THE NEWSLETTER

isarEXPRESS

Indian Society for Assisted Reproduction

ISSUE 1, 2020-2021

BHARAT SERUMS & VACCINES LIMITED

Nurturing **HOPE** Delivering **RESULTS**

Nurturing hope with the safe gonadotropin, that's **virus-free** and **virus-validated**

HUMOG®

Human Menopausal Gonadotrophin 75 IU / 150 IU

SINCE 20 YEARS*

* Data on file.

Delivering **results** with **India's first*** recombinant FSH

FOLIGRAF

Recombinant FSH 75 IU / 150 IU

49% clinical pregnancy rate per retrieval cycle[1]

FSH: Follicle-stimulating hormone. * Data on file.
REF: 1. Petanovski Z, *et al. Med Arch.* 2011;65(3):153-156.

Stay confident with **virus-validated** India's No. 1† HCG

HUCOG HP

Highly Purified Human Chorionic Gonadotrophin (2000 I.U. / 5000 I.U. / 10,000 I.U.)

† AWACS MAY 2020.

HUMOG (Human Menopausal Gonadotrophin Hormone,) freeze dried for Injection 75 I.U./150 I.U. and HUMOG HD (Human Menopause Gonadotrophin Hormone High Dose) 225 I.U.

COMPOSITION: Each vial contains: Menotropins B.P. equivalent to activity of Follicle stimulating Hormone 75 I.U. / 150 I.U./225 I.U. Luteinizing Hormone 75 I.U. / 150 I.U./225 I.U. Each vial of HUMOG is accompanied by an ampoule containing 1ml of Sodium Chloride Injection I.P. One I.U. of human urinary FSH and one I.U. of human urinary LH are defined as the activities contained in 0.11388 mg and 0.13369 mg of the 1st International Standard, respectively. **INDICATIONS:** Women: HUMOG and subsequently HUCOG (Human Chorionic Gonadotrophin) are indicated for the induction of ovulation in the amenorrhoeic patient or anovulatory women with regular or irregular cycles. Men: HUMOG with concomitant HUCOG therapy is indicated for the stimulation of spermatogenesis in men who have primary or secondary Hypogonadotrophic hypogonadism. **DOSAGE AND ADMINISTRATION:** HUMOG is given by intramuscular injection. Reconstitute powder of vial in 1ml of Sodium Chloride Injection I.P. provided in the pack immediately prior to use. Up to 5 vials of HUMOG may be Reconstituted in 1 ml of Sodium Chloride Injection. Reconstituted solution should be used immediately after preparation. Any unused portion of solution should be discarded **Women: Schedule 1:** Alternate day therapy Three equal doses (225 units) of HUMOG are given on alternate days. In menstruating woman, the initial dose of HUMOG should be given on day 7, 8 or 9 of the cycle. A single dose of HUCOG 10000 I.U. is given one week after the first injection of HUMOG, provided the clinical and biochemical responses are adequate and not excessive. **Schedule 2:** Daily therapy Daily injections of HUMOG are given until an adequate response is achieved. This is judged based on daily oestrogen determinations. In the absence of a response, the dose of HUMOG may be increased or the course abandoned. A single HUCOG injection of 10000 I.U. is administered 24 - 28 hours after the last dose of HUMOG. **Men:** Treatment should begin with HUCOG 2000 I.U. 2 - 3 times a week to produce evidence of adequate masculinisation. If the response to HUCOG is only androgenic, HUMOG (1 vial 3 times a week) and HUCOG 2000 I.U. (twice a week) are required to be administered. **CONTRA-INDICATIONS AND WARNINGS: Women:** HUMOG therapy is precluded when an effective response cannot be obtained e.g. Ovarian dysgenesis, Absence of uterus, Premature menopause, Tubular occlusion. **Men:** Patients with elevated endogenous FSH levels indicative of primary testicular failure are usually unresponsive to HUMOG and HUCOG therapy. The incidence of multiple births following HUMOG / HUCOG therapy has been variously reported between 10% and 40%. Pregnancy wastes by abortion is higher than in a normal population but comparable with the rates in woman with other fertility problems. Prior hypersensitivity to HUMOG or its excipients, high levels of FSH, primary ovarian failure, pregnancy, uncontrolled, non-gonadal endocrinopathies, sex hormone dependant tumours of the reproductive tracts and accessory organ, pituitary gland/hypothalamus tumour, abnormal uterine bleeding, ovarian cysts of undetermined origin not due to PCOS, pulmonary and vascular complications, multiple pregnancy, ovarian torsion, ectopic pregnancy, congenital malformation and ovarian neoplasm. **SIDE EFFECTS:** Most common adverse reactions (>2) in ART include: abdominal cramps, enlargement, pain, headache, headache, injection site pain and inflammation, Ovarian hyperstimulation syndrome. In the female, a local reaction at the injection site, fever and arthralgia have been observed in rare cases. In the male, a combined treatment with HUMOG and HUCOG may cause gynecomastia. **STORAGE:** Vials of HUMOG should be stored between 2°C - 8°C. Do not freeze. Protect from light. Reconstituted solution of HUMOG should be used immediately after preparation. Discard any unused portion. **PRESENTATION: HUMOG is supplied in vial containing sterile, freeze dried white powder having 75 I.U. / 150 I.U./225 I.U. activity of each FSH and LH . Each vial is accompanied by an ampoule containing 1ml of Sodium Chloride Injection I.P.**

For further details, refer pack insert. Manufactured in India by: BHARAT SERUMS AND VACCINES LIMITED Plot No. K-27, Additional M.I.D.C., Ambernath (E) - 421 501. Updated on 30/05/2020.

FOLIGRAF™ (Recombinant – Human Follicle Stimulating Hormone Freeze Dried for subcutaneous use only) for Injection 75/150 I.U.; Multidose vial lyophilized freeze dried powder 1200 IU/vial; Prefilled syringes for Injection 75 /150/225/300 I.U. per 0.5 ml

Composition: Each vial contains: Recombinant – Human Follicle Stimulating Hormone 75 I.U./150 I.U. Each multidose vial contain sterile, freeze dried, white to off white lyophilized powder of rFSH 1200 IU accompanied with prefilled syringe containing m-cresol B.P. 6mg and water for injection I.P. 2ml. One Foligraf prefilled syringe contains rFSH 75/150/225/300 I.U. and water for injection I.P. q.s. **Therapeutic Indications:** Anovulation (including polycystic ovarian disease, PCOD) in women unresponsive to Clomiphene Citrate treatment. Stimulation of multifollicular development in patients undergoing superovulation for assisted reproductive technologies (ART) such as in vitro fertilization (IVF), gamete intra-fallopian transfer (GIFT) and zygote intra-fallopian transfer (ZIFT). **Posology and Method of administration:** Treatment with Foligraf should be initiated under the supervision of a physician experienced in the treatment of fertility problem. Foligraf (r-hFSH) is intended for subcutaneous administration. The powder should be reconstituted immediately prior to use with the solvent provided. **Women with anovulation (including PCOD):** A commonly used regimen commences at 75-150 IU FSH daily and is increased preferably by 37.5 or 75 IU at 7 or preferably 14-day intervals if necessary, to obtain an adequate, but not excessive, response. The maximal daily dose is usually not higher than 225 IU FSH. If a patient fails to respond adequately after 4 weeks of treatment, that cycle should be abandoned, and the patient should recommence treatment at a higher starting dose than in the abandoned cycle. When an optimal response is obtained, a single injection of 5 000 IU, up to 10 000 IU HCG should be administered 24 - 48 hours after the last FOLIGRAF™ (r-FSH) injection. **Women undergoing ovarian stimulation for multiple follicular developments prior to in vitro fertilization or other assisted reproductive technologies:** A commonly used regimen for superovulation involves the administration of 150-225 IU of r-hFSH daily, commencing on days 2 or 3 of the cycle and continued until follicular development is achieved. Dose adjustment done according to patient's response, the maximum dose being 450 IU daily. A single dose of injection 10000 IU HCG administered 24 – 48 hours after last Foligraf injection. In case of downregulation with GnRH agonists, Foligraf is initiated 2 weeks after the start of GnRH agonist, 150 – 225 IU for the first 7 days. Dose adjustments done based on ovarian response. **Contraindications, Warnings and Precautions:** Foligraf is contraindicated in case of hypersensitivity to Foligraf or to any of its components, hypothalamus or pituitary gland tumours, ovarian cyst or enlargement (not due to PCOD), gynaecological haemorrhage due to unknown aetiology and carcinoma of ovary, uterus and breast, and ovarian hyperstimulation syndrome (OHSS), multiple pregnancies, pregnancy wastage, reproductive system neoplasm, congenital malformation and thromboembolic events. **Drug Interactions:** Concomitant use of Foligraf with other agents used to stimulate ovulation may potentiate the follicular response, whereas concurrent use of GnRH agonists to induce pituitary desensitization may increase the dosage of Foligraf for adequate ovarian response. **Pregnancy and Lactation:** No indication for use in pregnancy. No teratogenic risk has been reported, following controlled ovarian hyperstimulation with gonadotrophin. Not indicated to use in lactation. **Adverse reactions: Very common (>1/10):** Ovarian cysts, mild to severe injection site reaction such as pain, redness, bruising, swelling and/or irritation, and headache; **Common (1/100):** Mild to moderate OHSS, abdominal pain and gastrointestinal symptoms such as vomiting,

ALSO AVAILABLE AS

HUMOG® HP

Highly Purufied Human Menopausal Gonadotrophin 75 IU / 150 IU

Humog HD

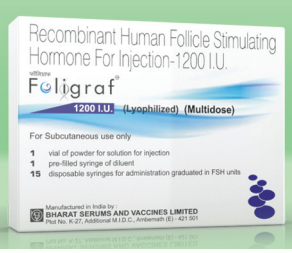
Human Menopausal Gonadotrophin 225 IU

AVAILABLE IN A WIDE RANGE

Foligraf™

Recombinant FSH

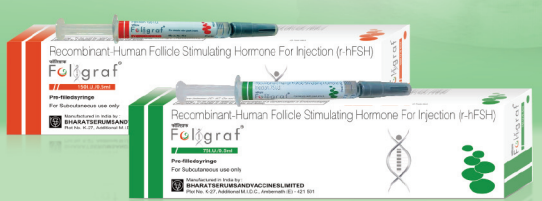
1200 I.U. (Lyophilized) (Multidose)



Foligraf™

Recombinant FSH 75 IU/150 IU/225 IU/300 IU

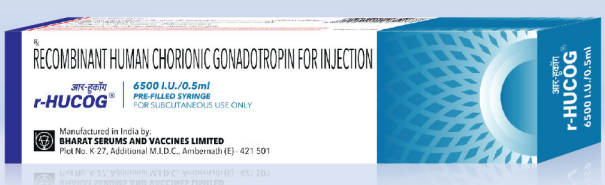
Prefilled Syringe



ALSO AVAILABLE

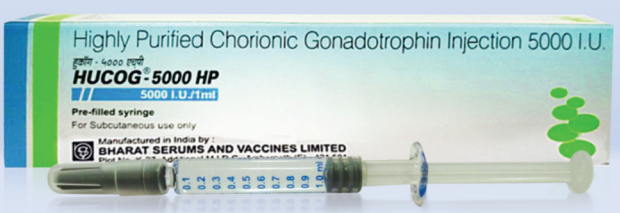
r-HUCOG®

Recombinant Human Chorionic Gonadotropin 6500 I.U. (250 µg) PREFILLED SYRINGE



HUCOG HP PFS

Highly Purified Chorionic Gonadotrophin Injection 5000 I.U./1ml



diarrhoea, abdominal cramps and bloating; **Uncommon (1/1000):** Severe OHSS; **Rare (< 1/10000):** Ovarian torsion; **Very Rare:** Thromboembolism usually associated with severe OHSS and mild systemic allergic reaction. **Overdosage:** Incompatibilities: Foligraf should not be mixed with other medicinal products except those mentioned. Reconstituted solution should be clear and used unless checked thoroughly. **Special precaution for storage:** Keep out of reach of children, stored between 2 – 8 degrees C in original pack. Do not freeze. Protect from light. For single and immediate use following first opening and reconstitution. **Presentation: Vial containing sterile, freeze dried, white to off white powder having recombinant – human Follicle stimulating hormone activity of 75 IU/ 150 IU. Each vial is accompanied by an ampoule containing 0.5 ml of Sterile water for injection I.P. Each prefilled syringes is supplied in single-use prefilled syringes containing 0.5ml of sterile colourless liquid having r-hFSH activity of 75 I.U. / 150 I.U. / 225 I.U. /300 I.U. Foligraf MD is available as multidose vial containing sterile, freeze dried white to off white powder having r-hFSH activity of 1200 I.U., accompanied by a pre-filled syringe. To be reconstituted with 2 ml diluent provided in pre-filled syringe along with this pack.**

For further details, refer the pack insert. In case of any adverse event, kindly contact pv@bharatserums.com. Manufactured in India by Bharat Serums and Vaccines Limited, Plot no. K – 27, Additional M.I.D.C., Ambernath (E) – 421501. Updated on 30/05/2020

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r-HUCOG PFS (Recombinant Human Chorionic Gonadotropin prefilled syringe) 6500 I.U. per 0.5 ml injection for subcutaneous use only.

Composition: Each PFS contains: Recombinant Human Chorionic Gonadotrophin 6500 I.U.*, Water for Injection I.P. q.s., Histidine, Glycine, Sucrose, Methionine, and Polysorbate 20. *250 µg is not less than 6500 I.U. **Indication:** r-HUCOG® is indicated in the treatment of: 1. Women undergoing superovulation prior to assisted reproductive techniques (ART) such as in vitro fertilization (IVF): r-HUCOG® is administered to trigger final follicular maturation and luteinisation after stimulation of follicular growth. 2. Anovulatory/ oligo-ovulatory women: r-HUCOG® in the treatment of anovulatory infertility where its administration is used would form part of recognized treatment regimen involving the prior stimulation of follicular maturation and endometrial proliferation. **Posology and method of administration:** Dose in Assisted Reproductive Technology: r-HUCOG® 6500 I.U. should be administered one day following the last dose of the follicle stimulating agent. Dose in Infertile women undergoing Ovarian Induction: hCG is also indicated for the induction of ovulation (OI) and pregnancy in anovulatory infertile patients in whom the cause of infertility is functional and not due to primary ovarian failure. r-HUCOG® 6500 I.U. should be administered one day following the last dose of the follicle stimulating age nt. r-HUCOG® administration should be withheld in situations where there is an excessive ovarian response, as evidenced by multiple follicular development, clinically significant ovarian enlargement or excessive estradiol production. **Method of administration:** Recombinant human Chorionic Gonadotrophin is given by subcutaneous injection only. **Contraindications:** r-HUCOG® should not be given if following conditions are present: 1. Prior hypersensitivity to hCG preparations or one of their excipients. 2. Primary ovarian failure. 3. Uncontrolled thyroid or adrenal dysfunction. 4. An uncontrolled organic intracranial lesion such as a pituitary tumor. 5. Abnormal uterine bleeding of undetermined origin. 6. Ovarian cyst or enlargement of undetermined origin. 7. Sex hormone dependent tumors of the reproductive tract and accessory organs. 8. Pregnancy. **Incompatibilities:** This medicinal product must not be mixed with other medicinal products except those mentioned. The reconstituted solution should not be administered if it contains particles or it is not clear. Shelf life: 24 months. For immediate and single use. **Storage: Store between 2°C -8°C. Do not freeze. Protect from light. Store in the original package. Keep out of the reach of children. Presentation: 0.5 ml of solution in a pre-filled syringe (USP Type 1 glass) with a plunger stopper (halobutyl rubber) and plunger (plastic), and with a needle for injection. (stainless) – pack of 1. Instructions for use/handling: The prefilled syringe is for single use only. Any remaining drug should be discarded. For the use only of a Registered Medical Practitioner or a Hospital or a Laboratory. For more details refer to pack insert. To report the occurrence of any adverse event with this product, please visit https://www.bharatserums.com/adverse.html or write to pv@bharatserums.com. Manufactured and Marketed by: Bharat Serums and Vaccines Ltd.**

HUCOGHP (Human Chorionic Gonadotrophin – Highly Purified) Injection 2000 I.U./5000 I.U./10000 I.U. For Subcutaneous/Intramuscular Injections only. HUCOG HP PFS (Human Chorionic Gonadotrophin – Highly Purified Prefilled syringes) Injection 5000 I.U. For Subcutaneous Injections only.

Composition: HUCOG HP and HUCOG HP PFS: Each ml contains chorionic gonadotrophin highly purified I.H. 5000 I.U.; water for injection I.P. q.s.; excipients and stabilizers. One IU of chorionic gonadotrophin (HCG) is defined as the activity contained in 1.279 mg of the 2nd International Standard Preparation. **Indications:** In women with anovulatory infertility it is administered as a part of recognised treatment regimen involving prior stimulation of follicular maturation and endometrial proliferation; Hypogonadotrophin hypogonadism and cryptorchidism. **Dosage and Administration:** HUCOG HP can be given subcutaneously or intramuscularly injection only. HUCOG HP PFS is given subcutaneously injection only. Anovulatory infertility: 10000 I.U. is administered in mid-cycle, following treatment with menotropin injection according to a recognised treatment regimen. Hypogonadotropin hypogonadism: 2000 I.U. twice weekly concomitant with menotropin injection for a minimum period of four months. Cryptorchidism: Dosing schedule is age dependent and can be modified as per the physicians discretion according to response. Dose of 250 I.U. twice weekly for children less than 1 year of age, 500 I.U. for children between 1 and 5 years of age, and 1000 I.U. for children above 5 years of age. **Contraindications and Warnings:** Superovulation and hyperstimulation syndrome. In males, high doses may lead to oedema. If sexual precocity is observed, reduced dose should be used. **Side effects:** Headache, irritability, restlessness, depression, fatigue, edema, gynecomastia, sexual precocity, pain at the site of injection. The adverse reactions for use in infertility are: (1) Ovarian hyperstimulation (OHSS), a syndrome of sudden ovarian enlargement, ascites with or without pain, and/or pleural effusion, (2) Rupture of ovarian cysts with resultant hemoperitoneum, (3) Multiple births, and (4) Arterial thrombo-embolism. **Precautions:** HCG should be used in conjunction with human menopausal gonadotropins only by physicians experienced with infertility problems. Drug interactions: If used concomitantly with agents to stimulate ovulation (e.g. HMG, clomiphene citrate) may potentiate follicular response. **Overdose:** Can result into ovarian hyperstimulation syndrome (OHSS). Usually, OHSS resolves spontaneously with the onset of menses. If severe OHSS occurs, gonadotrophin treatment should be stopped if still ongoing, the patient hospitalized and specific therapy for OHSS started. Storage: Vials of HUCOG HP® should be stored between 2°C - 8°C. Do not freeze. Protect from light. Any unused portion should be discarded. **Presentation: HUCOG HP is supplied in vials containing sterile having activity of 2000 I.U. / 5000 I.U. / 10000 I.U. HUCOG HP PFS is supplied in pre-filled syringes containing sterile having activity of 5000 I.U. To report the occurrence of any adverse event with this product, please visit https://www.bharatserums.com/adverse.html or write to pv@bharatserums.com. Manufactured and Marketed by: Bharat Serums and Vaccines Ltd.**

For more details, refer to pack insert.

PRESIDENT'S MESSAGE

Dear Colleagues

"Difficult times comes to those whom God trusts will manage, a true genius is not born but they are born to exhibit in crisis"

I am blessed as the 13th President of ISAR, and it is my honor & privilege to write a message for the first ISAR Express, after the new ISAR team got installed on 7th March'20 at Hyderabad, during the Silver Jubilee Congress. The Congress at Hyderabad was just like a miracle slipped into history with 2500 delegates attending, thanks to planning & hard work by Dr. Shantha Kumari, Dr Mamata Deendayal & team, well supported by Dr. Jaideep Malhotra, ISAR President and the 2019-2020 ISAR team. Just within few days not only India, but the whole world was gripped by the Covid 19 pandemic & later the lockdown.

My theme for the year was "Advanced Infertility & ART treatment at doorsteps". ISAR 2020-2021 team & all state chapters have truly stood way ahead by relentlessly imparting international standard Webinars, a historic 1st ISAR Conference plus many other activities, reaching to more than 25000 Fertility treatment enthusiasts from India & the world.

ISAR 2020-2021 is a formidable team with dynamic Secretary Kedar Ganla. This ISAR express with a change to scientific style is a clear testimony of excellent work done by Nandita Palshetkar, Kedar Ganla, Madhuri Patil, Fessy Louis & Sudesh Kamath.

From the archives of ISAR to the depth of Scientific content, the issue will make every reader engrossed in every page. JHRS the Journal of ISAR deserves a special applause and starts the subject rolling from Scientific abstracts then covering different aspects reaching all corners of ISAR education.

Understanding, consolidating and dispelling myths related to Fibroids Infertility-ART. Covering the difficult territory of evaluating Male Infertility. Embryology is important specially what a clinician should know and Art difficulties explored with a thick endometrium. Few subjects like Chronic Endometritis, oocyte quality in Poor responders are always a challenge needing new answers.

Sonography Folliculometry needs clarity in Infertility & ART. Never ending issue of PCOS and lifestyle change, Ovulation induction in PCOS, Luteal phase support with progesterone and advances in sperm selection needs depth of knowledge. But nothing can end without 'ART & the Law'.

Please enjoy the wealth of knowledge, expertise, depth and clarity of experts from all over India giving you the much-deserved break to read just as lock down eases.

We set our sails as the wind blows because without losing the sight of old shores, we can't find through the rough ocean new land & dreams which come after every dark night. We along with Sun reinstate our faith in our permanent quest of a dawn of best beginnings for our patients

Always indebted in service for ISAR

Regards & have safe times

Dr. Prakash Trivedi
President ISAR 2020-2021

MERCK FERTILITY PORTFOLIO

A portfolio of clinically established therapeutics[1-6] and state-of-the-art technologies[7-15]

Long-standing expertise

Continuous innovation

Designed with you and your patients in mind

THERAPEUTICS

GONAL-f® FOLLITROPIN ALFA

Luveris® LUTROPIN ALFA

OVITRELLE® choriogonadotropin alfa

Cetrotide® CETRORELIX

Crinone® progesterone gel 8%

TECHNOLOGIES

Genea BIOMEDX Gavi

Genea BIOMEDX Gidget

Eeva™

Genea BIOMEDX Geri

Genea BIOMEDX Gems

#TWO PINK LINES

REFERENCES: 1. GONAL-f ® (follitropin alpha) EU Product Information, August 2018. 2. Ovitrelle® (choriogonadotropin alfa) EU Product Information, September 2018. 3. Crinone® MRP Summary of Product Characteristics, August 2017. 4. Pergoveris® (follitropin alpha, lutropin alfa) EU Product Information, August 2018. 5. Cetrotide® (cetrorelix acetate) EU Product Information, August 2018. 6. Luveris® (lutropin alfa) EU Product Information, August 2018. 7. QFRM422 Geri User Manual. 8. QFRM168 Gavi User Manual. 9. QBOX IVFTM User Manual. 10. FU 3618_Instructions for Use Eeva System 3.0. 11. US Patent No:8,859,283. 12. Swain, JE. "Decisions for the IVF laboratory: comparative analysis of embryo culture incubators." Reproductive biomedicine online. 28.5 (2014): 535-547. 13. Adamson GD, et al. Improved implantation rates of day 3 embryo transfers with the use of an automated time-lapse-enabled test to aid in embryo selection. Fertility and sterility 105.2 (2016): 369-375. 14. Behr B, et al. Non-invasive technology combining time-lapse Imaging and statistical modeling: Bringing automation into the lab to improve blastocyst selection. ASRM. 2015. 15. Roy TK, et al. Embryo vitrification using a novel semi-automated closed system yields in vitro outcomes equivalent to the manual Cryotop method. Human Reproduction. 2014;19(11):2431-2438

For further information refer to full prescribing information or write to:

Merck Specialities Pvt Ltd., Godrej One, 8th Floor, Pirojsha Nagar, Eastern Express Highway, Vikhroli (East) Mumbai - 400079. www.merck.co.in

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Your partner in Healthcare

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Lab Air sterilisers & inlet air cleaners for AHU/HVAC

ZANDAIR 100C

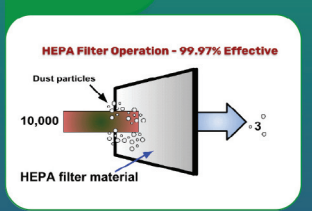
A portable unit specifically developed to meet the demands of IVF laboratories and patient waiting rooms. Eliminates VOC's, particles, Viruses and bacteria in the air.

