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CARING FOR WOMEN'S HEALTH : EVIDENCE, ATTITUDE & PRACTICE

Dedicated Issue:

Infertility and ART: Management guidelines and counselling points for the busy clinician

AOGD SECRETARIAT

Institute of Obstetrics & Gynaecology,
Sir Ganga Ram Hospital
Sarhadi Gandhi Marg, Old Rajinder Nagar, New Delhi-110060
Tel.: 011-42251768, 1789
E-mail: secretaryaogdsgrh2020@gmail.com

Website: www.aogd.org

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Sarhadi Gandhi Marg, Old Rajinder Nagar, New Delhi-110060
Tel.: 011-42251768, 1789
E-mail: secretaryaogdsgrh2020@gmail.com | www.aogd.org

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Editors
Dr Geeta Mediratta
Dr Chandra Mansukhani
Ph. No. 011-42251768, 1789; Email: secretaryaogdsgrh2020@gmail.com

From the President's Pen

Greetings from AOGD

We hope that all our AOGD members are healthy, happy and progressing. The corona crisis in Delhi keeps on increasing and frightening both, the patients & health care workers. It is sad that some of our front line warriors are losing battles against this pandemic while performing their duties sincerely. Quite a few of our young doctors and health care workers are also becoming prey to this disease but the good news is that they are fighting the virus out.

This issue of bulletin speaks about the management of ART during the corona crisis. There is discussion about the guidelines and counselling points for the clinicians. So that they can learn the tips & tricks of ART. These valuable lessons may help them to achieve enhanced pregnancy rates during this covid era.

We have an excellent guidance from Dr. Abha Majumdar on "How to reduce time to pregnancy through IVF". There are Do's and Dont's on Intrauterine insemination. The article on "Oocyte Cryopreservation-Freezing the biological clock" speaks about the scientific advancements.

Our young embryologist writes about "PGD: Eliminating heritable diseases from the family". There is description of "Micro TESE & ICSE: Boon for obstructive azoospermia" and "Evidence for medical treatment of male infertility". The pregnancies followed by ART are special with their unique problems and different outcome which is nicely highlighted in the article "Perinatal outcome after ART".

The problems of thin endometrium is nicely dealt with. There are points to guide the clinician about the psychological issues in couples dealing with repeated pregnancy failure. Covid crisis brings lots of preparations and sacrifices from the health care workers perspective. These concerns are nicely penned down by one of our great front line warriors.

Our editorial team headed by Dr. Geeta Mediratta and Dr. Chandra Mansukhani have nicely knitted and accumulated articles from star writers and presented a nice bouquet of scientific deliberations.

Hope these tough times will pass away soon. Our academics should continue and will continue unabated. Learning is always a mutual process. Nothing can defeat the enthusiasm of hard working gynaecologists. Long live AOGD.

Dr Mala Srivastava
President, AOGD

From the Vice President's Pen

Greetings to all members of the association!

As we welcome the monsoons in Delhi, we normally expect to see freshly bathed plants and greenery all over, with a pleasant cool breeze and relief from the heat of the summer. But with our country now in the unlock phase 1.0, we are seeing a tremendous upsurge in the number of CORONA positive cases in our city.

People are trying to self restrict themselves at home as much as possible to safeguard their family and themselves from the disease. Our Health Care Workers are fighting endlessly to win this battle. I bow my head in grief for the **Corona Warriors** who lost their lives in their service to mankind.

We at AOGD, are trying to keep up with the Academic activities through virtual platforms in the form of E-Webinars, Panels and E-CMEs and trying to keep our members abreast with the latest in our field. In the month of June, there were Poster, Slogan and picture competitions organised by FOGSI in which there was active participation from our AOGD members too.

Our July E-Bulletin on **Infertility and ART: Management guidelines for the clinician**, is an excellent academic feast, brought out by the hard work of our Editorial team. We hope it'll be of great use to all our members as we at AOGD are trying our best to fulfil our objectives of providing a platform for increasing knowledge, skills and awareness to our members in the field of Obstetrics and Gynaecology.

Hope that we all **stay healthy, happy and safe.**

With warm regards,

Dr Kanika Jain
Vice President, AOGD (2020-21)

From the Secretary's Desk

Greetings to all !

Hope you all are keeping Safe and Healthy. I pay my heartfelt tribute to CORONA WARRIORS and Healthcare workers, who lost their lives fighting tirelessly with COVID infection.

We appreciate the enthusiasm and spirit of our AOGD members and Sub Committee Chairpersons in keeping the academic activities alive through virtual platforms during this pandemic while maintaining a high standard of academic deliberations.

Our editorial team has brought the AOGD E-Bulletin July version dedicated to **Infertility and ART: Management guidelines and counselling points for the busy clinician**. I hope the content will be of great interest and of immense use to our readers.

On the occasion of **World Population Day on 11th July**, let the public and private sector organisations work for provision of family planning services even in a crisis, for a self reliant nation and family as parallel to fight against corona goes on the fight against maternal morbidity and mortality due to unmet family planning needs.

Looking forward to your continued support.

Although the world is full of suffering, it is also full of the overcoming of it. – Helen Keller

Warm Regards

Dr Mamta Dagar
Hon. Secretary

Monthly Clinical Meeting

AOGD Monthly Virtual Clinical Meet will be organised by AIIMS Hospital, New Delhi on **Friday, 31st July, 2020 from 04:00pm to 06:00pm**.

From the Editor's Desk

Dr Geeta Mediratta
Chief Editor

Dr Chandra Mansukhani
Co-Editor

Dr Ruma Satwik
Guest Editor

Inability to conceive is a growing concern amongst our patients with one in seven of reproductive age couples likely to present themselves to our outpatient department with it. The raging COVID 19 outbreak in our city has only increased the anxiety around this issue as most fertility services remained suspended in favour of more essential services during the past three months. But as we make a foray into normalcy, it is likely that couples holding back their infertility treatments might begin to increase in our clinics. This month's AOGD bulletin is a dedicated infertility issue addressing problems likely to be encountered in everyday practice by the busy Gynaecologist.

Intrauterine insemination is a commonly performed procedure and clinicians should benefit from an easy reference guide on ten dos and don'ts of IUI. Similarly, semen analysis is a test that is widely ordered with approximately thirty percent of these results returning as abnormal. The article entitled "Evidence on medical management of male infertility" informs clinicians on what those abnormalities are and how to treat them medically. How to deal with thin endometrium is dealt with next.

Often the desperate couple would have no option other than in vitro fertilization to overcome childlessness. As their primary physicians, we ought to be able to counsel them on not just the in vitro fertilization technique but also the newer available technologies such as pre-implantation genetic testing and oocyte cryopreservation. This requires a knowledge of indications, its scope, and its pros and cons. Three articles deal with these subjects. Azoospermia was often considered a barrier to biological fatherhood. Surgical sperm retrieval changed the prospects for such men. However, the yield of sperm even after surgical retrieval could sometimes be nil. The article on Micro TESE, an improved technique of surgical sperm retrieval, shows the way to an improved sperm yield.

Finally, clinicians ought to be able to answer the question, "how would IVF and allied procedures affect my pregnancy and the child's health?". The article on perinatal effects of ART is on point in this regard. Whether it be infertility or subfertility, the distress of childlessness is similar. The article on "Psychological aspects of repeated pregnancy failure: ten counseling points" guides the clinician in providing a rounded care to the distressed couple.

And to keep it topical, those affiliated with the largest COVID-19 hospital of Delhi share their personal experiences and challenges faced while caring for those afflicted with the virus.

The journal scan for this month picks up a didactic guide on what should not be done when dealing with endometriosis.

Hope you enjoy reading this issue and please do go through the crossword puzzle and picture quiz at the end which should be a cakewalk for you!

Editorial Team

Intrauterine Insemination: Ten Do's & Don'ts

Shikha Jain
Director, Dreamz IVF, Delhi-110034, India

Intrauterine Insemination (IUI)

Intrauterine insemination involves deposition of processed semen (partner/donor) in the uterine cavity close to the time of ovulation in order to achieve a pregnancy. The rationale behind IUI is increasing the gamete density at the site of fertilization.[1] The first report of IUI was published by Cohen in 1962 and since then IUI has evolved through various innovations. Though the basic procedure remains the same, success rates in IUI cycles have grown from 5% to more than 20% per cycle with the advances in the stimulation protocols, cycle monitoring, timing of ovulation, semen preparation methods and luteal phase support.

Indications

IUI can be offered for wide range of indications (table 1).

Pre-Requisites

IUI is a level I assisted reproduction procedure which entails only semen handling outside the body and rest of the processes involved in human conception like ovulation, fertilization, embryogenesis and implantation takes place naturally. Hence it can be considered as an extension of natural conception. The prerequisites for a couple to undergo an IUI cycle are:

1. Ovulatory cycle (Natural or stimulated)
2. At least one patent functional fallopian tube (preferably both)
3. Total motile sperm count > 10 million/ml

Contraindications

IUI should be avoided in few conditions, either because it is not possible, less successful or moving directly to IVF is a better option.

Table 1:

Homologous Insemination		**Donor Insemination**
Physical or psychosexual dysfunction	**Infertility**	
• Hypospadias • Retrograde ejaculation • Erectile dysfunction • Vaginismus • HIV sero-discordant couple	• Unexplained infertility • Mild to moderate male factor • Minimal to mild endometriosis • Anovulatory infertility • Unilateral tubal disease • Cervical factor	• Azoospermia • Severe oligo-astheno-teratozoospermia (OATS) when IVF-ICSI is not affordable • Inheritable genetic disease in male • Lesbian/Single women

(Adopted with permission)[2]

Table 2:

Pre cycle (Non modifiable)	**During IUI cycle**	**Post IUI**
Age of female	Stimulated or Natural	Abstinence post IUI
BMI	Ovarian stimulation: CC/Letrozole/ Gn	Immobilization after IUI
Duration of infertility	Type of Gn: recombinant v/s urinary	Luteal phase support
Infertility etiology	Prevention of premature LH surge: use of GnRH analogues	
Single or multiple factor	Mono-follicular/ Multi-follicular	
Number of IUI cycle	Endometrial thickness	
	Ovulatory trigger	
	Timing of IUI: ovulation, semen collection, preparation & insemination	
	Semen parameters	
	Semen preparation method	
	Number of insemination	
	Easy or Difficult IUI	
	Type of catheter	

(Adopted with permission)[2]

1. Active pelvic infection: Cervicitis, Endometritis
2. Cervical stenosis, atresia
3. Blocked fallopian tubes
4. Severe Oligo-astheno-teratozoospermia (OATS) where IVF-ICSI is treatment of choice
5. Stage III/IV Endometriosis
6. Dense pelvic adhesions
7. Poor ovarian reserve

Prognostic Factors

Success of IUI depends upon a wide range of parameters (table 2).

Complications

Although IUI is a simple minimally invasive procedure, it is not without complications. (table 3)

Table 3:

Related to IUI procedure	Related to COS	Remote
Trauma Infection Bleeding Pain/ Cramping	Multiple Pregnancy Monitoring Cost OHSS	Ectopic pregnancy Abortion Pelvic inflammatory disease Psychological

(Adopted with permission)[2]

We present here the ten do's & don'ts of IUI for clinicians on the basis of two recent up to date publications on IUI, one of which is by the author of the current paper and other one provides the summary recommendations for the development of global evidence based guidelines for implementation of IUI, based on methodology established by World Health Organization (WHO).[2,3]

Ten Do's of IUI

1. ***IUI is a first line treatment option in unexplained infertility, mild male factor infertility & stage 1-2 endometriosis.***

   Evidence: In two recent Cochrane systematic reviews on unexplained infertility, IUI with or without OS results in higher cumulative live birth rate compared to expectant management.[4,5]

   Infertility due to suboptimal semen parameters can be overcome by IUI in males where the sperm count, motility or morphology is affected moderately while IVF-ICSI is the treatment of choice in cases where severe defect or a combination of them is present. In the two Cochrane reviews on male subfertility, IUI was not better than TI in a natural cycle or stimulated cycle.[6,7] Non-inferiority of IUI to IVF has been confirmed in a large RCT by Bensdorp et al.[8]

   COS with IUI is a viable treatment option in women with American Society for Reproductive Medicine (ASRM) Stage I and II endometriosis after laparoscopic correction of the disease.[9] IVF is a better choice in Stage III and IV endometriosis, after 2–3 failed IUI cycles in early stage disease, or if associated infertility factors are present, such as diminished ovarian reserve, advanced female age, compromised tubal function, or poor semen parameters.

2. ***Tubal evaluation through HSG or laparoscopy should be done before IUI. Infection screening must for any couple going for IUI.***

   Evidence: Hysterosalpingography (HSG) is the primary screening method to evaluate fallopian tubes unless laparoscopy is planned for other associated conditions.[10] The only exception to this is women undergoing donor insemination where 2-3 cycles of donor insemination can be done without checking tubal patency if clinical history is non-suggestive.

   As per Indian council of Medical Research (ICMR) & National Institute for Health and Care Excellence (NICE) guidelines for any ART procedure infection screening of infertile couple is must to reduce the chances of infection and cross contamination.[11,12]

3. ***IUI with COS is better than natural cycle IUI in non-male factor infertility. The approach of mild ovarian stimulation with Clomiphene citrate, Letrozole or low dose Gonadotropin (75 IU) with the aim of 1-2 lead follicles is optimal.***

   Evidence: Apart from male factor, physical or psychosexual dysfunction where natural cycle IUI can be offered, controlled ovarian stimulation (COS) is an integral part of IUI. The rationale of ovarian stimulation is to increase the number of fertilizable oocytes to increase the chances of conception. Adding COS to IUI significantly increases cumulative live birth rate (OR with 95% CI:3.4, 1.7-6.8) in unexplained infertility[13] and also in mild endometriosis (OR with 95% CI:5.6, 1.18-17.4).

Available evidence suggests Clomiphene citrate or Letrozole as the first line agent for COS in IUI cycles. Significantly higher pregnancy rate is achieved with the use of gonadotropins but at the cost of higher multiple pregnancy rate.[14] The latest NICE & ASRM guidelines recommend against the use of gonadotropin for COS with IUI in unexplained infertility.[12,15] In Indian perspective we would recommend use of COS- IUI for 3-4 cycles before moving to IVF, considering the cost implications and reasonable success rate in IUI cycles with proper selection of patients.

4. ***Cycle monitoring should be done with trans-vaginal ultrasound only. Criteria to give HCG trigger: at least 1 follicle >18 mm with endometrial thickness of 7-8 mm tri-laminar pattern.***

   Evidence: COS requires monitoring of serial follicular growth and endometrial thickness which is done through trans-vaginal ultrasound. As the approach in IUI cycles is mild ovarian stimulation, there is no need to monitor serum estradiol levels to decide for adequacy of ovarian response or HCG trigger. Multifollicular growth is associated with higher multiple pregnancy rate. Although pregnancy rates are higher in patients with endometrial thickness >8 mm, there is paucity of data on endometrial thickness in IUI cycles.

5. ***Timing of insemination done with Inj. HCG in stimulated cycles and LH surge in natural cycle IUI. Single insemination is recommended 24-40 hours post HCG or LH surge+ 1 day.***

   Evidence: The timing of insemination is one of the most important factors influencing the outcome of IUI. According to analysis of natural cycle by WHO, ovulation occurs 24 to 56 hours after onset of LH surge (mean time 32 h) and after HCG injection ovulation starts 32-38 h later and is sequential thereafter.[16] According to Cochrane review of 14 RCTs there was no difference in live birth rate between HCG vs LH surge or u HCG vs rec HCG and HCG vs GnRH agonist.[17]

   A mature oocyte is fertilizable for 12-24 hours after release; hence a single insemination should be performed close to ovulation to achieve success. We recommend single insemination 24-40 hours post HCG injection or LH surge + 1day. Documentation of ovulation on ultrasound before IUI is also reported to yield higher pregnancy rates (23.5% vs. 8.8%).[18]

   Equally important is time interval between semen processing and insemination. Semen processing should start within 30 min of collection and insemination to be done within 60-90 min of processing of semen sample because removal of seminal plasma during processing initiates sperm capacitation by changes in sperm acrosome.

6. ***The semen parameters for IUI in washed sample:***
   - ***inseminate volume 0.3-0.5 ml***
   - ***Inseminating motile count > 1 million***
   - ***>4% normal sperm morphologyas per strict criteria***

   Evidence: Inseminate volume < 0.2 ml would not be sufficient for IUI as it may not compensate for the dead space of an IUI catheter. Similarly higher volumes of inseminate increases the chances of back spill and there is no evidence that increasing inseminate volume can increase the chances of conception. Hence the optimal volume of inseminate should be between 0.3-0.5 ml.

   It is not possible to define clear lower cut off levels of pre and post wash semen parameters below which IUI should be withheld. Ombelet et al suggested that following cut-off values for semen parameters can be taken while considering IUI:[19]

   I. Inseminating motile count (IMC) > 1 million
   II. Sperm morphology using strict criteria > 4% normal morphology
   III. Total motile sperm count (TMSC) in native sample 5-10 million
   IV. Total motility in native sample (TM) >30% While using these cutoffs the sensitivity (ability to predict pregnancy) was limited but the specificity (ability to predict failure to conceive) was much better.

7. ***IUI is an OPD based procedure. It should be gentle and atraumatic with any type of catheter-soft/rigid, with or without stylet.***

   Evidence: Cochrane systematic review of 9 trials suggested that there was no evidence of significant difference in CPR or LBR with the choice of catheter type.[20] We recommend IUI

catheter should be atraumatic, non-toxic and easy to use. The insemination process should be quick, gentle and painless to the patient.

8. ***Immobilization of 10-15 min post IUI is recommended.***

   Evidence: The rationale of immobilization in supine position after IUI is to prevent any leakage of semen and to compensate for the absence of sperm reservoir in cervical mucus that forms after natural intercourse. Cumulative ongoing pregnancy rate (27% vs. 18%) and live birth rate (27 % vs. 18%) was significantly better after 15 minutes of immobilization in comparison to immediate mobilization post IUI.[21] The recent RCT in 2017, however showed non-significant difference in cumulative ongoing pregnancy rate between mobilization and immobilization group (OR=1.00, 95% CI: 0.74-1.33).[22]

   Our recommendation is to immobilize the patient for 10-15 min post IUI as it provides psychological support to the patient and may prove useful. Although prolonged bed rest or restriction of routine activities is not recommended.

9. ***In COS IUI cycles luteal phase support with vaginal natural micronized progesterone or oral Dydrogesterone should be given to overcome the defective luteal endocrine milieu.***

   Evidence: Progesterone is absolutely essential for establishment and maintenance of pregnancy. In COS IUI cycles, supra-physiologic estradiol levels send negative feedback at hypothalamus causing low luteal LH levels leading to defective implantation. In an updated systemic review and meta-analysis of 11 trials, the CPR (RR 1.56, 95% CI 1.21-2.02) and LBR (RR 1.77, 95% CI 1.30-2.42) were significantly higher with vaginal progesterone supplementation in patients undergoing gonadotropin IUI cycles.[23] In view of low cost ease of administration and side effects, we recommend use of vaginal natural micronized progesterone in IUI cycles. Dydrogesterone is an option for women who are reluctant to vaginal preparation. LPS is not recommended in natural cycle IUI.

10. ***Couples opting for IUI should be counseled for at least 3 consecutive IUI cycles.***

    Evidence: The clinical pregnancy rate per cycle was significantly higher in the first three cycles compared to the second three cycles (P < 0.001).[24] The INeS-trial from Bensdorp et al. showed that six cycles of IUI–OS is still cost-effective compared to direct IVF in patients with unexplained and mild male infertility.[8] We recommend IVF after 3 unsuccessful attempts of COS- IUI in unexplained infertility, mild male factor or minimal to mild endometriosis however donor insemination can be tried up to 6 cycles.[12]

Ten Dont's of IUI

1. ***IUI is not indicated in female with advanced age (>40yrs), poor ovarian reserve & with tubal factor infertility.***

   Evidence: Female age is the most relevant predictor of clinical pregnancy in natural as well as assisted conception. The limited success of stimulated IUI over immediate IVF in older women was shown in FORT-T trial.[25] Hence we do not recommend IUI in women > 40 years of age as declining ovarian reserve and oocyte quality may not give desired results.

   Bilateral tubal disease is a contraindication for IUI but opting IUI in unilateral tubal disease also results in lower pregnancy rate with higher incidence of ectopic pregnancy.

2. ***Routine use of Hysteroscopy or Endometrial injury prior to IUI cycle is not recommended.***

   Evidence: In the absence of any documented intrauterine pathology on pelvic ultrasound routine use of diagnostic hysteroscopy prior to IUI cycle is not recommended.

