All the Fuss About?

Dr. Pranay Ghosh

Director, Elixir Fertility Centre
Sci-ART (UK), Diploma Reproductive Medicine (Germany)
Specialist Training in Reproductive Medicine (Singapore)
ESHRE Certified Clinical Embryologist

INTRODUCTION

Since the birth of the first baby following in vitro fertilization in 1978, there have been few albeit significant breakthroughs in the field of assisted reproduction technology.[1] The serendipitous discovery of the technique of intracytoplasmic sperm injection by Palermo et. al in 1992 was another milestone, and this technology enabled men with severe male factor infertility to have their own genetic offspring.[2] The use of cryopreservation in assisted reproduction dates back to 1938 when Luyet et al. published the first successful vitrification of sperm using a 2M sucrose solution.[3] In 1949, while trying to replicate the work of Luyet, Polge et al discovered the cryoprotective properties of glycerol and ushered in the era of slow freezing. Following the work of Fahy et. al in 1985 involving the vitrification of mouse embryos, the first birth from vitrified human cleavage-stage embryos was reported in 1998 by Mukaida et al.[4] The advent of vitrification has emerged as a game-changer in the field of fertility treatment. The cryosurvival and pregnancy rates with vitrification are far superior as compared to slow-freezing. Vitrification, along with the gradual adoption of antagonist protocol for ovarian stimulation combined with agonist trigger, and the strategy of a deferred single embryo transfer has revolutionized the way clinicians practice fertility treatment today, as this circumvents the problem of ovarian hyperstimulation syndrome (OHSS).

The use of antagonist protocol for ovarian stimulation is associated with a significant reduction in the incidence of OHSS. This risk can be further reduced by replacement of hCG trigger for final follicular maturation by a GnRH agonist trigger which practically eliminates the risk of OHSS without affecting the oocyte yield and further embryonic development. However, following the triggering with GnRH agonist, there is drastic luteolysis which is associated with luteal phase defect owing to the excessive negative feedback resulting in suppressed pituitary LH release, and an inadequate luteal phase that is suboptimal to support implantation of embryos. Hence, the use of an antagonist protocol followed by GnRH agonist triggering is often followed by a "freeze-all" strategy and transfer of embryo(s) is carried out in a subsequent frozen-thawed cycle. This de-linking of oocyte retrieval followed by embryo culture & vitrification and subsequent embryo warming & transfer in a frozen-thawed cycle is referred to as "segmentation" of IVF treatment.[5]

Vitrification has emerged as an effective technique for preserving supernumerary embryos following a fresh embryo transfer. Apart from this, as already mentioned, it has excellent cryosurvival post warming when the embryo transferred is deferred, either due to an imminent OHSS, the presence of embryo/endometrial asynchrony or the need for performing preimplantation genetic diagnosis/screening (PGD/PGS). This allows the transfer of embryo(s) to a more physiologic environment, thereby resulting in higher pregnancy rates, as well as decreasing maternal and neonatal morbidity. However, whether this strategy of "freeze-all" can be applied to all cases including normoresponders and poor responders is still up for debate. It has been argued that a patient risk-based approach (to prevent OHSS) rather than a universal approach (freezing all embryos in all IVF cycles) would be more prudent. It is of paramount importance that a fertility centre should have a robust cryopreservation program to be able to adopt a selective or an elective "freeze-all" protocol and translate it into their clinical practice. A variety of cryopreservation devices and media are available in the market which have been used successfully for hundreds of thousands of frozen thawed cycles (Figures 1- 5).

Fig. 1: Cryotech vitrification & warming media and carrier device

Fig. 2: Kitazato vitrification & warming media and Cryotop carrier device

Fig. 3: Vitrolife vitrification & warming media

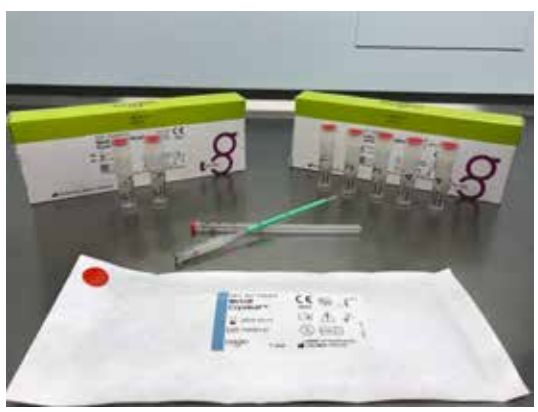

Fig. 4: Origio vitrification & warming media and McGill cryoleaf carrier device

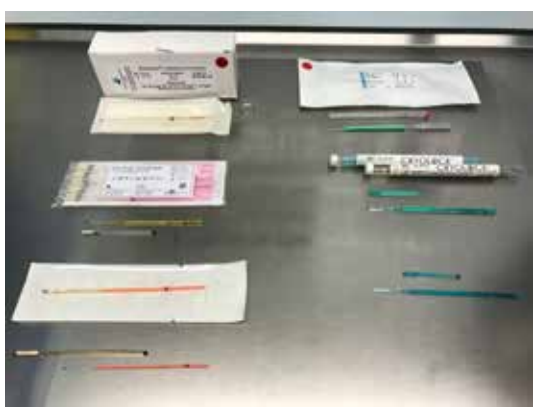

Fig. 5: Various vitrification carrier devices

EFFECTS OF CONTROLLED OVARIAN STIMULATION (COS)

Endometrial Receptivity

Embryo implantation depends upon the interaction of the embryo with a receptive endometrium. High estradiol level during the COS is inversely related to the chances of pregnancy and IVF outcome. Animal and human studies have shown

that superovulation causes histological changes at the time of implantation. In COS cycles, there is a decrease in the levels of specific integrins associated with WOI. Supraphysiological estradiol levels during COS may lead to an advancement in the window of implantation (WOI), as evidenced by endometrial transcriptomic studies that show an altered gene expression profile following superovulation. Endometrial advancement of ≥ 3 days is associated with failure of implantation. Even if the embryo successfully implants, the shift in the WOI may affect embryonic development which may be evident in defective placental formation and aberrant fetal growth.