ZANDAIR PCOC3

A powder-coated metal encased unit to be fitted into the existing HVAC/AHU unit or the air ducting of commercial buildings, sterilising the incoming air from Viruses bacteria and VOC's

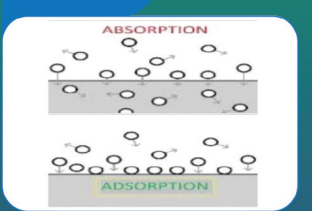
Zandair™ Systems Have A Four Step Air Purification Process

STEP 01



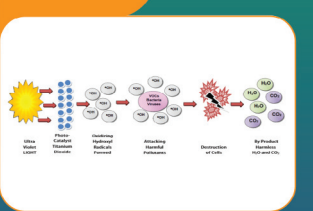
Hospital Grade High Efficiency Filter Removes Microparticles

STEP 02



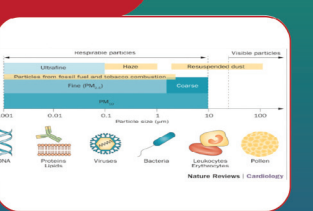
Carbon Filter Adsorbs Toxic Chemicals And Gases

STEP 03



Photocatalytic Oxidation (POC Chambers) Eliminates viruses and bacteria and destroys toxic chemicals

STEP 04



Ultraviolet Light Ultraviolet light attacks the molecular structure of viruses and bacteria

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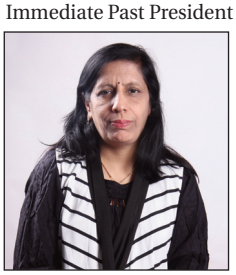

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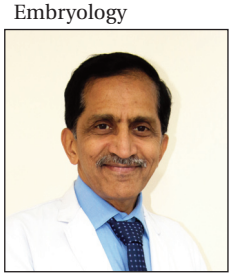

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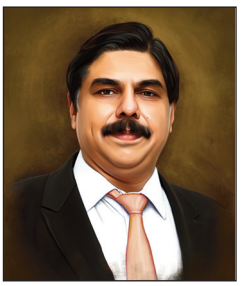
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ARCHIVES

1ST ISAR CONFERENCE AT MUMBAI, 1994

Indian Society of Assisted reproduction (ISAR) is a 29 year old , youthful & vivacious organization, which was founded in the year 1991 in Mumbai. In this column we shall attempt to bring to you rare pictures from the early days of ISAR.

First hands on ICSI workshop at Jaslok Hospital, in association with pioneering Belgian group of Prof. Andre Strategheim.

Trivector's booth at first ISAR conference at Hotel Oberoi Towers, Mumbai, 1994.

Our sincere thanks to Mr Dilip Patil of Trivector, who is a pioneer and been a part and parcel of the Indian IVF industry since the early years.
These rare images are from his personal collection.

25th ISAR Silver Jubilee Congress, Hyderabad, March 6th - 8th 2020

The silver jubilee meeting of ISAR 2020 was held in the pearl city of Hyderabad from 6th to 8th March with the theme “Better practices, Better outcomes” organized by TSAR. **Dr Prakash Trivedi took over as the 13th President of ISAR.**

The conference was by far the largest fertility conference in India. There were 13 pre congress courses with hands on experience. Two novel hands on workshops oocyte/ovary cryopreservation, oocyte pickup and embryo transfer.

Eminent speakers from USA, Europe, Australia and Africa enlightened delegates about their practices and research. The extensive pre-congress and congress included over 365 sessions of invited and sponsored sessions from ISAR industry partners. There were 130 e posters & over 200 oral abstract presentations.

DR. Sadhana Desai

"A person's most useful asset is not a head full of knowledge, but a heart full of love, an ear ready to listen and a hand willing to help others."

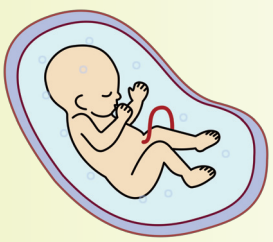
DR SADHANA DESAI ENDOWMENT FUND

FINANCIAL AID FOR COUPLES UNDERGOING IVF / ICSI

ABOUT THE FUND

• Considering the increasing number of couples seeking IVF treatment and the exorbitant costs involved, Dr Sadhana Desai established this fund. Indian Society for Assisted Reproduction (ISAR) graciously agreed to be part of it.

• **This Fund provides an aid of Rs 50,000/- per eligible couple.**



ELIGIBILITY CRITERIA

1. Couple with combined annual income below Rs 5 lakh
2. Undergoing IVF or ICSI treatment in centres registered with ICMR
3. Self cycles only (couple's own eggs and own sperms)
4. For couples of Indian Nationality only

APPLICATION PROCESS

1. Patient Application Form

a) To be downloaded from the ISAR Website at https://fertilityivffund.com/patient_application_form.php

b) To be filled by the couple and submitted to the IVF centre along with the following documents

c) Documents required:

i. PAN Card / Ration Card / Passport / any other document proving Indian Nationality

ii. Aadhar Card

iii. Marriage Certificate

iv. Last 3 years IT Returns or Bank Statements from all accounts for the last financial year

2. Clinic Registration Form - To be filled by the treating IVF Specialist and submitted online at https://fertilityivffund.com/application_form.php

3. The application is reviewed by the competent authority and approved by the panel.

4. Disbursement of Funds - Once approved, the payment will be made directly to the IVF centre

For Further Details please check https://fertilityivffund.com/our-process.php

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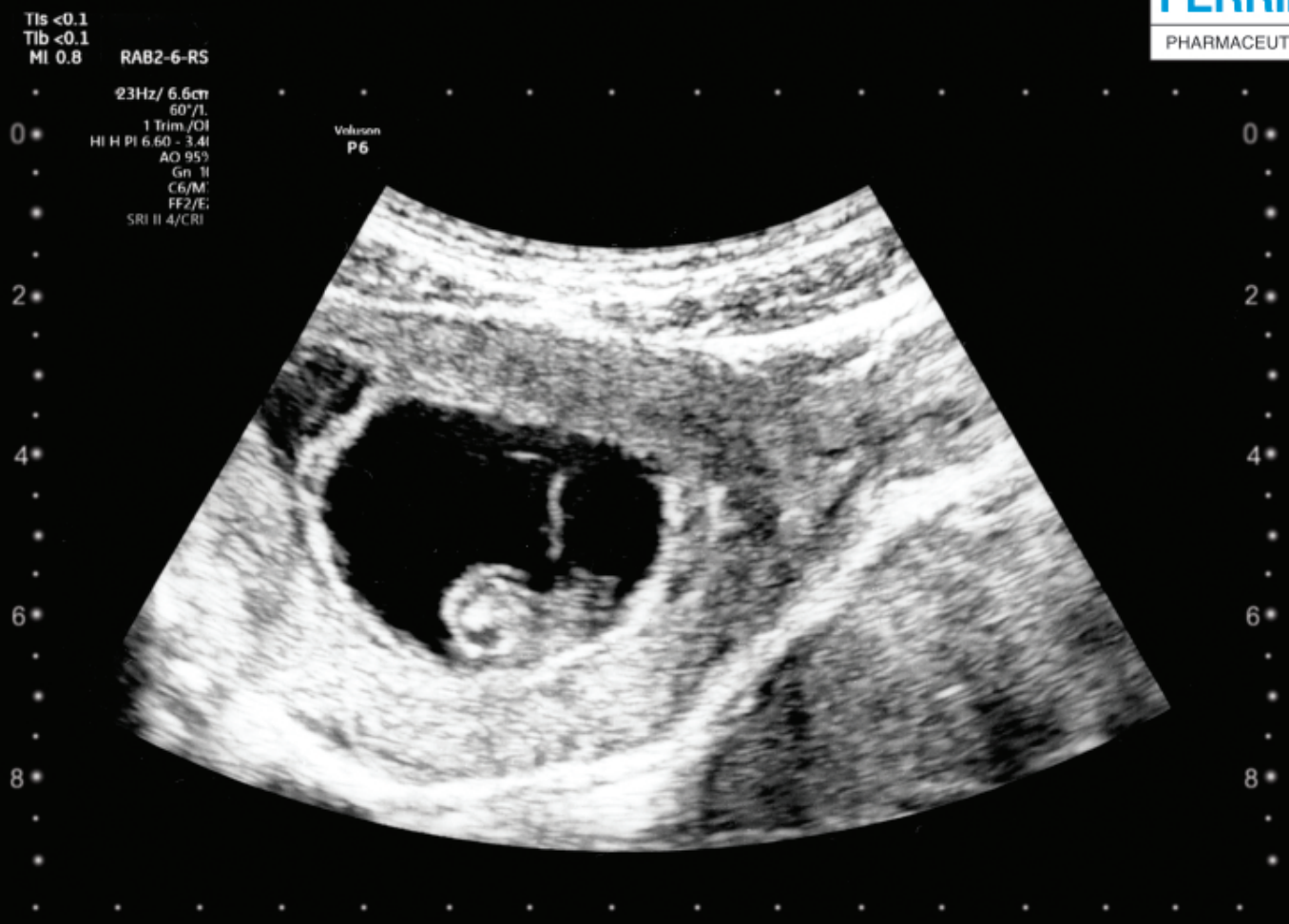

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FEW SELECTED BEST ARTICLES FROM THE ISAR JOURNAL

THIS JOURNAL IS PUBMED INDEXED.

Original Article:

CAUSES AND PREVALENCE OF FACTORS CAUSING INFERTILITY IN A PUBLIC HEALTH FACILITY

Priyanka Sanjay Deshpande, Alka Shanti Prakash Gupta
Department of Obstetrics and Gynaecology, Seth GS Medical College and KEM Hospital, Mumbai, Maharashtra, India

JOURNAL OF HUMAN REPRODUCTIVE SCIENCES, OCTOBER - DECEMBER 2019

ABSTRACT

Background: Infertility causes change according to local demographics. There is thus the need to find the causes of infertility in context to local population to aid and direct management strategies accordingly.

Aims: The aims were to study the causes of infertility and to calculate the proportion of the individual factors contributing in the population coming to a tertiary level public health facility.

Setting and Design: This cross-sectional, observational study was done in an infertility clinic in a medical college and government hospital.

Materials and Methods: Couples (n=120) who came for infertility evaluation and treatment were included in this study. Cause of infertility was determined on the basis of history and examination. The prevalence of each cause was evaluated.

Statistical Analysis: Prevalence of individual factors was calculated and analysis done using SPSS 16.0.

Results: Primary infertility (57.5%) was more prevalent than secondary infertility (42.5%). Female factor accounted for 46.6% with polycystic ovarian syndrome (PCOS) being the leading cause (46%). Infectious causes such as pelvic inflammatory disease and tuberculosis were significantly associated with tubal factor infertility (P = 0.001). In couples married for less than 5 years, PCOS was the main cause whereas later, male factor and unexplained infertility were the most common causes. Male factor contributed to 20% of the cases of infertility. Tobacco and alcohol consumption were significantly associated with abnormal semen reports (P = 0.001).

Conclusion: Causes of infertility vary according to the age of couple and duration of marriage. Although PCOS remains the main cause, infections are a major cause of tubal factor infertility, with tobacco and alcohol worsening semen reports. One-third of the cases still remain unexplained.

My comments

- The cross sectional observational study may be associated with many fallacies due to the difference in the class of patients as against private clinics.
- The data from most studies all over the world quote cause of infertility as 30% female factor, 30% male factor, 20% combined factor and 20% unexplained reason. In our study female pathology accounted for 47 %, male factor 10%, combined factor 10% while 33% unexplained infertility.
- The investigations should depend on age of the women, duration of infertility, clinical and trans vaginal ultrasound findings and semen analysis. Male factor infertility was seen in about 20%, based on semen analysis. One must remember that semen analysis has limited diagnostic value.
- There is a need for accurate, cost effective and targeted testing to improve the diagnostic accuracy and treatment outcome.

Original Article:

THE EFFECT OF SUBOPTIMAL SEMEN PARAMETERS ON MALE PARTNER'S ABILITY TO CONCEIVE. IS HE REALLY SUBFERTILE BECAUSE THE TEST SAYS SO?

Rubina Izhar, Zubaida Masood, Samia Husain, Suhaima Tahir, Mataa-E-Masood
Department of Gynaecology and Obstetrics, Aziz Medical Center, Karachi, Pakistan

JOURNAL OF HUMAN REPRODUCTIVE SCIENCES, OCTOBER - DECEMBER 2019

ABSTRACT

Context: Context: In many developing countries, subfertility treatment is not covered by government-funded institutions. It is observed that healthcare providers incriminate male factor for subfertility even when only a slight deviation from presumed normal criteria is observed.

Aim: This study aims to provide scientific evidence that pregnancies are possible at semen parameters that are below the generally accepted lower limits of normal.

Setting and Design: Retrospective cohort study (Jan 2014 - Dec 2018).

Materials and Methods: During the study period, couples who conceived without any treatment of male partner were included. The World Health Organization (WHO) reference values for semen analysis were utilized to assess the reports. The primary outcome measure was conception despite abnormal semen parameters.

Statistical Analysis Used: SPSS software program, version 15.0 (IBM, Armonk, USA).

Results: Of the 332 couples included, 233 (70.1%) couples conceived despite suboptimal semen parameters. The most common criterion not satisfied was rapid linear motility –200 (85.8%), 87 (37.3%) men were oligozoospermic, 94 (40.3%) were asthenozoospermic, and 21 (9%) were teratozoospermic. These abnormalities were more common in men having primary subfertility (71.7% vs. 28.3%, P = 0.002). The age group 40–44 years (n = 91, 39.1%) and overweight (n = 110, 47%) had more abnormalities.

Conclusions: A consensus for defining poor semen criteria is the need of the hour so that these males can be counseled satisfactorily. WHO criteria are a standard commonly employed, but they do not necessarily predict the fertility potential.

My comments

- WHO criteria for semen analysis is a standard commonly employed but they do not necessarily predict the fertility potential. A reliable consensus for defining poor semen criteria so that these males can be counselled satisfactorily is necessary.

Routine semen reports:

- Exhibit high degree of variability

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For Infertility in women caused by anovulation due to insufficient gonadotropin secretion, stimulation of follicle growth for IVF[1]

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Menotropin for Injection I.P. 75IU/600IU/1200IU

In randomized controlled trials[#] of HP-hMG (MENOPUR®) against rFSH [2,3]

	Live Birth Rate**		Ongoing Pregnancy Rate*	
Pituitary Down-regulation protocol ↓	**Menopur (n=737)**	**rFSH (n=743)**	**Menopur (n=737)**	**rFSH (n=743)**
GnRH agonist long protocol[2]	26%	22%	27%	22%
GnRH antagonist protocol[3]	29%	26%	30%	27%

MENOPUR® (Menotropin for Injection IP)
Abbreviated Prescribing Information
Composition: Menopur® 75 IU: Each vial with powder contains highly purified menotropin (human menopausal gonadotropin, HMG) corresponding to follicle stimulating hormone activity FSH 75 IU and luteinizing hormone activity LH 75 IU. **Menopur® multidose 600 IU:** One vial with powder contains: Highly Purified Menotropin (human menopausal gonadotropin, HMG) corresponding to Follicle Stimulating Hormone (FSH) activity 600 IU and Luteinizing Hormone (LH) activity 600 IU. One pre-filled syringe with solvent contains: 1.1 ml water for injection with m-cresol. **Menopur® multidose 1200 IU:** Highly Purified Menotropin (human menopausal gonadotropin, HMG) corresponding to Follicle Stimulating Hormone (FSH) activity 1200 IU and Luteinizing Hormone (LH) activity 1200 IU. One pre-filled syringe with solvent contains: 1.1 ml water for injection with m-cresol. **Indications:** Infertility in women caused by anovulation due to insufficient gonadotropin secretion, stimulation of follicle growth for IVF. **Dosage & Administration:** Dosage regimens are identical for SC and IM administration. **Women with Anovulation:** The recommended initial dose of MENOPUR® is 75-150 IU daily, which should be maintained for at least 7 days. Adjustments in dose should not be made more frequently than every 7 days. The recommended dose increment is 37.5 IU per adjustment, and should not exceed 75 IU. The maximum daily dose should not be higher than 225 IU. **Women undergoing Controlled Ovarian Hyperstimulation for stimulation of follicle growth for IVF:** In a protocol using down-regulation with a GnRH agonist, MENOPUR® therapy should start approximately 2 weeks after the start of agonist treatment. In a protocol using down-regulation with a GnRH antagonist, MENOPUR® therapy should start on day 2 or 3 of the menstrual cycle. The recommended initial dose of MENOPUR® is 150-225 IU daily for at least the first 5 days of treatment. Dose adjustment should not exceed more than 150 IU per adjustment. The maximum daily dose given should not be higher than 450 IU daily and in most cases dosing beyond 20 days is not recommended. **Method of administration:** MENOPUR® 75 IU is intended for subcutaneous (S.C.) or intramuscular (I.M.) injection after reconstitution with the solvent provided. The powder should be reconstituted immediately prior to use. After reconstitution with the solvent provided MENOPUR ® 600 IU and 1200 IU are intended for subcutaneous (S.C.) injection, as the syringe provided is for S.C. administration only . The reconstituted solution is for multiple injections and can be used for up to 28 days. **Contraindications:** Tumors of pituitary gland or hypothalamus; Ovarian, uterine or mammary carcinoma; pregnancy, lactation, gynaecological haemorrhage of unknown etiology; hypersensitivity to active substance or excipients; ovarian cysts or enlarged ovaries not due to polycystic ovarian disease. Menopur should not be administered in patients with primary ovarian failure, malfunction of sexual organs incompatible with pregnancy, fibroid tumors of uterus incompatible with pregnancy. **Warnings and Precautions:** MENOPUR® should only be used by physicians who are thoroughly familiar with infertility problems and their management. Adherence to recommended MENOPUR® dosage regimen of administration and careful monitoring of therapy will minimize the incidence of Ovarian Hyperstimulation Syndrome (OHSS). Due to high risk of multiple pregnancy as compared to natural conception, patients should be advised of the potential risk prior to treatment. The prevalence of ectopic pregnancy, congenital malformations and pregnancy wastage is higher with ART as compared to normal populations. It is unclear if baseline risk of reproductive system neoplasms is increased due to treatment with gonadotropins. Women with generally recognised risk factors for thromboembolic events, such as personal or family history, severe obesity (Body Mass Index > 30 kg/m2) or thrombophilia may have an increased risk of venous or arterial thromboembolic events, during or following treatment with gonadotrophins. **Adverse Reactions: Common (> 1/100 to < 1/10):** Nausea, abdominal pain, abdominal distension, headache, injection site reactions, OHSS, Pelvic Pain. **Uncommon (> 1/1,000 to < 1/100):** Vomiting, abdominal discomfort, diarrhea, fatigue, dizziness, ovarian cyst, breast complaints, hot flush. **Rare (> 1/10,000 to < 1/1,000):** acne, rash **Unknown:** Ovarian torsion, pruritis, urticaria, thromboembolism, hypersensitivity reactions, increased weight, musculoskeletal pain, pyrexia, malaise, visual disorders. The most frequently reported adverse drug reactions (ADR) during treatment with MENOPUR® in clinical trials are Ovarian Hyperstimulation Syndrome, OHSS, headache, abdominal pain, abdominal distension and injection site pain. None of these ADRs have been reported with an incidence rate of more than 5%. *For more details on undesirable effects, please see package insert* **Overdosage:** The effects of an overdose is unknown, nevertheless one could expect ovarian hyperstimulation syndrome to occur. **List of Excipients:** MENOPUR® 75 IU Powder: Lactose monohydrate, polysorbate 20, sodium hydroxide, hydrochloric acid Solvent: Sodium chloride, hydrochloric acid, water for injections. MENOPUR® multidose 600 IU and 1200 IU Powder: Lactose monohydrate, polysorbate 20, sodium phosphate dibasic heptahydrate, phosphoric acid Solvent: Metacresol, water for injection **Incompatibilities:** MENOPUR® should not be administered in the same injection with other products, except Ferring's urofollitrophin (FSH) BRAVELLE. **Storage Condition:** MENOPUR® 75 IU - Do not store above 25ºC. Do not freeze. Store in the original container in order to protect from light. MENOPUR® multidose 600 IU and 1200 IU - Store in a refrigerator (2ºC - 8ºC). Do not freeze. **Shelf Life:** MENOPUR® 75 IU - 2 years. For immediate and single use following reconstitution. MENOPUR® 600 IU and 1200 IU - 3 years. After reconstitution, the solution may be stored for a maximum of 28 days at not more than 25°C (preferably in a refrigerator). Do not freeze. **Presentation & Pack Size: MENOPUR® 75 IU:** 5 vials of powder and 5 ampoules of solvent. **MENOPUR® multidose 600 IU:** 1 vial of powder, 1 pre-filled syringe with solvent for reconstitution, 1 needle for reconstitution, 9 disposable syringes for administration graduated in FSH/LH units with pre-fixed needles. **MENOPUR® multidose 1200 IU:** 1 vial of powder, 2 pre-filled syringes with solvent for reconstitution, 1 needle for reconstitution, 18 disposable syringes for administration graduated in FSH/LH units with pre-fixed needles.
SCHEDULE H PRESCRIPTION DRUG - CAUTION
Not to be sold by retail without the prescription of a Registered Medical Practitioner.
Manufactured by: MENOPUR® 75 IU – Ferring GmbH, Germany
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For additional information on prescribing information, kindly refer to the package insert.
Date of Revision: 23rd December 2019

*Primary endpoint **Secondary endpoint # In RCT comparing efficacy and safety of Menopur vs rFSH (follitopin alfa) in women undergoing IVF following a long GnRH agonist protocol[2]; In RCT comparing efficacy and safety of Menopur vs rFSH (follitopin beta) in women undergoing ICSI following a GnRH antagonist cycle with compulsory single blastocyst transfer[3]

References

1. Menopur Prescribing Information 2. Andersen AN et al. Clinical outcome following stimulation with highly purified hMG or recombinant FSH in patients undergoing IVF: a randomized assessor-blind controlled trial. Human Reproduction. 2006; 21(12): 3217-3227 3. Devroey P et al. A randomized assessor-blind trial comparing highly purified hMG and recombinant FSH in a GnRH antagonist cycle with compulsory single-blastocyst transfer. Fertil Steril. 2012; 97:561-71

HP-hMG = highly purified human menopausal gonadotropin, rFSH = recombinant follicle stimulating hormone

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Ferring Pharmaceuticals Pvt Ltd., The Capital, Unit 509 / 510, A wing, 5th floor, Bandra Kurla Complex, Bandra (E), Mumbai 400051, India

FPPL/O3/20/MNP/06

For the use only of a registered medical practitioner or a hospital or a laboratory.

- Tell us about the function of testis but fail to predict sperm chromatin quality and sperm function
- Lacks information on sub cellular / molecular changes in spermatozoa
- Poor predictors of male fertility potential since 50% of infertile men have normal semen parameters
- Has low prognostic value
- Current WHO standards fail to meet rigorous clinical and statistical standards.

• Sperm function tests when indicated should be done to evaluate and stratify interventions. Evaluation of sperm DNA fragmentation index (DFI) may give some clue. High sperm DFI despite a normal semen analysis may have correlation with poor reproductive outcome.

• High DFI may provide a possible explanation for UI, RPL and IUI failure, therefore, SDF testing in these couples may prompt an early IVF or ICSI. DFI testing in patients with recurrent ART failure is indicated as it can provide useful prognostic information on subsequent ART cycles. The numbers needed to treat (NNT) to improve the ART outcome is about 4.9. ASRM (2013). However, AUA Best Practice (2011) guidelines do not recommend routine sperm DNA testing.

• Other tests that could be easily done are Hypo-osmotic swelling (HOS) test, Teratozoospermia index (TZI) and sperm survival test. Computer-assisted sperm analysis (CASA) though has a high precision and provides data on sperm density, motility, straight-line and curvilinear velocity, linearity, and average path velocity, but is not superior to conventional semen analysis (Grade A).

• Sperm function test may be useful for identifying a male factor contributing to unexplained infertility or for selecting therapy- but for whom and which one(s) is still unclear at present.

JHRS: Journal of Human Reproductive Sciences - **Editors**

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MS, Commonwealth fellowship (Reproductive Medicine), UK.
Professor of Reproductive Medicine, Reproductive Medicine Unit, Christian Medical College, Vellore

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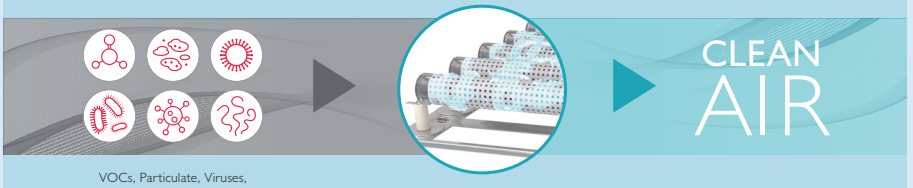

VOCs, Particulate, Viruses, Bacteria, Mould Spores, Odours

- Reduce VOCs
- Augment Existing Hygiene Protocols
- Improve Embryonic Development

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Maharashtra State Chapter Contribution

NEW UNDERSTANDING IN MANAGEMENT OF FIBROIDS IN INFERTILITY & ART

INTRODUCTION

"The capacity to learn is a gift, the ability to learn is a skill & the willingness to learn is a choice "

Leiomyoma of the uterus are benign, monoclonal uterine myometrial tumors, that affect 25 – 45% of reproductive age women.They are classified by FIGO leiomyoma subclassification system.

Though fibroids are known for more than 150 years, but our understanding is limited.The dilemmas pertaining to fibroids are :

- Do we know what really causes fibroids?
- Do we know the actual growth rate of fibroids?
- Do fibroids ever change to Leiomyosarcoma?
- What predisposes to fibroids?
- Which fibroids causes Infertility?

Unfortunately self imposed conclusions by many gynaecologists is surprisingly prevailing.