   Intentional endometrial injury is used as a technique to improve the probability of pregnancy in women undergoing IVF however the effectiveness of this procedure in women attempting to conceive via IUI remains unclear. In Cochrane systematic review of 9 trials there was low quality evidence that endometrial injury may improve clinical pregnancy rates (RR 1.98, 95% CI 1.51-2.58).[26]

3. ***In order to avoid multiple pregnancy & OHSS doubling the dose of gonadotropin or use of GnRH analogues is not recommended.***

   Evidence: The most feared complications of COS IUI cycle are multiple pregnancy & OHSS. Doubling the dose of gonadotropin or addition of GnRH agonist will add on to the cost without increasing the pregnancy rate but doubling

the multiple pregnancy rates.[14] The addition of GnRH antagonist offers no benefit.

4. ***Strict cancellation criteria: Cancel IUI if > 2 follicles of >15 mm or > 5 follicles of > 10 mm on the day of HCG Or ET < 6mm hyperchoic on the day of HCG.***

   Evidence: Multi-follicular growth is associated with higher pregnancy rates in COS-IUI. In a meta- analysis by Rumste et al, the chances of pregnancy was 5% higher with 2 follicles and 8% higher with 3 or 4 follicles. At the same time risk of multiple pregnancies was increased by 6%, 10% and 14% with 2, 3 and 4 follicles respectively.[27] In our previous study we found CPR of 19.7% in mono-follicular cycles while it was 29.4% in multi-follicular cycles.[28]

   We strongly recommend mono-follicular or bi-follicular cycles and cancellation should be considered if there are > 2 follicles of ≥ 15 mm or > 5 follicles of >10 mm on the day of HCG.[3] The HCG trigger is withheld and the couple is advised to abstain in that particular cycle to avoid multiple pregnancy.

   Optimal endometrial thickness is equally important for successful IUI outcome. Ideally IUI should not be performed if endometrial lining is < 6 mm or hyperechoic on the day of HCG.

5. ***Double insemination doesn't offer benefit in terms of pregnancy, hence not recommended.***

   Evidence: The rationale of double IUI in a multi follicular cycle and in male factor subfertility is to widen the fertilization window so as to deliver more spermatozoa for fertilization of multiple oocytes released sequentially. In the meta-analysis by Polyzos et al there was no benefit of double insemination in terms of clinical pregnancy rate in couples with unexplained infertility.[29] For Donor IUI as well, there was no difference in clinical pregnancy rates with double insemination (single 16.4% vs. double 13.6%).[30] We do not recommend double insemination in order to reduce the cost and psychological burden to the couple.

6. ***There is no difference in semen preparation method for IUI in terms of pregnancy rate.***

   Evidence: The rationale behind semen preparation in IUI is the separation of motile, morphologically normal spermatozoa from infectious agents, antigenic proteins, dead sperms, leukocytes and immature germ cells which may lead to production of free oxygen radicals. In the systemic review of 7 RCT there was insufficient evidence to recommend any semen preparation technique over another.[31] Amongst simple wash and centrifugation, swim up or double density gradient method, any method can be applied depending upon the quality of native semen sample. For donor inseminations frozen semen sample should be procured from ART bank after mandatory 6 months quarantine.[11]

7. ***Use of tenaculum, uterine sound or touching the fundus while doing IUI is not recommended. Routine use of ultrasound guided IUI not recommended.***

   Evidence: Using a tenaculum to hold the anterior lip of cervix, uterine sounding or touching the fundus may lead to release of prostaglandins and disturb the endometrial lining. Back spillage of semen, blood on the tip of IUI cannula, abdominal cramps or spotting post IUI are signs of difficult IUI and decreases the odds of pregnancy. In cases of difficulty, changing the position to extended lithotomy or doing IUI with partially full bladder or switching to metal cannula may help negotiating the internal os. Ultrasound guidance can be of help in few difficult cases only.[32]

8. ***Abstinence around IUI is not recommended in non-male factor infertility.***

   Evidence: In natural intercourse, cervical crypts acts as sperm reservoir, providing a supply of sperms for up to 72 hours but the fertilizable life span of washed sperms is only 2-3 hours, while it is 12-24 hours for a mature oocyte. In a multi follicular cycle, the ovulation of multiple oocytes is sequential, therefore to widen the fertilization window couples are advised to have sexual contact around IUI. We recommend sexual intercourse around the time of IUI in couples with non-male factor infertility.

9. ***Use of HCG or GnRH agonist as LPS not recommended.***

   Evidence: HCG is not recommended for luteal phase support in assisted reproduction considering risk of OHSS.[33] Although there is evidence of marginal benefit of using GnRH

agonist around the time of implantation in IVF, there is insufficient evidence in IUI cycles.[34]

10. ***There is insufficient evidence to recommend maximum number of IUI cycles although the chance of achieving pregnancy beyond 3 cycles is quite low.***

    Evidence: There is conflicting evidence regarding upper limit of number of IUI cycles. In a retrospective analysis of 15,303 IUI cycles, it was found that OPR/cycle decreased from 7.4% in first cycle to 4.7% and 4.6 % in sixth and ninth cycle respectively but the cumulative OPR was 18.3%, 30.3% and 41.2% after 3, 6 and 9 cycles respectively.[35] We recommend careful evaluation of patient's age, duration of infertility, ovarian reserve, pelvic factors and semen parameters before continuing with IUI treatment beyond 3 cycles. Timely referral for IVF should be discussed.

Conclusion

IUI should be offered as a first line treatment option in the management of unexplained infertility, mild male factor and mild endometriosis as well couples considering donor insemination. IUI is simple, patient friendly, non-invasive and cost effective over IVF. It offers less psychological burden for couples hence less dropout rate and good compliance to treatment is achieved. The approach of mild ovarian stimulation while minimizing the risk of multiple pregnancy & OHSS, proper timing to enhance success with thorough counseling should be adopted.

References

1. ESHRE Capri Workshop Group. Intrauterine insemination. Hum Reprod Update 2009;15:265–77.
2. Jain S. Intrauterine Insemination: Current place in Infertility management. EMJ. 2018;3[1]:58-66.
3. Cohlen B, Bijkerk A, Van der Poel S, Ombelet W. IUI: review and systematic assessment of the evidence that supports global recommendations. Hum Reprod Update. 2018;24(3):300-319.
4. Ayeleke RO, Asseler JD, Cohlen BJ, Veltman-Verhulst SM. Intra-uterine insemination for unexplained subfertility. Cochrane Database of Systematic Reviews 2020;3: CD001838.
5. Wang R, Danhof NA, Tjon-Kon-Fat RI, Eijkemans MJC et al. Interventions for unexplained infertility: a systematic review and network meta-analysis. Cochrane Database of Systematic Reviews 2019;9: CD012692.
6. Bensdorp A et al. Intra-uterine insemination for male subfertility. Cochrane Database Syst Rev. 2007;(4): CD000360.
7. Cissen M et al. Assisted reproductive technologies for male subfertility. Cochrane Database Syst Rev. 2016;2:CD000360.
8. Bensdorp AJ, Tjon-Kon-Fat RI, Bossuyt PMM, Koks CAM et al. The INeS study: Prevention of multiple pregnancies in couples with unexplained or mild male subfertility: randomised controlled trial of in vitro fertilisation with single embryo transfer or in vitro fertilisation in modified natural cycle compared with intrauterine insemination. Br Med J 2009;9:35.
9. Practice Committee of American Society for Reproductive Medicine. Endometriosis and infertility: A committee opinion. Fertil Steril. 2012; 98(3):591-8.
10. Practice Committee of the American Society for Reproductive Medicine. Diagnostic evaluation of the infertile female: a committee opinion. Fertil Steril. 2015; 103:e44–50.
11. The Assisted Reproductive Technology (Regulation) Rules – 2010. Indian Council Of Medical Research New Delhi, Available at: https://main.icmr.nic.in/
12. National Institute for Health and Care Excellence. Fertility: Assessment and treatment for people with fertility problems. 2013. Available at: http:// www.nice.org.uk/ CG156.
13. Farquhar CM, Liu E, Armstrong S, Arroll N, Lensen S, Brown J. Intrauterine insemination with ovarian stimulation versus expectant management for unexplained infertility (TUI): a pragmatic, open-label, randomised, controlled, two-centre trial. Lancet. 2018;391(10119):441-450.
14. Cantineau AEP, Cohlen BJ. Ovarian stimulation protocols (anti-oestrogens, gonadotrophins with and without GnRH agonists/antagonists) for intrauterine insemination (IUI) in women with subfertility. Cochrane Database Syst Rev. 2007;(2):CD005356.
15. Practice Committee of the American Society for Reproductive Medicine. Evidence-based treatments for couples with unexplained infertility: A guideline. Fertil Steril. 2020;113:305–22.
16. World Health Organization. Temporal relationships between ovulation and defined changes in the concentration of plasma estradiol-17 beta, luteinizing hormone, follicle-stimulating hormone, and progesterone. I. Probit analysis. World Health Organization, Task Force on Methods for the Determination of the Fertile Period, Special Programme of Research, Development and Research Training in Human Reproduction. Am J Obstet Gynecol. 1980;138(4):383-90.
17. Cantineau AEP et al. Synchronised approach for intrauterine insemination in subfertile couples. Cochrane Database Syst Rev. 2014;(12):CD006942.
18. Kucuk T. Intrauterine insemination: Is the timing correct? J Assist Reprod Genet. 2008;25(8):427-30.
19. Ombelet W et al. Semen quality and prediction of IUI success in male subfertility: A systematic review. Reproductive BioMedicine Online. 2014; 28(3):300-9.
20. van der Poel N et al. Soft versus firm catheters for

intrauterine insemination. Cochrane Database Syst Rev. 2010;11:CD006225.

21. Custers IM et al. Immobilisation versus immediate mobilisation after intrauterine insemination: Randomised controlled trial. BMJ. 2009;339:b4080.
22. van Rijswijk J, Caanen MR, Mijatovic V, Vergouw CG, van de Ven PM, Lambalk CB, Schats R. Immobilization or mobilization after IUI: an RCT. Hum Reprod 2017; 32:2218–2224.
23. Green KA et al. Progesterone luteal support after ovulation induction and intrauterine insemination: An updated systematic review and meta-analysis. Fertil Steril. 2017;107(4):924-33.
24. Aboulghar M, Mansour R, Serour G, Abdrazek A, Amin Y, Rhodes C. Controlled ovarian hyperstimulation and intrauterine insemination for treatment of unexplained infertility should be limited to a maximum of three trials. Fertil Steril 2001;75:88–91.
25. Goldman MB et al. A randomized clinical trial to determine optimal infertility treatment in older couples: The Forty and Over Treatment Trial (FORT-T). Fertil Steril. 2014;101(6):1574.
26. Lensen SF et al. Endometrial injury for pregnancy following sexual intercourse or intrauterine insemination. Cochrane Database Syst Rev. 2016;(6):CD011424.
27. Van Rumste MM et al. The influence of the number of follicles on pregnancy rates in intrauterine insemination with ovarian stimulation: A meta-analysis. Hum Reprod Update. 2008;14(6):563-70.
28. Jain S, Majumdar A. Impact of gonadotropin-releasing hormone antagonist addition on pregnancy rates in gonadotropin-stimulated intrauterine insemination cycles. J Hum Reprod Sci. 2016;9(3):151-8.
29. Polyzos NP et al. Double versus single intrauterine insemination for unexplained infertility: A metaanalysis of randomized trials. Fertil Steril. 2010;94(4):1261-6.
30. Zarek SM et al. Single-donor and double-donor sperm intrauterine insemination cycles: Does double intrauterine insemination increase clinical pregnancy rates? Fertil Steril. 2014; 102(3)739-43.
31. Boomsma CM et al. Semen preparation techniques for intrauterine insemination. Cochrane Database Syst Rev. 2019;(10):CD004507.
32. Oruç AS et al. Influence of ultrasoundguided artifcial insemination on pregnancy rates: A randomized study. Arch Gynecol Obstet. 2014;289(1):207-12.
33. Van der Linden M, Buckingham K, Farquhar C, Kremer JAM, Metwally M. Luteal phase support for assisted reproduction cycles. Cochrane Database of Systematic Reviews 2015, Issue 7. Art. No.: CD009154.
34. Bellver J et al. GnRH agonist administration at the time of implantation does not improve pregnancy outcome in intrauterine insemination cycles: A randomized controlled trial. Fertil Steril. 2010;94(3):1065-7.
35. Custers IM et al. Intrauterine insemination: How many cycles should we perform? Hum Reprod. 2008;23(4):885-8.

Calendar of Virtual Monthly Clinical Meetings 2020-21

31st July, 2020	AIIMS
Mid August	Lady Hardinge Medical College
28th August, 2020	Army Hospital- Research & Referral
Mid September	Apollo Hospital
25th September, 2020	DDU Hospital
23rd October, 2020	ESI Hospital
27th November, 2020	MAMC & LNJP Hospital
18th December, 2020	Sir Ganga Ram Hospital
29th January, 2021	Dr RML Hospital
26th February, 2021	UCMS & GTB Hospital
26th March, 2021	Lady Hardinge Medical College
23rd April, 2021	Apollo Hospital

Evidence for Medical Treatment of Male Infertility

Aanchal Agarwal
Senior consultant and Head, Deptt of Infertility, IVF and Reproductive Medicine, BLK Superspeciality Hospital, New Delhi

The overall prevalence of primary infertility ranges between 3.9% and 16.8%, as per WHO[1]. In Indian couples seeking treatment, the male factor is the cause in approximately 23%[2] and part of combined male and female factors in 40% - 50%.

Male infertility, in a broad perspective, refers to suboptimal reproductive potential of the male partner. It could be attributed to many causes. Initial evaluation of a male presenting with infertility mandates clinical examination followed by laboratory testing. Basic semen analysis is the simplest and most important laboratory test. To understand and interpret an abnormal semen analysis report, one must understand the process of spermatogenesis.

Physiology of Spermatogenesis

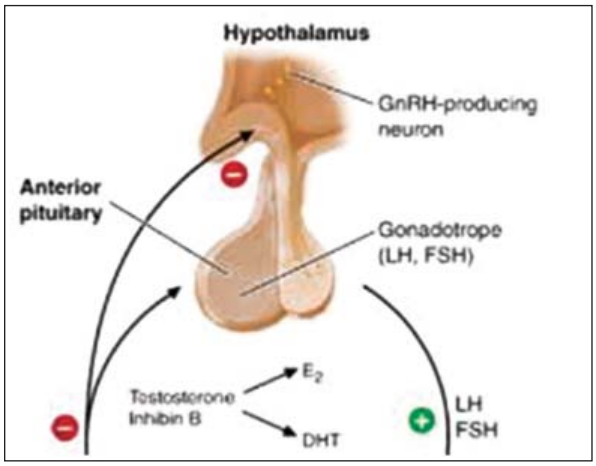

Fig 1:

Spermatogenesis is controlled by Hypothalamic-Pituitary axis. Pulsatile secretion of Gonadotropin releasing hormone (GnRH) by Hypothalamus stimulates anterior Pituitary to secrete gonadotropins Follicle Stimulating Hormone (FSH) and Leutinizing Hormone (LH).

FSH stimulates Sertoli cells to support sperm growth and stimulate production of Inhibin as well as Androgen binding globulin.

LH stimulates Leydig cells to produce Testosterone(T) which is then secreted into seminiferous tubules as well as circulation[3].

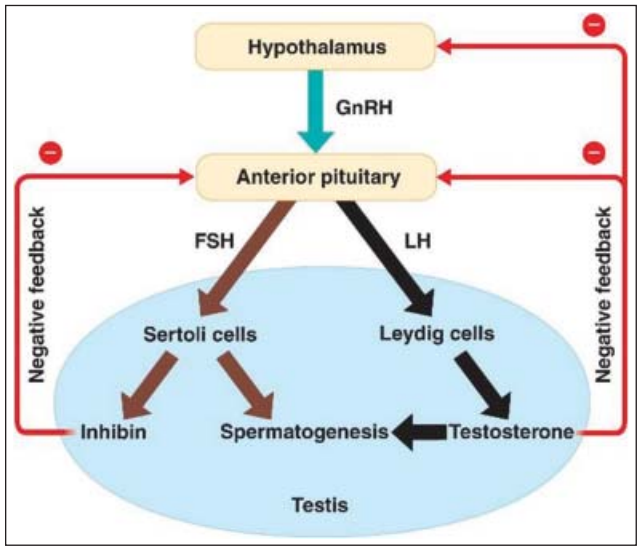

Fig 2:

Role of Testosterone(T) in Spermatogenesis

Testosterone levels in seminiferous tubules are 80-100 times more concentrated than in the general circulation. This high intratesticular concentration is required for spermatogenesis in Sertoli cells. It also Inhibits germ cell apoptosis[4].

Testosterone in the circulation provides negative feedback at hypothalamus and pituitary to inhibit secretion of GnRH, LH and FSH. Circulatory Testosterone is also converted to Estradiol (E2) by Aromatase found in testes, prostate, bone, brain and adipose tissue. E2 also provides negative feedback at hypothalamus and Pituitary just like Testosterone.

Etiological Classification of Male Infertility[5]

Table 1:

Pre testicular	Testicular	Post testicular
Endocrine • Gonadotropin deficiency • Kallman syndrome • Thyroid dysfunction	• Cryptorchidism	• Epididymal obstruction or dysfunction • Vasectomy

Pre testicular	Testicular	Post testicular
• Hyper prolactinoma • Hypothalamic/ pituitary tumors	• Environmental gonadotoxins like heat / smoking / metals / organic solvents/ pesticides	• Ejaculatory dysfunction due to spinal cord/ autonomic disease
Genetic • Single gene mutations • 47XXY Y chromosome deletion	• Klinefelter syndrome • Y chromosome deletions • Single gene mutations	• Congenital bilateral absence of vas deferens • kartagener syndrome • young syndrome
Drugs • GnRH analogues / androgens / estrogens / glucocorticoids / opiates	• Cimetidine • Alcohol • antiandrogens	
Others • Infiltrative diseases like Sarcoidosis/ Hemochromatosis • Chronic systemic illness or malnutrition • Injury • Infections • Obesity	• Radiation • Infections • Varicocele • Chronic illness like cirrhosis, cancer, renal insufficiency	Infections causing obstruction of vas deferens **idiopathic**

Evaluation of endocrine parameters i.e. serum levels of FSH, LH, Testosterone (T) help us in establishing the type of male infertility. Decision regarding further medical treatment also depends on the type of infertility thus established.

Table 2: Algorithm for establishing cause/etiology of male infertility

Etiology →	**Pre testicular**	**Testicular**	**Post testicular**
Clinical/lab ↓			
FSH	↓	↑	N
LH	↓	↑	N
Testosterone	↓	↓	N
Testes size	↓	↓	N

Medical treatment is indicated in the following conditions:

1. Hypogonadotropic Hypogonadism
2. Idiopathic hypogonadism
3. Hyperprolactinemia

Hormonal Treatment

1. **GnRH:**

   Indication: Hypogonadotropic Hypogonadism

   Pulsatile release of GnRH in the Hypothalamus stimulates the release of FSH and LH from anterior pituitary. Normal levels of FSH and LH are responsible for maintaining high levels of intratesticular Testosterone and for induction of spermatogenesis. (6). In infertile men with Hypogonadotropic Hypogonadism (HH) due to a lack of secretion from the Hypothalamus (e.g. Kallman's syndrome, Idiopathic HH), pulsatile exogenous administration of GnRH may be an effective therapy.

   Dosage: The most effective dose is between 5-20 microgram every one to two hours delivered by a subcutaneous pump or needle. (7) Currently, its use is limited only to specialty centres or research centres.

2. **Gonadotropins:**

   FSH/LH/hCG/combined therapy is indicated in following conditions:

   a. Primary HH

   b. Infertility with pituitary insufficiency e.g. pituitary adenoma, Hemochromatosis, Sarcoidosis.

   c. Pituitary insufficiency secondary to surgery/ radiation etc.