Higher estradiol levels are correlated with earlier rise of progesterone, before the hCG trigger for final follicular maturation. Elevated levels of progesterone (>1.5 ng/ml) are associated with endometrial advancement and decreased pregnancy rates following fresh embryo transfer. Bosch et al. demonstrated in a retrospective analysis that women who had an elevated progesterone level (>1.5 ng/ml) on the day of hCG administration had a significantly lower ongoing pregnancy rate of 19% compare with 31% in women with progesterone level < 1.5 ng/ml.[6] When the embryos are frozen and transferred in a subsequent cycle, the pregnancy rates are comparable to fresh transfers, indicating that the detrimental effect of elevated progesterone is at the level of the endometrium and not the embryo.

Endometrial gene expression

COS induces a functional genomic delay of the endometrium as evidenced by a 2-day delay in activation/repression of two clusters composed by 218 and 133 genes belonging to the class of WOI genes.[7] The molecular signature of COS cycles during the WOI differs from the natural cycles. Though a change in the stimulation protocols may favorably alter the endometrial gene expression, currently it is unknown whether there exists an optimal regimen to maximize favorable endometrial gene expression profiles.

Embryonic development and fetal growth

The supraphysiological peri-implantation environment following COS is associated with altered placental vasculogenesis. In animal models, the offspring obtained from superovulated females have been found to weigh significantly less than the control population. The placental histology from the superovulated females has suggested signs of altered trophoblast differentiation. Additionally, the expression of several imprinted genes regulating fetal growth has been found to be significantly higher in the superovulated group as compared to controls.

The first meta-analysis comparing fresh and FET cycles suggested a significantly higher implantation, clinical and ongoing pregnancy rate in FET cycles.[8] These results may be due to the improved embryo-endometrium synchrony, a negative consequence of ovarian stimulation on endometrial receptivity, which has been previously reported by many authors.[9]

It has been shown that the incidence of antepartum haemorrhage (APH) is more in pregnancies following fresh transfers rather than FET. Healy et. al showed that the risk of placenta previa was 6.7% vs 3.6% and the risk of abruptio placentae was 2.6% vs 1.1% as compared to control population.[10]

Perinatal outcomes

Studies have shown that singletons born after frozen embryo transfer (FET) had a lower risk of low birth weight (LBW), preterm birth and small for gestational age (SGA) compared with singletons born after fresh embryo transfer following IVF. A recent systematic review and meta-analysis concluded that antepartum hemorrhage (RR = 0.67, 95% CI 0.55-0.81), preterm birth (RR = 0.84, 95% CI 0.78-0.90), small for gestational age (RR = 0.45, 95% CI 0.30-0.66), low birth weight (RR = 0.69, 95% CI 0.62-0.76), and perinatal mortality (RR = 0.68, 95% CI 0.48-0.96) were lower in women who received frozen embryos as compared to fresh embryo transfers.[11] However, it was noted that due to the low absolute differences and the potential impact of unadjusted confounders, the author's conclusions may not be reliable. Wennerholm et al. performed the largest study of somatic health to date and found that the postnatal growth and health of FET children was normal and similar between fresh ET and spontaneously conceived (SC) groups.[12] Moreover, they analysed the prevalence of chronic diseases, which did not differ between the FET, fresh ET, and SC groups (18.0%, 15.3%, and 16.7%, respectively). In a Danish register study, no differences were found for cerebral palsy, intellectual disability, imprinting diseases, or malignancies in FET singletons compared with both fresh ET and spontaneously conceived children.[13] However, due to the size of the study group (957 FET children), the low prevalence rate of these rare diseases does not allow detailed analysis.

Incidence of ectopic pregnancy

It has been suggested that the incidence of ectopic pregnancy is increased following fresh embryo transfers as compared to transfer in a frozen thaw cycle. One meta-analysis comparing ectopic pregnancy rates in fresh vs frozen embryo transfer showed similar outcomes between the two methods.[14] However, multiple studies have been performed since then, adding significantly to the available data for analysis. Recently, Londra et al have shown that embryo transfers in cycles without ovarian stimulation, such as frozen or donor cycles, were associated with lower rates of EP compared with fresh autologous cycles, and that the odds of ectopic pregnancy were 65% lower in women with frozen embryo transfers.[15] However, a meta-analysis by Acharya et. al revealed no significant difference between ectopic pregnancy rates in fresh versus frozen embryo transfer. Similarly, there was no difference between ectopic pregnancy rates in natural-cycle frozen embryo transfer versus programmed cycles.[16]

Freeze-all strategy: strengths and weaknesses

A recent SWOT (strength-weakness-opportunity-threat) analysis by Blockeel et. al based on the available evidence showed that the "freeze-all" strategy has the potential to increase maternal safety by eliminating OHSS using a GnRH agonist trigger.[17] It also showed that there may be improved or similar pregnancy rates following a frozen embryo transfer. It has been argued that frozen embryo transfers were associated with lower ectopic pregnancy rates and better obstetrical and perinatal outcomes. However, despite the potential advantages of a "freeze-all" strategy, the benefit of the elective cryopreservation of all embryos in terms of pregnancy outcomes has only been verified in a few small and heterogeneous RCTs restricted mostly to high responders. Such limitations were also inherent to the meta-analysis published later, which while confirming that FET cycles seem to be associated with better ongoing and clinical pregnancy rates, was based on only a few events deriving from heterogeneous studies.[18] In this regard, high-quality RCTs are urgently required, and currently registered RCTs aiming to test the abovementioned hypothesis of the so-called 'freeze-all' strategy are ongoing (e-FREEZE multi-centred randomised controlled trial which is aiming to recruit around 1086 patients, and has already recruited 285 couples). Another argument against the case of "freeze-all" strategy for complete avoidance of OHSS is the presence of case reports in the literature where severe cases of OHSS have been reported following an agonist trigger, or even in frozen thawed cycles. Fatemi et al reported the incidence of severe OHSS in 2 patients after GnRH agonist trigger and "freeze-all" approach in GnRH antagonist protocol, and concluded that even the sequential approach of ovarian stimulation followed by a "freeze-all" strategy and a deferred embryo transfer does not totally completely eliminate OHSS in all patients.[19]