A research of 9230 cases of fibroids over 27 years on understanding fibroids with laparoscopic removal in 8344 cases was conducted at ***Dr.Trivedi's Total Health Care Centre & Aakar IVF - ICSI Centre Mumbai, India***

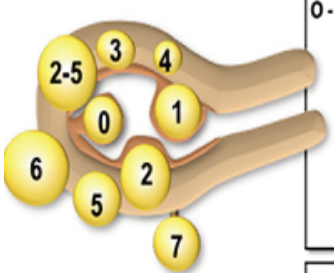
Parameters evaluated:

- Risks & Preventive factors
- Growth & actual sarcomatous changes
- Hormonal impact
- Impact on Infertility & ART ~ 27%
- Outcome of pregnancy with Fibroids
- Post Myomectomy outcome
- Rupture uterus

UNKNOWN FACTS OF FIBROIDS & INFERTILITY KNOWN THROUGH THIS RESEARCH:

- The prolactin level was high in the fibroid tissue especially in the follicular phase. Prolactin acts as a stimulator of the proliferation of the leiomyoma cells by the mitogen activated protein kinase cascade. This was suppressed by anti-prolactin antibodies and progesterone as well showed suppressive effect.
- Fibroids express Aromatase enzyme in strikingly high levels than the surrounding myometrium, nearly 3 times, this then converts the estrogen into an active estradiol which promotes growth of fibroids.Aromatase mRNA was detectable in 91% of myomas, 75% at 2 cm from the myoma and not detectable in disease-free myometrium. Thus fibroids, irrespective of size could produce a situation of local hyperprolactinemia and local hyperestrogenemia.Both are detrimental to conception leading to infertility and also increases abortion rates. Leiomyoma reported in 27% of infertile women and 50% of women with unexplained infertility became pregnant after myomectomy.
- 40% of fibroids need no treatment at all and medical management only treats symptoms.

Leiomyoma subclassification system



SM - Submucosal	0	Pedunculated intracavitary
	1	<50% intramural
	2	≥50% intramural
O - Other	3	Contacts endometrium; 100% intramural
	4	Intramural
	5	Subserosal ≥50% intramural
	6	Subserosal <50% intramural
	7	Subserosal pedunculated
	8	Other (specify e.g. cervical, parasitic)

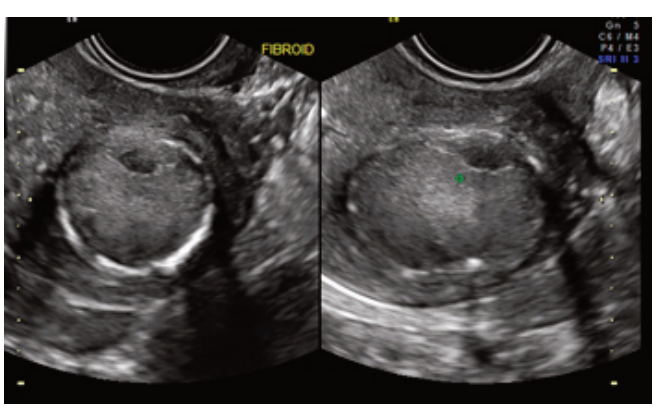
Hybrid leiomyomas (impact both endometrium and serosa)	Two numbers are listed separated by a hyphen. By convention, the first refers to the relationship with the endometrium while the second refers to the relationship to the serosa. One example is below	
	2-5	Submucosal and subserosal, each with less than half the diameter in the endometrial and peritoneal cavities, respectively.

HOW FIBROIDS AFFECT FERTILITY & ART OUTCOME?
• Altered peristalsis
• Distortion of the endometrial cavity
• Affects endometrial vascularity
• It is reported that endometrial receptivity markers significantly decrease in submucosal fibroids, while the same is evident for intramural fibroids especially for the HOXA 10 gene.

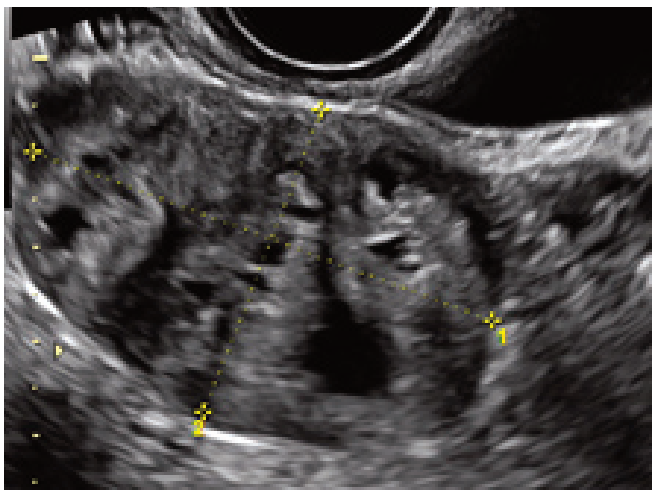
TOPOGRAPHY AND PENETRATION:
• Fibroids with their inner margins closer to the endometrium are more likely to interfere with implantation.In these cases, in addition to the mechanical effect and the vascular compromise,the pseudocapsule,a sort of neurovascular bundle surrounding leiomyoma plays a neuroendocrine-biological role.
• Several studies highlight a new endocrine function of such structure, which may have a potential role in the uterine healing and fertility, especially after myomectomy.
• Even when fibroid is far from the endometrial cavity, it reduces HOXA 10-11 genes expression, leukemia inhibitory factor and Beta 3 integrin throughout the endometrium.
• Leiomyoma derived transforming growth factor beta 3 impairs bone morphogenic protein type 1 and 2 receptors which are essential for endometrial receptivity.

What matters?
• Size
• Geography of the lesion
• Residual myometrial walls
• Junctional zone derangement.
• Symptomatic relevance to lesion

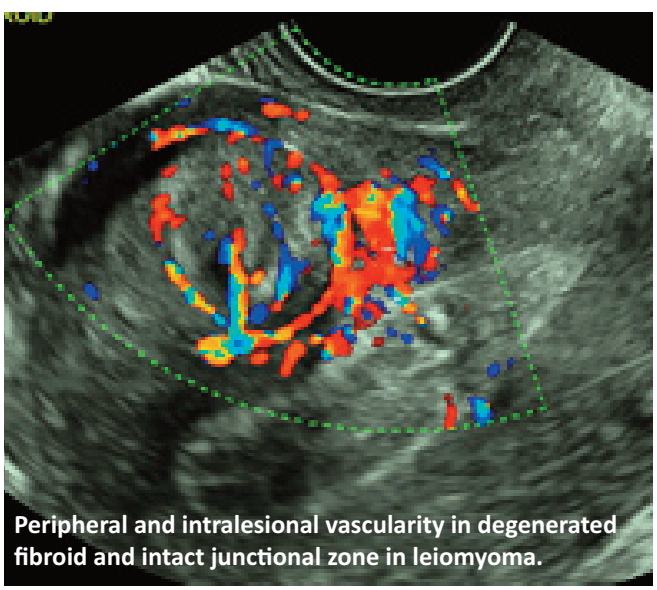
IMPORTANT ULTRASONOGRAPHIC ASSESSMENT POINTS:
• Size of the fibroid is measured in 3 longest orthogonal diameters



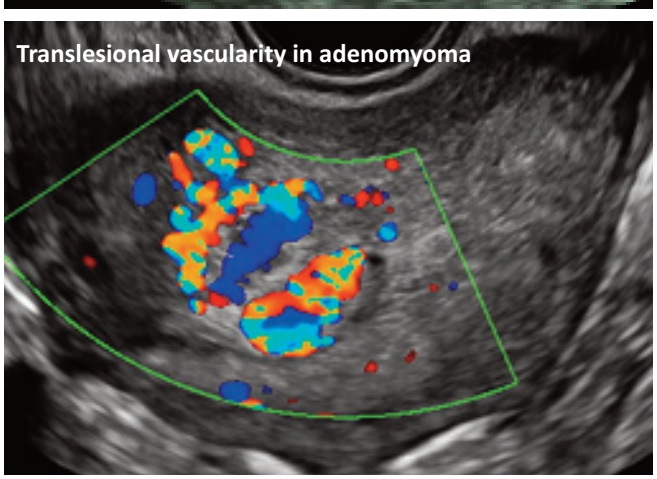
• Location & Mapping of lesions is done by systematic scanning technique.
• Fibroid echogenicity: Degeneration of fibroids results in increase heterogenecity due to presence of fibrous tissue



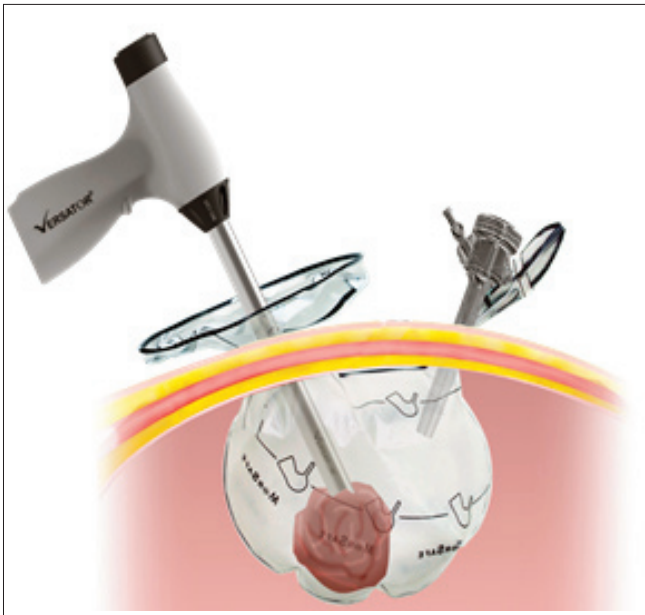
Degenerated fibroids can be differentiated from adenomyoma:



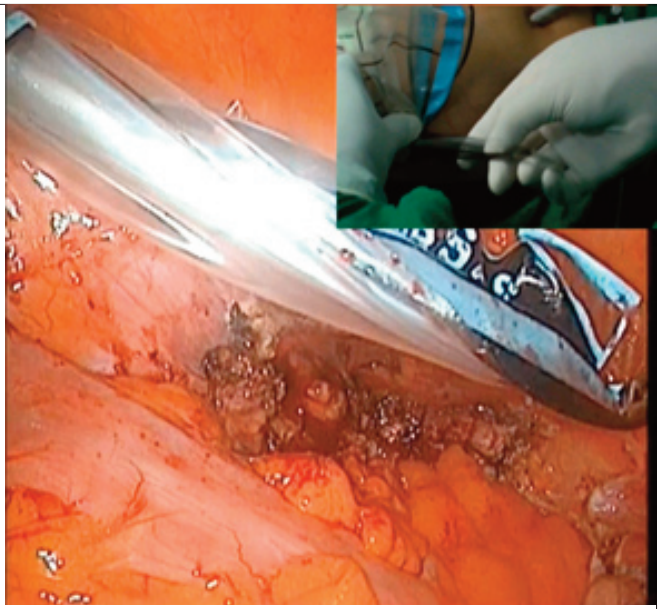
Peripheral and intralesional vascularity in degenerated fibroid and intact junctional zone in leiomyoma.



Translesional vascularity in adenomyoma



Schematic representation of In-Bag



Introduction of Bag with sleeve

• Elastography is a new modality that can help differentiate fibroid from adenomyosis

MANAGEMENT:

Surgical removal of fibroids

In the hands of expert endoscopist, laparoscopic or hysteroscopic removal of fibroids give better results than open myomectomies in terms of postoperative recovery, avoidance of abdominal scar & possibility of infection or hernia of the same. Further in infertile patients the postoperative adhesions with laparoscopic surgery are less dense not affecting future fertility adversely. The authors however agree that with multiple or very large fibroids, the surgical time taken for laparoscopic procedure is definitely longer than open surgery.

Accepted indications for myomectomy

• The presence of a submucous fibroid 2 - 5 cm
• Intramural fibroid > 4 cm, distorting the cavity, infertility of >3 years
• Large fibroid altering tuboovarian relationship
• Multiple large fibroids
• Large Fibroid causing back-pressure to kidneys
• Low large cervical fibroid likely to obstruct labour
• Previous abortion or pregnancy loss

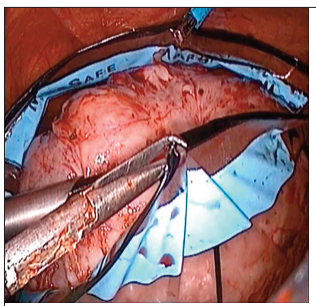
Hysteroscopic Myomectomy:

Removal of intra-cavitory fibroids or those indenting the cavity improves pregnancy rate, increases live birth rates, decreases miscarriages & preterm delivery beyond doubt.

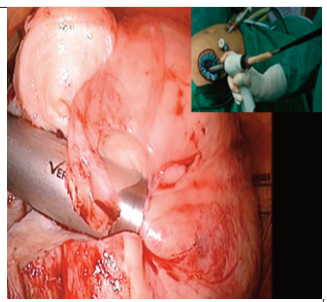
Laparoscopic Myomectomy:

Insights on current controversy regarding laparoscopic morcellation

• USFDA restricted use of laparoscopic morcellation with black box warning in 2014 following a case of leiomyosarcoma in which morcellation led to spread of the disease.
• In the wake of this controversy,we conducted the biggest study on laparoscopic in bag morcellation vs conventional morcellation with 720 fibroid uterus cases Trivedi et al 2019. A unique technique of Visual In-bag morcellation of specimen is introduced by us. A polyurethane bag in the shape of a stomach is used for morcellation and the specimen is put in it. The mouth of the bag is drawn out through the left lower port and the other ear shaped end of the bag is taken out from the main optic port and the laparoscope reintroduced from it. The pneumoperitoneum is created inside the bag and the morcellator is introduced from the left lower port. Morcellation is carried out as usual. Not a drop of blood or any specimen fragment spills over into the peritoneum. This reduces the chances of spread of uterine leiomyosarcoma. However, one should note that even after an open surgery for ULMS, the disease can recur as part of its natural course, thus showing that morcellation is not the only cause of the recurrence. The dilemma here is lack of proper diagnostic parameters pre-operatively. Further a 24 years study in Oncology Multi Institution in Vienna found that out of 71 Leiomyosarcoma none started from fibroid. Even latest Novak's edition clearly mentions that genes for fibroids are totally different compared to leiomyosarcoma. Further it's not possi-



Specimen loading & drawing bag edges



Morcellation within the bag

ble to have a small leiomyosarcoma amongst multiple or large fibroids.

OUR EXPERIENCE WITH LAPAROSCOPIC MYOMECTOMY (~8344 CASES):

Parameter Evaluated	Percentage
Pregnancy rate	42%
Caesarean section	64%
Vaginal delivery	31%
Miscarriage rate	5%
Laparotomy conversion	2.5%
Scar rupture	0%

Pregnancy rate after laparoscopic myomectomy of 42% with active fertility management including IUI, IVF–ICSI. Surprisingly the highest pregnancy rate was 50% in the group of donor oocyte IVF or ICSI.

EFFECT OF FIBROIDS ON FERTILITY & ART OUTCOMES, BASED ON LOCATION:

Intramural fibroids ~5cm lowers implantation rates (13.6% vs 20.2%), pregnancy rates (34.4% vs 47.5 %) and delivery rates (22.9% vs 37.7 %)

Sub-serosal <7 cm have very little effect

Presence of non-cavity distorting intramural fibroids reduced LBR by 21% & CPR by 15% compared to no fibroid

In a retrospective comparative study of 88 patients and 106 ART cycles, 33 subserosal, 46 intramural without cavity distortion & 9 sub mucosal compared to 318 ART cycles in patients without fibroid

Pregnancy rate/Transfer- 34.1, 16.4, 10 & 30.1% respectively

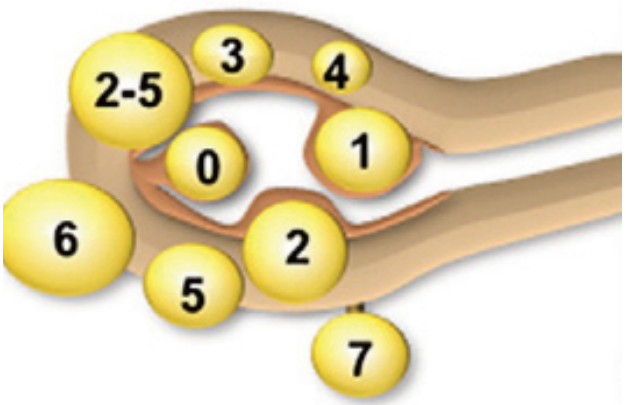
Implantation rate- 15.1, 6.4, 4.3 & 15.7% respectively

Sub mucous fibroid cases had 70% decrease in pregnancy rate mainly due to impaired implantation and increased abortion rates

Women with intracavitary distortion and undergoing myomectomy had significantly reduced midtrimester miscarriage rates in subsequent pregnancies from 21.7 to 0%. This result have been translated to an increase in the live birth rate from 23.3 to 52.0%

WHICH FIBROIDS INTERFERE WITH FERTILITY?

Peripheral and intralesional vascularity in degenerated fibroid and intact junctional zone in leiomyoma.



- Type 0, type 1, type 2: distortion of endometrial cavity and vascularity.Especially those involving > 1/3rd of the endometrial surface
- Type 3 interfering with vascularity
- Type 4 affecting vascularity and peristalsis
- Fibroids in the upper half of the endometrial cavity
- Fibroids close to the cornu
- Intramural fibroids of > 4cms

TAKE HOME MESSAGES:

- **40% of Fibroids need no treatment**
- **Presence of fibroids affect fertility and regardless of the location impair implantation**
- **Intramural fibroids impinging on endometrial cavity have negative impact on implantation, clinical pregnancy, miscarriages, live birth and ongoing pregnancy rates**
- **All large myomas >7 cm and cavity distorting myomas should be removed, if planning for ART**
- **Hysteroscopic Myomectomy has no alternatives**
- **Laparoscopic surgery in the hands of expert is rewarding. Recurrences are ~10%**
- **Open Myomectomy has same results as Laparoscopic Myomectomy in infertility and ART outcome**
- **Post Myomectomy risk of rupture uterus is not high however 70% elective LSCS is preferred**
- **Medical treatment holds promise in symptomatic treatment of fibroids prior to surgery or ART to build haemoglobin.**

Sonography photographs courtesy: Dr.Sonal Panchal


Dr Sathy M Pillai


Dr Venugopal Menon


Dr Ramgopal M Pillai


Dr Janaki Thankam M Pillai

Kerala State Chapter Contribution

EVALUATION OF THE INFERTILE MALE

INTRODUCTION

Approximately 8-15 % couples are unable to conceive by 1 year of unprotected intercourse. Male factor is solely responsible in 20-30 % cases of infertility and contributes in another 30-40% of couples.

GOALS OF EVALUATION

1. To check if semen is normal or abnormal
2. Identify sexual dysfunction

INDICATIONS FOR EVALUATION

A. Couple who fail to conceive after ≥12 months of regular unprotected intercourse
B. Earlier evaluation based on medical history and physical findings
C. After 6 months in couples where female partner > 35yrs
D. Men with concerns about their future fertility

Minimum initial screening of the male partner includes a reproductive history and at least one semen analysis.

HISTORY

Present	Duration of infertility Previous conceptions
Past	Recent febrile illness Diabetes Mellitus Childhood illness Developmental anomalies (Cryptorchidism, urethral stricture) Testicular trauma Infections Pelvic surgeries Chemo/radiotherapy Chest infection (h/o cystic fibrosis) Anosmia (Kallman's syndrome) Kartagener's / Young's syndrome (immotile cilia) Hypogonadotropic hypogonadism
Sexual	Coital timing, frequency, technique STDs Use of lubricants/contraceptives
Personal	Smoking Alcohol Drugs (Anabolic steroids) Stress Gonadotoxins exposure (heat, chemicals, pesticides, environmental toxins) Working shift of couple
Partner	Any evaluation Menstrual abnormality
Family	Infertility Congenital anomalies/genetic diseases

EXAMINATION

Height, build, virilization	Tall, thin, poor virilization ⏩ Klinefelter's syndrome Poor virilization ⏩ androgen insensitivity syndrome
General examination	Gynaecomastia ⏩ low testosterone, high prolactin, Dextrocardia (Situs inversus) ⏩ Kartagener's syndrome **Penis –** length, opening of urethral meatus (r/o hypospadias), curvature (chordee), phimosis
Local examination	**Scrotum –** examine in a warm environment (to prevent cremasteric muscle contraction), Look for sinuses (TB epididymitis), lumps, hydrocele (may indicate past infection which can cause obstructive azoospermia) **Testis –** size, consistency, masses <10ml - testicular failure small and firm – Klinefelter's syndrome small and soft – atrophic testes **Epididymis –** firm, distended ⏩ distal obstruction - Only head palpable ⏩ absent vas deferens - Epididymal nodules ⏩ inflammation including TB **Spermatic cord & Vas –** CUAVD & CBAVD* ⏩ vas deferens not palpable, hence can be diagnosed clinically Varicocele ⏩ examine in standing and supine position
Inguinal examination	Inguinal scars (previous surgeries) Lymphadenopathy Hernia
Per Rectal examination	Prostatic infection Infection of seminal vesicle Cysts of prostate & seminal vesicles are rarely palpable

**Congenital Unilateral Absent Vas Deferens & Congenital Bilateral Absent Vas Deferens*

SEMEN ANALYSIS

Semen analysis is the cornerstone of laboratory evaluation of the infertile man and helps to define the severity of the male factor. A pre-test abstinence period of 2-5 days is advised. Severe oligospermia patients can have equal or better sperm concentration with short (hours) period of abstinence.

Collection is by masturbation into a specimen cup or by intercourse with use of special non-toxic condoms. Ideally specimen should be collected at the laboratory. If collected at home, sample should be kept at body or room temperature during transport and examined within 1hr of collection.

If first semen analysis is normal, there is no need for repeat analysis. Repeat analysis is advised to be done a few weeks apart, if abnormal report is obtained.

Diagnosis of azoospermia can only be confirmed after centrifugation of the sample (at 3000g) for 15 minutes and examination of the pellet.

Semen parameter	2010 WHO lower reference limit
Semen Volume (mL)	1.5 (1.4-1.7)
Total motility PR+NP (%)	40 (38-42)
Progressive motility PR (%)	32 (31-34)
Vitality (% live spermatozoa)	58 (55-63)
Sperm number (10^6 sperm/ejaculate)	39 (33-46)
Sperm concentration (10^6 sperm/mL)	15 (12-16)
Morphology (% normal)	4 (3.0-4.0)
pH	=7.2
Peroxidase positive leucocytes (x10^6 /ml)	<1
MAR (motile spermatozoa with bound particles, %) test	<50
Immunobead test (motile spermatozoa with bound beads, %)	<50
Seminal zinc (micromol/ejaculate)	=2.4
Seminal fructose (micromol/ejaculate)	=13
Seminal neutral glycosidase	=20

Analysis, endocrine evaluation, Post ejaculatory urinalysis, Ultrasonography, specialized tests on semen and sperm, and genetic screening.

COMPONENTS OF A COMPLETE EVALUATION

When initial screening reveals abnormal reproductive history or demonstrates abnormal semen parameters, a thorough evaluation by a male reproductive specialist is indicated. Based on the results obtained additional tests and procedures may be recommended including serial Semen Analysis, endocrine evaluation, Post ejaculatory urinalysis, Ultrasonography, specialized tests on semen and sperm, and genetic screening.

ENDOCRINE EVALUATION

FSH

- Severe oligospermia (<5-10 mill/ml)
- Non-obstructive azoospermia
- Abnormal spermatogenesis on testis histology

FSH/Testosterone/LH

- Clinical features of hypogonadism
- Cryptorchidism

When the Total Testosterone level is below <300ng/ml more detailed evaluation is indicated and should include a second early morning measurement of total Testosterone and measurements of Free Testosterone, LH and PRL. Although serum gonadotropin concentrations vary as they are secreted in a pulsatile manner, a single measurement is usually sufficient to determine the clinical endocrine status. Measurement of TSH is also done.

CONDITION	***FSH***	***LH***	***T***	***PRL***
Normal Spermatogenesis	Normal	Normal	Normal	Normal
Abnormal Spermatogenesis	High/Normal	Normal	Normal	Normal
Hypogonadotropic Hypogonadism	Low	Low	Low	Normal
Complete Testicular failure/ Hypergonadotropic hypogonadism	High	High	Normal/Low	Normal
PRL -secreting pituitary tumour	Normal/Low	Normal/Low	Normal/Low	High

GENETIC TESTING

3% of infertile men have genetic abnormalities. In men with non-obstructive azoospermia (NOA), this rises to above 12%. Men with NOA should undergo genetic testing before resorting to ART. Screening usually includes karyotyping and testing for Y chromosome microdeletions.

Post-orgasm urine

Men with aspermia or low volume ejaculate may have retrograde ejaculation. If the post-orgasmic urine is cloudy in appearance with presence of total number of sperms in urine ≥ number of spermatozoa in semen, retrograde ejaculation can be diagnosed. Only presence of sperm in post-ejaculatory urine is not enough. Retroejaculators have Total Sperm Number in Urine (TSNU) > 3.8 x 106 and Retroejaculation index or RI >2.16%. [3]

$$RI = \frac{\text{total sperm in urine}}{\text{total sperm in urine} + \text{total sperm in semen}} \times 100$$

SPERM DNA FRAGMENTATION

Defects in the sperm DNA can be assessed using different tests – COMET (Single cell gel electrophoresis assay), TUNEL – Terminal deoxy Nucleotide Transferase-mediated dUTP nick end labelling assay, SCSA – Sperm Chromatin Structure Assay. Sperm DNA damage can cause miscarriages. This test, though not a routine test, maybe done following varicocele repair or prior to IVF-ICSI.