Dosage Schedule[8,5]

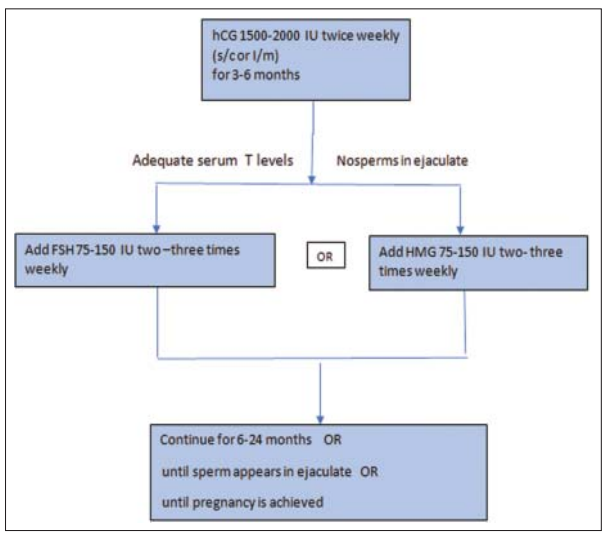

Fig 3:

Treatment should always begin with hCG alone (as a substitute for LH), without FSH, for following reasons:

1. hCG stimulates Leydig cells to produce Testosterone(T) resulting in high concentrations of intratesticular T required to stimulate and support spermatogenesis.
2. hCG alone may be sufficient to stimulate spermatogenesis but FSH alone is not.
3. Cost of hCG is much lower than FSH or HMG[5]

During treatment, serum Testosterone should be measured every 1-2 months for the first 3-4 months, aiming for a level between 400-900 ng/mL and doses of gonadotropins adjusted accordingly. Sperm count should also be evaluated regularly.

Most studies have shown induction of spermatogenesis in 80% of treated men and pregnancy in 38% to 51% of treated couples[9,10,11]. It should be noted here that urinary or recombinant gonadotropins are equally effective for this therapy.

Poor response is expected in men with Cryptorchidism, small testicles, elevated BMI and extreme Gonadotropin insufficiency. No benefit was shown in normo gonadotropic oligoasthenoteratozoospermia (OAT), Idiopathic OAT and hypergonadotropic Azoospermia (Testicular failure).

Exogenous Testosterone should never be used to treat Hypogonadism with Infertility. When given as a supplement, it can lead to negative feedback inhibition of LH secretion and further reduction in Testosterone secretion from Leydig cells as well as spermatogenesis.

3. Aromatase Inhibitors (AI):

Indications:

a. Idiopathic OAT

b. Idiopathic Azoospermia

c. Obesity related OAT

Importance of Estrogen and Aromatase

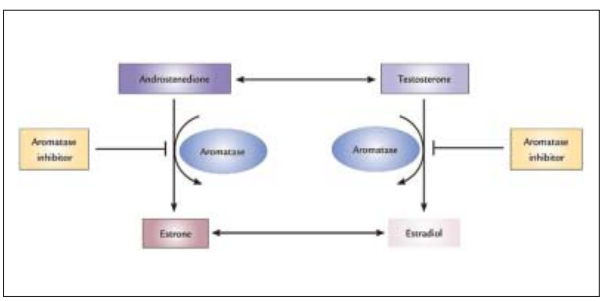

Fig 4:

As explained in Fig 4., circulating Testosterone is converted into Estradiol by Aromatase found in testes, prostate, bone, brain and adipose tissue[12].

Estrogen in optimal levels has positive effects on growth, development, and function of Leydig cells and promotes maturation of spermatogonia and inhibit germ cell apoptosis. Also, lack of aromatase leads to impaired spermatogenesis.

On the contrary, high levels of Estrogen has many detrimental effects as follows:

a. Negative feedback on Hypothalamus-pituitary axis leading to ↓ FSH and ↓ LH and finally ↓ spermatogenesis

b. Inhibits spermiogenesis related genes and promotes spermatocyte apoptosis

c. Exerts Inhibitory effects on Sertoli and Leydig cell functions

Needless to say, a fine regulation of Estrogen levels needs to be maintained for maintenance of spermatogenesis.

Mechanism of action of AI:

As shown in Fig 4., AI inhibit the conversion of Testosterone to Estrogen by acting on Aromatase enzyme. This leads to increase in circulating levels of T and decrease in circulating levels of E. This is quantitatively measured as the ratio between T and E (T/E ratio).

Recent studies have identified a potential specific endocrine defect in men with severe male factor infertility[13]. Some men with severely impaired sperm production have a relatively high Testosterone/Estradiol (T/E) ratio. Pavlovich et al. (2001) characterized men with severe male infertility as having a T/E ratio of 6.9, whereas men with normal spermatogenesis had a mean T/E ratio of 14.5. Based on these observations, they proposed a cutoff point of 10 as the lower limit of normal T/E ratios in men (calculated using T in ng/dL, and Estradiol as pg/mL).

Since males have testosterone levels detected by the pituitary by Estrogen levels rather than Testosterone alone, inhibition of Estrogen production by an aromatase inhibitor can be a potent stimulant for increased LH production and hence intratesticular and circulating Testosterone levels[14]. For men with a low serum Testosterone and low T/E ratio, treatment

with an aromatase inhibitor to increase sperm production would be more rational.

Saylam B etal in their study with Letrozole in 27 men with oligospermia or azoospermia reported improvement of sperm count and attainment of pregnancy in 20% of oligospermic men and identification of sperms in 24% of previously azoospermic men[15].

Types of Aromatase Inhibitors (AI)

Table 2:

Steroidal AI	Non-Steroidal AI
Irreversible enzyme inhibition	Reversible enzyme inhibition
Examples are Testolactone (50-100mg/d)	Examples are Letrozole (2.5mg/d) Anastrozole(1mg/d)
Advantages of Non-steroidal AI over Steroidal AI • Less interruption of androgenic steroid production • More effective in increasing T/E ratio • Fewer side effects	

The comparison of effects of Letrozole with Anastrozole were studied by Gregoriou etal (16) in infertile men with low T/E ratio and they concluded that these are equally effective in improving T levels and seminal parameters.

Good prognostic factors are low T/E ratio, low FSH, high BMI and high testicular volume.

AI are better compared to SERMs as they increase endogenous testosterone production without increasing circulating estrogen. (13)

4. Steroidal Estrogen Receptor Modulators (SERMs)

These are a class of compounds that act on Estrogen receptors as agonists or antagonists. Commonly used SERMs are Clomiphene Citrate (CC), Tamoxifen, Toremifene and Raloxifene.

Mechanism of action:

Competitively bind to Estrogen receptors in hypothalamus and pituitary and block the negative feedback effect of circulating Estrogen. This leads to increased secretion of FSH and LH by pituitary which stimulate spermatogenesis and Testosterone secretion by testes[17].

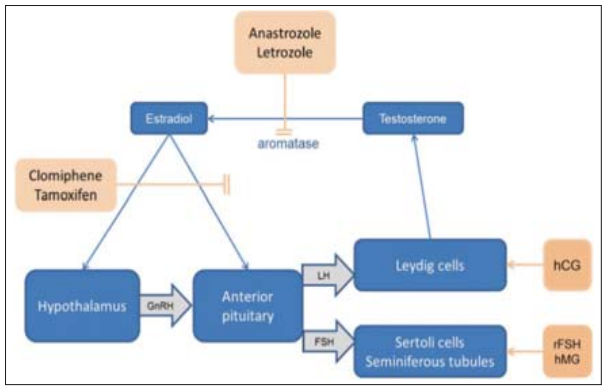

Fig 5:

Indications

1. Idiopathic oligospermia associated infertility
2. Idiopathic Hypogonadism

Dosage

1. Clomiphene Citrate: 25 mg once daily or 50 mg every other day
2. Tamoxifen: 20mg/day
3. Toremifene: 60mg/day
4. Raloxifene: 60mg/day

CC has many advantages like it is relatively inexpensive, can increase serum FSH, LH and T levels significantly, is well tolerated and can increase pregnancy rates. Recent meta-analysis done by Chua etal.[18] involving 11 RCT evaluating SERMs showed a significant increase in sperm conc. Of 5.24 mill/ml and 4.55% increase in sperm motility. It also revealed an increase in pregnancy rates (OR 2.42; 95% CI, 1.47-3.94; P=.004)

Cochrane review of clomiphene and Tamoxifen for idiopathic oligoasthenospermia, done in 2000 showed that there is some improvement in endocrine /hormone parameters (OR 1.56, 95% CI 0.99- 2.19) after 3 months of therapy but there was no change in pregnancy rates[19].

However, SERMs like CC may not be the best choice in patients with elevated FSH since they increase FSH levels further. Due to their agonist properties, they may exert negative feedback effect in pituitary leading to inhibition of FSH and LH secretion and thus reducing spermatogenesis.

5. Antioxidants

Mechanism of action: Oxidative stress is a well-established cause of male infertility and increased rates of infertility have been found in men with seminal fluid containing high levels of

reactive oxygen species (ROS)[20]. These ROS are associated with sperm dysfunction and germ cell DNA damage with the possibility of impaired fertility. These associations have led clinicians to treat infertile men with antioxidants. In addition, many clinical trials have suggested beneficial effect of antioxidants in improving sperm function and DNA integrity.

A systematic review of 17 randomized trials, including 1665 infertile men was conducted to evaluate the effects of oral antioxidants (vitamins C and E, zinc, selenium, folate, carnitine and carotenoids) on sperm quality and pregnancy rates in infertile men. Fourteen of the 17 (82%) trials showed an improvement in either sperm quality or pregnancy rate after antioxidant therapy. Ten trials examined pregnancy rate and six showed a significant improvement after antioxidant therapy[21]. This systematic review had multiple limitations: most of these studies were not controlled and differed in study design. The combined data differed in population, dosage and duration of antioxidants used. Currently there are no specific recommendations on the use of antioxidants in the treatment of male infertility, and the use of these products is completely empirical.

6. **Dopamine agonist**

   Indications: Men with Hypogonadism and infertility with elevated levels of prolactin

   Dose: Cabergoline (0.125 -1.0 mg twice weekly) is more effective than Bromocriptine.[22]

Conclusions

1. Goal of infertility treatment in infertile men is to optimize LH levels to stimulate Testosterone production and FSH levels to stimulate spermatogenesis.
2. Medical management should only be administered in highly selective cases e.g. Hypogonadotropic Hypogonadism, Idiopathic Hypogonadism with infertility, Hypogonadism in obese with T/E ratio <10.
3. Estrogen excess, if present, should be eliminated. Aim is to maintain T/E ratio>10. Aromatase inhibitors may be used for this purpose in obese men.
4. SERMs have a limited role in Idiopathic infertility with hypogonadism.
5. Exogenous Testosterone should never be administered to cure Hypogonadism or infertility.
6. Currently, there are no recommendations for use of antioxidants in male infertility and their use is entirely empirical.

References

1. Calverton, Maryland, USA: ORC Marco and the World Health Organization;2004. World Health Organization. Infecundity, Infertility, and childlessness in Developing countries. DHS Comparative Reports No9
2. Zargar AH, Wani AI, Masoodi SR, Larvay BA, Salahuddin M. Epidemiologic and etiologic aspects of primary infertility in the Kashmir region of India. Fertil Steril 1997; 68: 637-43
3. Bhasin et al. Harrison's principles of internal med. 2015 APPENDIX C 13

Table 3: Consolidated medical treatment of male infertility

Drug	Indication	Site of action	Dosage	Route of adm.
GnRH	HH	Pituitary	5-20mcg every 1-2hrs	s/c infusion pump
hCG	HH Pituitary insufficiency	Leydig cells	1500-2000 IU twice weekly	s/c or I/m
FSH/HMG HH Pituitary insufficiency		Sertoli cells	75 - 150 IU two-three times weekly	s/c or I/m
Aromatase inhibitors	Idiopathic OAT/AZO Obesity with OAT	Aromatase enzyme in adipose, brain, testes	Letrozole 2.5mg/d Anastrozole 1mg/d	oral
SERMs	Idiopathic oligo Idiopathic hypogonadism	Hypothal, pituitary	CC 50 mg/d Tamoxifen 20mg/d	oral
Dopamine agonist	Hypogonadism and infertility with hyperprolactinoma	Pituitary	Cabergoline 0.5 -1 mg twice weekly Bromocriptine 2.5 -5mg twice weekly	oral

4. Fode etal. Pathophysiology of disease. 2014. Lange Publishing.
5. Speroff's clinical Gynecologic Endocrinology and Infertility. Ninth Edition.2020. Wolters Kluwer.
6. Conn PM, Crowley WF Jr. Gonadotropin –releasing hormone and its analogues. N Engl J Med 1991; 324 : 93-103.
7. Happ J, Ditscheid W, Krause U. Pulsatile gonadotropin-releasing hormone therapy in male patients with Kallman's syndrome or constitutional delay of puberty. Fertil Steril 1985; 43: 599-608.
8. Warne DW, Decosterd G, Okada H, et al. A combined analysis of data to identify predictive factors for spermatogenesis in men with hypogonadotropic hypogonadism treated with recombinant human follicle stimulating hormone and human chorionic gonadotropin. Fertil Steril 2009; 92:594-604.
9. Burgués S, Calderón MD. Subcutaneous self administration of highly purified follicle stimulating hormone and human chorionic gonadotrophin for the treatment of male hypogonadotropic hypogonadism. Spanish Collaborative Group on Male Hypogonadotropic Hypogonadism. Hum Reprod 1997;12:980-6.
10. Büchter D, Behre HM, Kliesch S, et al. Pulsatile GnRH or human chorionic gonadotropin/human menopausal gonadotropin as effective treatment for men with hypogonadotropic hypogonadism: a review of 42 cases. Eur J Endocrinol 1998; 139:298-303.
11. Bouloux PM, Nieschlag E, Burger HG, et al. Induction of spermatogenesis by recombinant follicle-stimulating hormone (puregon) in hypogonadotropic azoospermic men who failed to respond to human chorionic gonadotropin alone. J Androl 2003; 24:604-11.
12. Use of anastrozole in the chemoprevention and treatment of breast cancer: A literature review Rev. Assoc. Med. Bras. vol.63 no.4 São Paulo Apr. 2017.
13. Pavlovich CP, King P, Goldstein M, et al. Evidence of a treatable endocrinopathy in infertile men. J Urol 2001;165:837-41.
14. Santen RJ. Feedback control of luteinizing hormone and follicle-stimulating hormone secretion by testosterone and estradiol in men: physiological and clinical implications. Clin Biochem. 1981;14:243–51.
15. Saylam B, Efesoy O, Cayan S. The effect of aromatase inhibitor letrozole on body mass index, serum hormones, and sperm parameters in infertile men. Fertil Steril 2011;95:809-11
16. Gregoriou O, Bakas P, Grigoriadis C, Creatsa M, Hassiakos D, Creatsas G. Changes in hormonal profile and seminal parameters with use of aromatase inhibitors in management of infertile men with low testosterone to estradiol ratios. Fertil Steril. 2012;98:48–51.
17. Goldstein SR, Siddhanti S, Ciaccia AV, et al. A pharmacological review of selective oestrogen receptor modulators. Hum Reprod Update 2000;6:212-24
18. Chua ME, Escusa KG, Luna S, et al. Revisiting oestrogen antagonists (clomiphene or tamoxifen) as medical empiric therapy for idiopathic male infertility: a meta-analysis. Andrology 2013;1:749-57.
19. Clomiphene and Tamoxifen for idiopathic oligoasthenospermia. Cochrane Database Syst Rev. 2000; (2): CD000151. doi: 10.1002/14651858.CD000151
20. Sharma RK, Agarwal A. Role of reactive oxygen species in male infertility. Urology 1996;48:835-50.
21. Ross C, Morriss A, Khairy M, et al. A systematic review of the effect of oral antioxidants on male infertility. Reprod Biomed Online 2010;20:711-23.
22. Webster J, Piscitelli G, Polli A, et al. Dose-dependent suppression of serum prolactin by cabergoline in hyperprolactinaemia: a placebo controlled, double blind, multicentre study. European Multicentre CabergolineDose-finding Study Group. Clin Endocrinol (Oxf)1992;37:534-41.

Step-wise Approach to Management of Thin Endometrium

Monica Gupta, Reeta Bansiwal, Reeta Mahey
Assistant Professor, DM Fellow Reproductive Medicine, Additional Professor, Department of Obstetrics & Gynecology, AIIMS, New Delhi

Introduction

Presence of receptive endometrium is a vital factor for successful embryo implantation in spontaneous conceptions and in assisted reproductive technique (ART). Endometrial thickness (EMT) and pattern, is a simple and non-invasive measure of receptivity. The optimal EMT for conception remains controversial among clinicians. Thin endometrium often defined as EMT <7 - 8 mm is a frequently encountered problem in ART with incidence of 1.5-9%[1]. EMT less than 7 mm on ultrasound is generally considered sub-optimal for embryo transfer and is correlated to a decreased probability of pregnancy[2].

Evaluation of Endometrium

The various modalities for evaluation of endometrium are transvaginal ultrasound (TVS), hysteroscopy, sonosalpingography and MRI. Among these TVS is well established and first line for assessing endometrium as it is non-invasive and technically easy. Other modalities are either invasive or need expertise and are costly. During TVS, EMT is assessed by measuring maximum thickness from one stratum basalis interface to other stratum basalis interface excluding the surrounding inner myometrial lucency, in the sagittal plane or long axis[3]. Usually this measurement lies 1 cm from the fundal tip. Figure 2b shows the role of 3-D USG in evaluation of thin endometrium which may be because of adhesions, mullerian anomalies or intrauterine adhesions.

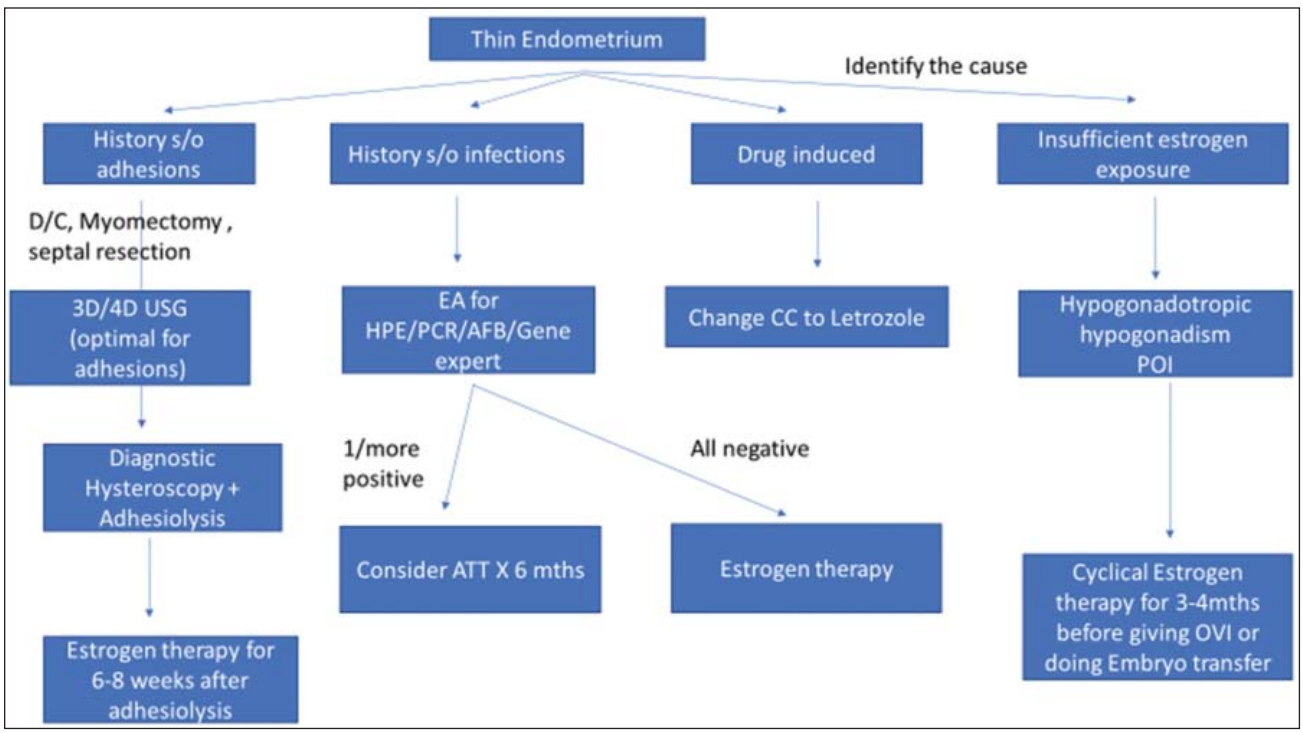

Fig 1. Management flow chart according to etiology.

Abbreviations: D/C, dilatation & currettage, EA, endometrial aspiration, HPE, histopathology, PCR, polymerase chain reaction, AFB, acid fast bacilli, ATT, anti-tubercular therapy, CC, clomiphene citrate, POI, premature ovarian insufficie ncy, OVI, ovulation induction.