The potential opportunities offered by a "freeze-all" strategy include the flexibility of scheduling and the avoidance of weekend oocyte retrievals. Stimulation may be started on any day of the cycle, which is otherwise deemed as a last-resort treatment and until now mostly applied to oncofertility patients. Recent published evidence has found comparable outcomes with stimulation initiated in the luteal phase.[20] In terms of patient friendliness, the "freeze-all" strategy could also allow for an alternate approach to prevent premature LH surges, i.e. the use of oral medroxyprogesterone acetate (MPA) instead of injectable GnRH analogs. Replacement of an injection by an oral medication would mean an enormous improvement in the quality of life for women

Fig. 6: SWOT analysis of a "freeze-all" strategy (adapted from Blockeel et al.17)

Table 1: Details of the 3 trials included in Meta-analysis by Roque et al[18]

Study	Patients (fresh/frozen)	Duration of trial	Day of ET	Outcome
Aflatoonian et al.[21]	374 (187/187) High responders	Feb 2007-Feb2009	Day 2	Ongoing pregnancy Implantation Clinical pregnancy Miscarriage rate
Shapiro et al[22]	137 (67/70) Normal responders	October 2007-October 2010	Day 5	Ongoing pregnancy Implantation Clinical pregnancy Miscarriage rate
Shapiro et al[23]	122 (62/60) High responders	July 2007-July 2010	Day 5	Ongoing pregnancy Implantation Clinical pregnancy Miscarriage rate

undergoing IVF and lead to more patient friendly protocols (Figure 6).

However, since the available evidence showing higher pregnancy rates in FET cycles mainly based on studies including high responders, the extrapolation of this data to the general population needs caution. The authors of the meta-analysis examining the outcome of fresh and frozen embryo transfers included only three trials (Table 1) with 633 cycles, two of which included only high responders.[18]

One of these three trials (Aflatoonian et al.[21]) was later retracted based on the results of an investigation which found serious methodological flaws in the study. Moreover, the quality of the available evidence for the other two studies included in the meta-analysis is also questionable. The author of the meta-analysis has gone on to further state that it is not clear if all normal responders and poor responders may benefit from the "freeze-all" strategy. This further solidifies the notion that patients may benefit from a selective "freeze-all" strategy rather than an elective "freeze-all" approach.[24]

The other potential weaknesses of a "freeze-all" strategy is the increased risk of large for gestational age babies following frozen embryo transfers. The results of a Nordic cohort study showed that the children born after FET were significantly at risk of being born with macrosomia (birthweight > 4500 gms) and post-term (> 42 weeks).[25] This may have implications for the offspring later in their adult lives (e.g. risk of developing obesity, diabetes mellitus, coronary heart disease etc.) according to the theory of developmental origin of health and disease (DOHAD). Though the numbers included in this study are large, this observation need to be confirmed by a well-designed RCT.

CONCLUSION

Though there are many advantages in adopting a "freeze-all" approach over fresh embryo transfers, this strategy is not suited for all IVF patients. Embryo transfers in fresh cycles have good success rates and the risk for obstetrical and perinatal complications has not been substantiated yet by any randomized controlled trials. There is an immediate need to conduct larger studies to compare the costs, time to pregnancy and cumulative pregnancy rates between the two approaches. The selection of patients who have an altered endometrial receptivity owing to superovulation, and determining the consensus for threshold of supraphysiological estradiol levels during COS above which the patients may benefit from a "freeze-all" approach needs to be done. There is a need for a non-invasive clinical tool to evaluate endometrial receptivity during fresh cycle, to enable selection of patients for a "freeze-all" approach.

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Timelapse Monitoring : Busting Myths

Dr. Keshav Malhtora
MBBS MCE
Director Rainbow IVF Agra/New Delhi

INTRODUCTION

Time-lapse monitoring systems (TLS) take digital images of embryos at set time intervals. The system can either be installed into an existing embryo incubator or can exist as a combined time-lapse incubation system. The images are compiled using specialist software to create a time-lapse sequence of embryo development, thus negating the need for the embryologist to remove embryos from the incubator for morphological assessment. Some TLS also utilize computer-assisted assessment of developmental milestones of embryos, also known as morphokinetic parameters, to offer a semi-quantitative process of embryo evaluation (Conaghan et al., 2013). These cell-tracking software algorithms have evolved as a non-invasive, non-subjective way of attempting to improve the selection of embryos with the highest implantation potential.

There are a number of TLS (Figure 1) on the market developed by various manufacturers, including Embryoscope®, Primo Vision (Vitrolife) and Eeva, Miri etc. Despite the technology being novel, numerous fertility clinics worldwide already have adopted TLS, often charging patients an additional fee (from several hundreds to well over one thousand U.S. dollar) for its use. The media have enthusiastically reported on TLS based on preliminary scientific publications (Campbell et al., 2013). Claims of tripling of IVF success rates by TLS, leading to live birth rates of 78%, helped to fuel the hype around the technology, without acknowledging limitations in study design of the available scientific publications for establishing the effectiveness of the novel intervention (Campbell et al., 2013). The industry behind TLS has largely driven the widespread adoption of the technology citing 'improved success rates', the advantage of 'bringing the latest technology to patients' and 'adding value to the treatment cycle' (FertiliTech).

Limitations of existing models

Usually the embryologists remove embryos from the incubator once per day to assess cleavage and morphology, but this type of monitoring only gives them a snapshot of a dynamic process. The embryos do not tolerate removal from optimal culturing conditions, which limits the number of observations that can be made. This problem is a significant one for the embryologists, and time-lapse technology may offer a solution.

With this technology, the embryos can be monitored without removing them from the incubator. A camera is built into the incubator and takes pictures of the embryos at preset intervals (Figure 2). With the help of the proper software, a video can be made that depicts their development. This type of monitoring allows for the collection of much more information on the timing of the cleavages and the dynamics of the morphologic changes. Payne and colleagues [Ciray et al 2012] were among the first to describe the early events of human embryonic development, and then, Mio and Meada described the kinetics of the events up until the blastocyst stage [Cruz et al 2013]. Their work was followed by observations made by several other groups that tried to correlate these kinetic and morphologic markers with embryo development, implantation potential, pregnancy rate and genetic health.