SEMEN CULTURE

Presence of leucocytes may not be an indication of bacterial infection especially when there are no clinical symptoms. Men with symptoms or persistent pyospermia or positive cultures should be treated with antibiotics. Routine semen culture should be avoided.

UNNECESSARY TESTS TO BE AVOIDED

- Open testicular biopsies – they cause scarring of the testicular tissue.
- Routine use of vasograms are more harmful than useful.
- Estimation of FSH is useful in some patients, but routine hormonal analysis other than TSH is rarely required.
- Genetic tests need be done only in indicated cases.

CONCLUSION

The male and female partners should be evaluated simultaneously for infertility. Usually in the case of the male, history, physical examination and semen analysis can diagnose the cause for infertility. If the semen parameters are abnormal, multiple semen analyses should be done to confirm the abnormality. Open testicular biopsies should be avoided. Genetic tests and hormonal evaluation should be done only if indicated.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Madhya Pradesh Chapter Contribution

WHAT A CLINICIAN SHOULD KNOW ABOUT EMBRYOLOGY?

INTRODUCTION
Embryology as a field is in a period of unprecedented change in its knowledge base. Like any other subject, embryology must justify its place in an increasingly crowded medical curriculum. This is because of the following reasons:

1. Understanding how male and female gametes arise and mature: how they are transported; and how the process of fertilization ultimately results in the fusion of the male and female genetic material. Any real understanding of these processes requires an integration of the basic genetics of mitosis and meiosis with the histology of gametogenesis and knowledge of the hormonal control of maturation. The phenomenon of parental imprinting should be studied and understood.

2. Recognition of the timing of embryologic events.

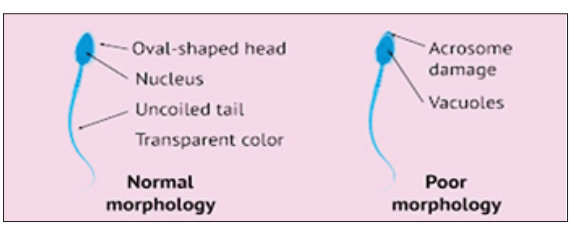
3. Understanding fundamental mechanisms and processes in embryo development.

4. An understanding of the molecular and genetic basis of development.

5. Understanding the interactions between the embryo and its mother: Starting with cleavage, the embryo is exposed to maternal fluids, and with implantation there is a cell–cell interaction between the embryonic trophoblast and the uterine epithelium. With the invasion of the embryonic trophoblast into the uterine lining, a new level of interaction and exchange between embryo and mother occurs. This would include the protection of the embryo complex from rejection by the mother's immune system.

Practically, the clinician should know the following minimum details about embryology. This is important for two reasons:
a) If the clinician is ready for oocyte retrieval and the embryologist fails to arrive. Similarly, if embryo transfers are scheduled and the embryologist doesn't arrive.
b) In many circumstances, the clinician needs to assess and explain success as well as failure rates to the patients. Here, he/she should have accurate knowledge about the intricacies of that particular IVF/ICSI cycle(s) at every stage of the procedure. That way the patient's queries can be handled more confidently by the treating physician.

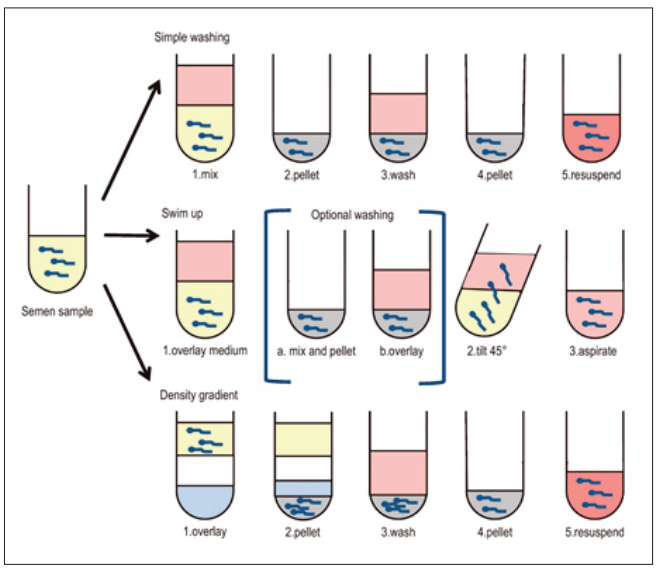
The following are the key areas where sufficient working knowledge is mandatory for clinicians.
1. MALE GAMETE-SPERMATOZOA: NORMAL MORPHOLOGY AND COUNT WITH DIFFERENT SEMEN-PREPARATION TECHNIQUES.
(WHO 2010 CRITERIA)
Normal semen parameters required for IVF and ICSI should be known. Different semen preparation techniques should also be known in detail. All clinicians should be familiar with the andrology laboratory procedures along with knowledge of various media used.



WHO 2010

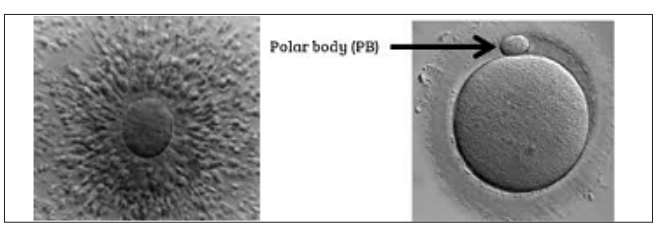
Parameter	1992	Lower Reference Limit 2010
Semen volume	2 ml	1.5 ml
Sperm concentration	20 M	15 x 10^6/ml
Total sperm number		39 x10^6/ejaculate
Progressive motility	>50 %	32 % A
Total motility		40 % A+B
Vitality (live sperms)		58 %
Sperm morphology	>15 %	4 %
pH	>/=7.2	>/=7.2
Leucocyte	<1M	<1 x10^6/ml
MAR/Immunobead test	<10 %	<50 %

DIFFERENT SPERM PREPARATION TECHNIQUES:



2. FEMALE GAMETE-OOCYTE: NORMAL OOCYTE IDENTIFICATION AND MORPHOLOGY.

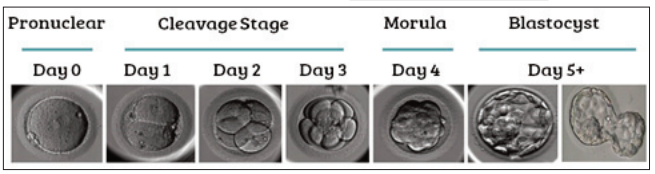
Embryo competence is most likely due to the quality of the originating gametes. Therefore, the morphologic appearance of the oocyte may indicate developmental potential of the subsequent embryo. The evaluation of oocyte morphology before standard IVF is difficult because of the presence of cumulus and corona cells. On the other hand, after cumulus-corona-cell removal, nuclear maturity of the oocytes can be easily assessed by visualizing the presence of the first polar body. Abnormal zona pellucida, large perivitelline space, vacuoles, refractile bodies, increased cytoplasmic granularity, smooth endoplasmic reticulum clusters, and abnormal, fragmented, or degenerated polar bodies can all be observed after oocyte denudation.



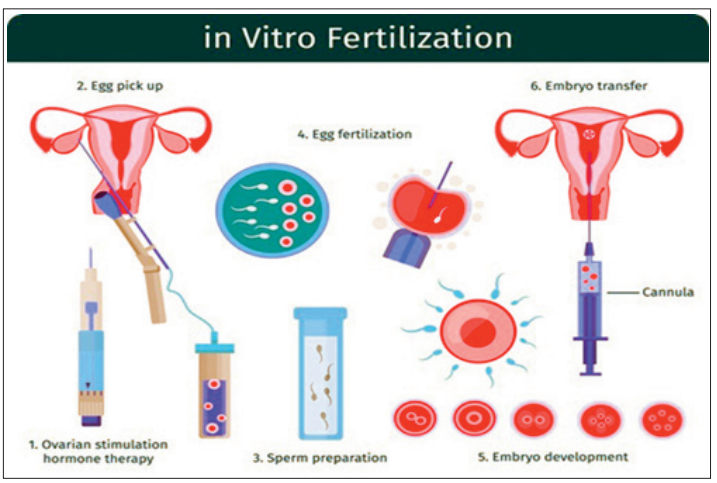
3. STAGES OF EMBRYO DEVELOPMENT:

STAGES OF EMBRYO DEVELOPMENT DURING IN-VITRO FERTILIZATION (IVF):

1. Fertilized Pronuclear Embryo- A fertilized pronuclear embryo showing syngamy (or coming together) of the male and female pronuclei at about 16-24 hours.
2. Fertilized 2 Cell Embryo.
3. Fertilized 4 Cell Embryo.
4. Fertilized 8 Cell Embryo.
5. Morula.
6. Blastocyst.



4. BASIC STEPS OF IVF:

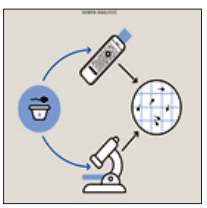


LAB AND EQUIPMENT PREPARATION: The clinician has to prepare the required media and tubes according to manufacturer's guidelines, one day prior to the scheduled oocyte retrieval.

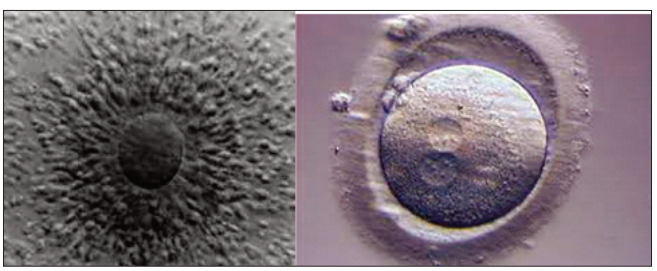
OOCYTE ASPIRATION: During the course of a follicular aspiration, it is vital that the clinician be able to communicate and discuss the source of the aspirate, i.e., the size and appearance of the follicles, the character of the aspirate, whether or not the aspirate contains granulosa cells, a portion of the cumulus mass, and/or the oocyte itself. Heating blocks, collection tubes and petri dishes are to be pre warmed to 37 degrees centigrade. Follicular aspirates are checked for the presence of oocyte-cumulus complexes under a microscope, as soon as possible.

PREPARATION OF THE SPERMATOZOA FOR INSEMINATION:

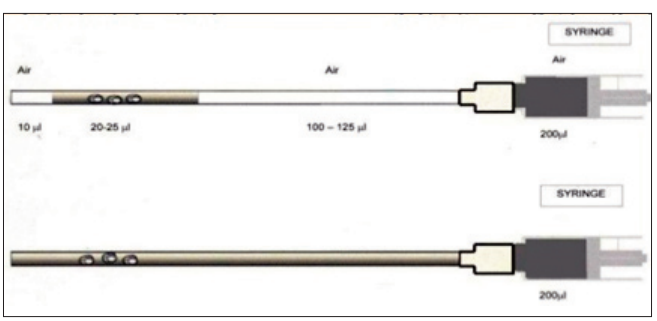
Different semen preparation methods are used as indicated by the previous semen reports.
For IVF, a record has to be kept of the time of insemination and the sperm concentration used. (Minimum 50,000-1,00,000 sperm count is required per oocyte for IVF).



FERTILIZATION CHECK:
-Fertilization check is done around 16-18 hours after insemination for the presence of two pronuclei (2PN Stage).
-Status of each oocyte should be recorded.
-The normally fertilized oocytes are removed from the insemination medium and transferred into new dishes with pre-equilibrated fresh medium.
-Denudation is done and media changed if required.
If Blastocyst culture if opted for, then on day 2/3, Cleavage media is changed to Blastocyst media.

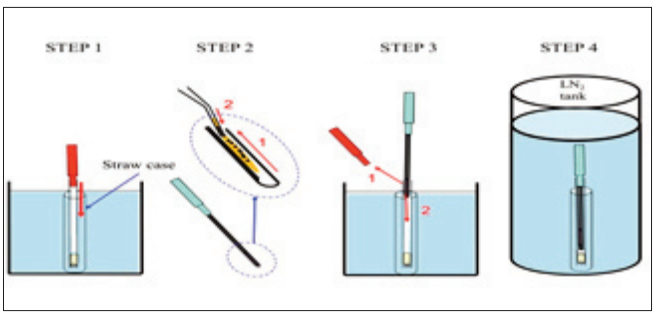


5. EMBRYO TRANSFER: BASIC STEPS:
The clinician should be able to load the embyo(s) in the Embryo Transfer Catheter. Sterile disposable ET catheters should be used with which the clinician is familiar and comfortable.

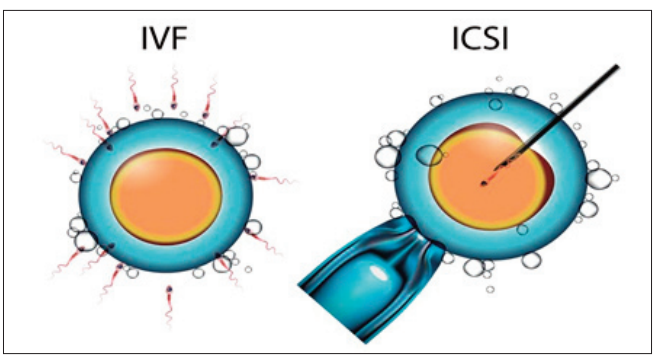


DIFFERENT LOADING TECHNIQUES :
Minimum loading culture media with air and media column in three or two .
A. Load in ET catheter: air, medium, air
B . Load in ET catheter: medium, air

6. CRYOPRESERVATION: BASIC STEPS
The clinician should know how to vitrify the cleavage stage embryos and blastocyst. Spare embryos can be vitrified as well as those in patients who are nor deemed fit for transfer in that cycle. Highly fragmented embyos and those with a very slow cleavage rate should be not vitrified.
- Transfer of embryos to receptacles should be by a method which avoids contamination of the external surface.
- Sealing should be checked carefully before freezing.
- Documentation of stored embryos should be done.



7. ICSI: SPECIFIC INDICATIONS (When to shift from IVF to ICSI)
It is not a must that a busy clinician needs to learn to do ICSI as well because it is a highly technical and time consuming procedure. But a thorough knowledge of the indications of ICSI is mandatory.



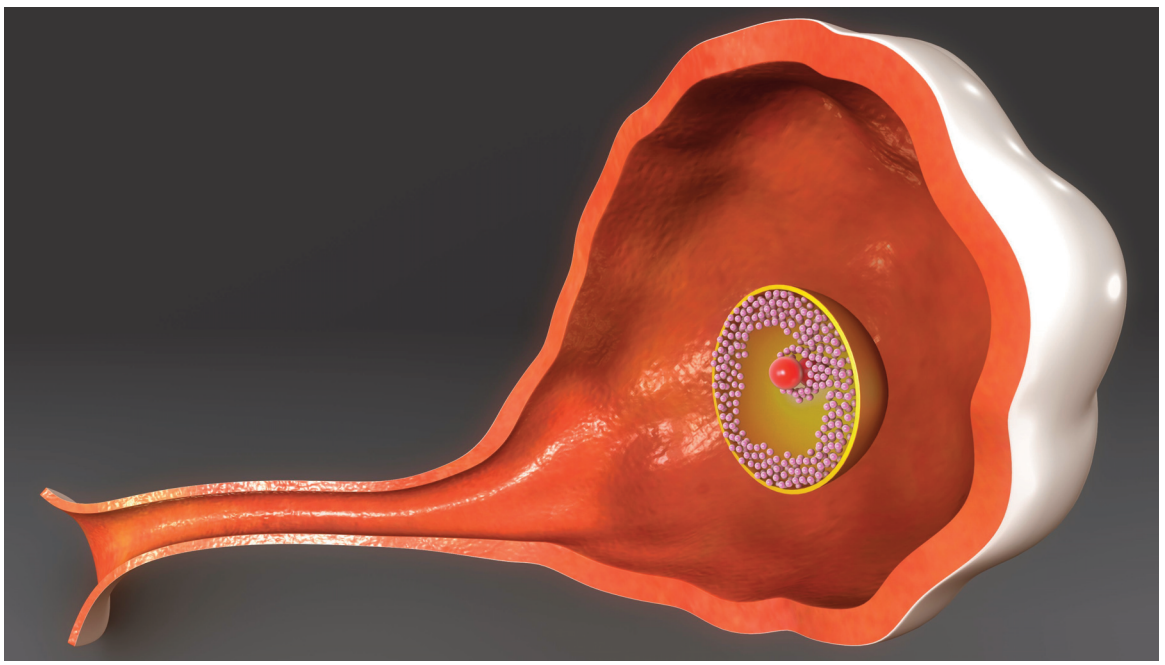
8. EMERGENCY SITUATIONS TO BE DEALT WITH:
a) Power supply to the laboratory
b) Gases in the laboratory (O2, CO2, N2O)
c) Instrument Calibration and breakdown
In these situations, the clinician should have back-up and should know the management of all required equipments in Lab.

As a final remark, in todays's ART practice, the clinician and embryologist work in unison, but for providing the very best results to patients, any untoward shortcoming should be eliminated. It is strongly advisable that the clinician learn the basics of embryology in order to gain confidence and expertise in emergency situations.

HUMOUR

Dr. Madhuri Patil
Dr. Patil's Fertility & Endoscopy Clinic, Bangalore

THICK ENDOMETRIUM AND ART OUTCOME



INTRODUCTION

Implantation failure due to an endometrial deficiency apart from embryo quality is an important reason for IVF failure in many cases. Knowledge on endometrial predictive factors for the occurrence of pregnancy is essential. Of the endometrial parameters, endometrial thickness assessment has become an integral part of standard monitoring during fertility treatment. It is easy to measure and has some prognostic value though it has limited capacity to identify women who have low chance to conceive after IVF. Frequently reported cut-off of less than 7 mm and more than 14 mm are related to a lower chance of pregnancy.

Use of endometrial thickness as a tool to decide on cycle cancellation, freezing of all embryos or refraining from further IVF treatment seems unjustified based on the current meta-analysis. In the presence of thick endometrium it is important to exclude intrauterine pathology before IVF or have evidence of a normal uterine cavity by a 3D ultrasound, normal hysterosalpingogram (HSG) or hysteroscopy before treatment.

As intrauterine pathology can influence reproductive outcome. Moreover the time period elapsed between an normal HSG and hysteroscopy and IVF will also make a difference. An transvaginal ultrasound done immediately before starting ovarian stimulation may exclude newly developed endometrial changes. Endometrial thickness alone on the day of hCG is not an independent prognostic parameter for treatment success in IVF though certain studies have reported significantly reduced implantation and pregnancy rates if the endometrial thickness was more than 14 mm while other studies have shown no such relationship. Two measures of uterine receptivity that are commonly used for predicting pregnancy are the thickness and pattern of the endometrium, measured by transvaginal ultrasound on the day of hCG trigger. Apart from the baseline endometrial evaluation and evaluation of endometrial thickness and pattern on day of hCG trigger, evaluation of blood flow and volume may help in predicting treatment success.

The mechanism leading to this poor outcome may be related to an increased risk of endometrial trauma due to the transfer catheter at the time of ET.

In the presence of thick endometrium it is also important to differentiate between endometrial hyperplasia without atypia and with atypia.

CAUSES OF ENDOMETRIAL HYPERPLASIA

1. Hormone Imbalance: May be due to relative excess of estrogen to progesterone which is seen in anovulatory cycles associated with polycystic ovarian syndrome (PCOS) and perimenopausal states

2. Obesity: Androgens are converted to estrogens in the fat and this excess of estrogens can stimulate the endometrium which results in excessive endometrial thickness.

3. Exogenous Hormones : Estrogen replacement therapy can result in endometrial hyperplasia. Another hormonal medication that can cause an abnormal thickening of the endometrium is Tamoxifen which is used for ovulation induction in few cases and for treatment of hormone-sensitive breast cancers

4. Estrogen producing ovarian tumours: Granulosa cell tumor and thecoma

5. Other causes of thick endometrium: Polyps may be endometrial mucous polyps which appear echogenic or they may be fibroid polyps which appear hypoechoic compared to the surrounding endometrium. Polyps are detrimental to implantation and need to be removed prior to ART. Endometrial polyps being echogenic are often difficult to detect by ultrasound during the second half of the cycle due to the echogenic nature of the surrounding endometrium.(Figure 1) This is when Doppler helps. One can look for feeder vessels to confirm the presence of a polyp. 3D helps to objectively determine and document the position of the polyp.

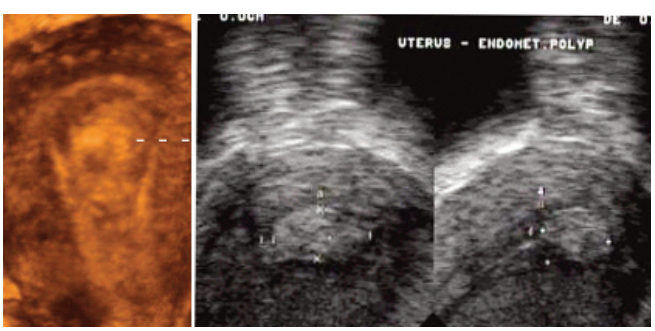


Figure 1 a and b: 3 D and 2 D Ultrasound picture of endometrial polyp

Diagnosis

History: medical history

Transvaginal ultrasound: Both 2D and 3D which helps in evaluating thickness and pattern of endometrium and also view myometrium and ovaries.

Endometrial thickness (Figure 2)

- measured in the sagittal plane
- distance between the hyperechogenic interfaces between the endometrium and myometrium should be recorded approximately 1cm beneath the uterine fundus .

When a collection of fluid is found in the uterine cavity the thickness of the fluid layer should be subtracted from the overall measurement. Abnormal (non-homogenous) echogenic patterns of the endometrium which may be either isoechoic or hyperechoic on day of hCG may be a sign of adverse ART outcome. (Figure 3)

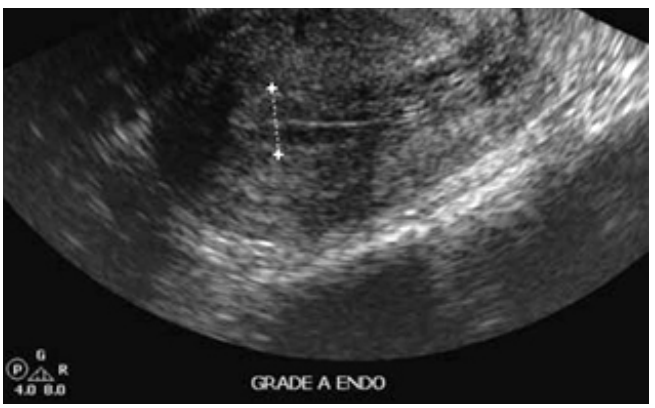


Figure 2 : Measuring endometrial thickness in sagittal plane

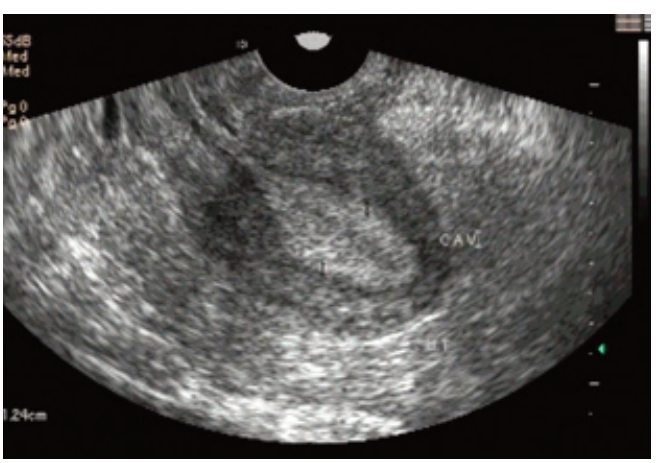


Figure 3 : Abnormal (non-homogenous) echogenic patterns of the endometrium on day of hCG

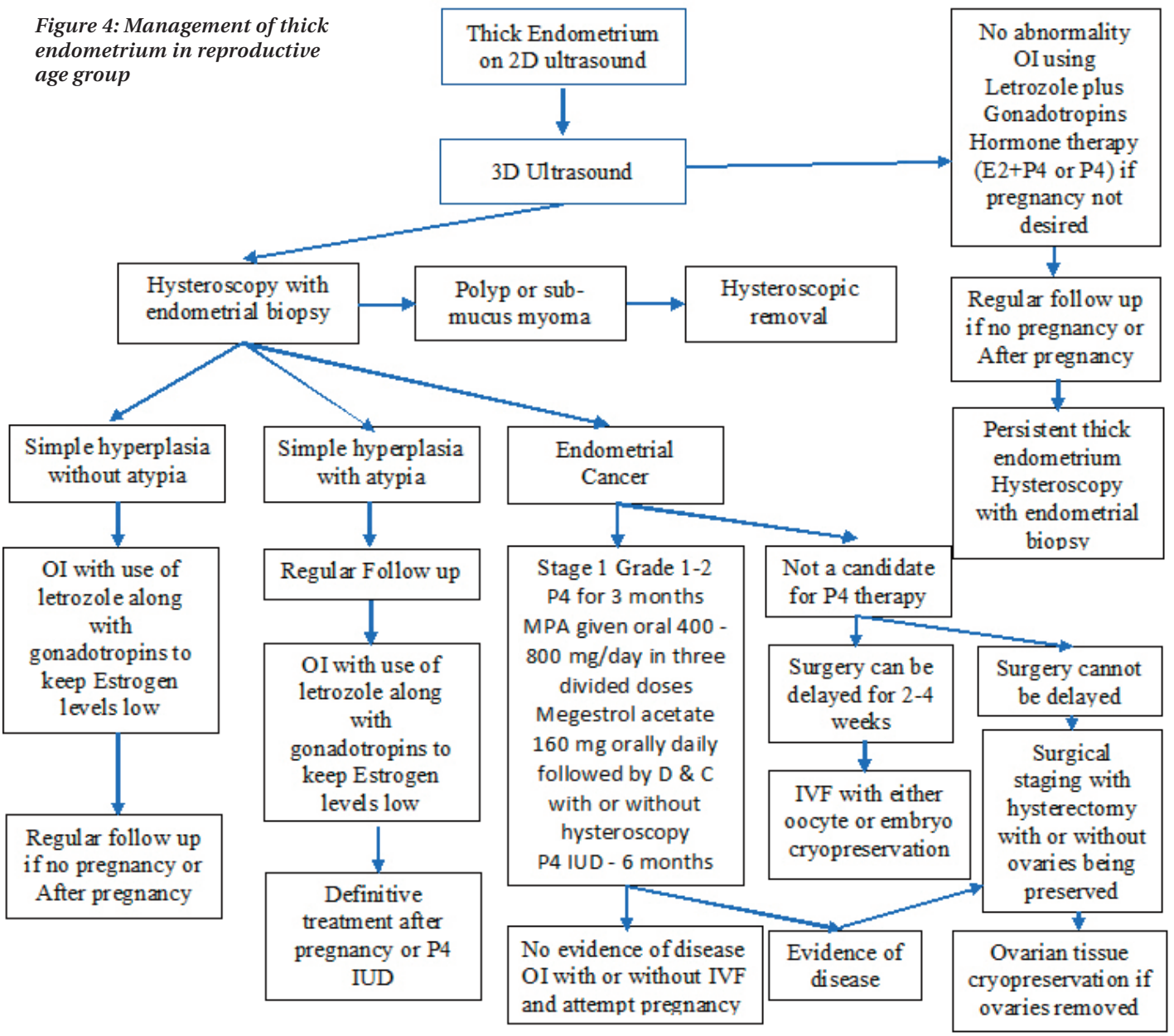
ULTRASOUND PARAMETERS THAT INDICATE A GOOD RECEPTIVE ENDOMETRIUM

o Endometrial morphology – "triple line" pattern
o Endometrial thickness of 8 – 14 mm
o Endometrial perfusion – presence of sub-endometrial and endometrial flow. Presence of sub-endometrial vascularisation is an indicator of endometrial receptivity
o Low RI of sub-endometrial vessels – good pregnancy outcome in embryo transfer into optimal areas of cavity under USG guidance

o Uterine Vascularity – Mean uterine artery PI between 2 – 3 and uterine artery PSV - 15 -20 cm/s
o Volume of more than 2ml has a significantly higher pregnancy rates

HYSTEROSCOPY: Hysteroscopy is a safe and reliable procedure for evaluating benign lesions of endometrium such as polyps or submucosal myomas and to rule out endometrial hyperplasia and cancer.