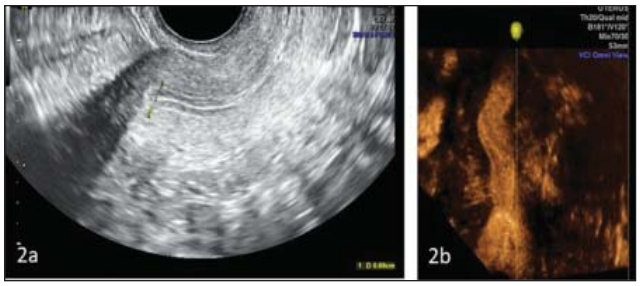

Figure: 2a. Thin endometrium, **2b.** Unicornuate Uterus

Pathophysiology and Management of Thin Endometrium

Thin endometrium is supposed to be caused by impaired endometrial growth, though there is little evidence with regards to its reasons. The first step to manage thin endometrium is to identify the likely cause of poor endometrial growth, as treatment, and response to treatment depends upon the underlying aetiology and extent of damage of basal layer of endometrium which gets replaced by fibrous tissue. The various causes of thin endometrium are presented in table 1.

Table 1: Causes of thin endometrium

Asherman syndrome 1. Uterine surgeries 2. Pelvic irradiation	• Aggressive dilatation and curettage • Hysteroscopic myomectomy/ polypectomy • Thermal ablation • Laparoscopic myomectomy (cavity opened)
Infective causes	• Tubercular endometritis • Septic abortion • Postpartum endometritis
Drugs	Clomiphene Citrate
Other causes	• Fibroids and adenomyosis involving endometrium • Mullerian anomalies (Figure 2) • Hypogonadotropic hypogonadism • Premature ovarian insufficiency

Various medical and surgical treatment modalities have been described in literature albeit with limited evidence of efficacy and successful pregnancy outcome. Sometimes its treatment is very challenging. Figure 1 illustrates the possible treatment strategies according to the aetiology.

If no medical or surgical risk factors are identified as a cause of thin endometrium, it is advisable to do a routine diagnostic hysteroscopy which enables us to evaluate the vascularity and glandular pattern of endometrium and to diagnose and treat adhesions if any. Various adjuvants that have been tried in the literature as detailed below.

I. Hormonal approach

a. Estrogen – This is the only drug proved to be beneficial in management of thin endometrium. It can be given by oral vaginal, transdermal and parenteral routes.

- Oral route is most common and preferred route as it is convenient and cheap though efficacy is reduced due to first pass metabolism which leads to higher estrone (E1) levels and higher ratio of estrone (E1) and estradiol(E2).
- Vaginal route bypasses the first pass metabolism and is more efficacious but preferred when there is poor response to oral estrogen.
- Transdermal route also bypasses first pass metabolism but absorption is unreliable leading to poor efficacy. The commonly available preparation is a 17-beta estradiol gel, and single application of 2.5 g of gel is equivalent to 2 mg of oral tablet.

Estrogen supplementation is most commonly used to prepare endometrial lining in frozen embryo transfer (FET) and rarely during fresh transfer when there is lag of endometrial growth during ovarian stimulation. During frozen embryo transfer estrogen supplementation is given in the form of oral estradiol valerate 2 mg three times a day from day 1 of menses for 12 days and upto 18 mg may be given depending upon the response unless patient is intolerant. Duration of estrogen therapy is important and up to 82 days of stimulation have been used in literature[4]. A newer form of estradiol (hemihydrate) is also widely used and in comparison to estradiol it has slower absorption and less systemic absorption, therefore suitable as vaginal tablets or transdermal application.

In patients who have long standing hypoestrogenic state (premature ovarian insufficiency, hypogonadotrophic hypogonadism), cyclical estrogen therapy (2-8mg per day) along with

progesterone (day 15-25) may be given for 3-4 months to improve vascularity of receptivity of endometrium before trying any infertility treatments.

Patients who have undergone hysteroscopic adhesiolysis or septal resection, estrogen therapy 4-8mg per day may be given for 6-8 weeks followed by progesterone withdrawal. This helps to prevent endometrial adhesions in postoperative period[5].

b. Human chorionic gonadotropin (hCG) priming in follicular phase- hCG is a glycoprotein secreted from syncytiotrophoblast and has many local and systemic functions during implantation. Since its receptors have been found on the endometrium even in follicular phase, suggesting action on angiogenesis and endometrial proliferation, it has been tried as 150 IU subcutaneous injection during follicular phase (from day 7 to day 11-12 once daily). However, if used in patients with normal EMT, adverse effect on endometrium was observed[6].

c. Gonadotropin releasing hormone (GnRH) agonist in luteal phase - Low dose GnRH agonist (0.1mg Triptorelin) during luteal phase (three doses: at oocyte pickup, embryo transfer (ET) and ET + 3days) has been recently explored to improve EMT and pregnancy rates[7].

***d.* Tamoxifen-** Tamoxifen is a selective estrogen receptor modulator with estrogenic effects on lower genital tract. Tamoxifen in a dose of 40 mg/day from day 3 of the menstrual cycle for 7 days with gonadotropins resulted in significantly increased endometrial thickness and pregnancy rate in comparison to clomiphene (100 mg/day for 5 days) in patients undergoing intrauterine insemination[8]. Similar results were seen in FET cycles where tamoxifen was given 20 mg per day from day 3 of cycle for 5 days along with 1mg /day of intravaginal 17-β estradiol tablets or 2mg/day oral estradiol from day 5 to day of ovulation, with most beneficial effect in PCOS patients[9].

II. Vascular approach

a. ***Low dose aspirin***

Low dose aspirin (75 -100 mg/day) has been commonly used as an adjuvant for thin endometrium as it improves uterine blood flow thus improving implantation. Some studies reported positive impact of low dose aspirin (100 mg aspirin from day 1 of menstrual cycle till pregnancy test) on EMT, pattern, and endometrial blood flow whereas, other have claimed no effect on implantation rate[10].

b. Sildenafil citrate- It is a phosphodiesterase type 5 (PDE5) inhibitor, which prevents breakdown of cGMP and increases effect of nitric oxide on vascular smooth muscles. This in turn improves uterine blood flow and promotes estrogen induced endometrial proliferation. Sildenafil citrate oral tablet 50 mg per day in addition to estrogen from day 1 of cycle till the day of progesterone in FET cycles resulted in significant increase in EMT and non-significant increase in pregnancy rates in comparison to estrogen alone[11].

III. Growth factor approach

a. ***Granulocyte colony stimulating factor (G-CSF)***

G-CSF is synthesized in humans to promote development of neutrophils. Recombinant G-CSF is most commonly used to treat bone marrow failure and myelosuppression. G-CSF intrauterine infusion 300 mcg was found beneficial in improving EMT and pregnancy rate in some small studies but not recommended as standard therapy[12].

b. ***Platelet-rich plasma (PRP) -*** PRP is blood plasma enriched with platelets, several growth factors and cytokines, and is prepared from autologous fresh whole blood[18]. A small pilot study showed promising results in terms of improvement in EMT and 50% increase in pregnancy rate when 0.5 ml of PRP was instilled in uterine cavity on day 10/11 and repeated again if required on day 13/14 along with estrogen therapy in FET cycles[13] but prospective randomised trials are required to conclusively establish its efficacy[14].

c. ***Stem cell therapy -*** As endometrial stem cells are present in both basalis and functionalis layers of the human endometrium, and these cells play a key role in regenerating

endometrial lining during each menstrual cycle, this formed the basis for stem cell therapy. In a most recent experimental study, 16 patients with refractory Asherman's syndrome or endometrial atrophy received autologous CD133+ bone marrow derived stem cells through the spiral arterioles by catheterization. Endometrial thickness increased about 2.4 mm and 1.5 mm in Asherman's syndrome and endometrial atrophy patients respectively. Three patients conceived spontaneously and seven pregnancies were achieved with ART[15].

The use of platelet-rich plasma or stem cells has only been described in patients with thin endometrium resulting from Asherman syndrome. Although these preliminary studies are promising for a population which has a poor prognosis and few options for treatment, further research and controlled studies are required given the invasiveness and expense of stem cell treatment.

Conclusion

- Thin endometrium is an infrequent but challenging occurrence in assisted reproduction.
- It is important to evaluate every patient individually to know the possible etiology to guide individualised management.
- Except for estrogen therapy, currently, there is minimal evidence to support any specific protocols or adjuvants to significantly improve pregnancy outcomes in patients with thin endometrium

References

1. Kimberly E. Liu, Michael Hartman, Alex Hartman. Management of thin endometrium in assisted reproduction: a clinical practice guideline from the Canadian Fertility and Andrology Society. Reprod Biomed Online. 2019 Jul;39(1):49-62.
2. Yuan X, Saravelos S.H, Wang Q, Xu Y, Li T.C, Zhou C. Endometrial thickness as a predictor of pregnancy outcomes in 10787 fresh IVFICSI cycles. Reprod Biomed Online. 2016;33:197–205.
3. Persadie R.J. Ultrasonographic assessment of endometrial thickness: a review. J Obstet Gynaecol Can. 2002;24:131–136
4. Chen M-J, Yang J-H, Peng F-H, Chen S-U, Ho H-N, Yang Y-S. Extended estrogen administration for women with thin endometrium in frozen-thawe din-vitro fertilization programs. J Assist Reprod Genet. 2006;23:337–42.
5. Xiong Q, Zhang T, Su S. A Network Meta-Analysis of Efficacy of Different Interventions in the Prevention of Postoperative Intrauterine Adhesions. Clin Transl Sci 2020 Mar;13(2):372-380
6. von Wolff M, Berkholz A, Wildt L. Evidence for cycle dependent expression of full-length human chorionic gonadotropin / luteinizing hormone receptor mRNA in human endometrium and decidua. Fertil Steril. 2003;79(Suppl 1):718–23.
7. Qublan H, Amarin Z, Al-Qudah M, Diab F, Nawasreh M et al. Luteal phase support with GnRH-a improves implantation and pregnancy rates in IVF cycles with endometrium of <or=7 mm on day of egg retrieval. Hum Fertil (Camb). 2008;11:43–7.
8. C.W Wang, S.G Horng, C.K Chen, H.S Wang, H.Y Huang, C.L Lee, et al. Ovulation induction with tamoxifen and alternate-day gonadotrophin in patients with thin endometrium, Reprod Biomed Online. 2008;17(1):20– 26.
9. H Ke, J Jiang, M Xia, R Tang, Y Qin, Z.J Chen. The effect of tamoxifen on thin endometrium in patients undergoing frozen-thawed embryo transfer. Reprod Sci. 2018;25(6):861-866.
10. Y.Y Hsieh, H.D Tsai, C.C Chang, H.Y Lo, C.L Chen. Low-dose aspirin for infertile women with thin endometrium receiving intrauterine insemination: a prospective, randomized study. J Assist Reprod Genet. 2000;17(3):174–177.
11. Dehghani Firouzabadi R, Davar R, Hojjat F, Mahdavi M. Effect of sildenafil citrate on endometrial preparation and outcome of frozen-thawed embryo transfer cycles: a randomized clinical trial. Iran J Reprod Med. 2013;11: 151–158
12. Kunicki M, Lukaszuk K, Liss J, Skowronska P, Szczyptanska J. Granulocyte colony stimulating factor treatment of resistant thin endometrium in women with frozen-thawed blastocyst transfer. Syst Biol Reprod Med. 2017;63:49–57.
13. S Zadehmodarres, S Salehpour, N Saharkhiz, L Nazari, Treatment of thin endometrium with autologous platelet-rich plasma: a pilot study. JBRA Assist Reprod. 2017;21(1):54–56.
14. Noemie Ranisavljevic, Jade Raad, Tal Anahory, Michaël Grynberg, Charlotte Sonigo. Embryo transfer strategy and therapeutic options in infertile patients with thin endometrium: a systematic review. J Assist Reprod Genetics. 2019;36(11):2217-2231.
15. X Santamaria, S Cabanillas, I Cervello, C Arbona, F Raga, J Ferro, et al. Autologous cell therapy with CD133+ bone marrow-derived stem cells for refractory Asherman's syndrome and endometrial atrophy: a pilot cohort study. Hum Reprod. 2016;31(5):1087–1096.

How Can One Reduce Time to Pregnancy by IVF

Abha Majumdar
Director & Head, Centre of IVF and Reproductive Medicine

It Is A Reality to Reduce Time to Pregnancy by IVF!

Ever since its inception, the primary aim for IVF has been to, perhaps, reduce time to pregnancy for infertile couples. As clinicians, referring doctors and infertility specialists, one expects a pregnancy to be achieved as soon as possible after starting an IVF cycle. Historically, ovarian hyper stimulation followed by transfer of multiple embryos was the first step towards achieving this goal. Nevertheless, in the bargain, we also increased the rate of multiple pregnancies and its antecedent complications along with pregnancy losses. This in fact lead to a delay in achieving pregnancy and further lengthened the time to live birth. Hence forth, scientists have been in search of a strategy which could truly reduce time to pregnancy in terms of live birth rate, without raising the complication rates. This has now been made possible to some extent by Preimplantation genetic screening (PGS) or Preimplantation genetic testing for aneuploidy (PGT-A) of embryos generated during IVF. PGT-A, helps identify karyotypically normal embryos known as Euploid embryos from the abnormal ones called Aneuploid. Euploid embryos, having a higher implantation potential, thus, can reassuringly be placed back into the uterus singularly, giving the highest chance of a singleton live birth in the shortest period of time.

What is PGS?

PGS is a genetic test designed to screen embryos for numerical as well as structural chromosomal abnormalities. In PGS, embryos are biopsied mostly at the blastocyst stage (day 5 or 6 of embryonic development in vitro) followed by comprehensive chromosomal screening for all 24 chromosomes (22 pairs of autosomes and two sex chromosomes). Embryos that are found to have either an extra or a missing chromosome(s), termed as Aneuploid, are deselected for transfer as they can potentially result in failure of implantation, early pregnancy loss or birth of a baby with a congenital malformation or mental retardation. By transferring only euploid embryos, with the correct number of chromosomes, in the uterus, PGS aims to improve the likelihood of implantation and pregnancy in couples undergoing IVF. PGS could especially benefit those infertile couples who are at a higher risk of producing aneuploid embryos such as those in the advanced reproductive age group or having a history of recurrent pregnancy losses or who have had a previous aneuploidy conception.

Why Do Women with Advanced Age Benefit from IVF with PGS?

Women are born with a fixed number of oocytes, they will ever have, at birth. Over time, the number of oocytes steadily decrease by the process of atresia and apoptosis, till eventually one day, the ovary gets completely depleted. It is at this stage that menopause sets in and women stop menstruating. Simultaneoulsy, as a woman's age progresses, the genetic component of these oocytes i.e. chromosomes also become more and more prone to damage. As a result of ageing, a genetically abnormal oocyte even though, still fertilizable by a sperm, will result in a genetically non-viable aneuploidy embryo. As, we do not have any objective markers for qualifying egg quality, we mostly use age as a marker to indicate where a woman's egg quality stands. While theoretically, oocyte quality would be expected to constantly recede throughout a woman's life, through practice we know that it stays stable until age 34. During these years, about 60% of a woman's oocytes are still healthy and viable, while the other 40% are probably of poor quality that will not result in a live birth. At age 35, this ratio is down to 50% and it continues to deteriorate at a much more rapid rate as age advances towards late thirties. By the age of 38 years, more than 70% of oocytes may have become prone to numerical chromosomal abnormalites and beyond the age of 40, 80 to 90% of oocytes may have been rendered aneuploid. This biological phenomenon may render Indians, who

are born with a smaller genetic pool of oocytes as compared to Caucasians, to a more rapid decline in numbers as well as quality of oocytes, which may therefore result in an earlier menopause.

How Does PGS Reduce The Time to Pregnancy?

Let us take an example to understand this; a 39-year old woman undergoing IVF has 3 blastocyst stage embryos on day 5 of embryonic development in vitro. As per available data, using her age as marker of her egg quality, at 39, we would assume only one out of the 3 embryos to be chromosomally normal and capable of implantation. Also, ideally, one would want to transfer only one of these three embryos back into the uterus, as more than one, would again increase her risk of having a higher order pregnancy, which itself would impose an increased risk to the health of the pregnant mother as well as to the babies. Unfortunately, we know that having a good morphology doesn't preclude an embryo of the ill effects of advanced age, which means that, at 39, there is a significant chance that even the most 'ideal' looking embryo selected for transfer will be aneuploid. However, in case, the woman does get pregnant but the pregnancy ends in a miscarriage, it becomes a lengthy process as she may end up wasting as much as 6 months of crucial time before trying again with the remaining embryos. Time is especially important for a woman of advanced age, as with passing time there may be further decline in oocyte numbers and quality both. Therefore, in such patients who are at a high risk of aneuploidy, PGS may become a pertinent and effective tool to identify and select a 'normal' embryo, thus lowering the possibility of a miscarriage at this crucial juncture. Therefore, the expected benefits of offering PGS in women with advanced age would manifest in the form of the following:

- Higher implantation and clinical pregnancy rates
- Reducing the chance of having a miscarriage
- Lesser number of abnormal or aneuploid conceptions
- Improved outcomes in elective single embryo transfer cycles
- Reduction in multiple pregnancy rates
- Decreased time to pregnancy which increases the cost effectivity of IVF

Does PGS Have Any Down Sides?

PGS is used in conjunction with in vitro fertilization (IVF) and uses sophisticated and scientifically validated technology. PGS examines all 24 chromosomes, the 22 non-sex chromosomes, plus the two sex chromosomes (X & Y) for any gains or losses, otherwise known as aneuploidy. Only embryos with the correct chromosome complement are transferred. Sometimes PGS detects embryos with a condition labelled as *mosaicism* which describes a situation in which different cells in the same embryo have different numbers or arrangements of chromosomes. Mosaicism imposes the risk of labelling the embryo normal or abnormal depending on the proportion of such cells in the cluster of 6 to 8 cells taken out for biopsy. Many a times a normal embryo may be discarded based on mosaicism and vice a versa. Therefore, when only mosaic embryos are available for transfer, genetic counselling is advised to assess the chance of implanting or discarding such embryos prior to embryo transfer.

What is the Process for PGS?

Typically, the entire procedure consists of five different steps, usually performed by different experts and different laboratories. However, in our IVF centre the whole procedure is done under one roof not needing to transport biopsy samples to other laboratories.

1. Patients having PGS undergo ovarian stimulation and IVF-ICSI to create embryos which are cultured to the blastocyst stage. This process is managed by the combined team of the IVF clinician and embryologist.
2. The second step involves embryo biopsy performed by an embryologist at the appropriate stage of the embryo. As per international norms, in our centre, biopsy is done only on competent blastocyst stage embryos on day 5 or 6 of embryonic development.
3. The biopsied cells are then usually transported to an appropriate genetic laboratory for further genetic testing. In 2014, we successfully set up an in house genetic testing facility using qPCR technology to screen embryos for aneuploidy. Therefore, no transportation is required.
4. CCS is performed using a specially designed qPCR

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based genetic platform by a team of scientists from our genetic and embryology laboratory. At the end of testing, results are carefully analysed in consultation with our collaborator genetics laboratory in Italy, and each embryo is marked with a diagnosis as 'normal' or 'abnormal'.

5. This method takes only up to 4 hours to complete the test, therefore, allowing the possibility of performing a fresh embryo transfer, if required. All other methods used for CCS such as NGS or array CGH do not afford this benefit as it takes much longer for the test to be completed (~24 h or more), thereby necessitating an embryo transfer only in a subsequent cycle post cryopreservation.

Will PGS also Help in Building a Family Later in Life after the First IVF?

As a fertility specialist, this is certainly to be kept in our minds, that most women come back after 2 or 3 years to have another child. However, having a second child may not even be on the radar of a woman in her late 30s or early 40s who is trying for her first child. Also, in case a woman was having trouble getting pregnant at the age of 39, it will almost certainly be harder for her to have another baby at 41, when the oocyte number and quality may have drastically declined further. With the help of IVF and PGS, if done at the first instance, any supernumerary cryopreserved euploid embryos could come in very handy while trying for a second child a few years down the lane. Therefore, embryo banking following IVF coupled with PGS would provide a very good opportunity for women of advanced age to try for a second child.

In my opinion it would be good to counsel women to opt for PGS with IVF especially when of advanced age or repeated IVF failures with an ovarian reserve good enough to still make 3 to 4 blastocysts following one good controlled ovarian stimulation. This would not only increase their chances of getting a live birth in the smallest possible time but would also prevent the high cost of multiple cycles of IVF.

Answer: July 2020 Issue

Crossword

1. Glycerol
2. Kallman
3. CONUTA
4. Klinefelters
5. Ulipristal
6. Inhibin
7. Synechiae
8. Edwards
9. Pergonal
10. EFI
11. HFEA
12. Fifteen
13. ERA

PGD: Eliminating heritable diseases from the family

Gaurav Majumdar
Chief Clinical Embryologist, Center of IVF, Sir Ganga Ram Hospital, New Delhi

Preimplantation Genetic Diagnosis (PGD) is a procedure, in which a couple, carrying an inherited genetic mutation, goes through *in-vitro* fertilization (IVF), followed by preimplantation genetic analysis of the resulting embryos to look for the mutant gene and implant only those embryos that do not carry it. Ordinarily IVF is a treatment meant for infertile couples trying to conceive. However, IVF in conjunction with PGD is normally sought by fertile couples, but who are carriers of a genetic disorder, looking to conceive a disease-free child. The challenging part of the whole process is to successfully perform genetic testing on the DNA from single cells removed from each embryo, to determine the presence of the mutation.