The relevance of predicting aneuploidy and blastocyst development is founded on the assumption that embryo development and aneuploidy represents reasonable end points for the birth of a healthy child (Figure 3). Using embryos and developmental potential instead of pregnancy as the end point allows for smaller studies, shorter time interval, and reduced costs due to a smaller number of treatment cycles needed. The validity depends, however, on the extent to which end points being measured are associated to chance of pregnancy. In particular, for blastocyst development, it is important to bear in mind that a large proportion of

Fig. 1: Time-lapse Monitoring Systems

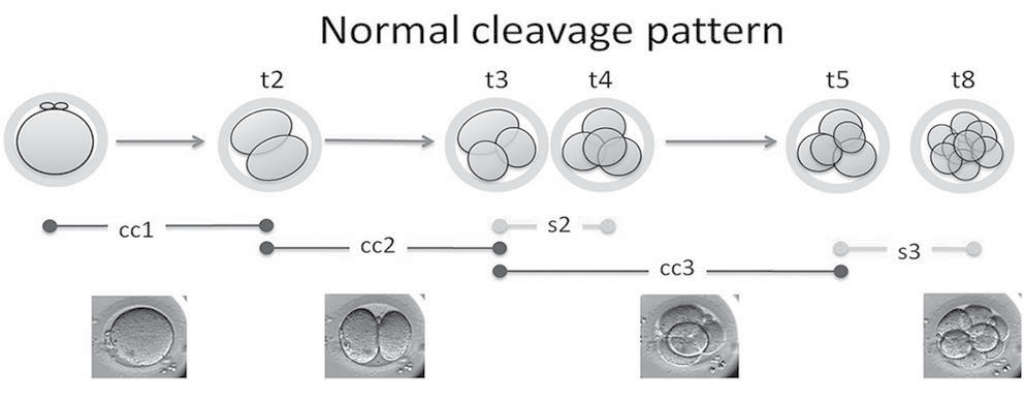

Fig. 2: Embryo development and important endpoints to assess implantation potential

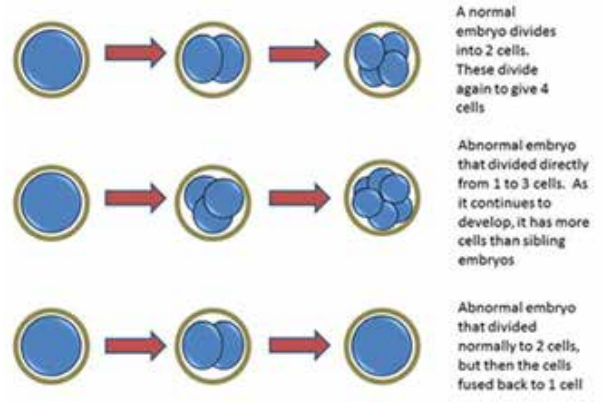

Fig. 3: Normal and Abnormal embryo development patterns

blastocysts do not implant. Also, it is relevant to distinguish between development of a blastocoel alone and blastocyst quality, of which the latter is a better end point, being more strongly correlated to clinical outcome

LIMITATIONS OF TIME LAPSE TECHNOLOGY

The concept of continuous embryo observation improving IVF outcome seems sound at first look. The technology has been shown to exert no harmful effects on the embryos. Most of the reviewed studies already show promising results but suffer from methodological issues. First, essentially all of the cited studies have a retrospective design. Retrospective study design cannot account for differences in the patient populations or culturing conditions. It is well-known that patients with similar characteristics could have a different treatment outcome in different clinics. This fact has been shown in the study by Meseguer et al (Dal Canto et al 2012) in which the impact of time-lapse monitoring on clinical pregnancy rate ranged between a few percent decrease to a 50% increase among the clinics participating in a multi-center trial (Table 1). It is not known how much of this difference can be attributed to patient characteristics and how much to the different culture conditions. Culture conditions in a given lab (e.g., oxygen tension, culture medium used) could affect embryo development [Campbell et al 2013, Campbell et al 2014]. The genetic integrity of the embryo is, however, expected to have an even more profound effect on the early development of the embryo. During embryo culture, the most crucial task is to differentiate embryos that will implant from those that will not. We can rephrase this statement and say that we need to differentiate the healthy, euploid embryos from the unhealthy, aneuploid embryos (Figure 4).

Time-lapse technology has already shown us that euploid embryos follow a much tighter division pattern and aneuploid embryos tend to fall out of range (Boue et al 1975). Therefore, each lab should test whether the proposed kinetic parameters are appropriate for their lab or whether they need to modify them based on their own results rather than adopting them automatically. It is also well-known that the treatment outcome depends on the stage at which embryo transfer occurs (Hum Reprod 2006). The different studies used different stage (day 2 to blastocyst stage) transfers (Fleming et al 2008; Hashimoto et al 2012; Hlinka et al 2012), which may interfere with their conclusions regarding the kinetic markers due to their impact on implantation and pregnancy rates.

Blastocyst formation and implantation rate are important markers of treatment efficacy, but neither can be used to replace the live birth rate or at least the ongoing pregnancy rate. Furthermore, in some of the discussed studies that are considered landmark studies (Oxford University Press; 2000) in the field of time-lapse technology, embryos have not been transferred and therefore clinical data are not available. Another problem with the cited studies is that most of them draw conclusions based on small number of patients involved, as noted in a commentary by Wells et al 2010. Data on the associations between ongoing pregnancy rate and live birth rate are limited at this stage. The above discussed studies involve mostly retrospective data analysis, and the time ranges for certain kinetic markers as well as the hierarchical models have not been properly tested prospectively.