Figure 4: Management of thick endometrium in reproductive age group



o Persisting presence of fundo-cervical (FC) waves until hCG administration after which wave direction switch occurs from FC to cervico-fundal (CF). This has a higher the likelihood of pregnancy

Biopsy: Taking endometrial biopsy with histopathological examination is recommended in both presence and absence of endometrial lesions when thick endometrium is persistent on ultrasound.

Treatment: It is very important that all endometrial hyperplasia be closely followed or treated. (Figure 4)

ENDOMETRIAL HYPERPLASIA WITHOUT ATYPIA: When there are no atypical cells present, the chance of endometrial hyperplasia eventually becoming endometrial cancer is very unlikely. The evidence suggests that only about 5% of women with endometrial hyperplasia without atypia will develop endometrial cancer. It is also likely that this type of endometrial hyperplasia will resolve on its own over time.

TARGET RISK FACTORS : The first line of treatment is to look for risk factors that are modifiable. For example, if significantly overweight or obese, losing weight will help decrease the excess estrogen produced by fat cells. Similarly, if on hormone replacement therapy the dose and drug needs to be adjusted or stopped.

PROGESTERONE: Progestin treatments counteract the thickening effect of the excess estrogen on endometrium. The two types of progesterone suggested for the treatment of endometrial hyperplasia without atypia are oral progesterone or the progesterone-containing IUD.

Follow up with endometrial sampling is essential.

ENDOMETRIAL HYPERPLASIA WITH ATYPIA: There is a much more significant risk of developing endometrial cancer in presence of hyperplasia with atypia. The management may be more aggressive because of increased risk of developing cancer. Hysterectomy is recommended as the first line treatment for atypical hyperplasia in women who are done having children. If still planning for pregnancy treatment is elucidated in figure 4. In this case more frequent endometrial sampling is required to assure that the atypical hyperplasia has been treated adequately. Hysterectomy is recommended after having children because of the high likelihood of recurrence of atypical endometrial hyperplasia.

CONCLUSION

Although the effect of endometrial thickness on IVF success has been addressed in the literature. Most literature discusses on the minimal thickness compatible with pregnancy. Endometrial thickness assessment has become part of standard monitoring during fertility treatment, which is easy to measure. Both thin and thick endometrium has been associated with lower IVF success rates but the results from studies that investigated the relationship between endometrial thickness and IVF outcomes are conflicting. Lack of consensus possibly explained by fact that, no exact definition of thin or thick endometrium as assessed by ultrasound exists.

The impact of increased endometrial thickness at the time of hCG trigger or ET, on implantation and pregnancy rates has been controversial. Several studies have suggested a poor outcome when the endometrium exceeded a thickness. Of more than 14 mm while others have shown no difference in the clinical pregnancy rates. Therefore it is necessary to counsel patients about the uncertainties of transfer in the setting of extreme endometrial thickness. Cryopreservation of embryos with transfer in subsequent cycle may be an option but reports of successful outcomes are reassuring for proceeding with ET in a fresh cycle.

Further research is needed to investigate the real independent significance of thick endometrium in IVF. Possible interrelation of the factors like female age and oocyte number with endometrial thickness should be established. Newer ultrasound modalities (e.g. spatio-temporal image correlation for assessment of blood flow) should be investigated along with endometrial thickness. Further histology studies might be of value to unravel the pathophysiology at the endometrium level.

HUMOUR

I'm so classy even my ovaries wear a string of pearls.

-PCOS-

Maharashtra State Chapter Contribution

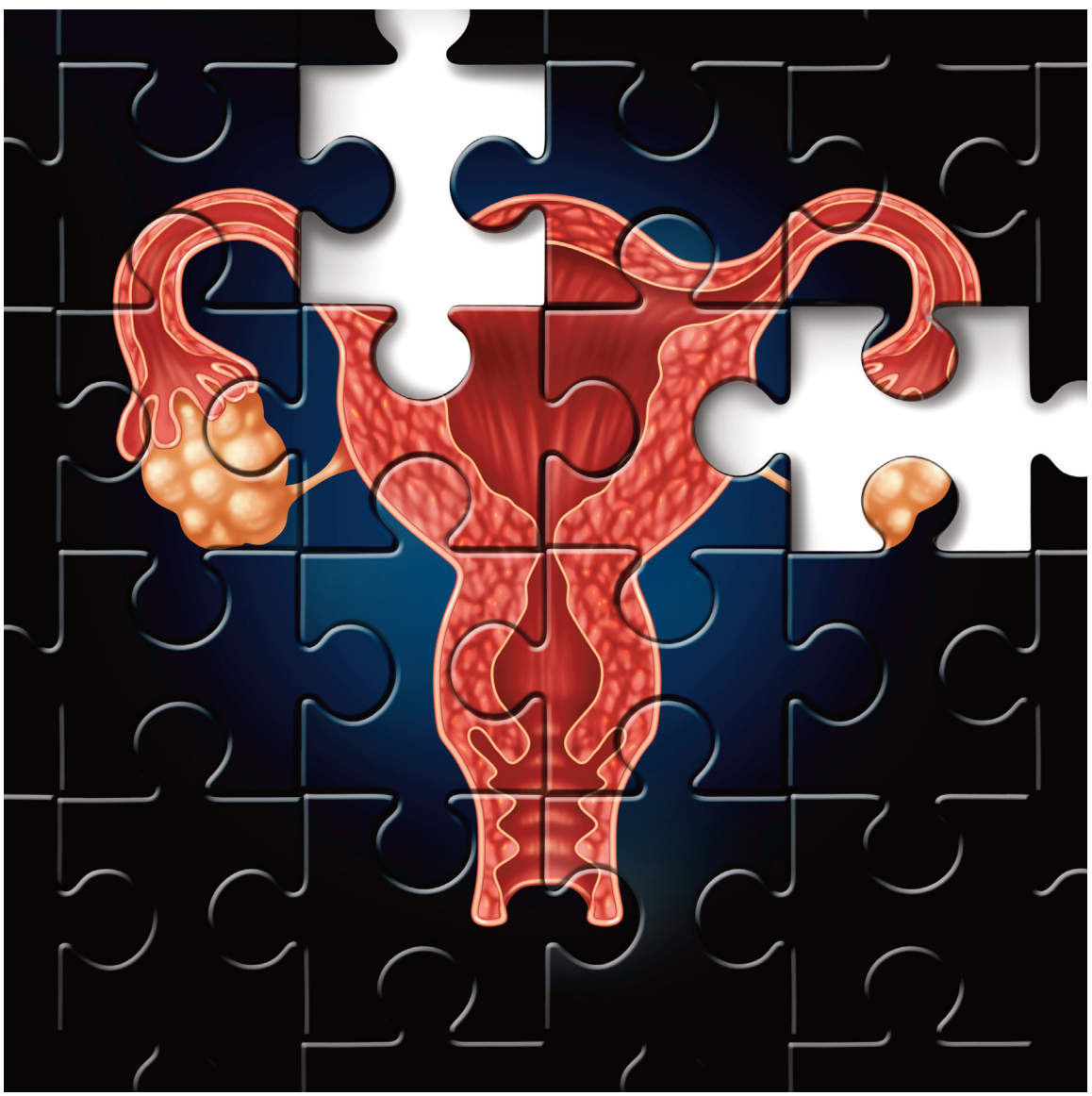
CHRONIC ENDOMETRITIS


Dr Sheetal Sawankar


Dr Mohit Saraogi


Dr Shrutika Thakkar


Dr Neelam Bhise



DEFINITION

Chronic endometritis (CE) is a disease of continuous and subtle inflammation OF endometrium characterized by the infiltration of plasma cells in the endometrial stromal area.

PATHOLOGY

Breakdown of the peaceful co-existence between microorganisms and the host immune system in the endometrium.

PREVALANCE

Infertile Women	Recurrent Implantation Failure	Recurrent Pregnancy Loss
2.8 TO 56.8%	14 TO 67.5%	9.3 TO 67.6%

MICROORGANISMS INVOLVED - Mainly Gram negative bacteria
- E. Coli
- Streptococci
- Ureaplasma Urealyticum
- Staphylococci
- Enterococcus faecalis
- Klebsiella Pneumoniae

SOURCE OF INFECTION
1. Ascending infection from the vaginal tract .
It occurs due to
    - Break in cervical mucous barrier
    - Break in the defence mechanism of endometrial epithelium
    - Altered immune response of the endometrium
2. Haematogenous spread from other chronic infections of the body, eg. Chronic otitis media
3. Direct peritoneal spread of gut organisms through the fallopian tubes.

SYMPTOMS
1. Mostly Asymptomatic
2. Occasionally mild symptoms:
    - Abnormal uterine bleeding- polymenorrhea to amenorrhea
    - Pelvic pain
    - Leucorrhoea
    - Dyspareunia
    - Difficulty in conception

PATHOPHYSIOLOGY

Endotoxin of gram negative bacteria

↓

Shift in immune response at the level of the decidua

↓

TH2 to TH1 cells shift
More circulating NK cells
More Lymphocyte B cell levels
More CD3+ NK cells

↓

Less NK cells in endometrium
Less CD56 bright and CD16 – NK cells

↓

Micropolyps formation

↓

Menstrual secretions with increased proinflammatory cytokines of IL-6, IL-1 beta, TNF alfa

↓

Genetic modulations rise in IFGP1 & fall of IL-11, IGF-1, CCL4

In summary, chronic endometrial inflammation can alter endometrial cytokine production, damage endometrial function, result in the formation of abnormal patterns of lymphocyte subsets in the EM, and induce the altered secretion of paracrine factors, which ultimately may reduce the receptivity of embryos in the endometrium.

EPIDEMIOLOGY AND CLINICAL FEATURES
The prevalence of CE ranges from 8% to 72% in women of reproductive age.
Tubercular endometritis merits special mention. This is usually seen secondary to respiratory or abdominal localisation, with a clear predilection for adnexal localisation.

Associated Conditions:
- Intrauterine Device usage
- Endometriosis
- Bacterial Vaginosis
- Endometrial polyps

Impact on Fertility:
- Decreased spontaneous conception rates
- Lower Implantation rates
- Recurrent Pregnancy loss
- Recurrent Implantation Failure
- Adverse obstetrical outcomes- intra uterine infections, preterm delivery and postpartum endometritis.

DIAGNOSIS
Endometrial Biopsy (Definitive diagnosis)
- Quantitative polymerase chain reaction or next generation sequencing of 16s RNA gene in endometrial sample is highly sensitive in picking up small colonies of bacteria as well.
- Immunohistochemical staining (IHC)- It has a sensitivity of close to 58%

IHC stain is capable of detecting CD38 and CD138 plasma cell specific surface antigen.
- H & E staining – Has low sensitivity for plasma cells (<13 %)

Histological Features On H&E staining:
- More plasma cells in the stromal area of the endometrium
- High stromal cell proliferation
- Dissociated maturation between the epithelium and stroma
- Pronounced pre-decidual reaction

• 'Spoke wheel' or 'clockwork' pattern of plasma cells

Although such pathological features can be confirmed with stains such as hematoxylin and eosin (HE), it is hard for even experienced pathologists to detect plasma cells in the endometrium because of monocyte infiltration, stromal mitosis, plasmacytoid appearance of stromal cells, and predecidual reaction, which are morphologically difficult to distinguish. Thus, immunohistochemistry (IHC) for detection of the plasma cell marker CD138 (also known as syndecan-1) is used clinically to diagnose CE, since it stains well on the surface of plasma cells.

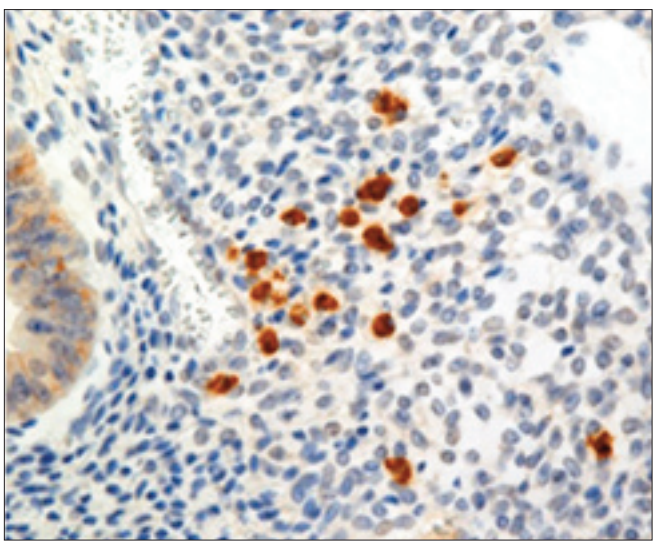


FIGURE 1. Immunohistochemistry for detection of plasma cells with CD138. CD138 is stained on the surface of plasma cells in the stromal compartment of chronic endometritis

2. Ultrasound and Colour Doppler

• Thick and hyperechoic endometrial stripe difficult to delineate with respect to the surrounding myometrium
• Multiple cones of shadow from the endometrium obscuring the posterior uterine wall
• Intracavitary/cul-de-sac fluid
• Gas formation within the endometrial cavity
• Hematometra/pyometra
• Intracavitatory synechiae
• Increased vascularity on Doppler ultrasound

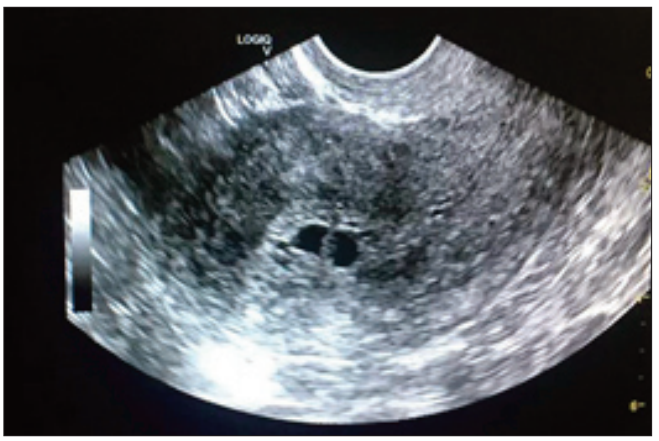


FIGURE 2- USG shows fluid with band/adhesion

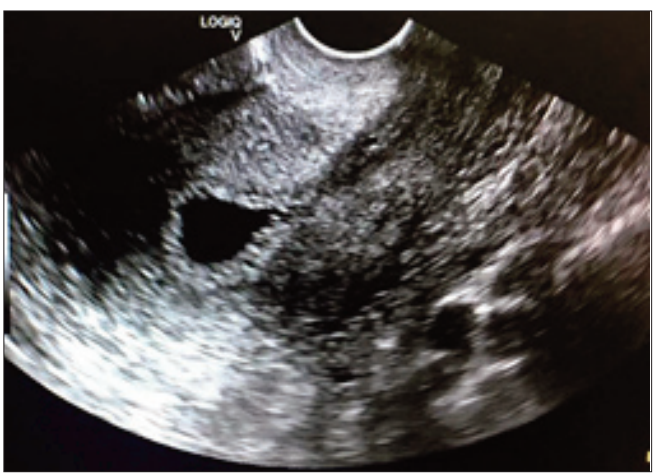


FIGURE 3- USG shows fluid in the endometrial cavity

3. Fluid Hysteroscopy- has 93.4% diagnostic accuracy

• Micropolyps
• Stromal edema
• Diffuse or focal hyperemia- bright red with white central dots called 'strawberry pattern'
• Uterine synechiae

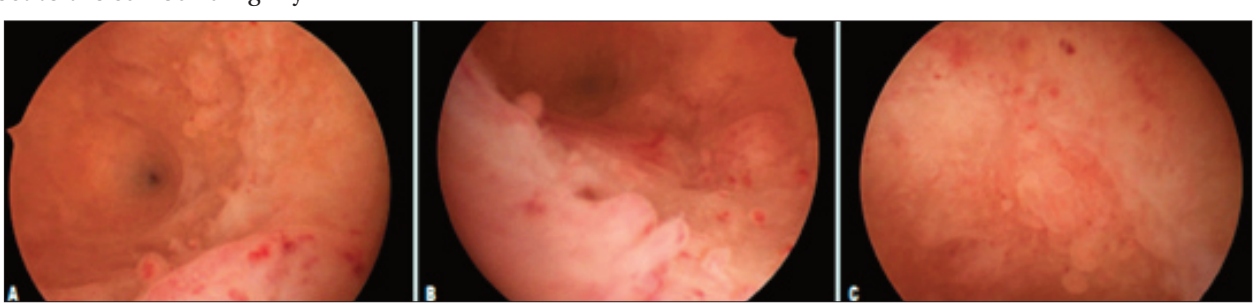


FIGURE 4 – The stromal edema is clearly evident on the posterior wall (A) and micropolpys are detected on the uterine walls (A-C)

4. Culture of endometrial sample for bacterial colonization

5. Magnetic Resonance Imaging (MRI)
• T2: the uterus may be enlarged with overall high signal intensity
• T1 C+ (Gd): can show intense enhancement of the uterus

COMPLICATIONS
• Progression to a pyometrium
• Pelvic septic thrombophlebitis

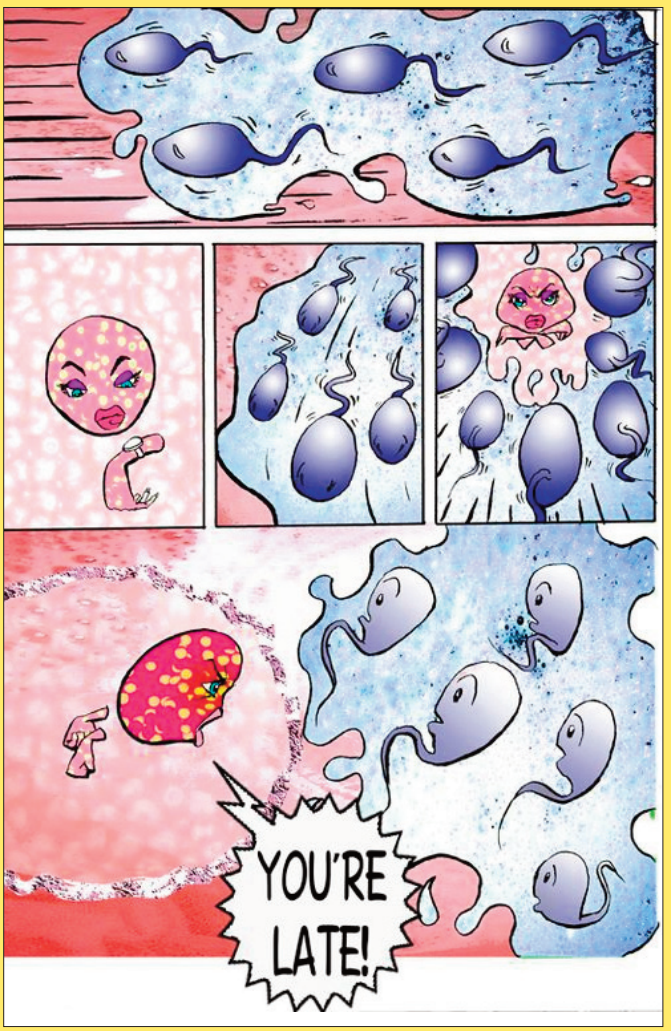
TREATMENT
The therapy for chronic endometritis is pharmacological and is based on the administration of broad spectrum antibiotics.
• DOXYCYCLINE 100 mg twice a day for 14 days has shown to have a cure rate of 96%.
• Combination of Ornidazole 400 mg BD plus metronidazole 500 mg BD has shown to have a cure rate of 73%.
• In case of persistence of signs of CE at subsequent hysteroscopy, the protocol can be repeated up to three times.

In a retrospective study including patients with unexplained RPL, the patients in whom hysteroscopic findings normalized after antibiotic treatment for CE, regardless of the results of endometrial cultures, showed a significantly higher incidence of successful pregnancy than the patients who did not show normalization

TAKE HOME MESSAGES
1. Highly prevalent condition in cases of poor reproductive outcomes but difficult to diagnose
2. Immune abnormalities and impaired decidualization of the endometrium are seen
3. Histological diagnosis with endometrial biopsy is the gold standard investigation.
4. Antibiotic therapy may improve uterine receptivity
5. Timely treatment of this chronic inflammatory condition improves reproductive and pregnancy outcome

As gynecologists it is our job, to keep this condition in mind and actively search for it when dealing with cases of infertility, especially those associated with a poor reproductive outcome.