PGD is being offered for a host of single gene disorders around the world thus enabling numerous people to use this technology to avoid passing genes for serious disorders to their children. PGD involves taking a biopsy of embryonic tissue from an embryo followed by its genetic analysis for the detection of either an inherited mutation that affects the functioning of a specific gene, or even numerical chromosomal abnormalities, followed by subsequent replacement of the genetically 'normal' embryos back into the patient, therefore providing a high probability that the resulting pregnancy is healthy. The basic principle behind PGD is to be able to distinguish healthy embryos from those affected with genetic abnormalities. In essence, PGD is very similar to prenatal diagnosis (PND), since in both situations embryonic tissue undergoes genetic analysis to determine the presence of disease or abnormality. But in contrast to PND, where measures are taken to abort a pregnancy only once the 'damage' has been done, PGD helps in pre-empting disease before pregnancy is established and hence helps in avoiding the task of pregnancy termination in case an affected foetus is conceived. In surveys, a majority of couples at risk for severe genetic diseases indicate they prefer PGD to getting prenatal testing later via amniocentesis and facing the wrenching decision of whether to terminate a pregnancy.

Evolution of PGD Technologies

In the PGD procedure, IVF is used to generate embryos, from which single cell(s) are biopsied for genetic testing. The genetic analysis, to begin with, relies on the use of polymerase chain reaction (PCR) for amplification of the biopsied single cell DNA, to a level that is sufficient as well as necessary for conducting any standard genetic tests, with the main challenge being the successful amplification of the single cell DNA without any contamination. Handyside, Winston and Hughes described the first clinical application for PGD in 1990, for sexing embryos from couples with sex linked disorders (Handyside et al. 1990) typically affecting only hemizygous males, have been identified. In many of these, prenatal diagnosis is possible by chorion villus sampling (CVS. They used PCR to amplify a Y chromosome repeat sequence and therefore were able to detect the female embryo by the absence of the Y chromosome on gel electrophoresis. Subsequently PGD was offered to couples that were known carriers of single gene defects (Verlinsky et al. 1990, 1992) genetic analysis of the first polar body allows the identification of oocytes that contain the maternal unaffected gene. These oocytes can be fertilized and transferred to the mother without risk of establishing a pregnancy with a genetically abnormal embryo. We have demonstrated that removal of the first polar body has no effect on subsequent fertilization rates or embryonic growth to the blastocyst stage. We have developed a PCR technique to successfully analyze the PI type Z and PI type M genotypes of alpha-1-antitrypsin deficiency and applied this technique for a couple at risk for PI type ZZ alpha-1-antitrypsin deficiency. After standard IVF treatment to stimulate multiple follicle development, eight oocytes were aspirated transvaginally. Polar bodies were removed by micromanipulation from seven oocytes and

fertilization occurred in six cases. PCR analysis was successful in five oocytes. One was PI type M, two were PI type Z and two were heterozygous MZ due to crossing over. Embryos from the two oocytes containing the unaffected gene (polar body PI type Z or chromosomal translocations (Munné et al. 2000) including telomeric probes. Design: Retrospective study. Setting: Clinical IVF laboratory. Patient (s. As the testing was performed to detect a known genetic disorder, the methodology was termed as preimplantation genetic diagnosis (PGD or PIGD).

Gradually, PGD technologies incorporated screening for numerical chromosomal abnormalities or aneuploidies by infertile patients undergoing IVF to try and improve treatment outcomes. Therefore, to differentiate the use of the term 'PGD' in those patients who did not have or carry genetic disorders, the term PGD for aneuploidy screening (PGD-AS) or preimplantation genetic screening (PGS) was adopted. These patients had indications where the risk of aneuploidy was high, and thus warranted 'screening' all the chromosomes as opposed to its earlier use in patients with existing genetic disorders. PGS was initially performed in 1993 by Munne and colleagues (Munné et al. 1993). They used fluorescent in situ hybridization (FISH) for detecting chromosomal abnormalities, the first instance of using PGS, for five chromosomes X, Y, 13, 18 and 21.

How is the Technology Used?

Following controlled ovarian hyper stimulation with gonadotropins, eggs are retrieved from the mother and fertilized by the father's sperm in a petri dish in the IVF laboratory. The resulting preimplantation embryos are then grown in culture dishes for 3 to 5 days in vitro before which an embryo biopsy is performed to remove a few cells from each embryo. In the beginning, embryo biopsy for PGD was performed on day 3 of embryonic development when the embryo is at the 8-cell stage. However, now clinics wait till day 5 or 6 before which a few cells are biopsied from the embryo now termed as a blastocyst (Fig 1.). As the cells that are removed for testing belong to the trophectoderm, the layer that later develops into the placenta, and not from the inner cell mass or the 'embryo proper', the latter approach is considered to have a considerably lower impact on embryo viability.

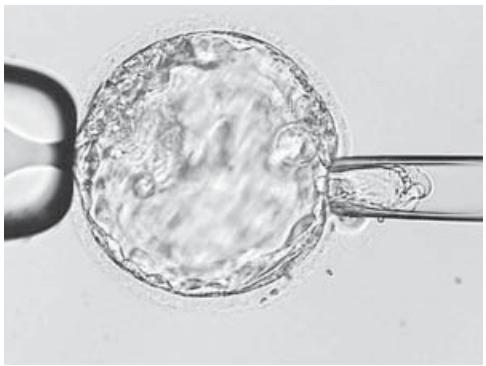


Fig 1: A clump of trophectoderm cells being biopsied from a day 5 blastocyst for PGD analysis.

After biopsy, each embryo is individually labelled and cryopreserved, and the corresponding biopsies are sent to the genetics laboratory for PGD analysis. Once the results of genetic analysis are obtained after approximately 7 to 10 days, and the presence of healthy embryos devoid of the genetic mutation are confirmed, an embryo transfer is planned for transfer of 1 or 2 normal embryos back into the mother. In the past, clinics often recommended two, but now some clinics are adopting a policy of single embryo transfer, believing that a single-embryo pregnancy has a much greater chance of success in these precious PGD conceptions, avoiding the added complications and co-morbidities linked with twin or triplet conceptions.

Treatment Spectrum

With the use of PGD growing steadily over the past few years in the country, PGD is now offered for a host of single gene disorders including autosomal recessive diseases that may appear early in life such as blood disorders like beta – thalassemia and haemophilia, some late onset dominant conditions like fragile X syndrome and hypertrophic cardiac myopathies, as well as some X-linked disorders like Bruton Type A gammaglobulineamia, thus enabling more people to use this technology to avoid passing genes for serious disorders to their children. Few of the disorders that have been treated successfully with PGD and IVF thus far at Sir Ganga Ram IVF, and resulted in births of babies born free of disease include thalessemia, citrullinemia, retinitis pigmentosa, ADPKD, leukodystrophy, congenital erythropoietic porphyria, hypertrophic cardiacmyopathy, Aicardi-Goutieres Syndrome, Charcot Marie Tooth 2A and Leigh Syndrome. Unfortunately, since embryo sexing is not allowed

in our country, PGD for X-linked diseases cannot be used to reveal the carrier status of embryos, which therefore doesn't serve to eliminate the mutant gene from the family germline and thus acts as a deterrent for such couples who are seeking PGD treatment. Many such couples who do not desire a girl child only for the fear of a continued legacy of the disease in the family have no choice but to opt out of treatment. On the other hand, the use of PGD for adult-onset neurological disorders remains quite limited. In the United States, only a small percentage of Huntington's families have used it, and a mere handful of families with autosomal dominant Alzheimer's disease are known to have done so, mainly due of lack of awareness. While some ethicists argue, that for most late onset diseases it is not fair to deny life to embryos carrying genes that could lead to a disease only after 30 to 50 years of healthy life, most reproductive medicine societies around the world support the use of PGD by fertile couples to prevent serious adult onset diseases. Recently we reported the birth of a healthy baby through PGD and IVF to a family carrying the mutation for an adult onset, neurological disease Charcot Marie Tooth A2, being one among only a handful more reports published in scientific literature.

Ethical Issues Concerning PGD

Since embryo biopsy is a prerequisite towards preimplantation testing, in the beginning, clinical embryologists, who traditionally have been trained to minimize any form of disturbances during embryo culture, had been understandably reluctant in performing invasive micromanipulation procedures on embryos in order to remove cells for the fear of causing potential damage to an embryo. However, in due course of time, it has become clear that in the context of a couple trying to avoid a pregnancy affected by an inherited disease, the risks to the embryo get overshadowed by benefits of the testing, as prevention of a serious inherited disease is the ultimate aim.

References

1. Munné, S, Lee A, Rosenwaks Z, Grifo J, and Cohen J. 1993. "Diagnosis of Major Chromosome Aneuploidies in Human Preimplantation Embryos." *Human Reproduction* 8 (12): 2185–91.
2. Munné S, Sandalinas M, Escudero T, Fung J, Gianaroli L, and Cohen J. 2000. "Outcome of Preimplantation Genetic Diagnosis of Translocations." *Fertility and Sterility* 73 (6): 1209–18.
3. Munné, S., T. Dailey, K. M. Surtan, J. Grifo, and J. Cohen. 1995. "The Use of First Polar Bodies for Preimplantation Diagnosis of Aneuploidy." *Molecular Human Reproduction* 1 (2): 94–100.
3. Handyside A, Kontogianni E, Hardy K, and Winston R. 1990. "Pregnancies from Biopsied Human Preimplantation Embryos Sexed by Y-Specific DNA Amplification." *Nature* 344 (6268): 768-770.
4. Verlinsky Y, Ginsberg N, Lifchez A, Valle J, Moise J, and Strom C. 1990. "Analysis of the First Polar Body: Preconception Genetic Diagnosis." *Human Reproduction* 5 (7): 826–829.
5. Verlinsky Y, Rechitsky S, Evsikov S, White M, Cieslak J, Lifchez A, Valle J, Moise J, and Strom C. 1992. "Preconception and Preimplantation Diagnosis for Cystic Fibrosis." *Prenatal Diagnosis* 12 (July 1991): 103–110.

Ans 1. Grade 4AA blastocyst
Ans 2. Picture of Intracytoplasmic sperm injection showing
From left to right: Holding pipette, M2 oocyte, Injection Pipette

Oocyte Cryopreservation- Freezing the biological clock!

Neeti Tiwari
Consultant, Centre of IVF and Human Reproduction, Sir Ganga Ram Hospital, New Delhi

Introduction

Cryopreservation of gametes and embryos brought a revolution in the field of reproductive medicine. It opened the avenues for individuals who were facing potential loss of reproductive capacity e.g. those requiring gonadotoxic therapy, to have their own biological offspring in future. The first human birth with frozen sperm was reported in 1953 but freezing the oocyte was technically more challenging. In 1986, the first human birth with frozen oocyte was reported.[1] Since then there has been extensive research going on in the field of oocyte cryopreservation and vitrification has gradually taken over the slow freezing process making it more efficient. Another important development is the use of this technology for women who for social reasons want to freeze their oocytes so that they can delay childbearing without losing on to their biological clock.

Oocyte Cryopreservation Technology

Cryopreservation refers to bringing down the temperature of cells or tissues to sub-zero levels so that all biological processes come to a standstill and the cells can be preserved for future use. (Figure 1) The challenge in cryopreservation is to prevent cellular damage by intra- and extracellular ice formation and excessive dehydration for which cryoprotectants like propanediol, ethylene glycol and dimethyl sulfoxide (DMSO) are used. Oocyte freezing is done at M II stage (metaphase II) which is a large cell with more water content and it has metaphase chromosomes lined up by the meiotic spindle along the equatorial plate (Figure 2). All these make a mature oocyte more liable to cryodamage. This explains the delay in development of successful oocyte freezing programs and utmost need of expertise and experience of embryologist handling it. Many modifications have been made in the oocyte cryopreservation techniques which have attributed to the improved post thawing survival of the frozen oocytes. Newer developments have been made in the concentration and composition of cryoprotectants. Vitrification has gradually taken over the process of 'slow freezing' for embryos and oocytes. In slow freezing the freezing occurs at a slow enough rate to allow sufficient cellular dehydration to prevent intracellular ice formation and it requires programmable freezers. In vitrification, on the other hand, cryopreservation is done by using higher concentrations of cryoprotectants and ultra-rapid cooling so that an intracellular 'glass' like state is achieved without ice formation. Though with time oocyte recovery rate after thawing has improved with both slow freezing and vitrification recent studies suggest that vitrification yields better results in terms of oocyte recovery rate and pregnancy rates.[2] Hence, today most of the IVF laboratories worldwide are freezing oocytes by vitrification.

Fig 1: M2 oocyte with extruded polar body

Fig 2: Cryo tech device used for freezing oocytes and a cytoplasmic inclusion

Indications of Oocyte Freezing

1. **Patients receiving gonadotoxic therapies:** Fertility preservation in women undergoing

gonadotoxic chemotherapies and radiotherapy is slowly becoming popular and acceptable with the advances in cancer therapy leading to improved longevity of young cancer survivors. In addition, there are also women who undergo bilateral oopherectomy for benign and malignant conditions who can benefit. For women who are married or have a stable partner, embryo cryopreservation is a viable option. For others especially young post-pubertal girls mature oocyte banking is an attractive strategy to preserve their own genetic pool for future use. Though data is still limited on the pregnancy and live birth rates with use of these cryopreserved oocytes in cancer survivors it is imperative to discuss this option with patient and her family.

2. **Certain genetic conditions:** There are certain genetic conditions which are associated with premature ovarian failure e.g. Turner's syndrome and Fragile X premutation whereby when these women plan childbearing their ovaries are already exhausted. If an early diagnosis is made while still some ovarian reserve is left these women may become a candidate for oocyte freezing. Whenever fertility preservation is offered to these women there is a concern about risk of chromosomal abnormalities in the offspring and safety of future pregnancies. In addition, there are women who are carriers of BRCA gene mutations which puts them at higher risk of breast and ovarian cancer, hence are advised prophylactic salpingo-opherctomy. Mostly this prophylactic surgery is done after the family is complete, in later part of reproductive life. But in case it is advised before childbearing, oocyte freezing can be recommended for fertility preservation. There is a concern amongst BRCA mutation carriers that ovarian stimulation during fertility preservation may increase their chances of developing breast cancer. A case control study on 1380 matched pairs of women with BRCA 1 and BRCA 2 gene mutations showed no adverse effect of fertility preservation on development of breast cancer as compared to controls.[3] Ovarian stimulation protocols based on aromatase inhibitors e.g. letrozole keep the serum estradiol levels low and further ameliorate this concern. The risk of transmission of BRCA mutation to the offspring can be avoided by performing PGD on the resulting embryos. Hence fertility preservation is necessary and safe in BRCA gene mutation carriers and oocyte cryopreservation is the most reliable procedure.[4]
3. **Failure to obtain sperms on day of oocyte retrieval:** All of us who are doing IVF know that despite all our precautions like prior sperm freezing we do encounter this situation of unavailability of partner's sperms or sufficient number of sperms on the day of oocyte retrieval. It mostly happens where testicular sperms have to be used in cases of nonobstructive azoospermia and hence prior counseling of such couples is important. The options available for the couple is either to use donor sperms the same day to fertilize oocytes or to cryopreserves the oocytes. If oocytes are frozen a repeat attempt can be made in future to obtain partner's sperms or the couple gets time to reconsider the decision of use of donor sperms.
4. **Unable to cryopreserve embryos:** In certain countries like Germany cryopreservation of embryos are not allowed. Some couples themselves may not want to freeze their extra embryos left after fresh transfer due to religious or social reasons. In such conditions oocyte cryopreservation can take care of the supernumerary eggs for future use and hence can improve the cumulative success rate of the cycle.
5. **Oocyte banking – The future:** Egg donation opens the doors of fertility to many women for whom otherwise it would have been very difficult to realize their dream of parenthood e.g. women with premature ovarian failure, advanced age, transmissible genetic conditions and recurrent implantation failure. According to ART (Regulation) Bill framed by ICMR, oocyte donation should be anonymous i.e. the ART clinic under no circumstances (except when asked by court of law) reveal the identity of donor to recipient couple or to anyone else.[5] This anonymity of oocyte donors can be maintained only when we start cryopreservation of donor oocytes. Oocyte cryopreservation offer other advantages also in a donor oocyte program. It allows oocytes from one donor to be donated to multiple recipients permitting optimal usage of oocytes. Donor oocytes can be quarantined and

screened for all sexually transmissible diseases before donation (presently donor is screened). Recipients will not have to be in a waiting list and can obtain donor oocytes from an 'oocyte bank' whenever they want. Presence of sperms of recipient couple at the time of oocyte retrieval of the donor also will not be a pre-requisite making the procedure less tedious. Oocyte banks are still not a reality in India because of lack of required technology and expertise amongst embryologists but it definitely holds promise for future.

6. **Social egg freezing (*Planned oocyte cryopreservation*):** Last few decades have seen an increased access of women to higher education and they have emerged as an important workforce. These changing social structure has put women in a new predicament, as in their 20s and 30s when they are busy in education and career that is also the best time for childbearing. There is a progressive loss of oocyte quantity and quality with women's age and this pattern accelerates after 35 years making the women prone to infertility and pregnancy losses. To begin with oocyte cryopreservation was done only as an 'emergency' procedure in women heading for gonadotoxic therapies but soon it opened the door for women who wanted to 'freeze the biological clock' or defer childbearing. Technology like egg freezing could help them to have their own biological children later in life. Initially this idea met with lot of criticism and international societies like American Society of Assisted Reproduction (ASRM) and Society of Assisted Reproductive Technologies (SART) in 2013 in a guideline concluded that data on safety, efficacy, cost-effectiveness and emotional risks are not sufficient to recommend elective cryopreservation for the purpose of deferring childbearing. Despite this many women were willing to opt for elective egg freezing and many physicians were keen to provide this opportunity to women. Meanwhile the technology improved and concerns regarding the efficacy of the procedure were alleviated to some degree. Finally, in 2018, ASRM came out with an ethics committee opinion which approved oocyte cryopreservation to help women who would have faced infertility or increased risk of chromosomal abnormalities due to high maternal age. They also commented that the word 'social' egg freezing sounds trivial and insignificant hence in such circumstances it should be called 'planned oocyte cryopreservation'.[6]

Advantages of Planned Oocyte Cryopreservation

1. Planned oocyte cryopreservation allows women to have reproductive autonomy. If they wish they can concentrate on their education or career without the fear of losing on their biological clock. It also allows them time to establish suitable relationships and circumstances for childbearing and rearing a family.
2. It also potentially eliminates the need of third party reproduction i.e. oocyte donors with its financial and emotional implications. To be able to bear one's own genetic progeny is a big advantage.
3. If embryos are frozen both partners have rights over it while a woman has sole rights over her gametes. This becomes important if the male partner retracts consent for future use of embryos after separation and can lead it to a legal dispute. Hence planned oocyte cryopreservation gives an opportunity to woman to freeze her own gametes if she doesn't wish to preserve embryos even if partner is present.
4. Planned oocyte cryopreservation promotes social justice and contributes to equality between men and women at workplace. It takes away the disadvantage woman faces because her reproductive life being smaller than man's.

Challenges with Planned Oocyte Cryopreservation

1. There is an expected decline in success rates when oocyte cryopreservation happens at an advanced age. Often women consider oocyte freezing after the age of 37 years when the ovarian reserve is already compromised. A large Italian retrospective study on 450 couples who used previously vitrified oocytes (supernumerary in this case) in oocyte thaw cycles found that live birth rates were inversely correlated with maternal age at the time of oocyte retrieval.[7]
2. Limited data exists on the effect of duration

of storage on the survival rate of oocytes and pregnancy rates.

3. Since there have not been many births after use of frozen oocytes long term safety of offspring is still not well established. Though short-term data shows no increased risk of congenital anomalies in infants born with frozen oocytes as compared to conventional IVF.[8]
4. The expense and the risk taken of an invasive procedure might prove futile if the woman never returns to use the oocytes or conceives naturally in future.
5. It might give a 'false sense of security' to women that once their oocytes are frozen they can have a successful healthy childbirth at any age. Hence counseling and informed consent is very important prior to enrolling women in this program. For example if they come at the age of 38 years they should be told that they would need 25-30 oocytes cryopreserved to have a reasonable chance of one live birth and since ovarian reserve is low they would require multiple cycles.[9]
6. There is also a concern that planned oocyte cryopreservation may promote late childbearing. Women may feel that they can plan pregnancy at any age and may deliberately postpone childbirth for other priorities and life plans. It might be unjust for the children thus born to have 'old' parents. Besides the medical risks of pregnancy at advanced age to mother and neonate also should be clearly mentioned to the woman interested in egg freezing.