Two RCTs have been published that compared standard incubation and embryo selection based on morphology with time-lapse incubation and morphokinetic embryo selection. Both trials included good prognosis patients or egg donation cycles. The larger study by Rubio et al. (Ver Milyea et al 2014), reported an increase in the ongoing pregnancy rate among those couples who had their embryos cultured in the time-lapse system. In addition to the method of embryo selection (morphology alone vs morphokinetic markers based on various time-lapse parameters) there were significant differences in the culture conditions as well and this could have affected the results too. The proportion of good quality day 3 and day 5 embryos was significantly higher in the time-lapse system and this suggests more optimal incubation conditions. Due to differences in the culture conditions the exact role of morphokinetic selection in improving outcome cannot be determined. Both studies included good-prognosis patients only. Therefore, we can apply these results primarily in good prognosis patients. If the morphokinetic parameters are predictive of embryo health (and therefore implantation potential) then

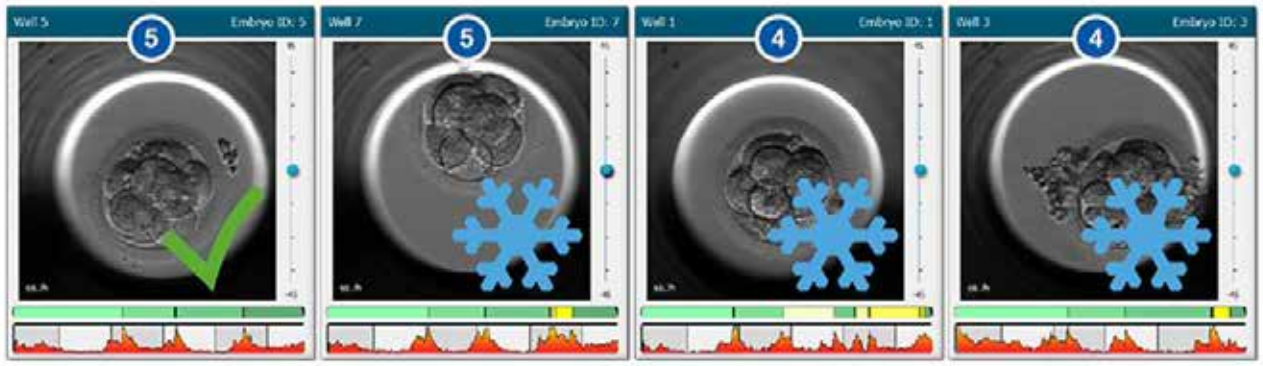

Fig. 4: Decision making using Kids score on embryoscope

Table 1: The optimal time interval of kinetic markers predictive of different clinical outcomes by various groups

	Wong et al. predictive of BC formation [32]	Meseguer et al. predictive of implantation [33]	Cruz et al. predictive of good morphology blastocyst development [35]	Conaghan et al. predictive of blastocyst formation [21]	Basile et al. predictive of euploidy [42]	Chavez et al. predictive of euploidy [41]	Campbell et al. predictive of euploidy [43],[44]
S_1	14.3 ± 6 min					14.4 ± 4.2 min	
CC_2	11.1 ± 2.2 h	≤ 11.9 h		9.33 – 11.45 h		11.8 ± 0.71 h	
$_S2$	1 ± 1.6 h	≤ 0.76 h	≤ 0.76 h	≤ 1.73 h		0.96 ± 0.84 h	
t_5		48.8 – 56.6 h	48.8 – 56.6 h		47.2 – 58.2 h		
t_{5-2}					>20.5 h		
CC_3					11.7 – 18.2 h		
t_{BC}							<122.9 h (and <96.2 h time to start of blastulation)

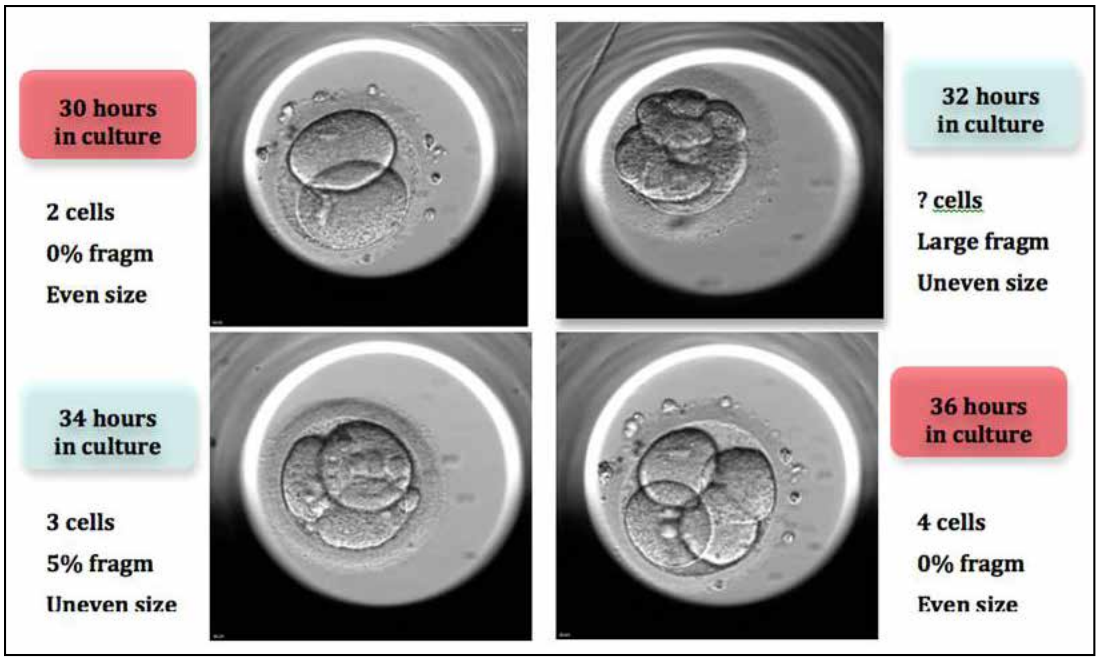

Fig. 5: Dynamic changes in Fragmentation during embryo development

we should expect the models to work in a different subset of patients as well. In older patients or poor responders however, the proportion of embryos that are identified as having a higher implantation potential is expected to be lower though.

CONCLUSIONS

Time-lapse embryo observation allows us to monitor the dynamic events of embryo development as they happen rather than just evaluate snapshots of it. A lot has already been learned of the events of early embryonic development, and it has also been shown that if observations are made only once a day, some of the important changes the embryo undergoes (e.g., changes in fragmentation pattern - Figure 5) will be missed, which may result in the false identification of the best embryo for transfer [Reinzi et al 2015, Ahlstrom et al 2011).

While its full impact on clinical care needs to be explored, the technology could be useful for research and industry purposes as the steps of embryo development can be precisely standardised. Furthermore time-lapse technology could revolutionise quality control in the lab.