HUMOUR



OPTIMIZATION OF OOCYTE QUALITY IN POOR RESPONDERS

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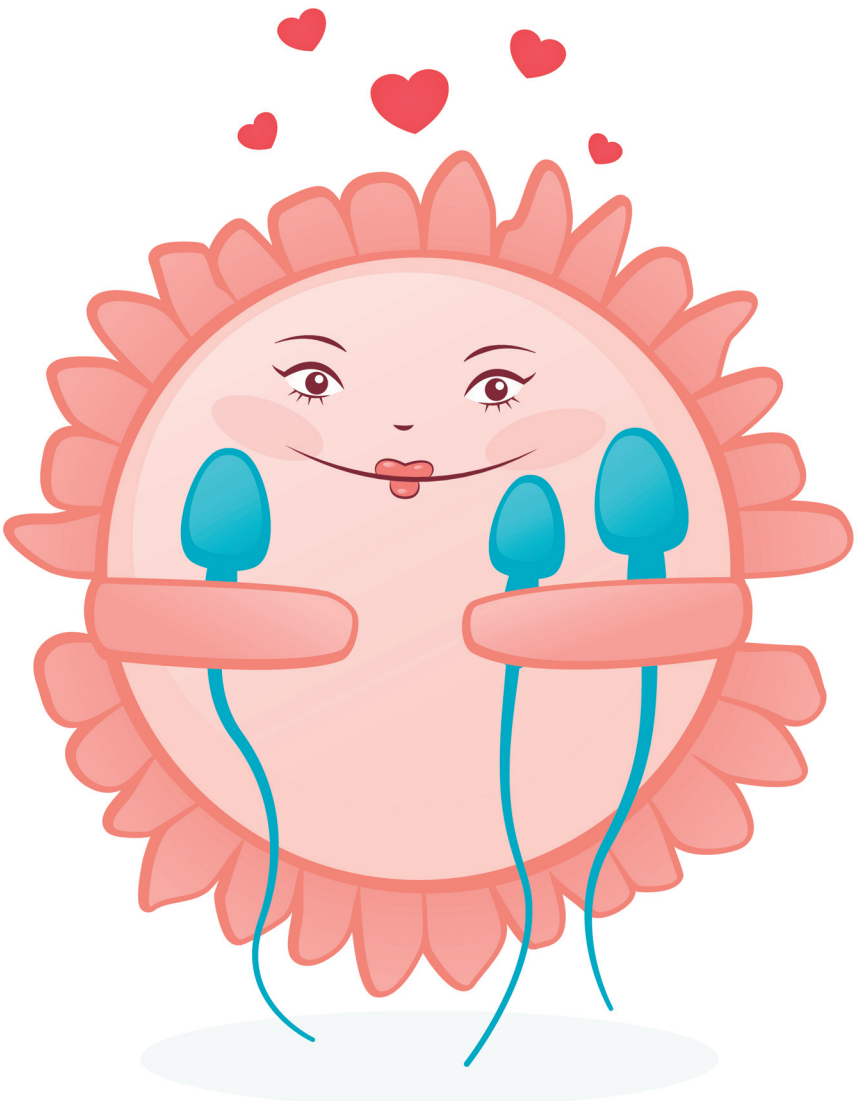


INTRODUCTION

Despite numerous advances in the field of assisted reproduction, poor ovarian response to controlled ovarian stimulation still remains a major problem in IVF practise. The incidence of POR varies from 9–24% according to various studies. Despite using different stimulation protocols and multiple treatment courses of IVF, the pregnancy outcome remains poor in these women, which is exasperating for clinicians as well as patients. Over the years, numerous techniques and therapies have been developed in an effort to optimise the quality and quantity of oocytes and embryos and thus improve the suc-

Pretreatment / Adjuvants	Stimulation Protocols& Triggers	Newer Modalities
• Growth Hormone • Androgens- ➢ DHEA ➢ Testosterone • Antioxidants ➢ Co-enzyme Q ➢ Melatonin	• Gonadotropins-dose and type • Addition of LH • Minimal/ mild stimulation • Dual stimulation • Dual triggers	• Autologous Stem Cell Ovarian Transplant • PRP • Mitochondrial transfer • Triple Parenting

Table-1 summarises the various strategies adopted in poor responders to increase oocyte quality and quantity.

cess rates in these patients. Though many of these interventions result in an enhanced oocyte yield, with more number of good grade embryos, very few have actually translated into improved live birth rates, the ultimate desired outcome of ART.
The various strategies adopted to enhance the oocyte quality and quantity in poor responders can be categorised as pre-treatment or adjuvants, ovulation stimulation and ovulation trigger strategies. Besides these there are few newer techniques being evaluated which are still considered experimental.

ADJUVANTS TO OPTIMISE OOCYTE QUALITY
Over the years many adjuvants have been proposed along with ovarian stimulation so as to increase LBR in poor response patients.

Growth Hormone in ART
Growth hormone (GH) as adjuvant treatment along with gonadotropins to facilitate follicular development has been in use since many years. GH is an anabolic peptide which acts via IGF-1 and augments the effect of gonadotropin action on both the granulosa and theca cells and plays an essential role in follicular development, oocyte maturation, and steroidogenesis. It can also improve follicular survival and granulosa cell proliferation by directly inhibiting follicle apoptosis. Various studies and meta-analysis have shown that adjuvant therapy with GH increases the number of oocytes retrieved, improves the embryo quality and pregnancy outcomes. However, several clinical trials have failed to demonstrate significant benefits regarding clinical pregnancy and live birth rates.
In a recent randomized control trial, it was observed that supplementation of GH in poor responders resulted in pregnancy rates comparable to normo-responders without GH. A systematic review and meta-analysis conducted by Zhang et al observed that, of the various adjuvant treatments used in poor responders, supplementation with GH, DHEA and CoQare the only ones to improve clinical outcomes in terms of achieving pregnancy.
There is no standard protocol regarding GH regimen and dosage to date. The use of GH ranges from 4 to 24 IU, and is usually started from previous cycle day 21 until the day of hCG administration. It is usually injected daily or on alternate days. Most studies have shown a positive impact of GH on ovarian response and pregnancy outcome, without any adverse effects. However, an increased economic burden is inevitable since GH is very expensive.
Though the results in many studies are promising, more studies and RCTs are needed to prove the efficacy of GH in improving the live birth rates in poor responders.

Androgens
Androgens as adjuvants has been used for POR patients in several trials. Androgens are known to have a role in early folliculogenesis before the follicle becomes sensitive to gonadotropins. Testosterone induces FSH receptors on granulosa cells thereby increasing the growth and recruitability of pre-antral and antral follicles. It has been observed that androgens level decline with increasing age. Using androgens as adjuvants thus seems rational for women with poor ovarian reserve (POSEIDON grp 3 and 4) in whom POR is expected.
Several studies have shown that androgen supplementation improves the ovarian response, LBR and decreases the chances of aneuploidy and miscarriages. Similarly, the latest Cochrane meta-analysis reported moderate quality evidence supporting that DHEA and testosterone pre- treatment may improve LBR in POR patients.
Androgens have been used either transdermally as testosterone gel or spray or orally as DHEA which is a precursor of testosterone. The transdermal testosterone is usually started on day 21 of the previous cycle and continued till day 2 of stimulation cycle in doses of 25-50mg. DHEA is usually supplemented in doses of 75 mg daily and given for atleast 60 days prior to stimulation.

Anti-oxidants as adjuvants in ART
Though the physiology of poor ovarian response has not

been fully understood, mitochondrial dysfunction and oxidative stress are amongst the most investigated possibilities. Oocytes and early embryos have abundance of mitochondria. Higher levels of ROS accumulating in mitochondria during multiple physiological conditions contribute to mitochondrial dysfunction and increase in oxidative stress. This, in turn, leads to oxidative damage to DNA and other intra-cellular aberrations.
Furthermore, ART procedures can result in oocyte and embryos being exposed to high levels of free radicals. Ovarian stimulation protocols are associated with altered follicular environment with decreased levels oxygen scavengers. Also, oocytes and embryos are exposed to higher oxygen concentrations in incubators and during handling during the IVF/ ICSI procedure. The high oxygen concentration can be associated with high ROS levels which can affect the oocyte and embryo quality, causing a detrimental effect on fertilisation and success rates. Thus, anti-oxidants supplementation has been proposed as one of the strategies to enhance reproductive performance.Importantly, antioxidants are promising adjuvants keeping in mind that they seem to cause no or very limited adverse reactions and side effects.

Melatonin
Melatonin is a potent endogenous scavenger of free radicals. It has a direct free radical scavenging effect, and also has several antioxidative effects through its receptors. Unlike other antioxidants, it is a suicidal terminal antioxidant as it reacts with free radicals and generates several stable anti-oxidant end products in a scavenging cascade reaction. It also enhances the activity of other endogenous antioxidants like glutathione peroxidase and super- oxide dismutase.
An inverse correlation has been observed between follicular fluid melatonin concentration at the time of oocyte retrieval and products of free radical damage, confirming its protective role against oxidative stress. Further it has been observed that melatonin levels are higher in mature follicles compared to immature follicles.
Melatonin being a potent anti-oxidant can protect granulosa cells and oocytes from ROS and can thus improve the oocyte and embryo quality. Melatonin has been used both in vivo (oral supplements) and in vitro (as a supplement in culture media).
A study by Tamura et al showed that 3 mg oral administration of melatonin to patients with poor oocyte quality helps improve the fertilisation and pregnancy rates in these women. Many other researchers have similarly observed improved number of mature oocytes, better fertilisation rates and better embryo quality with oral melatonin supplementation in women with poor oocyte quality. Mojaverrostami et al in their study observed that melatonin improves oocyte and embryo quality in patients with PCOS. Similarly, Espino et al improved better pregnancy rates with melatonin administration in women with unexplained infertility. Further, it has also been observed by various researchers that supplementing culture media with melatonin improves oocyte maturation, decreases apoptosis, improve blastocyst formation and increases implantation rates. These findings suggest melatonin supplementation could become a new treatment for improving oocyte quality and it may benefit women who suffer from infertility.

Coenzyme Q10 (CoQ10)
CoQ10 acts as an antioxidant by inhibiting lipid peroxidation and DNA oxidation, and thus is capable of strengthening endogenous antioxidant system within the cell. It has been proposed that CoQ10 reduces mitochondrial oxidative stress and thus, improve oocyte competence. A pretreatment for 60 days prior to ovarian stimulation has been shown to improve the number of mature oocytes retrieved, reduce total gonadotropin dosage and higher number of good quality embryos. There is a possible beneficial effect on clinical pregnancy and live birth rates, but this needs to be confirmed in larger randomized controlled studies. Though, the optimal timing, duration and dose of CoQ10 supplementation remain unclear, it is usually prescribed in a dosage of 200mg three times a day for 60 days prior to ovarian stimulation.

STIMULATION PROTOCOLS
Various stimulation protocols have been suggested for poor responders from time to time. However, none is found to be superior to others. Though many of these protocols result in higher oocyte yield, improvement in live birth rates has not been observed.

High Dose Gonadotropins
The use of high dose gonadotropins for patients with poor response is questionable as the limiting factor in these women is the number of recruitable follicles. Also,it has been suggested that high doses of gonadotropins can result in very high levels of estrogen, which can have a negative impact on oocytes quality and endometrium

leading to compromised success rates in these patients.It has been observed that doses above 300IU daily do not increase LBR. In fact, a large retrospective study reported that daily dosing above 300 IU of FSH (including both uFSH and rFSH) significantly decreased the odds of a live birth.
The only subset of patients which can probably get benefitted from higher gonadotropin dosages per day are those with FSH receptor polymorphism.

Mild and minimal stimulation
Various studies have shown that mild stimulation protocols using combination of clomiphene citrate or letrozole with gonadotropins and GnRh antagonists work as effectively as conventional high dose protocols in poor responders. Few recent RCTs have actually shown improved live birth rates with use of mild protocols. Use of minimal stimulation, along with embryo pooling and transfers in remote cycle is another strategy adopted in poor responders and has shown promising results in these patients.

LH supplementation
LH enhances endogenous intra-ovarian androgen production in the theca cells and thus stimulates follicular growth. The androgens in turn increase FSH receptor expression on granulosa cellsand also acts synergistically with IGF1 for the growth of the follicle. LH also binds to granulosa cell LH receptors–expressed from the mid-follicular phase and sustains FSH dependent activities, including aromatase induction, release of growth factors and regulation of final oocyte maturation. It has been observed in various studies that rLH supplementation is beneficialin terms of a higher LBR and a lower miscarriage rates especially in elderly women (>35 years of age, POSEIDON group 4) and those with poor response.

Dual stimulation
Dual / double stimulation ("DuoStim") allows for accumulation of more number of embryos within a short span of time and increases the probability of having at least one euploid blastocyst for subsequent elective frozen embryo transfer. Approximately 65.5% percent of poor prognosis patients (POSEIDON 4) were observed to have atleast one euploid blastocyst after one cycle of Dual stimulation, with an ongoing pregnancy rate of 20.7%. However, there are currently no results from prospective randomized trials comparing DuoStim to two conventional stimulation cycles with cumulative live birth rate and time to live birth as end points. Importantly, a freeze-all policy is mandatory in dual stimulation, which adds on to the cost.

Dual/Double triggers
An ideal ovulation trigger (OT) should be able to reach an optimal LH activity resulting in retrieval of more than 75% mature oocytes. A low follicle:oocyte ratio (FOI) in a previous cycle could reflect inappropriate follicular response to trigger and can be associated with ovarian aging, poor ovarian reserve or even mutations in LH receptors. This can be improved by changing the OT strategy.
OT strategies such as "dual trigger" and "double trigger" have been explored in patients with poor ovarian response. Dual trigger involves administration of GnRHa and a low-dose hCG simultaneously, whereas in double trigger GnRHa and HCG are administered 40 and 34 hrs prior to oocyte retrieval. These trigger strategiescombine the advantages of agonist and HCG. HCG enhances the by intrafollicular LH activity and supports the luteal phase, while the agonist simultaneously induces the endogenous FSH surge. Double trigger also allows for more time between trigger and oocyte retrieval which may help to retrieve more mature oocytes in some patients. However, the current literature about use of these ovulation strategies in POR is limited to few small studies and further large studies are needed to prove its efficacy in POR.

NEWER MODALITIES
It is evident that the poor ovarian response is because of the limited number of gonadotropin sensitive recruitable follicles in the ovaries. However, it has been suggested that there might be a subset of secondary follicles in reserve which are not responsive to gonadotropins and are awaiting transition. The newer modalities to treat poor responders bank on these secondary follicles and aim to transform these to recruitable pre-antral and antral follicles. However, most of these techniques are still experimental and have been evaluated in only small studies and so should be used judiciously. These include:

Platelet Rich Plasma (PRP)
PRP is a highly-concentrated solution of plasma which is prepared from patients own blood and it is known to be very rich in growth factors, hormones, and cytokines. These factors are known to have potent therapeutic nature and help in tissue regeneration. It has been observed that PRP infusion into the ovaries can lead to resumption of menses and retrieval of mature oocytes in women with premature menopause. Another small study showed that autologous intraovarian PRP infusion results in increase in AMH and decrease in FSH, and better IVF response in women with low ovarian reserve. It has been suggested that the ideal candidates for ovarian regeneration with PRP are menopausal or perimenopausal women less than 50 years, women with POF and women with low ovarian reserve.

Autologous stem cell ovarian transplant (ASCOT)
Few researchers have shown that ovarian infusion of

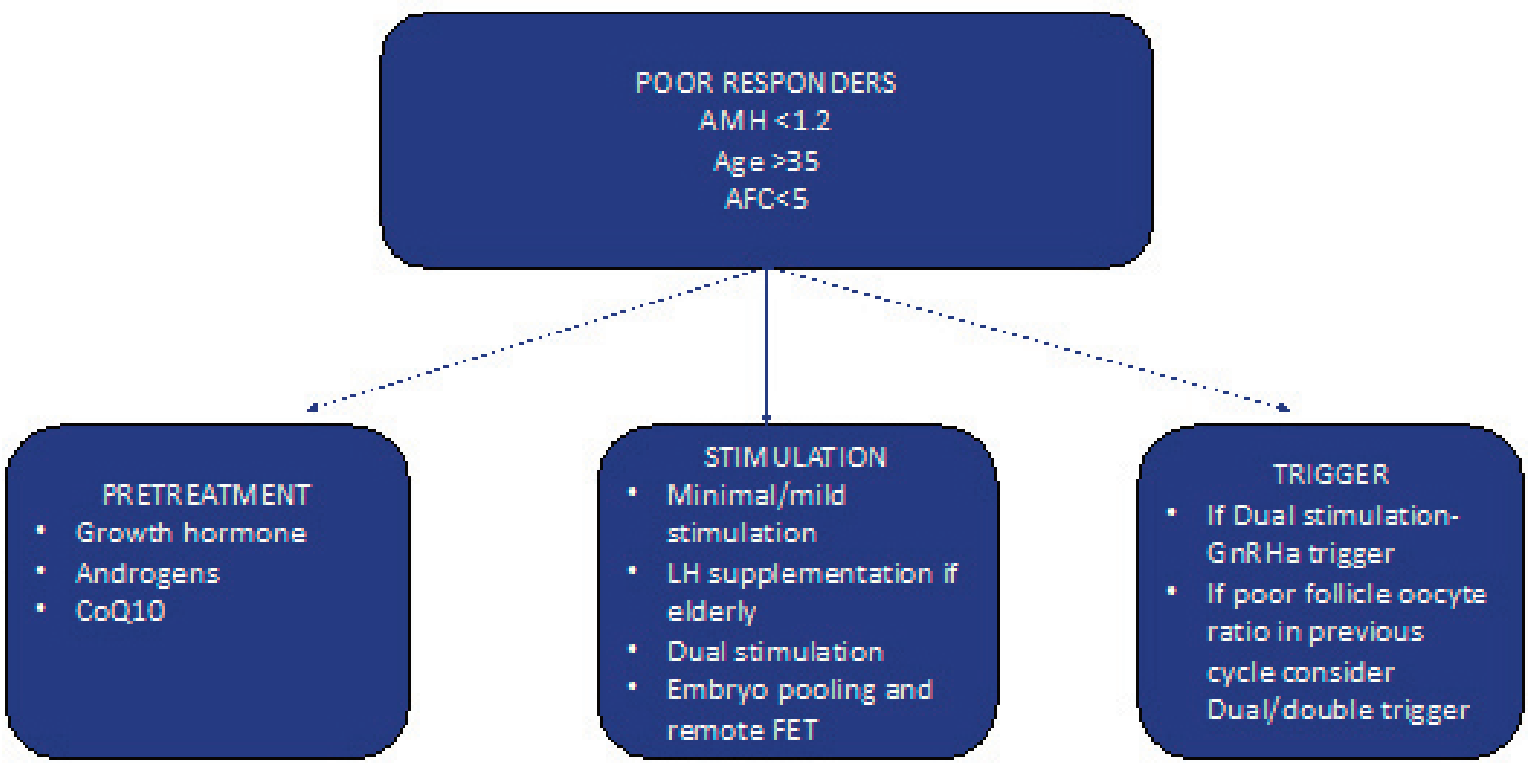
bone marrow derived stem cells results in significant improvement in antral follicle count and serum AMH levels in women with poor ovarian response, enabling them to have their own biological child and avoid oocyte donation. The positive effects of stem cells have been associated with presence of Fibroblast growth factor (FGF-2) and thrombospondin. Also, it was observed that though ASCOT improved the recruitable antral follicles and retrieved oocytes, the embryo euploidy rate still remained low.

Mitochondrial transfer

The quality and quantity of oocytes deteriorate with age. Mitochondrial dysfunction is presumed to be the reason for decline in the quality in the aged oocytes. Mitochondrial transfer is thus being evaluated as an option to treat elderly women with poor response. It has been suggested that adult oogonial stem cells (OSCs) might provide a source of patient-matched germline mitochondria and these can improve the oocyte quality. OSC are obtained from cortical biopsies, and mitochondrias are then separated by differential centrifugation. The mitochondrial suspension is the injected in the oocytes along with sperms while performing ICSI. The procedure has shown to improve pregnancy outcomes in women with prior poor results with IVF. However, bigger RCTs with appropriate controls are required to prove the efficacy of the technique.

Triple Parenting

It involved co- injection of small amounts of donor ooplasm along with sperms during ICSI in order to enhance the oocyte quality. Though the procedure led to many successful pregnancies and live births, it raised both ethical and genetic concerns as donor mitochondrial DNA was identified in the offspring (three genetic parents). The procedure has therefore been suspended by US FDA.



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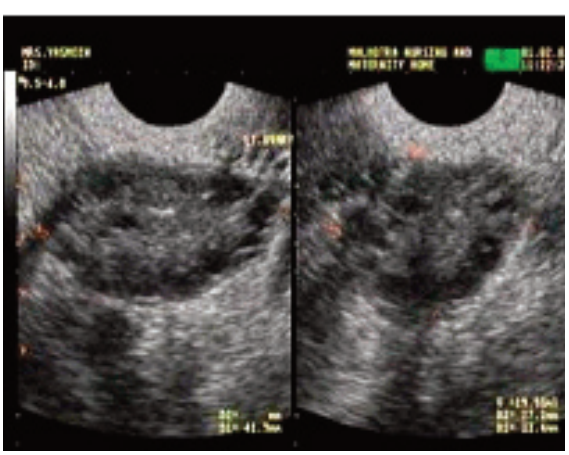
FOLLICULOMETRY

INTRODUCTION

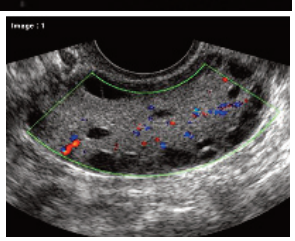
Folliculometry is the gold standard investigation to document ovulation, follicular development and growth, corpus luteum integrity, and endometrial growth and character. Ultrasound monitoring of follicular growth was first introduced in 1978 by Hackelöer and Robinson. [1] Since then, transvaginal ultrasound scan (TVS) has been the norm for routine monitoring of follicular growth whether it may be a natural cycle, ovulation induction program or controlled ovarian hyperstimulation (COH) in an ART cycles. Recently color Doppler and 3 D power Doppler parameters have been found to have added value in follicular monitoring. Color Doppler provides qualitative information, while the power Doppler (PD) signal can provide quantitative information. [2,3,4]

Follicular growth in a natural cycle:

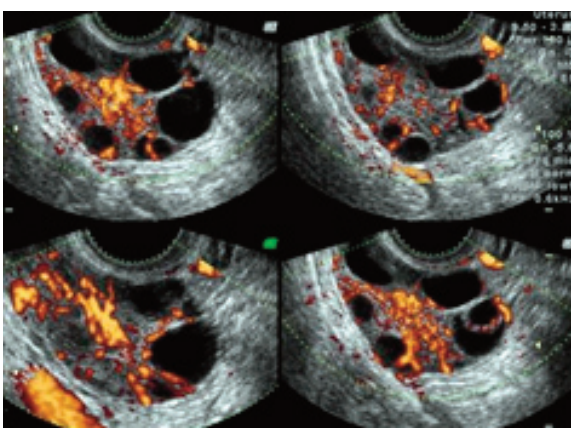
• Cohort of small antral follicles (2–5 mm in diameter) appears in the ovary early in the proliferative phase.



[Fig 1 – ANTRAL FOLLICLE COUNT]

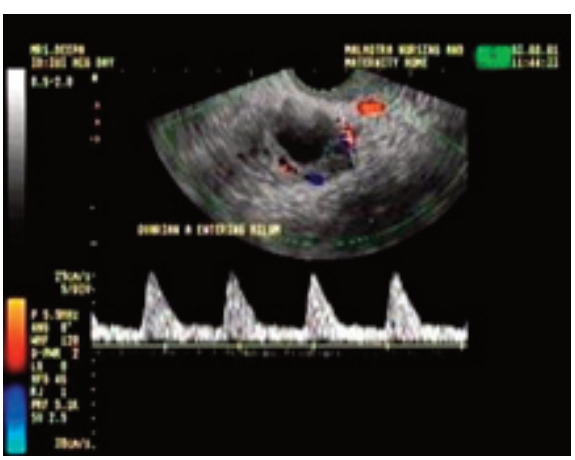


• Further growth of the follicles occur in relation to rising levels of FSH.



[Fig 2 – DAY 6-7 FOLLICLE STIMULATED OVARY]

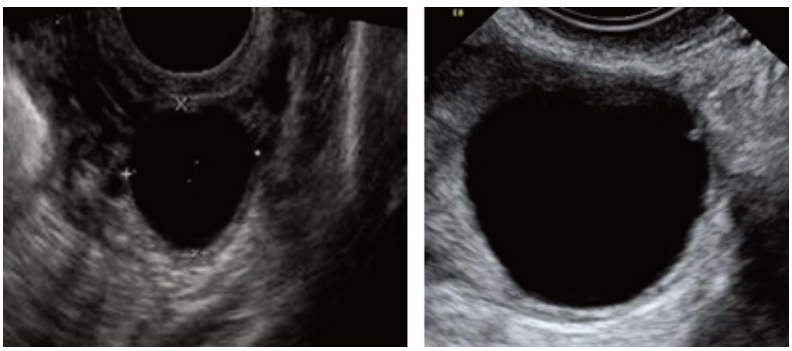
• Physiological decline in FSH level in the late follicular phase allows the selection of the single most sensitive follicle to continue to grow.



[Fig 3 – DOMINANT FOLLICLE]

• The follicle, which has developed maximum receptors for FSH and LH in response to FSH

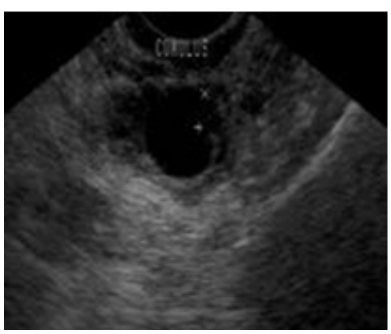
will continue to grow, while the other follicles will undergo apoptosis and atresia [Fig-3].
• Once the leading follicle reaches a diameter of approximately 14 mm, the daily growth rate is between 1.5 and 2.0 mm until a diameter of 22–25 mm is reached, when ovulation occurs.

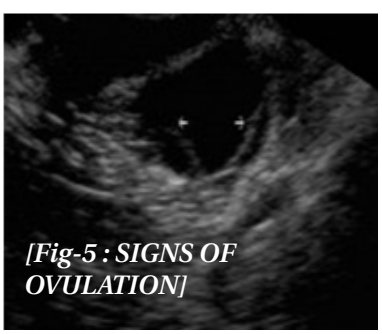


[Fig 4 – PERIOVULATORY FOLLICLE]

MONITORING A NATURAL CYCLE: [FIG 1-4]
• Baseline scan on day 2 or 3.
• first scan can be done either on day 9 or 10 of the menstrual cycle
• The scans can be repeated every 48 h till the follicles reach 14 mm and then repeated every 24 hours.
• Follicle can rupture at any time once the follicle becomes more than 16 mm.