Important Considerations before Social Egg Freezing

1. Single women in thirties who desire their own genetic child in future should consider oocyte cryopreservation before the age of 37 years to have best results.
2. The IVF centre needs to be honest and specific about their experience in oocyte freezing. They should be able to give data of their own centre's post-thaw survival rate of frozen oocytes, how many patients have used their frozen oocytes and how many pregnancies resulted thereafter.
3. Informed consent should be taken after thorough counseling before enrolling the woman for social egg freezing program. They should be told about the procedure of oocyte cryopreservation and the risks involved. She should be told her realistic chances of having a livebirth later with her frozen oocytes depending upon her age. Consent regarding future disposition of oocytes in the event of death, marriage or divorce should also be taken.
4. Currently follow up data is scarce because very few women have come back to use their frozen oocytes.

Our Experience at SGRH

At SGRH we started oocyte cryopreservation in 2008 and we reported our first livebirth with cryopreserved oocytes in 2009. Table 1 shows the number of cycles done so far with their indications and utilization.

Table 1: Data of oocyte cryopreservation at SGRH

Indications	Number (total= 46)	Utilization	Pregnancies
Social egg freezing- single women	24	0	0
Donor- recipient cycles	9	7	4
Emergency egg freezing	12	6	2
Fertility preservation	1	0	0

As we can see in this table majority of egg freezing cycles have been done for single women who wanted to preserve their oocytes for future use for varied reasons like higher education, career or lack of a stable partner. None of these women, till date have come to use their oocytes. Although few women who were about to receive gonadotoxic chemotherapy came to centre to enquire about fertility preservation only one got it done after the counseling. Emergency egg freezing in the event of unavailability of sufficient sperms were done in 12 cases while in 9 cases we did freezing of donor oocytes. Only in 13 patients the frozen oocytes were thawed for use out of which embryos could be formed in 9 patients. These 9 patients went through embryo transfer and pregnancy was reported in 6. Table 2 gives the data of the 78 oocytes in total which were thawed. Out of 78 oocytes, 76 survived the thawing process but ICSI could be done on 73.

Table 2: Oocyte thawing data of SGRH.

Key performance indicators	Number
Survival rate	76/78 (97.4%)
Fertilization rate	47/73 (64.4%)
Pregnancy rate per embryo transfer	6/9 (66.7%)
Pregnancy rate per patient	6/13 (46.2%)

With increasing experience we are trying to introduce more egg freezing in our oocyte donor cycles so that we can achieve anonymous donation and avail the advantages reported earlier. We are also collaborating with our hemato-oncology and surgical oncology departments to counsel women actively for fertility preservation though in our country there are still few takers as compared to our western counterparts.

Conclusion

With the rapid development in cryo-technology there is enough data to support that fertilization rates and pregnancy rates are similar when fresh or frozen oocytes are used for IVF-ICSI. The initial short-term data on safety in children born out of cryopreserved oocytes, in terms of birth defects is reassuring but long-term data on development of these children is awaited. In post-pubertal girls and young women facing gonadotoxic therapies fertility preservation by cryopreservation of oocytes should be offered after thorough counseling. The future of oocyte banking lies in the efficient oocyte cryopreservation program. As we can see from our experience, social egg freezing is emerging as an attractive option for women who want to delay childbearing for education or career needs. It has given new meaning to reproductive autonomy in women. At the same time there is also a concern that women should not get a 'false sense of security' and deliberately postpone childbirth. Informed consent after counseling is important where the centre should disclose their own success rates and experience in the oocyte cryopreservation.

Fig 3: Goblet for storing straws/devices

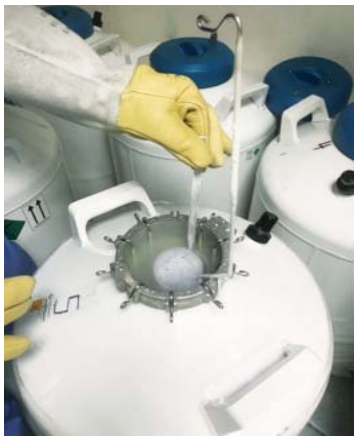


Fig 4: Cryocan filled with liquid nitrogen used for the storage of Goblets on which 1-3 oocytes have been frozen.

References

1. Chen C. Pregnancy after human oocyte cryopreservation. Lancet 1986;1: 884–6. Level III.
2. The practice committees of American Society of Assisted Reproduction and Society of Assisted Reproductive Technology. Mature oocyte cryopreservation: a guideline. Fertil Steril 2013 Jan;99(1):37-43.
3. Kasum M, von Wolff M, Franulić D, Čehić E, Klepac-Pulanić T, Orešković S, et al. Fertility preservation options in breast cancer patients. Gynecol Endocrinol. 2015;31(11):846–51.
4. Zhang X, Niu J, Che T, Zhu Y, Zhang H, Qu J. Fertility preservation in BRCA mutation carriers – efficacy and safety issues: a review. Reprod Biol Endocrinol. (2020) 18:11 https://doi.org/10.1186/s12958-019-0561-0.
5. Assisted Reproductive Technology (Regulation) Bill, 2010. ICMR.
6. Planned oocyte cryopreservation in women seeking to preserve future reproductive potential: an ethics committee opinion. Fertil Steril 2018 Nov; 110(6): 1022-1028.
7. Rienzi L, Cobo A, Paffoni A, Scarduelli C, Capalbo A, Vajta G, et al. Consis- tent and predictable delivery rates after oocyte vitrification: an observational longitudinal cohort multicentric study. Hum Reprod 2012;27:1606–12. Level II-2.
8. Noyes N. Over 900 babies born with cryopreserved oocytes with no apparent increase in congenital anomalies. Reprod Biomed Online 2009;18:769-76.
9. Goldman RH, Racowsky C, Farland RV. Predicting the likelihood of livebirth after elective oocyte cryopreservation: a counseling tool for physicians and patients. Hum Reprod 2017;32:853-9.

Micro TESE and ICSI: Boon for Non obstructive azoopermia

Shweta Mittal Gupta
Senior Consultant, Centre of IVF & Human Reproduction, Sir Ganga Ram Hospital, New Delhi

Azoospermia is defined as complete absence of spermatozoa in ejaculate after centrifugation. It affects 1-3% of male population and about 10% in infertile males[1]. Azoospermia can be obstructive or non-obstructive in nature. In obstructive azoospermia there is mechanical blockage, patients otherwise have normal sperm production. Main causes are congenital bilateral absence of vas deferens (CBAVD), post vasectomy, post infection, iatrogenic or trauma[1]. Sperm retrieval is easier in such cases with help of percutaneous epididymal sperm retrieval (PESA), microsurgical epididymal sperm aspiration (MESA)[2] or testicular sperm aspiration (TESA). The chance of retrieving sperms in such cases is almost 100%. Non obstructive azoospermia (NOA) is a more common cause of all azoospermia cases[3]. The commonest causes are testicular (genetic, varicocele, cryptorchidism, chemotherapy or radiation, infection) and pretesticular (hypogonadotropic hypogonadism)[1]. On clinical examination these patients have small testes, except those with early or late maturation arrest histology where the testicular sizes may be normal. These individuals may have elevated or high normal FSH. When we are dealing with hypergonadotropic hypogonadism it is an untreatable condition medically. These men may produce sperms, however since spermatogenesis is so scattered that these sperms are not seen in ejaculate also the geographic location is unpredictable. The method of choice for sperm retrieval is based on type of azoospermia, whether obstructive or non-obstructive.

The major question to be answered is that can we predict sperm retrieval in NOA? This is important as it will minimize the trauma or damage to testis during sperm retrieval and secondly it minimizes emotional and financial cost of IVF cycle. Ideal situation would be if any of the markers could predict spermatogenesis and finding live sperms. Verza jr. & Esteves et al in 2011[4] presented their work on predictability of FSH, testosterone and testicular volume in predicting sperm retrieval. However not a single factor was found to correlate with success. Invasive test of testicular biopsy had better predictability of about 81.9 % altogether when histopathology was either hypo spermatogenesis, maturation arrest or Sertoli cell syndrome (Verza Jr. & Esteves,2011)[4,5]. All these markers and test reflect the global spermatogenic function but not the most advanced site of sperm production in a dysfunctional testis. Studies to date, have only shown poor correlations between testicular size and sperm retrieval rates. Thus, sperm retrieval is the only way out. Different sperm retrieval methods performed for NOA are testicular sperm aspiration (TESA) or open procedures like testicular sperm extraction(TESE). TESA is a closed method in which seminiferous tubules are not visualized. Even though in TESE, seminiferous tubules are exposed, however a very small area is visualized and segregating tubules by naked eye visualization without magnification hinders segmentation of tubules leading to uncertainty of finding live sperms as well as taking out bigger chunks of tissue[5,6].

In patients with non-obstructive azoospermia (NOA), micro-TESE (testicular sperm extraction) has become a recognized and more effective procedure in isolating sperm for intracytoplasmic sperm injection (ICSI). This is a method to identify sites of sperm production based on diameter of seminiferous tubules under magnification. It is a microsurgical approach to identify site of production.

Main Goals to be Accomplished during Sperm Retrieval[7]

- Acquisition of an adequate number of sperm for immediate use and cryopreservation
- Retrieval of the highest quality of sperm
- Minimizing damage to the reproductive tract, preserving testicular function

Advantages of Microsurgical Approach[7]

- Identifies site of sperm production
- Preserves vasculature of testis
- Small quantity of tissue is excised

Procedure of Microdissection TESE[7]

(Intraoperative pictures courtesy Dr. Manu Gupta, Senior consultant, urologist, Sir Ganga Ram Hospital)

Procedure of micro TESE is generally carried out under general anaesthesia or regional anaesthesia.

After cleaning, painting and draping, microscope is docked. Midline incision is made in the scrotum, and the scrotal content is pushed out preferentially from the side with the larger testis.

Fig 1: Docking of microscope

Fig 2: Tunica vaginalis is opened using cautery, minimizing bleeding and testis is uncovered . Tunica albuginea is visualized.

Fig 3: After the tunica albuginea is opened, direct examination of the testicular parenchyma is performed at 12 to 18× magnification. Open tunica albuginea to adequately expose the testicular parenchyma for dissection.

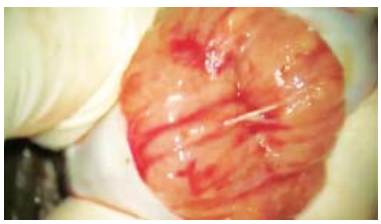
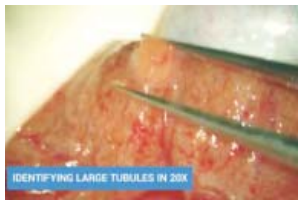


Fig 4: Exposing seminiferous tubules, avoiding excessive bleeding.



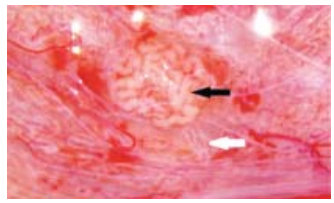


Fig 5: Small samples (1–3 mg) of the larger, more opaque tubules are dissected (picture taken from atlas by Verza and Esteves).

Fig 6: Tubule slicing and looking for sperm in 400X

Each sample is dropped into a suspension, passed through a 24g angio-catheter and examined immediately by a skilled embryologist for the presence of the testicular spermatozoa by placing a small droplet of dispersed tissue suspension on a glass slide under a phase-contrast microscope at 200× magnification.

If no spermatozoa are identified in the initial sample, subsequent samples are taken from the same testis and, if needed, from the contralateral testis.

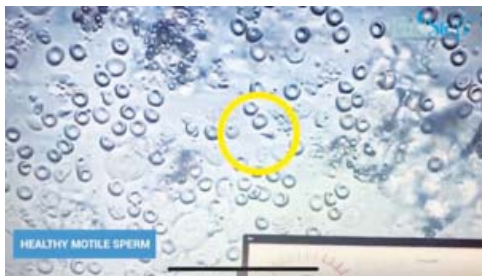


Fig 7: Identifying healthy sperm

Note: Dissection is performed through all regions of testicular tissue, preserving the testicular blood supply. After the TESE procedure, the best testicular samples are pooled in 5 mL tubal fluid medium and the suspension is subjected to centrifugation at 1800g and examined carefully for the presence of even a single spermatozoon. Advantage of using magnification is to identify seminiferous tubules with larger diameter, which increases probability of sperm retrieval.

Recent Advances in Micro TESE[9]

Recent advances in micro TESE involves objectively measuring the diameter of tubules and identifying sperm producing tubules. Generally even under magnification under microscope tubules are subjectively selected based on their appearance. However tubules with diameter of more than 100 um had higher chance of finding sperms (25% vs 3%). These patients had significantly larger testicular size and lower FSH levels.

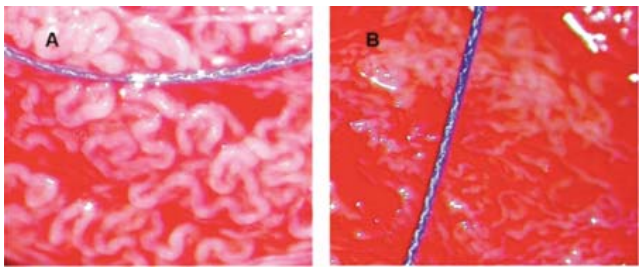


Fig 8: (a): Depicting tubules larger than the suture material (100 um diameter and above). **(B):** Tubules smaller than suture material (< 100 um).

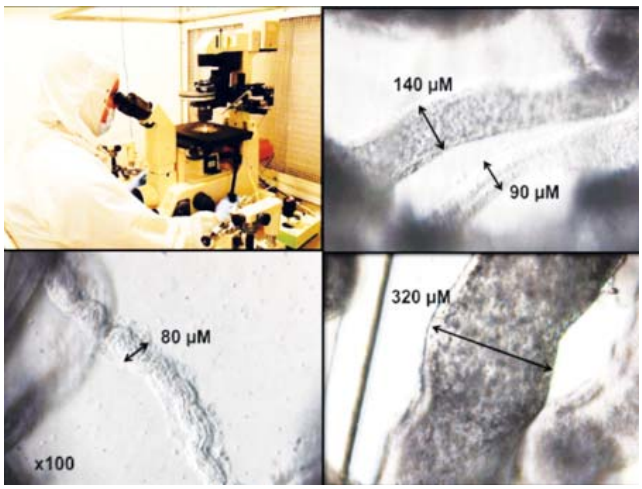


Fig 9: showing tubular diameter

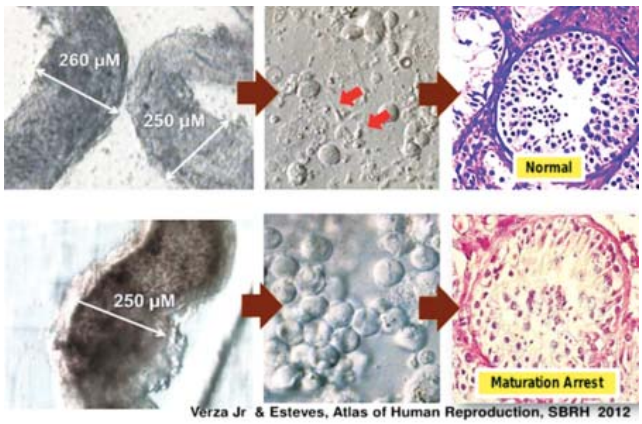


Fig 10: Correlation of tubular diameter to presence of sperms and histopathology.

Discussion[10-11]

Conventional sperm retrieval techniques in NOA have varied results. Friedlar et al suggested 11% sperm retrieval with needle aspiration vs. 43% by open biopsy. Ezeh et al found a 14 % chance by needle aspiration vs. 63 percent in open biopsy. Multiple studies have shown the chance of sperm retrieval was about 53%(Schlegel 1999, Amer et al 2000,Okada 2020, Okubu 2002, Tsujimura 2002, Ramon 2003, Esteves, 2001) vs 41% by conventional TESE.

Campell et all, In 2014 showed that testicular sperm were successfully retrieved in 56% of the men. Sperm retrieval rates (SRR) in men with testicular volumes of ≤ 2, >2–10, and >10 mL was 55%, 56% and 55% respectively. Of those men who had sperm retrieved, clinical pregnancy and live birth rates were similar in the three groups (55.2%, 50.0%, and 47.0%; and 47.2%, 43.0% and 42.2% respectively). Of the 106 men with average testis volume ≤ 2 mL bilaterally, men who had sperm retrieved were younger (31.1 vs. 35.2 years), and were more likely to have a history of Klinefelter syndrome (82.2% vs 55.6%) compared to those in whom sperm was not found ($p < 0.05$). There was no cut-point for age beyond which sperm could not be retrieved in men with small testes. On multivariable analysis, younger age was the only preoperative factor associated with successful sperm retrieval in men with small testes (<2mL).

Conclusion

In present scenario micro TESE results in higher sperm yields with less negative effects on testicular function and fewer post-operative complications. Before this procedure is offered, multiple factors need to be considered. For example, there is currently a lack of clinical predictors that can quantify the chance of finding usable sperm. Procedure of micro TESE is more complicated and expensive than conventional TESE and it is a procedure that should only be considered if a suitably experienced surgeon is available. The advent of micro TESE, in conjunction with ICSI, has revolutionised the management of men with NOA who desire a child, which is genetically their own and should be offered to all non-obstructive azoospermia cases irrespective of testicular size.

References

1. Sandro C Esteves; Ricardo Miyaoka; Ashok Agarwal. An update on the clinical assessment of the infertile male. Clinics. 2011; 66 (4):
2. Verza S, Esteves SC. Microsurgical versus conventional single-biopsy testicular sperm extraction in nonobstructive azoospermia: a prospective controlled study. Fertil Steril. 2011;96(3):S53.
3. Esteves SC, Miyaoka R, Agarwal A. Sperm retrieval techniques for assisted reproduction. Int Braz J Urol. 2011;37(5):570–83.
4. Friedler S, Raziel A, Strassburger D, Schachter M, Soffer Y, Ron-El R. Factors influencing the outcome of ICSI in patients with obstructive and non-obstructive azoospermia: a comparative study. Hum Reprod. 2002;17(12):3114–21.
5. Verza S, Esteves SC. Microsurgical versus conventional single-biopsy testicular sperm extraction in nonobstructive azoospermia: a prospective controlled study. Fertil Steril. 2011;96(3):S53.
6. Mulhall JP, Ghaly SW, Aviv N, Ahmed A. The utility of optical loupe magnification for testis sperm extraction in men with nonobstructive azoospermia. J Androl. 2005;26(2):178–81.
7. Esteves SC;Agarwal A,An update on sprem retrieval techniques for azoospermic males, clinics, 2013 Feb; 68 (Suppl 1): 99-110
8. Ramasamy R, Fisher ES, Ricci JA, Leung RA, Schlegel PN. Duration of microdissection testicular sperm extraction procedures: relationship to sperm retrieval success. J Urology. 2011;185(4):1395–8.
9. Silber SJ. Microsurgical TESE and the distribution of spermatogenesis in non-obstructive azoospermia. Hum Reprod. 2000;15(11):2278–84.
10. Bryson CF, Ramasamy R, Sheehan M, Palermo GD, Rosenwaks Z, Schlegel PN. Severe testicular atrophy does not affect the success of microdissection testicular sperm extraction. J Urol. 2014 Jan;191(1):175-8.
11. Yu Y, Xi Q, Wang R, et al. Intraoperative assessment of tubules in predicting microdissection testicular sperm extraction outcome in men with Sertoli cell-only syndrome. *J Int Med Res*. 2019;47(2):722-729.

Perinatal Outcomes after ART

Bhanupriya[1], A G Radhika[2]
[1]Assistant Professor, [2]Sr Consultant, Obstetrics & Gynecology, UCMS & Guru Teg Bahadur Hospital, Dilshad Garden Delhi-110095

Introduction

Reports of perinatal outcomes following ART are difficult to interpret due to lack of randomized trials and multiple factors that affect the outcome of pregnancy. In a Finnish population analysis, risk of adverse birth outcomes (low birth weight & preterm delivery) were increased when ART-conceived children were compared with those born through natural conception in general population but not when compared with siblings. Such observations cause one to question whether the observed outcome was entirely due to ART. Reasons for slightly raised adverse perinatal outcomes following ART could be related to the medications, manipulation of gametes and embryos, culture, effect of ovarian stimulation on endometrial receptivity etc although a physiological mechanism explaining the reason is unclear[1].