There is also a long way to go before the method's routine application for embryo selection can be recommended. Certain parameters have already been identified that are associated with very low implantation potential. There are other markers that can predict blastocyst formation and implantation potential, though different groups have identified different markers. There is little data about the predictive ability of these parameters for clinical pregnancy and live birth. A few hierarchical models (again based on different markers) have been proposed and tested in retrospective analyses. The predictive ability of these markers has to be tested prospectively and using clinically meaningful endpoints.

Thus far, the time-lapse technology has proven to be safe. However, pregnancy and neonatal outcome data must be collected as well.

Time-lapse technology is just one of the methods that is currently being evaluated for embryo selection. None of these technologies are perfect, and rather than looking at them as competing technologies, we should evaluate how they could complete each other and further improve embryo selection during IVF. In summary, time-lapse technology provides us with a safe, undisturbed, continuous embryo observation option that can aid embryo selection and could improve outcomes. However, the full benefit of the technology and its place among the other embryo screening tools remains to be determined.

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Dr. Puneet K. Kochhar
Consultant Gynecologist & IVF Specialist
Elixir Fertility Centre, Gujranwala Town, Delhi

1. **Effect of follicular diameter at the time of ovulation triggering on pregnancy outcomes during intrauterine insemination.**

   Maher MA, Abdelaziz A, Shehata YA.

   Int J Gynaecol Obstet. 2017. doi: 10.1002/ijgo.12291.

OBJECTIVE: To compare pregnancy outcomes when triggering ovulation at different follicle sizes during intrauterine insemination (IUI) cycles.

METHODS: A prospective observational study was undertaken at two collaborative fertility centers in Saudi Arabia between January 2014 and May 2016. Women of any age were enrolled if they met inclusion criteria: primary, secondary, or unexplained infertility (≥1 year); day-2 follicle-stimulating hormone less than 12 IU/mL; normal prolactin, thyroid function, and uterine cavity; at least one patent tube; and a male partner with normal semen count and motility. IUI cycles were subdivided by size of dominant follicle (17 to <18 mm, 18 to <19 mm, 19 to <20 mm, and ≥20 mm), and pregnancy outcomes compared.

RESULTS: Data from 516 IUI cycles were analyzed. Frequencies of clinical pregnancy, ongoing pregnancy, and live birth for a follicle size of 19-20 mm were 30.2% (39/129), 24.0% (31/129), and 28.7% (37/129), respectively; these rates were significantly higher than those in other groups (all P<0.05). Only endometrial thickness was found to also contribute to outcome: probability of pregnancy increased as thickness rose (odds ratio 1.148, 95% confidence interval 1.065-1.237; P<0.001).

CONCLUSION: The optimal follicular diameter associated with increased pregnancy rates in gonadotropin-stimulated IUI cycles was between 19 and 20 mm.

2. **The effect of endometrial scratch injury on pregnancy outcome in women with previous intrauterine insemination failure: A randomized clinical trial.**

   Ashrafi M, Tehraninejad ES, Haghiri M, Masomi M, Sadatmahalleh SJ, Arabipoor A.

   J Obstet Gynaecol Res. 2017. doi: 10.1111/jog.13401.

OBJECTIVE: Endometrial scratch injury (ESI) has been recently proposed to enhance the implantation rate in assisted reproductive technology cycles. The present study was conducted to determine the effect of ESI on pregnancy rate in women with intrauterine insemination (IUI) failure.

METHODS: This prospective randomized controlled study was carried out in Imam-Khomeini Hospital and Royan Institute, Tehran, during a 12-month period from January 2013 to January 2014. After assessment, 169 patients who had IUI failure twice or more (no chemical or clinical pregnancy) with normal uterine anatomy and hysterosalpingography, were enrolled. They were randomly assigned into two groups. In the experimental group, all patients underwent ESI at day 8 or 9 of stimulation phase in the present IUI cycle, whereas no intervention was performed on the control group. IUI outcome was then compared between the two groups.

RESULTS: A total of 150 patients completed the IUI cycle during the study. The chemical pregnancy rate was 10.7% and 2.7% in the experimental and control groups, respectively, without significant difference (P = 0.09). Also no significant differences were detected in terms of clinical pregnancy and miscarriage rates between the two groups (P > 0.05).

CONCLUSIONS: No significant beneficial effect of ESI on fertility outcome in patients with repeated IUI failure was detected when it was carried out on day 8 or 9 of the same IUI stimulation cycle. Also, however, no negative impact secondary to ESI was observed. Therefore, confirmation or refutation of this hypothesis requires further studies with a larger sample size.

3. **Specialized sperm function tests in varicocele and the future of andrology laboratory.**

   Majzoub A, Esteves SC, Gosálvez J, Agarwal A.

   Asian J Androl. 2016 Mar-Apr;18(2):205-12.

BACKGROUND: Varicocele is a common medical condition entangled with many controversies. Though it is highly prevalent in men with infertility, still it marks its presence in males who do have normal fertility.

OBJECTIVE: Determining which patients are negatively affected by varicocele would enable clinicians to better select those men who benefitted the most from surgery.

METHODS: Since conventional semen analysis has been limited in its ability to evaluate the negative effects of varicocele on fertility, a multitude of specialized laboratory tests have emerged. In this review, we examine the role and significance of specialized sperm function tests with regards to varicocele.

RESULTS AND CONCLUSION: Among the various tests, analysis of sperm DNA fragmentation and measurements of oxidative stress markers provide an independent measure of fertility in men with varicocele. These diagnostic modalities have both diagnostic and prognostic information complementary to, but distinct from conventional sperm parameters. Test results can guide management and aid in monitoring intervention outcomes. Proteomics, metabolomics, and genomics are areas; though still developing, holding promise to revolutionize our understanding of reproductive physiology, including varicocele.

4. **Obesity and male infertility.**

   Kahn BE, Brannigan RE.

   Curr Opin Urol. 2017 Sep;27(5):441-445.

OBJECTIVE OF REVIEW: The prevalence of obesity has risen steadily for the past 35 years and presently affects more than a third of the US population. A concurrent decline in semen parameters has been described, and a growing body of literature suggests that obesity contributes to the male infertility. The purpose of this review is to examine the effects of obesity on male fertility, the mechanisms by which impaired reproductive health arise, and the outcomes of treatment.