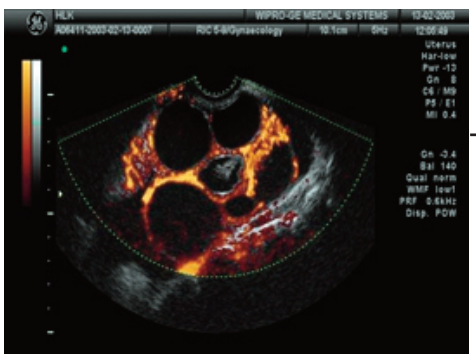
FEATURES OF OVULATION ON ULTRASONOGRAPHY: [FIG - 5]
• Diminution in the follicle size or sudden collapse of the follicle
• Blurring of the follicle borders, which become crenated
• Appearance of intrafollicular echoes, which are more isoechogenic with respect to surrounding ovary and
• Presence of a small amount of free fl uid in the pouch of Douglas (POD)
• Thereafter, an irregular, slightly cystic structure representing the corpus luteum shrinks throughout the luteal phase of the cycle until luteolysis occurs before menses.





[Fig-5 : SIGNS OF OVULATION]

MONITORING OF AN OVULATION INDUCTION CYCLE WITH ORAL AGENT:
• Baseline scan on day 2 or 3 should be done before initiation of any ovulation induction therapy.
• Ovulation induction drugs are initiated within 3 days of the menstrual cycle if the follicular size is <10mm, there are no ovarian cysts, endometrial thickness is <6mm, estradiol levels are less than 50 pg/mL, and progesterone level is less than 1.5 ng/mL.
• TVS is usually performed 4–5 days after the last dose of the oral ovulation agent and then, every other day till the follicle is 14 mm, and then daily until a follicle of approximately 20 mm in diameter is seen.
• Ovulation trigger is given
Monitoring for Ovulation induction with gonadotropins:



[Fig-6 GN STIMULATED OVARY]

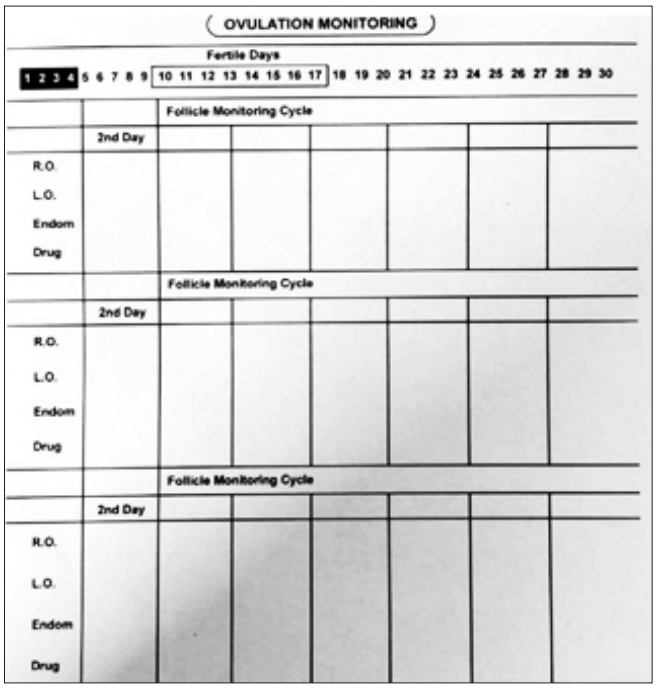
• The gonadotropins are initiated after a day 2 or 3 is normal baseline scan
• The first scan after initiation of gonadotropins is done on the 4th day.
• Further adjustment of the gonadotropin dose and/or administration of GnRH antagonist depends on serial USG findings and E2 levels.

A rough guide is as follows:

If on Day 4
- Number of follicles <4 dose increased by 37.5/75 IU
- Number of follicles 8 dose reduced by 37.5/75 IU

If on Day 7
- Rate of growth <2–3 mm/day and number of follicles <4 which are <12 mm in size dose increased by 37.5/75 IU
- Rate of growth 2–3 mm/day and number of follicles >10, which are > 12 mm in size the dose is decreased by 37.5/75 IU

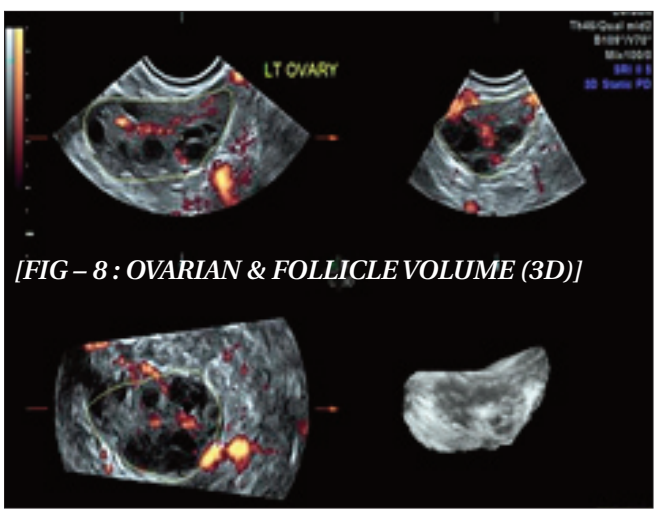
• At each scan, the size of all follicles documented
Daily scans once follicular diameter 12 mm or more



[FIG-7 : FOLLICLE MONITORING CHART]

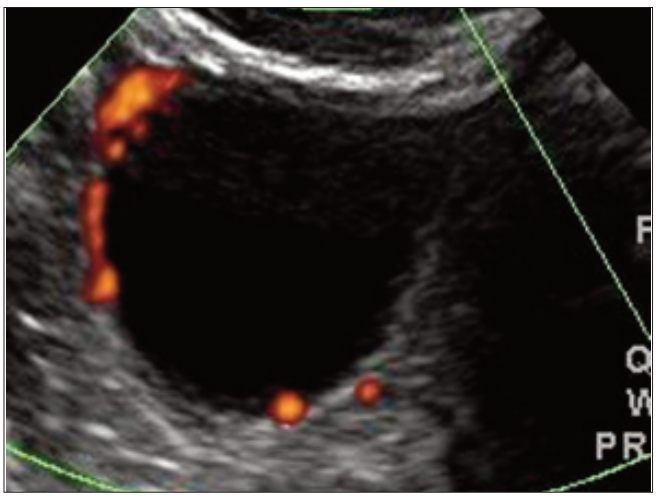
Administration of trigger:
General principle of trigger administration is as follows:
• In natural cycles and clomiphene citratestimulated cycles – when the dominant follicle is 20–24 mm
• In gonadotropin-stimulated cycles and IUI – when the dominant follicle is larger than 18–20 mm
• In IVF cycles – when three follicles above 16–18 mm are present

COLOR DOPPLER STUDIES OF OVARIAN CIRCULATION
Perifollicular microvascular network is an essential element for initiation and maintenance of follicular growth. [5,6]
Grading of Perifollicular Blood Flow (PFBF): Based on percentage of blood flow of the circumference of the follicle
Grade 1: Blood flow (BF) <25 %



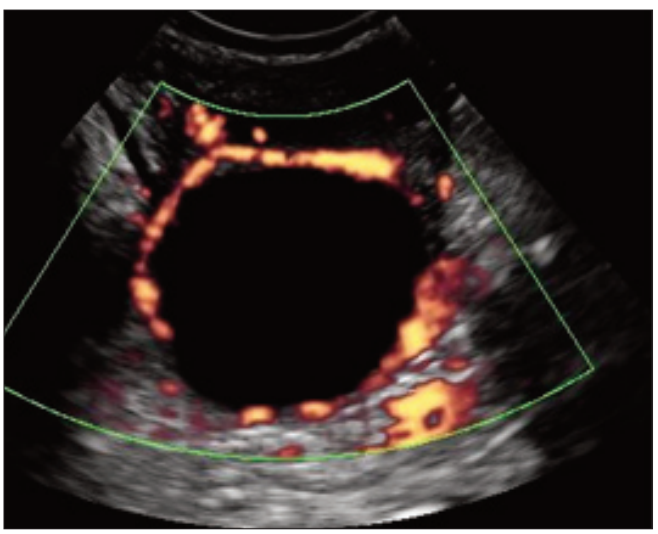
[FIG – 8 : OVARIAN & FOLLICLE VOLUME (3D)]

Grade 2: Blood flow ≥25 % but <50



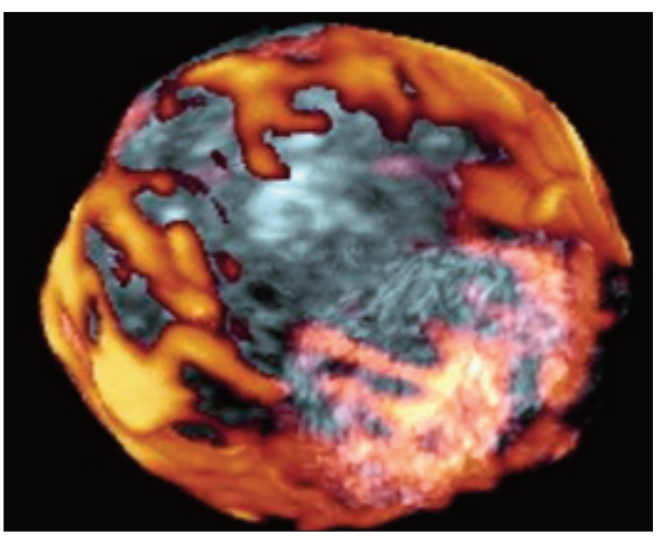
[FIG-9]

Grade 3: Blood flow ≥50 % but <75%



[Fig-10]

Grade 4: Blood flow ≥75 % *[Fig-11]*

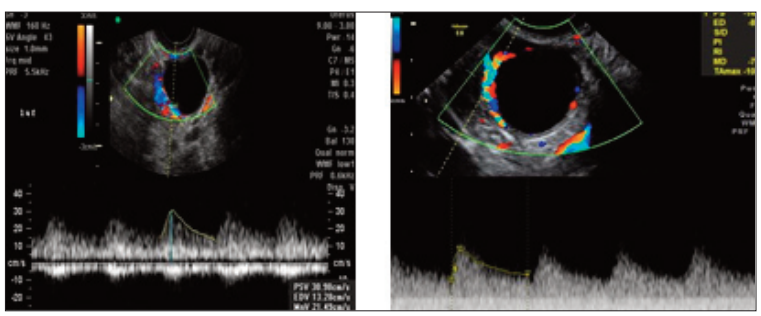


CLINICAL APPLICATION OF COLOR AND PULSE DOPPLER PARAMETERS OF OVARIAN FOLLICLES:

• Studies have shown that Doppler parameters follicles that have more than 75 % [Fig-11] of their surface perfused, ovarian stromal PSV of more than 10 cm/s, and RI of less than 0.4–0.48 contain mature oocytes of satisfactory quality and result in better grade of embryos.

• Follicles having a perifollicular blood flow of >50 % [Fig-10] have increased oocyte retrieval rate with more number of mature oocytes with high fertilization rate and lower triploidy rates.

• Rising PSV with steady low RI suggests that the follicle is close to rupture (follicular PSV goes as high as 45 cm/s an hour before ovulation),



[Fig-12 - COLOR DOPPLER FOLLICULAR FLOWS]

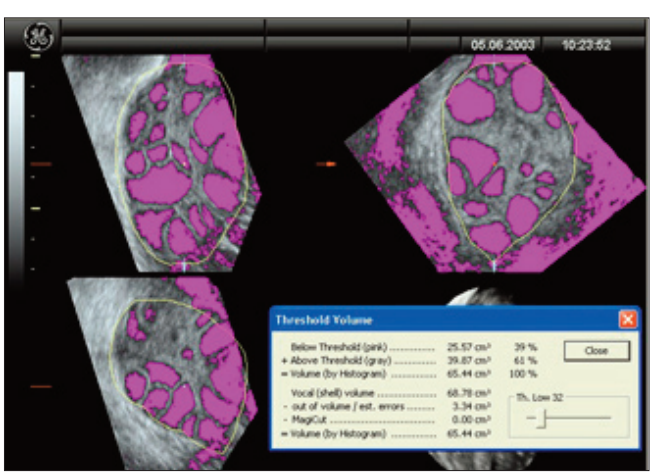
• On the other hand steady or decreasing PSV with rising RI suggests possibility of Lutenised unruptured follicle(LUF).

• It was also observed that fertilization of a follicle with PSV of less than 10 cm/s has high chances of the embryo being chromosomally abnormal. [Fig-13]

• Doppler in the secretory phase gives an idea about the function of corpus luteum (CL). Usually, the RI of the corpus luteum is between 0.35 and 0.50. In luteal phase deficiency (LPD), RI is 0.58 ±0.04, PI is 0.70–0.80, and PSV is between 10 and 15.

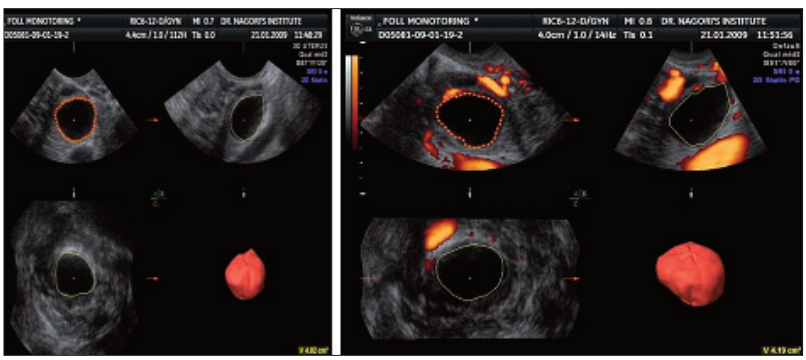
3-D volume and Doppler parameters:

• The follicular volume of 3 to 7 cc has been found to be optimum.



[Fig-13 - FOLLICULAR VOLUME]

• Follicles without visualization of cumulus in all three planes are not likely to contain mature oocytes. Appearance of the intrafollicular cumulus structures by 3D USG has a good correlation with the recovery rate of the mature oocytes.

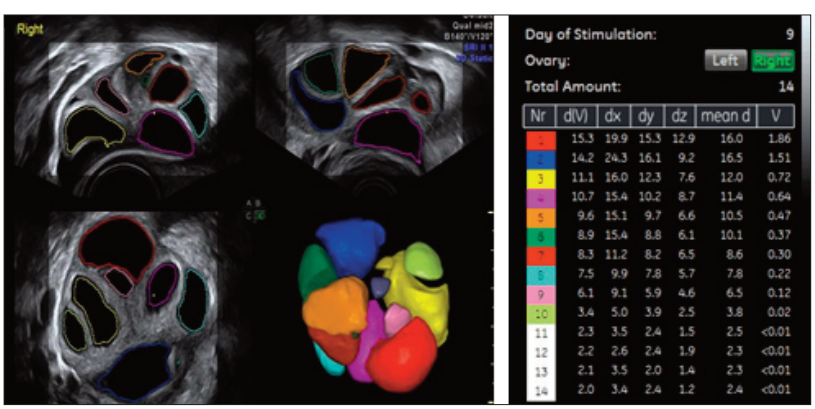


[Fig-14 - 3D FOLLICLE VOLUME]

• Follicular volume between 3 and 7 cc, presence of cumulus, the perifollicular VI between 6 and 20 and perifollicular FI > 35 are associated with a better pregnancy rate.

• The best predictors of IVF outcome are the ovarian flow index (FI) using 3D ultrasound and power Doppler angiography (PDA) on the hCG day and the transfer of grade 1 embryos.

• Assessment of multiple follicles is better done by Sonoavc

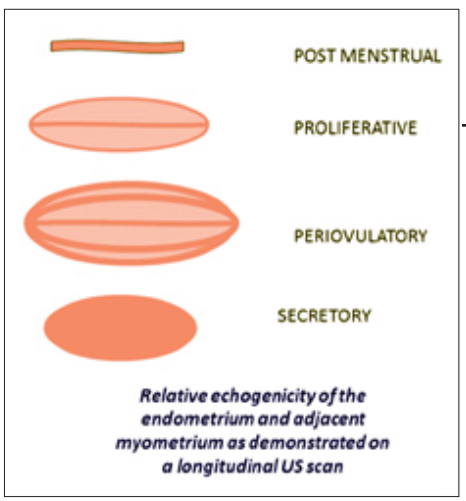


[Fig – 15, 16 - SONOAVC]

THE ENDOMETRIUM:

Endometrial receptivity is pivotal for successful implantation and hence for conception. Therefore in addition to ovarian follicles, endometrium also should be assessed for its morphological and functional characteristics.

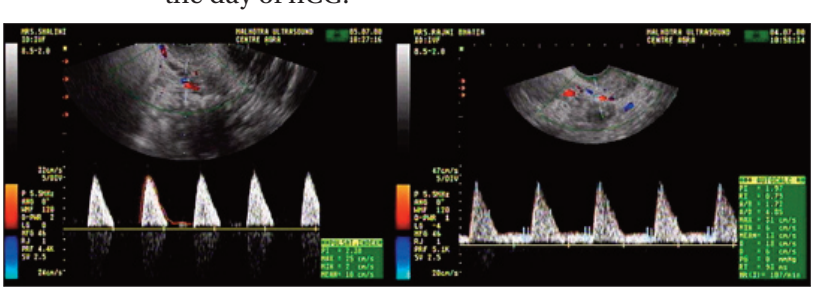
Endometrial Thickness:
Endometrial thickness is measured as the sum thickness of the two opposing endometrial layers in the mid-sagittal plane. Few pregnancies have been noted in gonadotropin-induced IUI cycles when the endometrium measured <7mm mm on the day of hCG (human chorionic gonadotropin)-induced ovulation.[15] However in several studies optimum endometrial thickness has been found out to be 8-14 mm.



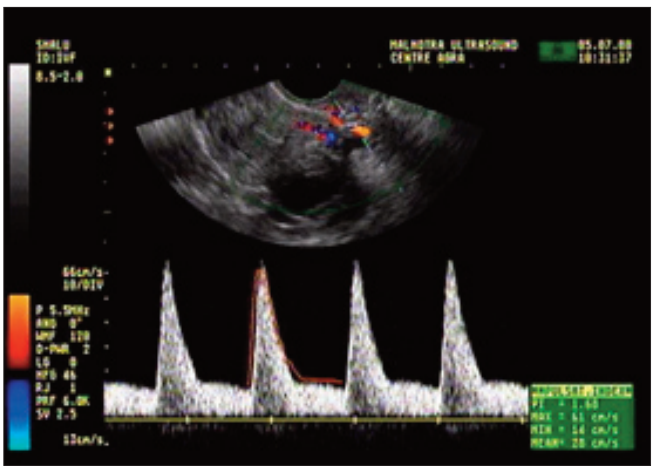
[FIG-16 - ENDOMETRIAL PATTERNS]

Morphology:
Morphologically, the endometrium has been graded as follows.
Grade A (best)- when it is a triple line endometrium with the intervening area is as hypoechoic as the anterior myometrium. Grade B(intermediate)-when it is multilayered or triple line with hypoechoic intervening area. Grade C (most unfavourable)- when it's a homogenous isoechoic endometrium.
Breach or irregularity of endo-myometrial junction is an indication of unhealthy endometrium and therefore poor receptivity.

Uterine Artery Doppler:
Several authors have shown that the optimum uterine receptivity was obtained when average pulsatility index of the uterine artery was between 2 and 3 and RI should be less than 0.9 on the day of transfer or on the day of hCG.

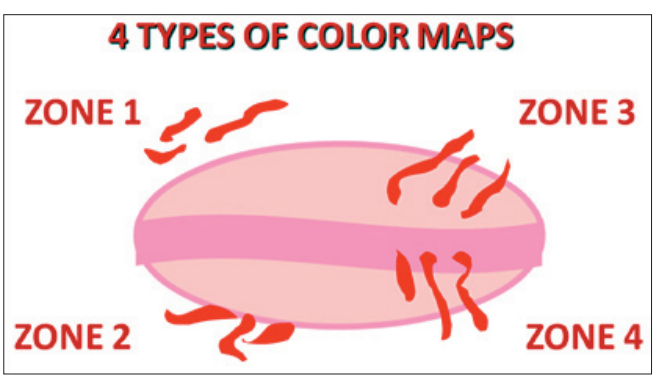


DAY 2 **DAY 6**



DAY 10
[FIG-17 - UT. A. IN A MENSTRUAL CYCLE]

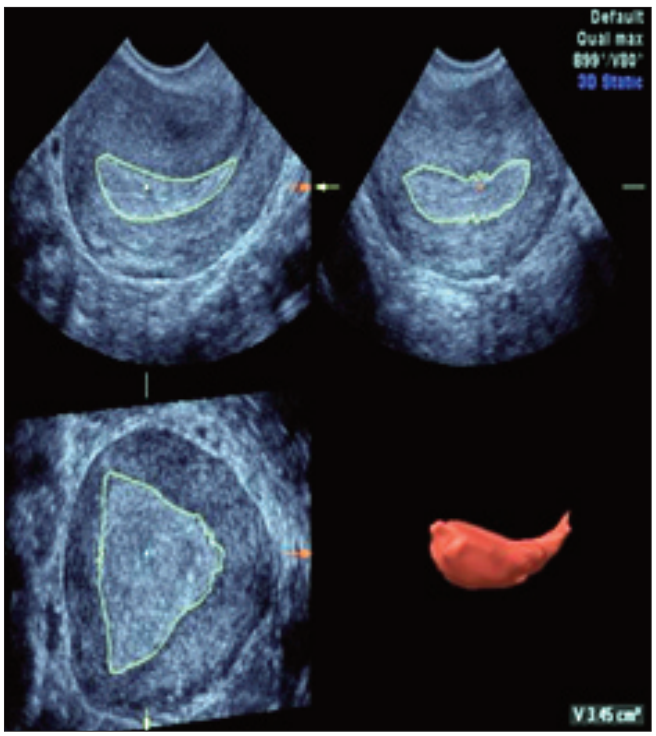
ENDOMETRIAL AND SUBENDOMETRIAL VASCULARITY
There are three zones of vascularity according to Applebaum:
Zone 1 when the vascularity on power Doppler is seen only at endometriomyometrium junction,
zone 2 when vessels penetrate through the hyperechogenic endometrial edge,
zone 3 when it reaches intervening hypoechogenic zone and
zone 4 when they reach the endometrial cavity.
Vascularity in zone 3 and 4 represent a mature endometrium and are correlated to have better implantation rates.



[Fig-18 - SPIRAL ARTERY PERFUSION]

ENDOMETRIAL VOLUME
The endometrial volume by 3D USG having optimum pregnancy rate has been described as 2–13 mL. The calculation of endometrial volume is particularly useful in cases of synechiae, adenomyosis, and uterine anomalies to predict the outcome of treatment. En-

dometrial and subendometrial volume increase rapidly during the follicular phase and then remain almost unchanged during the luteal phase.

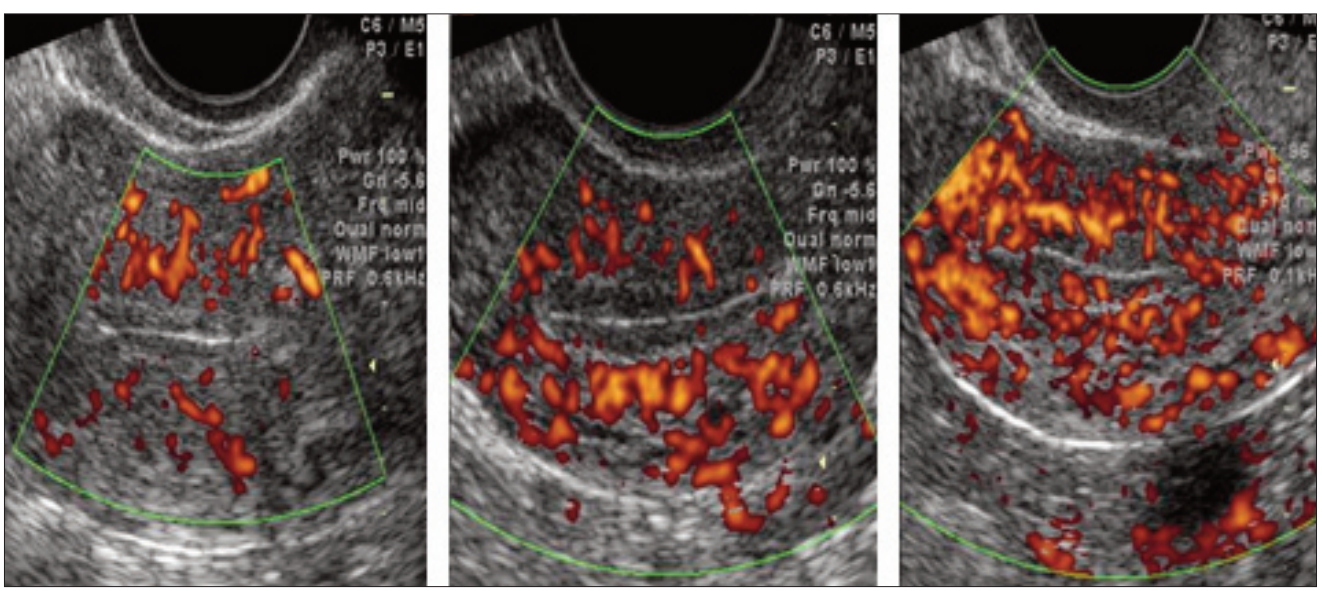


[Fig-19- ENDOMETRIAL VOLUME]

ENDOMETRIAL VASCULARIZATION USING 3D POWER DOPPLER

Endometrial vascularization is calculated by measuring the vascular index (VI), flow index(FI), vascular flow index(VFI), and flow vessel quotient. Higher subendometrial Doppler indices i.e. VI(>1), FI(>31), and VFI (>0.25) on the day of hCG have been found to have higher conception rate.