Elective single embryo transfer (eSET) is currently the first-line approach to reduce multiple pregnancies with ART[2]. While multifetal pregnancies following ART are higher, they have been found to have equivalent outcomes as spontaneous conceptions. Singleton pregnancies with ART have been found to have higher adverse effects. This observation has explained by the fact that high proportion of singletons result from vanishing twins or triplets. Patient's characteristics including the infertility/ subfertility and other preconception high risk factors also contribute to increase in the absolute and relative risk of obstetric morbidity[3].

The aim of the current document is to comprehensively discuss the role of ART as well as the expanding ART applications such as embryo biopsy [pre-implantation genetic diagnosis (PGD)], gamete donation and surrogacy to elucidate the evidence for their contribution to perinatal risks.

ART Conceived Pregnancies & Adverse Events

1. **Spontaneous miscarriage**
   Spontaneous abortion rate after ART (IVF with or without ICSI) is the same as for the general population though the rates may be slightly higher for thawed embryos.[4,5]
2. **Ectopic pregnancy** risk may vary according to the type of ART procedure and reproductive health of the mother. Slightly higher rates have been found with zygote intrafallopian transfer (ZIFT) and in women with tubal factor infertility. Heterotopic pregnancy is more common in ART pregnancies than in spontaneous conceptions (1/100 versus 1/30,000).[6]
3. The overall rates of **multiple births** are higher for ART births than natural births. In recent times, there is decline of multiple births from ART because fewer embryos are being transferred per cycle and centers are increasingly opting for elective single embryo transfer (eSET). However, the risk of monozygotic fetuses is increased from 0.4% to 1-5% following ART
4. **Preterm birth, low birth weight and small for gestational age**
   Singleton IVF pregnancies are at higher risk of preterm birth (double), LBW (≤2500 g) and SGA compared with spontaneously conceived pregnancies.[7] However, it appears that frozen embryo transfers (FETs) are associated with a reduction in all the three adverse effects. This reflects that the more natural endometrial preparation prior to FET plays a role in these conditions, possibly by allowing for more natural placentation than that which occurs in stimulated cycles.[8]

   A meta-analyses including several thousand IVF and approximately two million spontaneously conceived singleton births matched for maternal age and parity found IVF pregnancies were at significantly higher risk of preterm delivery, LBW and SGA infants.[9]
5. **Pubertal & Neurodevelopmental problems**
   Available evidence suggests that both the outcomes are similar in naturally versus ART conceived singleton children
6. **Congenital anomalies**
   The baseline risk of 2 to 4% for a congenitally anomalous fetus is potentially increased by

approximately 30% with ART (more with ICSI) though the reason in unclear. It is known that natural conceptions in subfertile couples may also have a somewhat elevated risk.

7. **Chromosomal & genetic abnormalities**
   Though data is deficient, studies have not shown a higher prevalence of karyotype abnormalities (including mosaicism) in offspring of IVF conceived pregnancies compared to naturally conceived pregnancies.

8. **Cancers**
   The risk of cancer in offspring conceived through ART is a matter of debate. The overall absolute risk appears to be slightly higher than that of the general population, but it is difficult to demonstrate a causal relationship with treatment and also to rule out the effect of the underlying fertility disorder.[10]

9. **Obstetric complications**
   Increased risk of obstetric complications from ART apply to twin gestations as well as singletons. In singleton pregnancies, IVF has been associated with an increased risk of placenta previa and abruption, gestational diabetes, preeclampsia, and cesarean delivery; however, the absolute increase in risk is small, and most such pregnancies have normal outcomes. The risk of early PTB and of very LBW was found to be two-fold higher in ART conceptions compared to natural conception.[11]

   There is recent evidence to demonstrate that FETs are associated with decreased risk of placenta previa and abruptio placentae.[12]

10. **Morbidity and mortality**
    IVF conceived pregnancy may increase the risk of maternal morbidity eg postpartum hemorrhage, ICU admission, and sepsis. Ovulation induction or intrauterine insemination which are less invasive are not associated with increased risk of either morbidity or mortality. In a cohort study that used propensity score matching and controlled for multiple factors, including maternal age and multiple gestation, IVF was associated with about 40% increase in risk of severe maternal morbidity compared with spontaneous conception though the absolute risk was low (30.8 [IVF] versus 22.2 [spontaneous] per 1000 births).[13]

11. **Long term outcomes**
    Long term outcomes in ART offsprings up to 28 years of age are reassuring. In a study which obtained data through military preinduction screening records comparing 253 ART-conceived adolescents (born between 1982 and 1993) with case-matched controls, there were no differences between groups for general and mental health, or cognitive ability (16 to 17 years).[14] Few reports have documented increased risk of asthma and vascular dysfunction in children conceived through ART but this needs confirmation.

ART Techniques & Perinatal Outcomes

a. **Oocyte Donation** Meta-analysis of 11 studies that compared oocyte donation (OD) pregnancies with those from autologous oocyte IVF or ICSI documents that women with OD pregnancies had a nearly 4 times increased risk of developing preeclampsia or pregnancy-induced hypertension (OR 3.92, 95% CI 2.62-5.16)[15]. Limitation of this study includes lack of information on the ART protocols and inability to adjust for biologic factors associated with infertility.

b. **Controlled ovarian stimulation**
   Though the evidence is limited, a study has demonstrated that severe OHSS, is not associated with any major adverse late pregnancy outcome except for PTB. Therefore, following resolution of the OHSS, pregnancies should be regarded as any pregnancy resulting from IVF treatment.[16]

c. **Blastocyst versus cleavage stage transfer**
   Systematic review and meta-analysis showed a higher risk of PTB (adjusted OR (aOR): 1.32, 95% CI: 1.19–1.46) in singleton pregnancies conceived following blastocyst stage transfers[17] & higher perinatal mortality though the incidence of LBW was lower.[18]

d. **Fresh versus frozen embryo transfer**
   The proportion of frozen ET has increased due to the better reproductive outcomes. A recent systematic review, involving 11 observational studies, suggested that the obstetric and perinatal outcome were better in singleton pregnancies arising from frozen embryos rather than after fresh oocyte cycles. There were reduced obstetric complications (APH, PTB, SGA, LBW and perinatal mortality) with frozen embryo transfer.[12]

e. **Pre implantation genetic diagnosis (PIGD)**
   Preimplantation genetic testing involves a biopsy of the embryos either at the cleavage or blastocyst stage, and whether such manipulation

affects perinatal outcomes has been addressed by studies. The European Society of Human Reproduction and Embryology (ESHRE) PGD Consortium 2012 reported no effects on pregnancies and baby born after ICSI/PGD and after ICSI treatment though long-term follow up data is lacking.

f. Surrogate pregnancy

In a recently published systematic review on gestational surrogacy, it was reported that the pregnancy and perinatal outcomes of surrogacy was comparable with those of standard IVF and OD cycles.[19]

Conclusion

Detailed preconceptional counselling is required to identify and treat modifiable high-risk factors. An effort should be made to optimize the infertility treatments to prevent or reduce the risk of pregnancy complications. Patients should be informed that pregnancies conceived through ART are associated with slightly increased risks of multiple gestations, congenital anomalies, preterm delivery, low birth weight, and the complications associated with these outcomes. The adverse effects are higher in ART conceived singleton pregnancy compared to natural conception though the adverse effects are comparable or lower to that compared with spontaneous pregnancy in subfertile women.

Since ART pregnancies are invariably associated with excessive parental anxiety there is the possibility of treatment bias. They are likely to undergo more intense monitoring and more frequent interventions including LSCS. However, very LBW (<1500 g) which is more common among singletons conceived through ART is unlikely to be due to elective LSCS and preterm birth. Subfertility seems to have an adverse effect on pregnancy outcome, independent of its treatment.

References

1. Goisis A, Remes H, Martikainen P, et al. Medically assisted reproduction and birth outcomes: a within-family analysis using Finnish population registers. Lancet 2019; 393:1225.
2. Bhattacharya S, Kamath MS. Reducing multiple births in assisted reproduction technology. Best Pract Res Clin Obstet Gynaecol2014;28:1919.
3. Seggers J, Pontesilli M, Ravelli AC, Painter RC, Hadders-Algra M, Heineman MJ, et al. Effects of in vitro fertilization and maternal characteristics on perinatal outcomes: a population-based study using siblings. Fertil Steril.2016;105:590–8
4. Kovacs GT, Breheny S, Maclachlan V, et al. Outcome of pregnancies achieved by in vitro fertilisation techniques and diagnosed as twins at the 6 week ultrasound. Aust N Z J Obstet Gynaecol 2004; 44:510.
5. Schieve LA, Tatham L, Peterson HB, et al. Spontaneous abortion among pregnancies conceived using assisted reproductive technology in the United States. Obstet Gynecol 2003; 101:959
6. Clayton HB, Schieve LA, Peterson HB, et al. Ectopic pregnancy risk with assisted reproductive technology procedures. Obstet Gynecol 2006; 107:595.
7. Jackson RA, Gibson KA, Wu YW, Croughan MS. Perinatal outcomes in singletons following in vitro fertilization: a meta-analysis. Obstet Gynecol 2004; 103:551.
8. Hwang SS, Dukhovny D, Gopal D, et al. Health outcomes for Massachusetts infants after fresh versus frozen embryo transfer. Fertil Steril 2019; 112:900.
9. Jackson RA, Gibson KA, Wu YW, Croughan MS. Perinatal outcomes in singletons following in vitro fertilization: a meta-analysis. Obstet Gynecol 2004; 103:551.
10. Spector LG, Brown MB, Wantman E, et al. Association of In Vitro Fertilization With Childhood Cancer in the United States. JAMA Pediatr 2019; 173:e190392.
11. Qin J, Liu X, Sheng X, Wang H, Gao S. Assisted reproductive technology and the risk of pregnancy-related complications and adverse pregnancy outcomes in singleton pregnancies: a meta-analysis of cohort studies. Fertil Steril. 2016;105:73–85.
12. Maheshwari A, Pandey S, Shetty A, Hamilton M, Bhattacharya S. Obstetric and perinatal outcomes in singleton pregnancies resulting from the transfer of frozen thawed versus fresh embryos generated through in vitro fertilization treatment: a systematic review and meta-analysis. Fertil Steril. 2012;98:368–77.
13. Dayan N, Joseph KS, Fell DB, et al. Infertility treatment and risk of severe maternal morbidity: a propensity score-matched cohort study. CMAJ 2019; 191:E118.
14. Fruchter E, Beck-Fruchter R, Hourvitz A, et al. Health and functioning of adolescents conceived by assisted reproductive technology. Fertil Steril 2017; 107:774.
15. Jeve YB, Potdar N, Opoku A, Khare M. Donor oocyte conception and pregnancy complications: a systematic review and meta-analysis. BJOG 2016; 123:1471.
16. Haas J, Yinon Y, Meridor K, Orvieto R. Pregnancy outcome in severe OHSS patients following ascitic/pleural fluid drainage. J Ovarian Res. 2014;7:56.
17. Dar S, Lazer T, Shah PS, Librach CL. Neonatal outcomes among singleton births after blastocyst versus cleavage stage embryo transfer: A systematic review and meta-analysis. Hum Reprod Update 2014;20:439-48.
18. Ginström Ernstad E, Bergh C, Khatibi A, Källén KB, Westlander G, Nilsson S, et al. Neonatal and maternal outcome after blastocyst transfer: a population-based registry study. Am J Obstet Gynecol. 2016;214:378.e1–378.e10.
19. Söderström-Anttila V, Wennerholm UB, Loft A, Pinborg A, Aittomäki K, Romundstad LB, Bergh C. Surrogacy: outcomes for surrogate mothers, children and the resulting families-a systematic review. Hum Reprod. 2016; 22:260–76

Overcoming Psychological Issues in Couples Dealing with Repeated Pregnancy Failures: Ten points to guide the treating clinician

Geeta Mediratta
Senior Consultant, Sir Ganga Ram Hospital, New Delhi

A. Recurrent pregnancy loss (RPL) is an enigma; etiology is often unknown, treating strategies are few and not well researched, diagnostic strategies have not been very clearly defined.

B. Studies on etiology & management of RPL have suffered from following weakness:-
   - Methodologic weakness
   - Varied criteria for diagnosis of RPL
   - Ascertainment bias
   - In proper selection of controls
   - Non exclusion of aneuploid fetuses.
   - Very high post randomization patient withdrawal.

C. Thus while dealing with couples with RPL we need to be empathetic rather than judgemental, as our own information bank is flawed. Counselling of such couple needs time and exhaustive knowledge of the patho physiology of RPL

D. Following issues should be discussed with the couple :-
   1. What is the definition of RPL:- (Recurrent pregnancy loss for 3 or more).
   2. What is the incidence of RPL. (1 in 300 pregnancies)

E. What are the risk factors / Etiology of RPL?
   These should be discussed in detail to enhance the patient's understanding of the disorder.
   - Previous pregnancy loss.
   - Uterine factors
   - Defective endometrial/ receptivity
   - Immunologic factors and APS
   - Endocrine factors
   - Genetic factors
   - Thrombophilias

F. After above counselling it is essential to give a reasonably positive prognosis to patients regarding her future obstetric outcome.

G. Investigate as per history /HPE/genetic analysis / USG report / blood workup

H. In the rare case of couple harbouring lethal genes in a heterozygous or balanced combination, that does not affect them, but causes pregnancy loss when embryo inherits it in a homozygous or
   Unbalanced state the pregnancy is lost. In such cases couple can be offered prenatal tests PGD options/ donor gametes--- to maximize their chance of a healthy offspring.

I. Stress factor needs to be addressed on a priority as it has been scientifically proven that women suffering from anxiety/ stress disorders have suboptimal pregnancy outcome.

J. Approach to allay the anxiety of the couple can be by using case anecdotes with positive pregnancy outcomes, sharing the current advances in therapy also help one couple to take a positive attitude (LITT, IVIG, Low aspirin dose LMWH) for enhancing positive pregnancy outcomes.

The following study clearly demonstrates the importance of good psychological counselling:

Posttraumatic stress, anxiety and depression following miscarriage and ectopic pregnancy: a multicentre, prospective, cohort study

Jessica Farren, PhD: Maria Jalmbrant, DClinPsy; Nora Falcomieri, MSc; Nicola Mitchell-Jones, MD; Shabnam Bobdiwala, MBBS; Maya Al-Memar, PhD; Sophie Tapp, BSc; Ben Van Calster, PhD; Laura Wynants, PhD; Dirk Timmerman, PhD; Tom Bourne, PhD

women suffering from anxiety/ stress disorders

Am J Obstet Gyneloi 2020 ; 222 : 367. E 1-22

Why was This Study Conducted?

Early pregnancy losses affect up to 1 in every 2

women in their lifetime; however, to date, the psychologic consequences have not been a major focus of research or treatment.

Key Findings

One month after early pregnancy loss, 29% of women met criteria suggestive of post-traumatic stress, 24% of women met criteria suggestive of moderate-severe anxiety, and 11% of women met criteria suggestive of moderate-serve depression. Post- traumatic stress, anxiety, and depression decline over time in those women without further losses or pregnancies, but they remain at clinically important levels at 9 months. Posttraumatic stress, anxiety, and depression symptoms were high after both ectopic pregnancies and miscarriage.

AOGD Events Held

- On 27th June 2020 – A webinar on **"Physiological Interpretation of CTG and Making Sense on Doppler"** by Dr. Manju Khemani, Dr. Rinku Sen, and Dr. Manisha Kumar.
- On 29th June 2020 – A webinar on **"Pregnancy Care During COVID19 & ANC Class"** by Life Cell & Sir Ganga Ram Hospital.
- On 1st July 2020 – A webinar on "**Management of Gynecological Malignancies and Tuberculosis"** by Dr. Vishakha Munjal and Dr. Manish Sharma.
- On 1st July 2020 – **AOGD Celebrates Doctor's Day** - Talk on **"Organ Donation"** by Dr. Mala Srivastava and **"Main Nahi Darungi"** by Dr. Mamta Dagar, Program Co-ordinated by Dr. Shivani Sachdev Gour and Dr. Jyoti Malik.
- On 3rd July 2020 – A webinar on -**"Complications of Second Stage of Labour A Case Based Panel Discussion"** moderated by Dr. Sujata Agarwal and Dr. Surekha Tayade.
- On 3/4/5/12/18th July 2020 – A webinar on **"Masterclass on Fertility Enhancing Endoscopic Surgery"** by AOGD Endoscopy committee & FOGSI Young Talent Promotion Committee.
- On 3rd July 2020-e-RTM on **"Menopause Hormonal Therapy - Dealing the Dilemmas of Menopause"** under the aegis of Delhi Gynaecologist Forum and Reproductive Endocrinology Committee AOGD.
- On 4th July 2020 – A panel discussion on **"Pap and LBC Laid Bare"** moderated by Dr. Saritha Shamsundar under the aegis of Indian Society of Colposcopy and Cervical pathology (ISCCP) in association with Oncology Committee of Association of Obstetricians and Gynaecologists, Delhi (AOGD)
- On 5th July 2020 – Sunday webinar series on **"Updating Skills in Cervical Cancer Prevention"** under ISCCP & FOGSI Oncology Committee in association with Oncology Committee of AOGD
- On 6th July 2020 – A webinar on **"FAQs in Pregnancy: In COVID Era"** by Dr. K. Gujral and Dr. Harsha Khullar.
- On 8th July 2020 – A panel on **"Urinary Incontinence-Don't be shy about the leak"** moderated by Dr. Ragini Agrawal.
- On 8th July 2020 – A webinar on **"The Surging Hormones"** by Reproductive Endocrinology Committee AOGD & DGF South West.
- On 11th July 2020 – A webinar on **"The Saga of Genital Tuberculosis – How I treat".**
- On 11th July 2020 – A panel discussion on **"Placenta Accreta Spectrum (PAS)"** moderated by Dr. Jyotsana Suri, Dr. Mrinalini Mani and Dr. Shashilata Kabra under the aegis of DGF SW and Safe Motherhood Committee AOGD.

COVID Crisis: Overcoming personal challenges
(From the Diary of a Corona Warrior)

Niharika Dhiman[1], Preeti Singh[1], Nuzhat Zaman[2]
[1]Associate Professor, [2]Postgraduate, Department of Obstetrics and Gynaecology, Maulana Azad Medical College & Associated Lok Nayak Hospital, New Delhi

"It is only in our darkest hours that we may discover the true strength of the brilliant light within ourselves that can never, ever, be dimmed." **-Doe *Zantamata***

It was that time of the year in the department when second year postgraduates were busy compiling their thesis work for submission, third year postgraduates had finished with their sent ups in February and were counting days before their final examination. Annual report for 2019-2020 from the department had been submitted; various projects were being finalized for the year ***2020,*** plans of academic activities for the year ***2020*** were in pipeline, least were we aware of how **2020** would change our lives forever. It was not more than a week later that Delhi had its first diagnosed case of COVID -19.

COVID crisis is a war-footing situation with preparations against the invincible enemy. The paucity of data, lack of definite treatment protocols for the disease was not the only foreseen hurdles but the virus was equally virulent in mutating the entire health care system. It not only affected our physical health but also affected us mentally and emotionally. *'Social distancing'* became the '*New Normal'*, distancing oneself from family, friends, colleagues, students and community as a whole.

The COVID Care Facility for Obstetrics and Gynaecology was ready within a weeks' time. *Were we prepared as healthcare workers?* A difficult question to be answered. May be physically – YES, but mentally and emotionally it was turmoil.

Days, before the start of duty, were spent in briefing the team, protocols of donning and doffing PPE, hand hygiene, sequence of steps were reiterated, floor plan of designated green and red zones revised. It was not like the usual drills in our field; safety of our frontline workers was of prime importance. The team was anxious, fear and nervousness was obvious as medical sciences till date was still struggling to crack even its basics. It had changed the ways of medicine that had been taught to us over the years. Entry to our own facility was restricted; the *'New Normal'* was to be followed. Apprehension of staying away from friends and family for the next twenty-eight days was a challenge in itself, families were equally worried, so in this war, our families had equal share of struggle.

The first few days of duty over exhausted us with mental, physical and emotional stress. Understanding the functionality of a new system and getting things streamlined was an uphill task but constant support from colleagues and seniors kept our morale high.

The PPE kit had limited our abilities, skills and strength. Once you are donned, you are longing for air, sweating profusely and dehydrated but there is a responsible job you must do. We realized that preparing and performing a caesarean section took longer than the usual, with each surgery we gained experience and streamlined our local protocols.