RECENT FINDINGS: Obesity alters the hypothalamic-pituitary-gonadal axis both centrally and peripherally, resulting in hypogonadotropic, hyperestrogenic hypogonadism. Adipose tissue-derived factors, like leptin and adipokines, regulate testosterone production and inflammation, respectively. Increased systemic inflammation results in increased reactive oxygen species and sperm DNA fragmentation. Increased testicular temperature because of body habitus and inactivity impairs spermatogenesis. The degree to which obesity affects hormone levels, semen parameters, sperm DNA integrity, and pregnancy rates is variable, which may be the result of other comorbid conditions. Treatment in the form of weight loss has also had inconsistent results.

SUMMARY: Multiple interdependent mechanisms contribute to the detrimental effect of obesity on male fertility. Large, randomized control trials are needed to better characterize the therapeutic benefits of weight loss to restore male reproductive potential.

5. **The Impact of laparoscopic surgery of peritoneal endometriosis and endometrioma on the outcome of ICSI cycles.**

   Guler I, Erdem A, Oguz Y, Cevher F, Mutlu MF, Bozkurt N, Oktem M, Erdem M.

   Syst Biol Reprod Med. 2017 Jun 13:1-7. doi: 10.1080/19396368.2017.1332114.

OBJECTIVE: Our objective was to assess the role of laparoscopic removal of ovarian endometriomas and ablation of peritoneal endometriosis on the outcome of intracytoplasmic sperm injection (ICSI) - Embryo Transfer cycles by comparing with the results of patients with untreated endometriomas and tubal factor without underlying endometriosis confirmed by laparoscopy.

METHODS: For this purpose, between 2002 and 2015, outcomes of 257 ICSI cycles of 150 patients, including 91 cycles of 48 patients in minimal endometriosis, 57 cycles of 25 patients in endometrioma removal, 65 cycles of 53 patients in non-operated endometrioma, and 44 cycles of 24 patients in tubal factor groups were retrospectively analyzed.

RESULTS AND CONCLUSION: Basal ovarian reserve was significantly lower, mean starting and total gonadotropin consumption was significantly higher, and mean serum E2 on the day of hCG injection, number of dominant follicles, number of retrieved total, and MII oocytes were all significantly lower in the endometrioma cystectomy group. Fertilization and embryo cleavage rates were also significantly the lowest in the endometrioma cystectomy group, whereas clinical pregnancy and live birth rates were comparable among all groups. The number of transferred embryos and duration of infertility were the most significant predictors of clinical pregnancy and live birth. Basal antral follicle count was also significant in predicting live birth.

6. **Revisiting the management of recurrent implantation failure through freeze-all policy.**

   Magdi Y, El-Damen A, Fathi AM, Abdelaziz AM, Abd-Elfatah Youssef M, Abd-Allah AA, Ahmed Elawady M, Ahmed Ibrahim M, Edris Y.

   Fertil Steril. 2017 Jul;108(1):72-77. doi: 10.1016/j.fertnstert.2017.04.020.

OBJECTIVE: To determine whether a freeze-all policy for in vitro human blastocysts improves the ongoing pregnancy rate in patients with recurrent implantation failure (RIF).

DESIGN: Prospective cohort study.

SETTING: Single private center.

PATIENTS: A total of 171 women with RIF divided into two groups: freeze-all policy group (n = 81) and fresh embryo transfer (ET) group (n = 90).

INTERVENTIONS: Freeze-all policy.

MAIN OUTCOME MEASURES: Ongoing pregnancy rate.

RESULTS: The clinical pregnancy rate (52% vs. 28%; odds ratio [OR] 1.86; 95% confidence interval [CI], 1.29-2.68) and ongoing pregnancy rate (44% vs. 20%; OR 2.2; 95% CI, 1.04-3.45) were statistically significantly higher in the freeze-all group than the fresh ET group, respectively. The implantation rate was also statistically significant (freeze-all group 44.2% vs. fresh ET group 15.8%; OR 2.80; 95% CI, 2.00-3.92).

CONCLUSIONS: The freeze-all policy statistically significantly improved the ongoing pregnancy and implantation rates. Thus, a freeze-all policy is likely to be the new key to helping open the black box of RIF. These findings also are useful for further investigating the adverse effect of controlled ovarian stimulation on in vitro fertilization outcomes.

7. **Abnormal ratio of CD57+ cells to CD56+ cells in women with recurrent implantation failure.**

   Jiang R, Yan G, Xing J, Wang Z, Liu Y, Wu H, Fan X, Zhou J, Ding L, Sun H.

   Am J Reprod Immunol. 2017 May 20. doi: 10.1111/aji.12708.

OBJECTIVE: To define a more precise parameter for a better understanding of natural killer (NK) cells and its relation with regulatory T cells (Tregs) in women with recurrent implantation failure (RIF).

METHOD OF STUDY: The percentages of CD56+ cells, CD57+ cells and Foxp3+ cells in the endometrium and blood from 23 normal controls and 32 women with RIF were measured by immunocytochemistry and flow cytometry.

RESULTS: Women with RIF had significantly increased ratio of CD57+ cells to CD56+ cells in both the endometrium (P<.01) and blood (P<.05), and decreased percentage of Foxp3+ cells in the endometrium (P<.05). There was a significant negative correlation between CD57+ cells to CD56+ cells ratio and the percentage of Foxp3+ cells in the blood of RIF patients (P<.05).

CONCLUSION: Our study provides a novel assessment parameter, CD57+ cells to CD56+ cells ratio, to evaluate NK cells and its relation with Tregs in RIF patients.

8. **Use of imaging software for assessment of the associations among zona pellucida thickness variation, assisted hatching, and implantation of day 3 embryos.**

   Lewis EI, Farhadifar R, Farland LV, J Needleman D, Missmer SA, Racowsky C.

   J Assist Reprod Genet. 2017 Jul 6. doi: 10.1007/s10815-017-0978-3. [Epub ahead of print]

OBJECTIVE: The aim of this study was to determine if zona pellucida thickness variation (ZPTV) is associated with

implantation and if this relationship changes with use of assisted hatching (AH).