Equally anxious were our patients and their attendants, patients felt lonely as they had to confine themselves to their cubicles, they could not meet their family members. We also faced challenges which were earlier not our direct concern; the team not only dealt with their clinical complaints but also with other needs and necessities. As an obstetrician, the challenge was enormous, protocols of care could not be followed as usual. The team had to develop a new dynamic plan to communicate and address the needs, keeping the exposure to the minimum. Communication became the vital key for implementation of treatment for the patients and the team worked as equal members with lines of hierarchy blurred, probably that made us an effective team. It made us realize the importance of teamwork along with the idea that everyone is instrumental and indispensable be it nursing staffs, orderly or cleaning staffs.

Working team being in close proximity with COVID positive cases and other healthcare workers (HCW) poses a risk of occupational exposure and raises concerns about physical and psychological health for all. There was a fear of improper use of personal protective equipment and constant worry of contacting the disease, being a source of infection, being quarantined and putting family members and HCWs at risk.

Staying calm and composed during the duty hours, constant support and appreciation from team members and seniors were immune boosters. The workload took a huge mental, physical and emotional toll but it also made us realize that as a health professional we are trained to take any challenge regardless of its grave impact.

To allay the anxiety and apprehension of the upcoming team, webinars and training sessions were organized a week before to prepare them for duties. During these webinars details of the new working system and experiences were shared among the present and upcoming teams. Zero infection rates amongst HCWs created positivity for the new team.

The fundamental of the medicine in treating the COVID-19 patients remains the same - the empathy and our role as healers. We are dealing with the patients who are abandoned and stigmatized by society and our role is to bring them back to the same societal norms as they were before. Here comes a role of sensitizing not only the patients and their relatives but also to the society at large.

At last, we were extremely overwhelmed by the support and confidence that our seniors and colleagues have braved upon us, which has kept our morale high even in the most difficult of situations.

AOGD Forthcoming Events

- On 12th July 2020, 19th July 2020 – Sunday webinar series on **"Updating Skills in Cervical Cancer Prevention"** under ISCCP & FOGSI Oncology Committee in association with Oncology Committee of AOGD
- On 12th and 18th July 2020 – A webinar on **"Masterclass on Fertility Enhancing Endoscopic Surgery"** by AOGD Endoscopy committee & FOGSI Young Talent Promotion Committee.
- On 17th July 2020 – A webinar on **"ART"** to be moderated by Dr. Geetendra Sharma.
- On 17th July 2020-A webinar on **"Near Missed Situations based on Case Scenario"** to be moderated by Dr. Geeta Mediratta.
- On 18th July 2020 – e-RTM on **"Menopause & Malignancies"** to be moderated by Dr. Sunita Malik.
- On 21st July 2020 – A webinar on **"Role of Luteal Support in IUI"** by Dr. Sunita Kumar & **"Role of Luteal Support in IVF"** by Dr. Ruma Satwik.
- On 23rd July 2020 – A webinar on panel discussion on **"Pre-eclampsia"** to be moderated by Dr. Chitra Setya.
- On 28th July 2020 – A webinar on **"UTI"** by Dr. Mala Srivastava.
- On 31st July 2020, 04:00-06:00 pm**,** AOGD Virtual Monthly Clinical Meeting will be organised by AIIMS, New Delhi.

Journal Scan

Ruma Satwik
Consultant, Centre of IVF and Human Reproduction, Sir Gangaram Hospital, New Delhi

ETIC Endometriosis Treatment Italian Club, When more is not better: 10 'don'ts' in endometriosis management. An ETIC* position statement, Human Reproduction Open, Volume 2019, Issue 3, 2019, hoz009

Abstract

Aim: To select *10 low-value medical interventions*, characterized by an unfavorable balance between potential benefits, potential harms, and costs, which should be discouraged in women with endometriosis.

Methods: A network of endometriosis experts from 16 Italian academic departments and teaching hospitals distributed all over the country made a critical appraisal of the available evidence and definition of 10 suggestions regarding measures to be de-implemented. Strong suggestions were made only when high-quality evidence was available.

Results: The following suggestions were agreed by all experts:

1. Do not suggest laparoscopy to detect and treat superficial peritoneal endometriosis in infertile women without pelvic pain symptoms.
2. Do not recommend controlled ovarian stimulation and IUI in infertile women with endometriosis at any stage.
3. Do not remove small ovarian endometriomas (diameter <4 cm) with the sole objective of improving the likelihood of conception in infertile patients scheduled for IVF.
4. Do not remove uncomplicated deep endometriotic lesions in asymptomatic women, and also in symptomatic women not seeking conception when medical treatment is effective and well tolerated.
5. Do not systematically request second-level diagnostic investigations in women with known or suspected non-subocclusive colorectal endometriosis or with symptoms responding to medical treatment.
6. Do not recommend repeated follow-up serum CA-125 (or other currently available biomarkers) measurements in women successfully using medical treatments for uncomplicated endometriosis in the absence of suspicious ovarian cysts.
7. Do not leave women undergoing surgery for ovarian endometriomas and not seeking immediate conception without post-operative long-term treatment with estrogen–progestins or progestins.
8. Do not perform laparoscopy in adolescent women (<20 years) with moderate–severe dysmenorrhea and clinically suspected early endometriosis without prior attempting to relieve symptoms with estrogen–progestins or progestins.
9. Do not prescribe drugs that cannot be used for prolonged periods of time because of safety or cost issues as first-line medical treatment, unless estrogen–progestins or progestins have been proven ineffective, not tolerated, or contraindicated.
10. Do not use robotic-assisted laparoscopic surgery for endometriosis outside research settings.

Comments

The field of evidence based medicine is riddled with uncertainty, not just because disease is a complex condition, large aspects of which lie uncovered; but also because not enough trials exist that deal with specific interventions in specific populations looking for specific outcomes. Rarely is medical literature so didactic and clear in its recommendations. But when didactic recommendations do make an appearance like in this one, one needs to be certain if these are backed by unequivocal evidence. The methods employed in

formulating these guidelines (not detailed here but accessible through https://doi.org/10.1093/hropen/hoz009) are very rigorous. The best available evidence was collated through a comprehensive literature search encompassing all large and credible databases, appraised and compressed into ten points for the reader's consumption.

The purpose of choosing this study in the journal scan is two fold. One is its utility value for gynaecologists, involved in the care of a woman with endometriosis. Second is to pass on the benefit of this knowledge through her treating gynaecologist, to the patient, for whom all medical science exists.

Congratulations to Newly Elected AOGD Sub - Committee Chairpersons

2020-2022			
Sub-Committee	**Chairperson**	**Contact No.**	**Email**
Breast and Cervical Cancer Awareness, Screening & Prevention Committee	Dr Sushma Sinha	9717691898	sushmasinha65@gmail.com
Infertility Committee	Dr Kavita Aggarwal	9990167888	drku93@gmail.com
Rural Health Committee	Dr Seema Prakash	9818225007	seemaprakash2502@gmail.com
Multidisciplinary Patient Sub-committee	Dr Shashi Lata Kabra Maheshwari	9718990168	drshashikabra@gmail.com

2019-2021			
Sub-Committee	**Chairperson**	**Contact No.**	**Email**
Endometriosis Committee	Dr Manju Khemani	9810611598	dr.manjukhemani@gmail.com
QI Obst & Gynae Practice Committee	Dr Manju Puri	9313496933	purimanju@rediffmail.com
Oncology Committee	Dr Amita Suneja	9868399728	Amita_suneja@yahoo.com
Urogynaecology Committee	Dr Achla Batra	9811105560	achla_batra@yahoo.com
Adolescent Committee	Dr Kiran Aggarwal	9312277346	dr_kiranaggarwal@hotmail.com
Reproductive Endocrinology Committee	Dr Anita Rajorhia	9711177891	dr.anitarajorhia@gmail.com
Safe Motherhood Committee	Dr Jyotsna Suri	9810858358	jyotsnasuri@gmail.com
Fetal Medicine and Genetics Committee	Dr Manisha Kumar/ Dr Reema Bhatt	9818014887 9560208571	manishaonly@gmail.com reemakumarbhatt@yahoo.co.in
Endoscopy Committee	Dr Richa Sharma	7011484180	Gautamdrricha1@gmail.com

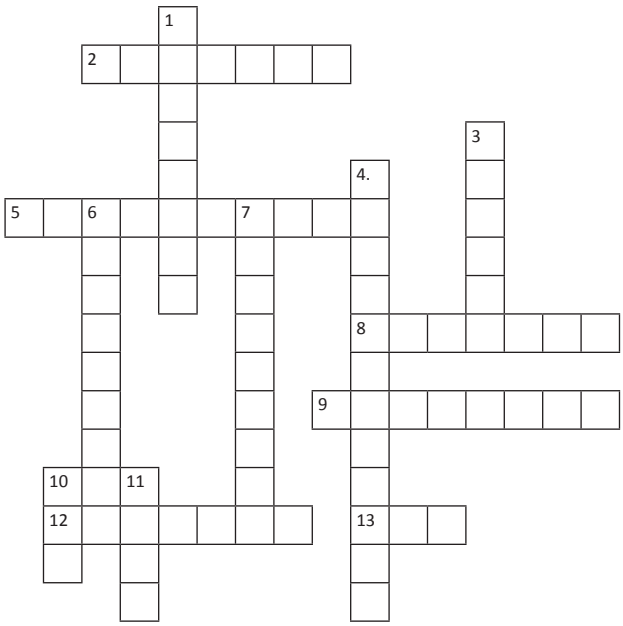
AOGD Members are Invited to Become Members of Various Sub-committee.
Please contact respective Chairperson.
Membership of Maximum Two Sub-committee can be Taken at a Time.

Cross Word Puzzle

Ruma Satwik
Consultant, Centre of IVF and Human Reproduction, Sir Gangaram Hospital, New Delhi

CROSSWORD

Test your knowledge of Reproductive Anatomy and Physiology



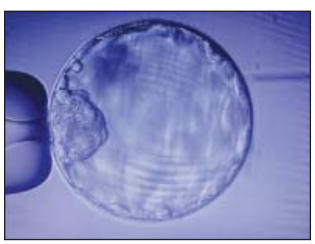
Across

2. Syndrome with features of anosmia and hypogonadotropic hypogonadism (7)
5. A selective progesterone receptor modulator (10)
8. Embryologist responsible for the birth of Louise Browne (7)
9. Name of a gonadotropin (8)
12. prevalence of infertility per hundred after one year of regular unprotected intercourse (7)
13. An molecular test for endometrial receptivity (3)

Down

1. Cryoprotectant for Sperm (8)
3. ESHRE ESGE classification for Mullerian Duct anomalies (6)
4. Genetic condition responsible for azoospermia in men (12)
6. Hormone indicative of ovarian reserve (7)
7. Responsible for uterine infertility (9)
10. Endometriosis Fertility index (3)
11. British Government regulatory body that regulates fertility clinic and research (4)

PICTORIAL QUIZ



A

B

Q1. Identify and grade

Q2. Identify all the three things seen in this picture.

Answer to July Pictorial Quiz given on Page no. 30

Answer: June 2020 Issue

Crossword

1. Hymenoplasty
2. 2. LEEP
3. Hysteropexy
4. Colposcopy
5. Sacrospinous
6. Wertheim
7. Outlet
8. Trachelectomy
9. Mersilene
10. Leopolds
11. Rubins
12. MIRENA
13. Mac Indoe

Answer to July Crossword given on Page no. 27

Clinical Proceedings of AOGD Clinical Meeting held at VMMC & Safdarjung Hospital, New Delhi on 26th June, 2020

An Unusual Case of Postpartum Thrombo-Embolism

Divya Pandey, Baishali Jain, Pratima Mittal, Jyotsana Suri, Rekha Bharti, Sumitra Bachani

The Virchow's triad of hypercoaguability, blood stasis and endothelial damage is active in COVID infection making the COVID infected individuals more prone for venous thromboembolic events(VTE). Deep Venous Thrombosis(DVT) and Pulmonary embolism (PE) are main manifestations of VTE. Here we describe a case of DVT in post-partum women who had no risk factor other than active COVID-19 infection.

25 yr old, P1L1 admitted from casualty on POD7 of LSCS done for failed induction of labour, with chief complaints of fever (~ 100 F) and pain in stitch line since 3 days. Her peri-operative period was uneventful and was discharged on POD3. There was no history suggestive of any other infection/ DVT/ COVID infection. She had Grade 2 SSI (Surgical Stitch line Infection). Rest all examination was normal. She was admitted and relevant fever investigations along with covid sampling done. Broad spectrum antibiotics (BSA) and supportive treatment were started. All reports were normal. Culture reports and COVID report were negative. Despite serial dressings and BSA, fever with low spikes continued. On day 11, she again had 103 F fever along with pain in right lower limb. Urgent USG with Doppler was done and anticoagulant treatment was started with speculation of DVT. USG revealed acute DVT of right lower limb. With high suspicion of corona infection, a repeat covid sampling was done which was positive. Chest X Ray revealed minimal infiltration in lower zone though no respiratory symptoms were present. She was managed with therapeutic anticoagulants, BSA, and supportive treatment. Serial dressing done and wound healed by secondary intention. She was discharged on tab warfarin 4 mg with advice to follow up in CTVS department.

Covid 19 is a hyper-coaguable state which can end up in drastic consequences like VTE. Repeat covid sampling must be done if there is high index of suspicion. All confirmed or suspected postpartum women of covid – 19 infection (within 6 weeks) should receive thromboprophylaxis for 10 days atleast. It can be extended to 6 weeks in the presence of persistent morbidity (RCOG guideline, COVID-19 infection in pregnancy, version 10.1:19 June 2020).

Care of Women with Covid-19 in Pregnancy- An audit from a tertiary care hospital in Delhi

Renu Arora, Sunita Malik, Sheeba Marwah, Divya Arora, Versha Dhama

Introduction

Novel coronavirus pandemic has overburdened the existing healthcare system in India with a precipitous upsurge in cases and deaths, eliciting unparalleled first time processes and actions. Data on COVID19 in pregnancy so far has been very sparse, with most being retrospective reviews and isolated case reports to comprehensively fathom risks attributable to COVID-19 infection. Not many accounts have been narrated in India so far too. In-fact, information on pregnancy is still in its incipient stage, with plausible consequences of the infection on mother and baby still rapidly evolving. Thus, a novel attempt was made to audit the initial experience in our hospital, with an objective to appraise the clinical characteristics and feto-maternal outcome.

Material & Methods

This was a single centered prospective observational study over a span of 3 months From March to June 2020, where all SARS COV2 suspect later turning out to be positive and known COVID-19 positive women coming to the department of gynecology & admitted in the hospital were included. Suspect and cases were defined as per current ICMR guidelines. All COVID19 positive women were managed as per MOHFW guidelines and hospital SOPs. Variables measured were sociodemographic and obstetric

attributes, risk factors for acquiring COVID-19, clinical presentation and feto-maternal outcome. All data were collected in predesigned pro forma, which was reviewed and analyzed at the end of this study.

Results

Amongst the 70 women included in the current study, 10 were known positive, while 60 were suspect who later turned out to be positive. 3% women presented in the third trimester. Most common symptom was fever in 89% women, followed by cough in 14.5% women. Severe anemia and hypertensive disorders of pregnancy were the most common associated comorbidities. 6.4% babies required nursery admission and one neonatal mortality was noted.

Conclusion

Most cases (67/70) had mild symptoms or were asymptomatic. They were managed conservatively, recovered and discharged as per the protocol. Further longitudinal studies warranted to document vertical transmission.

A Rare Case of Refractory Thrombocytopenia During Pregnancy: Management dilemmas

Manjula Sharma, Harsha S Gaikwad, Ankur Jain

Thrombocytopenia, defined as platelet count less than 150,000/ml complicates 10% of all pregnancies. Common causes are gestational thrombocytopenia (70-80%), hypertensive disorders of pregnancy(20%) and idiopathic thrombocytopenic purpura (3-4%). We are presenting a case of thrombocytopenia during pregnancy with severe thrombocytopenia which was refractory to the possible management options available to treat in such a patient. The patient was treated with steroids, platelet infusions, intravenous immunoglobulins, thrombopoetin receptor agonist and anti D immunoglobulin, however there was no satisfactory rise in the platelet count throughout her stay in the hospital. Inspite of very low levels of platelets (2000/mm3) the patient remained asymptomatic and had an uneventful delivery. The case was presented for its rarity.

Management Dilemma in a Case of AUB with Severe Anemia and Thrombocytopenia

Sowmya Mahesh, Ashu Bhardwaj, Achla Batra

Abnormal menstrual bleeding in adolescents can be caused by a number of conditions. Heavy menstrual bleeding may be the first manifestation of Immune thrombocytopenia (ITP). Since nutritional deficiency is very prevalent in India, B12 deficiency may coexist with ITP and present a diagnostic dilemma.This may delay the diagnosis of ITP, which can result in life threatening complications, as happened in this case.

A 15 year old girl presented with complaint of heavy menstrual bleeding for 15 days. There was no significant history except low grade fever for a month preceeding the bleeding episode She was very pale but there were no signs of failure, no hepatosplenomegaly. Genu valgum suggestive of rickets was present. No petechie or purpuric spots were present. Patient was investigated for anemia which revealed a Hb of 2.5gm%, TLC 7500/dl, platelet count of 9000/dl and peripheral smear showed dimorphic picture with no definite abnormal cells. She was started on tranexemic acid injections. Further investigation for thrombocytopenia such as coagulation profile, DCT, HIV, HBsAg, ANA were normal which ruled out hemolysis. B12 levels were very low 112 pg/ml, ferritin was also low 22ngm/ml. USG and X-ray chest were normal .In the absence of any bleeding manifestation and presence of iron and B12 deficiency, a diagnosis of nutritional deficiency with bicytopenia was made and patient was started injectable B12 and oral iron by haematologist. Meanwhile packed RBC and platelet rich plasma were transfused and norethistrone 10mg TDS was started. The platelet count remained above 15000 with transfusion and Hb also rose to 6gm%. After 7 days in the hospital, the patient had an episode of epistaxis and gum bleeding and also complained of blurring of vision. Referrals were taken from eye and ENT. Patient also started having somnolence by evening, and then became drowsy and unconscious. The platelet count report came out to be 5000/dl. NCCT revealed diffuse intracerebral bleed suggestive of systemic cause and MRI was advised to rule out structural cause. Hematologist now made a diagnosis of ITP

and advised Methyl prednisolone 1gm I/V daily and IVIG 1g/kg for 2 days. 6 Platelet rich plasma /day and packed RBC were given to keep Hb >8 gm%. The patient improved dramatically in 48 hours and became fully conscious after 72 hours. Her platelet count after 48 hours was 90000/dl and 1.5 lac /dl after 72 hours. Methyl prednisolone was stopped and she was put on wysolone 40mg /day, which was gradually tapered to 5mg/day. MRI revealed no anomaly. Patient is now under haematology care and is doing well with Hb 10.5gm% and platelet 1.75Lac/dl.

This case illustrates that how B12 deficiency can masquerade ITP, which is a diagnosis of exclusion with peripheral smear showing normal cell lines and macroplatelets. B12 deficiency makes the peripheral smear dimorphic and also causes thrombocytopenia and macroplatelets. The learning point from this case was that though heavy menstrual bleeding is a rare manifestation of ITP, it should be kept in mind and if peripheral smear is inconclusive with severe thrombocytopenia bone marrow aspiration should be done to rule out ITP. With thrombocytopenia and wet bleeding, one must be on watch for signs /symptoms of cerebral bleed such as somnolence, headache and blurring of vision. Prompt treatment of life threatening bleeding with intravenous methyl prednisolone 1mg/kg but maximum 1gmIV and IVIG 1gm/kg can increase platelets rapidly, meanwhile platelet transfusion to keep the count above 20000 and packed cell to keep Hb above 8 gm% can salvage the patient.

"Aapda mein bhi parivar niyojan ki tayari, Saksham rashtra aur parivar ki poori jimmedari"

जोड़ी ज़िम्मेदार
जो प्लान करे परिवार

World Population Day 11 July, 2020

Mobilization Fortnight
27June-10 July.

Population Fortnight
11 July - 24 July

"Provision of family planning services even in a crisis, for a self reliant nation and family"

Need of the Hour

- Rejuvenate of family planning services
- Male participation in family planning
- Use of promotion of long acting temporary contraceptive methods
- Post partum IUCD
- Post abortion family planning methods (PAIUCD, MPA, Mala-N, Chhaya etc.)

PARALLEL TO FIGHT AGAINST CORONA GOES ON THE FIGHT AGAINST MATERNAL MORBIDITY AND MORTALITY DUE TO UNMET FP NEEDS.

Directorate of Family Welfare
e-mail : dirdfw@nic.in, spofpdfw2@gmail.com

NATIONAL HEALTH MISSION
राष्ट्रीय स्वास्थ्य मिशन

दिल्ली सरकार
आप की सरकार