METHODS: Day 3 embryos from single or double embryo transfers (DETs) performed between 2014 and 2016 were included. ZPTV was assessed by examining photographs taken before transfer using an automated image processing platform to segment the zona pellucida (ZP) with an active contour technique. One hundred points were obtained of ZP thickness (ZPT) of each embryo to calculate ZPTV ([maximum ZPT-mean ZPT]/mean ZPT). Logistic regression was used to calculate the odds ratio (OR) and 95% confidence intervals (CI) of implantation by tertile of ZPTV. Maternal age and AH were adjusted for a priori. Other cycle and embryo characteristics were adjusted for if they altered the continuous effect estimate by >10%.

RESULTS: There was no statistically significant association between ZPTV and implantation across tertiles although embryos with greater ZPTV showed a trend of decreased implantation (Tertile 2 (T2) versus Tertile 1 (T1), OR = 0.80, CI = 0.50-1.28; Tertile 3 (T3) versus Tertile 1 (T3), OR = 0.75, CI = 0.47-1.20). While similar nonsignificant trends for the association between ZPTV and implantation were observed across tertiles after stratification of embryos hatched or not, embryos with the greatest ZPTV had slightly higher odds for implantation when AH was utilized (T3 vs. T1: with AH, OR = 0.89, CI = 0.49-1.62; without AH, OR = 0.61, 0.29-1.27).

CONCLUSION: ZPTV was not associated with implantation after day 3 transfer. This finding did not vary by use of AH.

9. The role of G-CSF in recurrent implantation failure: A randomized double blind placebo control trial.

Davari-Tanha F, Shahrokh Tehraninejad E, Ghazi M, Shahraki Z.

Int J Reprod Biomed (Yazd). 2016 Dec;14(12):737-742.

BACKGROUND: Recurrent implantation failure (RIF) is the absence of implantation after three consecutive In Vitro Fertilization (IVF) cycles with transferring at least four good quality embryos in a minimum of three fresh or frozen cycles in a woman under 40 years. The definition and management of RIF is under constant scrutiny.

OBJECTIVE: To investigate the effects of Granulocyte colony stimulating factor (G-CSF) on RIF, pregnancy rate, abortion rate and implantation rates.

MATERIALS AND METHODS: A double blind placebo controlled randomized trial was conducted at two tertiary university based hospitals. One hundred patients with the history of RIF from December 2011 until January 2014 were recruited in the study. G-CSF 300μg/1ml was administered at the day of oocyte puncture or day of progesterone administration of FET cycle. Forty patients were recruited at G-CSF group, 40 in saline and 20 in placebo group.

RESULTS: The mean age for whole study group was 35.3±4.2 yrs (G-CSF 35.5±4.32, saline 35.3±3.98, placebo 35.4±4.01, respectively). Seventeen patients had a positive pregnancy test after embryo transfer [10 (25%) in G-CSF; 5 (12.5%) in saline; and 2 (10%) in placebo group]. The mean of abortion rates was 17.6% (3), two of them in G-CSF, one in saline group. The implantation rate was 12.3% in G-CSF, 6.1% in saline and 4.7% in placebo group.

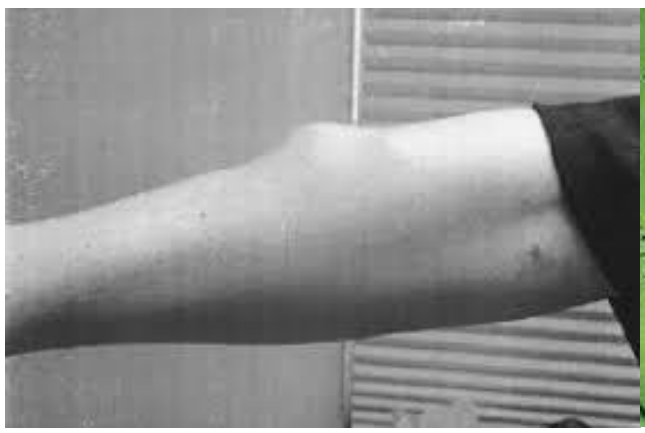
CONCLUSION: G-CSF may increase chemical pregnancy and implantation rate in patients with recurrent implantation failure but clinical pregnancy rate and abortion rate was unaffected.

Brain Teasers

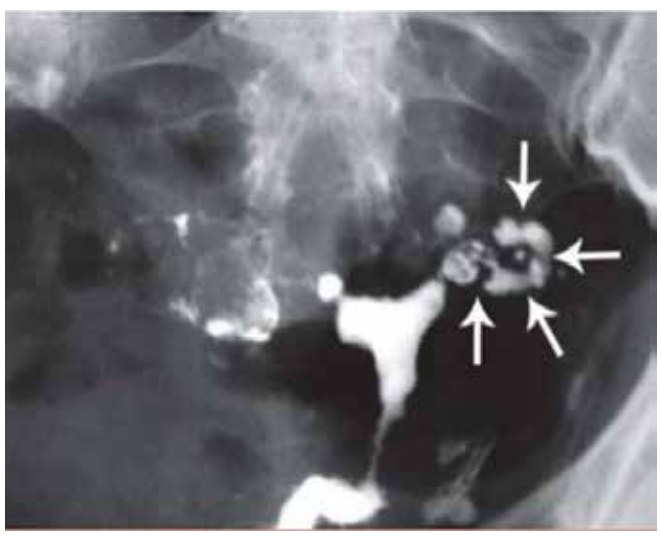
Dr. Abha Rani Sinha

Associate Professor, Obst & Gynae, Patna Medical College, Patna,
Chairperson Quiz Committee FOGSI (2015-2017)

Q. 1. Identify



Q. 2. Identify



Q. 3. Embryo grading does not include which one of the following

A. Rate of Embryo cleavage
B. Fragmentation of Blastomeres
C. Zona Pellucida Thickness
D. Equality of blastomeres

Q. 4. In Androgen insensitivity syndrome mutation of AR gene is encoded on the

A. Long arm of X chromosome
B. SRY genes
C. Autosomes
D. Long arm of Y chromosome

Q. 5. What is the commonly used sperm function test to differentiate dead sperms from immobile sperms during semen analysis?

ANSWERS TO BRAIN TEASERS – JUNE ISSUE

1. MRI guided focused ultrasound surgery
2. Uterine artery embolization. Post embolisation syndrome consists of pain, nausea, fever and malaise.
3. MRI
4. S- Size of fibroid, T- topography, E- extension of the base of fibroid, P- penetration into myometrium, W- wall
5. TRUE