CONCLUSION: Folliculometry is the cornerstone of a infertility management programme. Ultrasound can alone be used to accurately monitor OI therapy for both in vivo and in vitro fertilization by successfully measuring endometrial thickness and size of ovarian follicles and correlates strongly with serum estradiol concentrations. Color Doppler and 3D power Doppler parameters can have adjunctive role to usual folliculometry. The follicular and endometrial physiological status can be better understood with these newer parameters.



[Fig- 20, 21, 22 - ENDOMETRIAL PERFUSION]

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Uttar Pradesh State Chapter Contribution

LIFE STYLE MANAGEMENT OF PCOS

Dr Anupam Gupta
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Past vice president FOGSI

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Secretary of UP state ISAR
Chairperson of Endocrine committee of FOGSI

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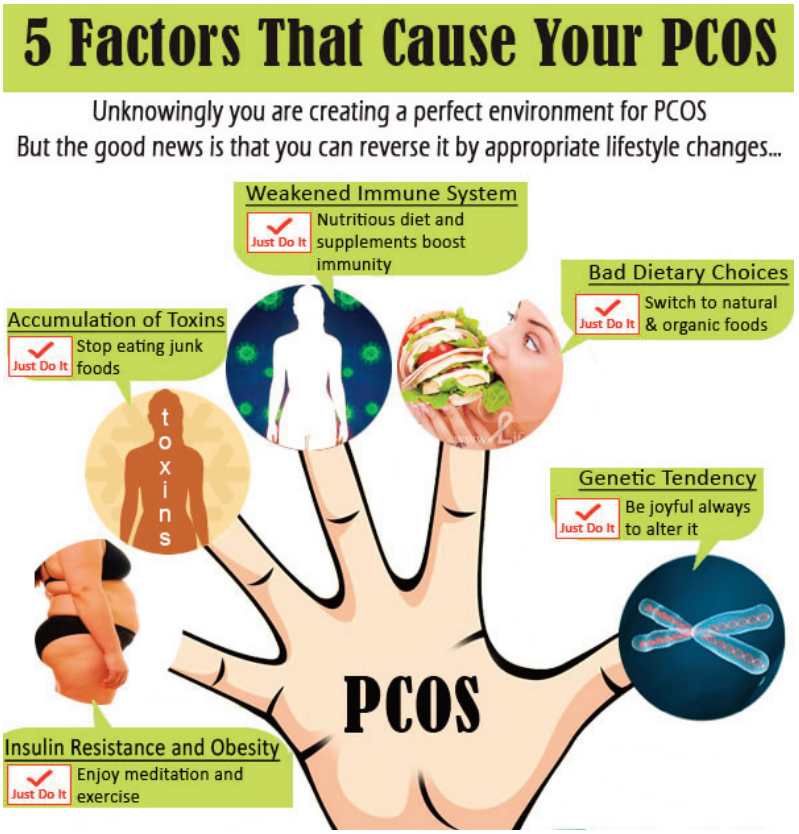
PCOS is one of the most common endocrine disorders in women of reproductive age. Estimated prevalence varies widely from 2.2% to as high as 26% because of differences in diagnostic criteria employed. The prevalence of PCOS when diagnosed by the National Institute of Health (NHI) criteria was less than half that found when the Rotterdam criteria were used to diagnose PCOS.

The prevalence of PCOS may be different according to ethnic background. For example, compared to Caucasians, a higher prevalence is noted among women of south Asian origin where it presents at a younger age and has more severe symptoms.

PCOS often presents with chronic anovulatory infertility and hyperandrogegism with the clinical manifestations of oligomenorrhea, hirsutism and acne. Obesity is common with this condition and have a higher prevalence of impaired glucose tolerance, type II diabetes and sleep apnea than in the general population. They often exhibit an adverse cardiovascular risk profile, features of cardiometabolic syndrome as presence of higher incidence of hypertension, dyslipidaemia, visceral obesity,



insulin resistance and hyperinsulinemia. Gynaecologist frequently diagnosed PCOS and therefore it is important to have good understanding of the long-term implications of the diagnosis for a holistic approach to the disorder.

PCOS should be diagnosed according to the Rotterdam consensus criteria. Women diagnosed with PCOS should be informed of the possible long-term risks to health that are associated with their condition by their healthcare professional like:

1. Gestational diabetes: Screening is must at first visit and at 24-28 weeks of gestation, with referral to a specialist obstetric diabetic service if abnormalities are detected.

2. Type II diabetes: Women with PCOS who are overweight (BMI ≥ 25 kg/m2) and women with PCOS who are not overweight (BMI< 25 kg/m2), but have additional risk factors such as advanced age (> 40 years), personal history of gestational diabetes or family history of type II diabetes, should have a 2-hour post 75 gm oral glucose tolerance test performed. In women with impaired fasting glucose (fasting plasma glucose level from 6.1mmol/l to 6.9 mmol/l) or impaired glucose tolerance (plasma glucose of 7.8 mmol/l or more but less than 11.1 mmol/l after a 2-hour oral glucose tolerance test), an oral glucose tolerance test should be performed annually (grade B recommendation).

3. Sleep apnoea: Women with PCOS should be enquired about snoring and daytime fatigue/somnolence and offered investigation and treatment when necessary (grade B recommendation).

4. Cardiovascular disease: All women with PCOS should be assessed for CVD risk by assessing individual CVD risk factors (obesity, lack of physical activity, cigarette smoking, family history of type II diabetes, dyslipidaemia, hypertension, impaired glucose tolerance, type II diabetes) at the time of initial diagnosis.

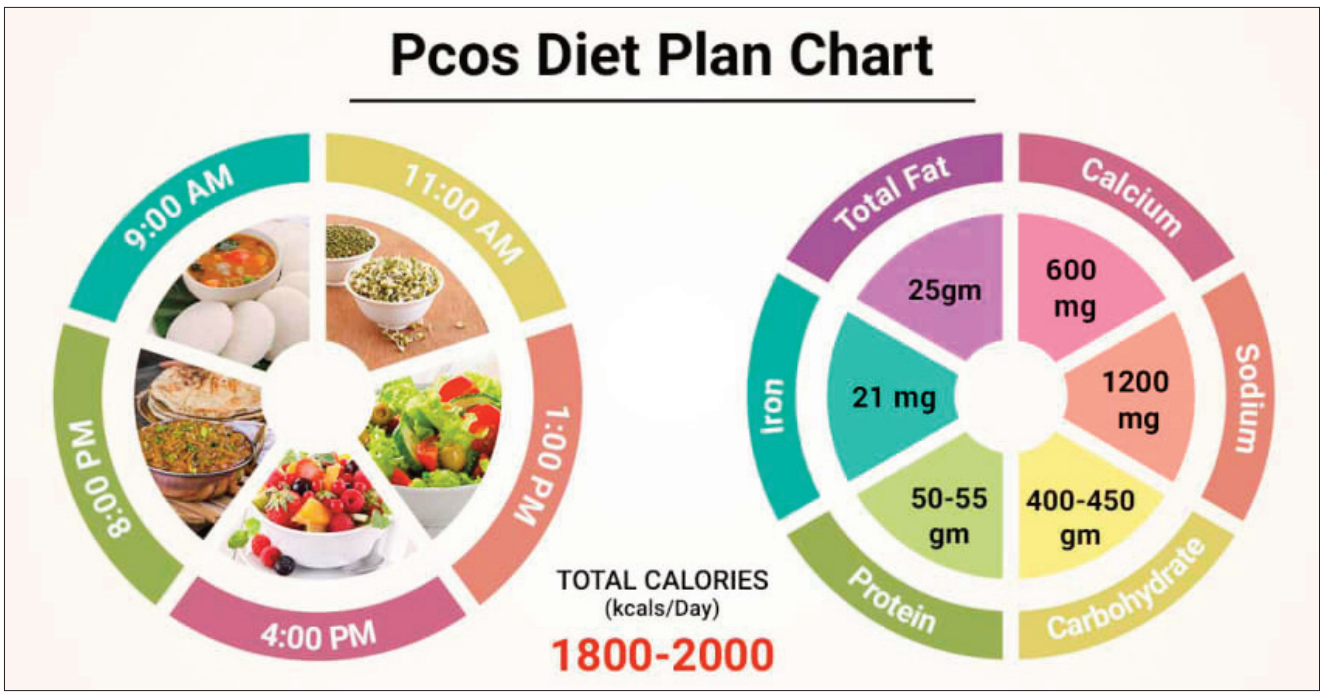
5. Psychological issues: These issues should be considered in all women with PCOS. Depression and / or anxiety should be routinely screened for and, if present, assessed. If a woman with PCOS is positive on screening, further assessment and appropriate counselling and intervention should be offered by a qualified professional.

6. Cancer and PCOS: Oligo- or amenorrhoea in women with PCOS may predispose to endometrial hyperplasia and later carcinoma. It is good practice to recommend treatment with progestogens to induce a withdrawal bleed at least every 3 to 4 months. Transvaginal ultrasound should be considered in the absence of withdrawal bleeds or abnormal uterine bleeding. In PCOS, an endometrial thickness of less than 7 mm is unlikely to be hyperplasia. A thickened endometrium or an endometrial polyp should prompt consideration of endometrial biopsy and /or hysteroscopy. There does not appear to be an association with breast or ovarian cancer and no additional surveillance is required (grade C recommendation).

Counselling: Women should made aware of the long-term implications of their condition and should be made aware of the positive effect of lifestyle modification, including weight loss, for improving their symptoms, especially those who are overweight or obese. Women should be counseled that there is no strong evidence that PCOS by itself can cause weight gain or that having PCOS makes weight loss difficult or impossible. Many patients find great benefit from support groups (e.g. http: //www.verity-pcos.org.uk) and details of these, and sources of information, should be provided.

STRATEGIES FOR REDUCTION OF RISK:

1. Exercise and weight control by lifestyle modifications: It is recommended that lifestyle changes, including diet, exercise and weight loss, are initiated as



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the first line of treatment for women with PCOS for improvement of long-term outcomes and should precede and/or accompany pharmacological treatment. In women with PCOS and excess weight, a reduction of as little as 5% of total body weight has been shown to reduce insulin resistance and testosterone levels as well as improving body composition and cardiovascular risk markers.

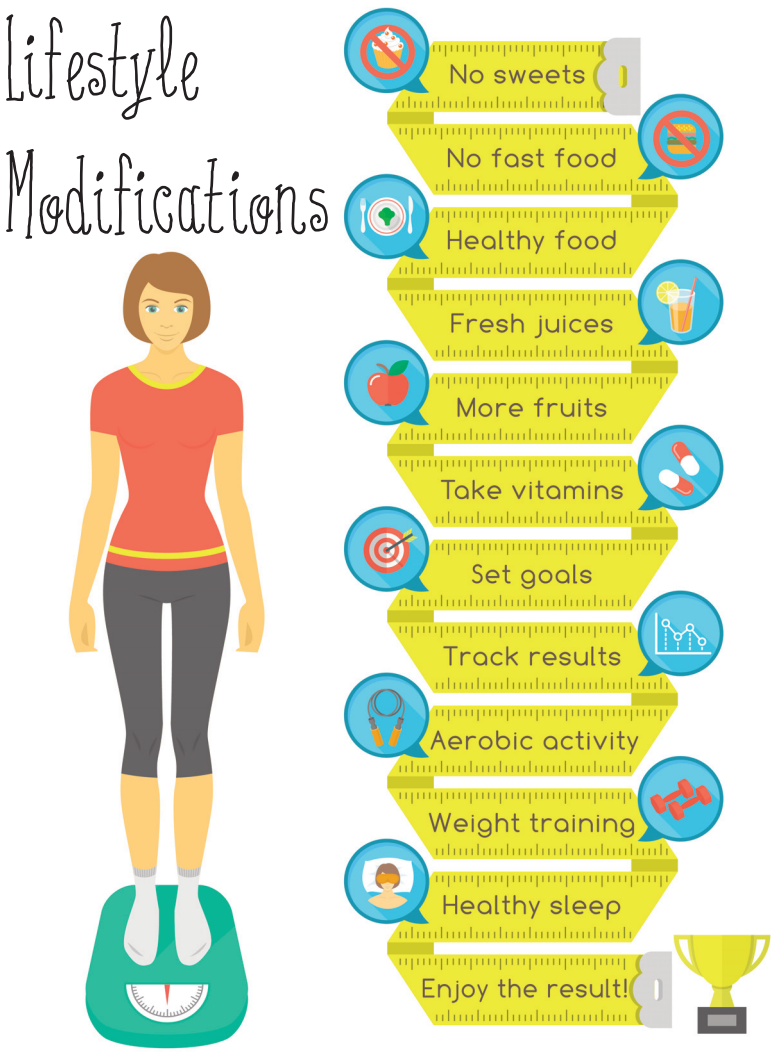
Lifestyle management targeting weight loss (in women with a BMI of 25 kg/m2 or more) and prevention of weight gain (in women with a BMI of 18.5-24.9 kg/m2) should include both reduced dietary energy (caloric) intake and exercise. This should be the first-line therapy for all women with PCOS for managing long-term consequences. Prevention of weight gain should be targeted in all women with PCOS through monitored caloric intake and in the setting of healthy food choices, irrespective of diet composition.

Behaviour change techniques should target prevention of weight gain in all women PCOS. Women who have failed to lose weight with lifestyle strategies and who have a BMI of 40 kg/m2 or more or who have a BMI of 35 kg/m2 or more with a high-risk obesity-related condition (such as hypertension or type II diabetes) should be considered for bariatric surgery.

2. Drug therapy: The demonstration of the potential long-term health consequences of PCOS has been accompanied by the use of insulin-sensitising agents such as metformin and the thiazolidinediones to reduce insulin resistance and thereby reduce the risk of developing diabetes and other metabolic sequelae. However, there is no strong evidence regarding the long-term benefits for the use of sensitising agents in women with PCOS. Metformin has been shown to have beneficial short-term effects on insulin resistance and other cardiovascular risk markers in women with PCOS without type II diabetes. There is evidence that metformin may modestly reduce androgen levels by around 11% in women with PCOS compared to placebo and modest reductions in body weight have been reported by some, but not all, studies. Women with a BMI of more than 37 kg/m2 may not respond well to the standard dose of metformin therapy.

There is no current robust evidence to support the use of these drugs for prevention of CVD in PCOS and future research in this area is required. Inference from the diabetes prevention trial that examined a cohort of patients who had similar metabolic profiles to women with PCOS suggested that lifestyle intervention was superior to metformin in improving cardiometabolic risk factors and progression to type II diabetes

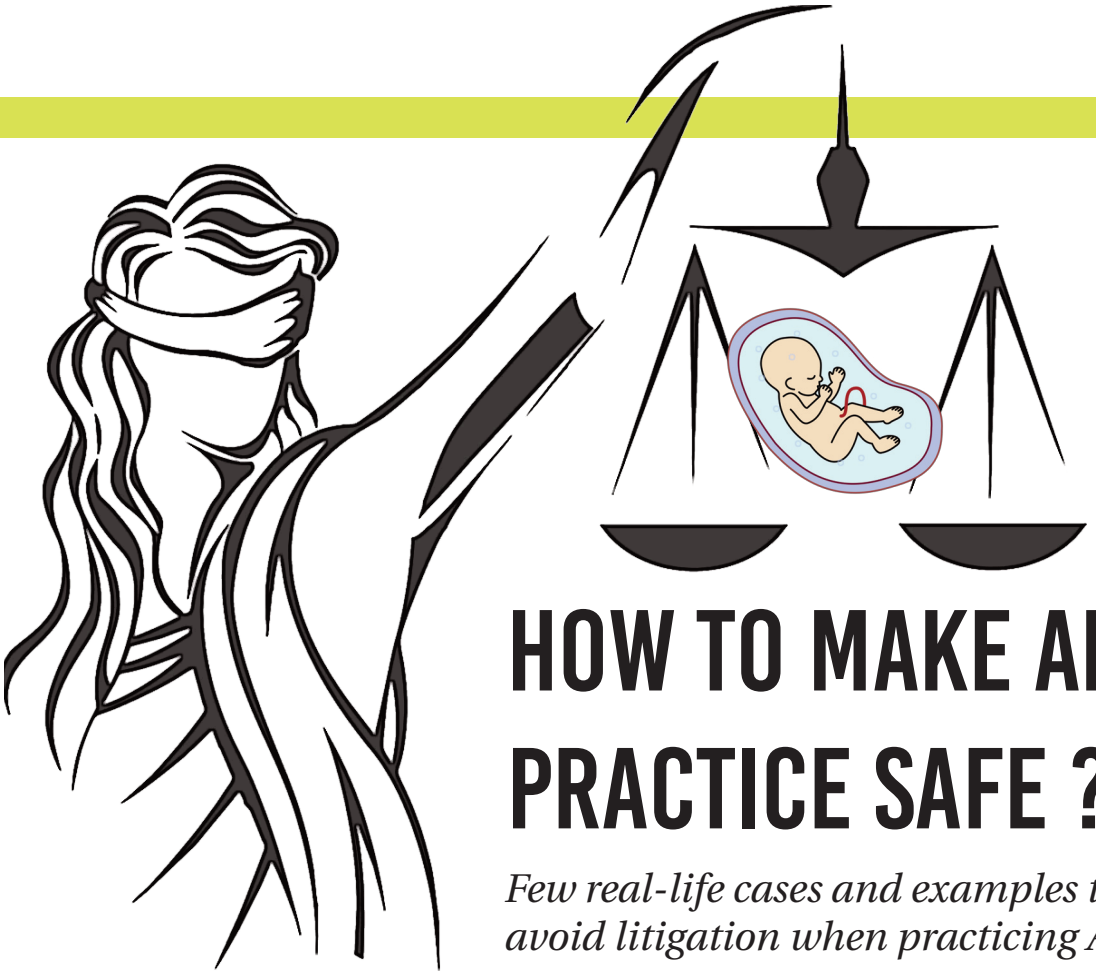
Metformin can be considered in women with PCOS who are already undergoing lifestyle treatment and do not have improvement in impaired glucose tolerance and in those women with impaired glucose tolerance. The use of metformin in induction of ovulation in women with PCOS will not be discussed here as it is beyond the remit of this article.



Incretin hormone-based therapies such as exenatide have shown to reduce weight and improve insulin resistance in women with PCOS. However, the clinical experience with these agents in PCOS is limited and significant side effects may occur, therefore, routine use of incretin-based therapies in PCOS is not recommended.
Orlistat induces a small weight reduction and improves biochemical hyperandrogenaemia but without changing glucose-insulin homeostasis or lipid patterns.

3. Bariatric surgery: It may be indicated in selected women with PCOS and morbid obesity. Bariatric surgery may induce a significant weight loss (up to 60% of excess weight) and improve diabetes, hypertension and dyslipidemia, reducing mortality from CVD and cancer when compared with lifestyle modification. Long-term weight loss of 14-25% may result.28 In women with PCOS, bariatric surgery has been shown to be effective. In 12 morbidly obese women with PCOS, an average postoperative weight loss of 41 kg in the first year improved hyperandrogenism, insulin resistance, dyslipidaemia and hypertension and reversed the PCOS diagnosis.
Bariatric surgery may be an option for morbidly obese women with PCOS in whom long-term diet-based strategies have failed. However, surgically induced weight loss must be balanced against the risk of surgery. These risks include a 0.1-1.1% mortality rate, bowel obstruction, infection, oesophagitis and nutritional abnormalities29 and hence bariatric surgery should be performed only after standard weight loss strategies have failed in women with PCOS with a BMI of 40 kg/m2 or more or a BMI of 35 kg/m2 or more together with a high-risk obesity-related condition.

CONCLUSION:
Based on recommendations, the audible standards are considered below:
1. 100% of women with PCOS should have an accurate diagnosis of PCOS as defined by at least two out of three Rotterdam criteria.
2. 100% of overweight (BMI greater than or equal to 25) women with PCOS and lean subjects with other risk factors such as advanced age (over 40 years), personal history of gestational diabetes or family history of type II diabetes should have a 2-hour post 75 gm oral glucose tolerance test performed.
3. 100% of women with PCOS should be assessed for CVD risk by assessing individual CVD risk factors (obesity, lack of physical activity, cigarette smoking, family history of type II diabetes, dyslipidaemia, hypertension, impaired glucose tolerance, type II diabetes) at baseline.
4. 100% of women with PCOS should be assessed for obesity with measurement of the BMI and waist circumference at every visit.
5. 100% of women with PCOS have their blood pressure checked routinely at every visit.
6. 100% of overweight women with PCOS should be provided with dietary and lifestyle advice.
7. Psychological issues should be considered and addressed in 100% of women with PCOS.



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HOW TO MAKE ART PRACTICE SAFE ?

Few real-life cases and examples to avoid litigation when practicing ART.

INTRODUCTION:

Assisted Reproductive Technology (ART) makes the intervention of law inevitable as the constitution of family unit or childbirth happens with gametes handled outside the body and sometimes with third party assistance. The Technological advancement in this field has its own nuances sometimes posed by the patients, sometimes errors at the facility or may be different scenarios which may not anticipate in the course of regular practice.

The best way to educate is not only from what is written or codified as a law but also to understand how the same is applied and/ or interpreted. Case laws or Case Scenarios which have happened or may happen when discussed, helps in imparting invaluable understanding and drawing up conclusions to establish the norms of good clinical practices. Writing from the Legal Desk of ISAR, is exactly the idea to help the doctors to understand, value and appreciate the law thereby protecting their interests and help in establishing good clinical practices.

CASE NO.1 (ACTUAL CASES WITH LITTLE VARIATION IN FACTS / PROPOSITION)

Facts of the Case:

A married couple having two daughters, conceived for the third time but the women underwent abortion. The husband wanted another child, but his wife refused stating that she could not go through the trauma of another abortion. The couple went to an IVF/ Infertility consultant and after that the wife agreed to go ahead with treatment. The ART consultant suggested surrogacy as an option to which the Husband readily agreed but the wife was not too sure. She told her sister that she does not want to have another child and thus not comfortable with surrogacy. Her sister supported and confronted the husband. The couple had a massive fight and the husband told his wife to leave the house. The wife left the house with both daughters. The wife went to police station, but they refused to file a complaint stating this as family dispute. The couple started contesting divorce. While the divorce was pending, the Husband opted for single parent surrogacy and a baby boy was born. The Husband executed a Single parent Affidavit witnessed by the doctor. The wife approached child right commission and interalia sought action as well compensation against the doctor and the parent.

The allegations primarily made were:

• The Husband was wanting male child and thus,

there were abortion/s and went ahead for surrogacy i.e., violation of PCPNDT Act and ICMR.
• The doctor connived with the patient despite knowing that he was married and the divorce is yet not concluded and helped him (the husband) in getting surrogacy and sex selection, basis false statement and false documents.
• Her and the child rights were deprived and violated as the Surrogacy procedure was done without the consent of wife as it is a violation of ICMR guidelines.
Defence of Doctor rejected that:
• The doctor could not remember each and every patient's history. Even if the husband consulted previously along with wife, the doctor does not remember the same.
• The husband gave the affidavit of being single.

Child Rights Commission passed an order with following recommendations.
• Police was recommended to take appropriate action including imprisonment for the husband for giving a false affidavit as he had committed both civil and criminal wrong.
• The recommendation of establishment of special task force to assess if the doctor/hospital is complying with ICMR guidelines.
• Sex selection and criminal liability of the hospital was not established.

Concluding Remark: In the above case, the medical record, and the affidavit of the patient as single were contrary. The argument that doctor could not remember each, and every case is not a valid and legally sustainable. Once the medical record shows that the husband came with wife and later as a single parent, it becomes the duty of the doctor to ask / seek divorce order (as in this particular case) and should not rely merely on an legal instrument like affidavit which is drawn contrary to the true facts and have strong legal implications.

CASE NO.2 (LEGAL QUERY BASED MOCK CASE)
A couple underwent IVF treatment and few embryos transferred in the wife, while remaining were frozen with the consent that after the death of the Husband, the embryos can be used / released/ handed over to the wife. The Husband died in an accident. His wife did not have cordial relationship with her in laws (i, e., the deceased Husband's parent). The parents of the deceased husband went to the clinic to get the embryos released. The clinic refused saying that they will either handover to the wife of the deceased or the parents get the consent of the wife of the deceased. The parents preferred knocking the doors of the court for seeking the relief of directing the clinic to hand over the embryos to the parents and compensation for harassment.

Concluding Remark: The Doctor / clinic/ hospital has taken the right decision else the very purpose of getting the consent for handing over the embryos in case of death of husband would get defeated. The parents cannot claim the embryos and as per our understanding and experience the case will get dismissed.

CASE NO.3 (ACTUAL LEGAL CASE)
In 2004, a California woman settled for $1 million with the fertility clinic that transferred the wrong embryo into her (created by another couple using the husband's sperm and an unrelated egg donor). A judge ordered the gestational mother to split custody with the genetic father, but not technically with his wife, who was not genetically related.
There have been many similar such cases of Embryos mix up and related custodial issues of the child so born who is unrelated to the birth mother and due to the error of clinic results into Involuntary Surrogacy by the birth mother.
Though there has been no settled rule of custody, however, the fertility clinics had to shell out huge amount as compensation due to mix up.

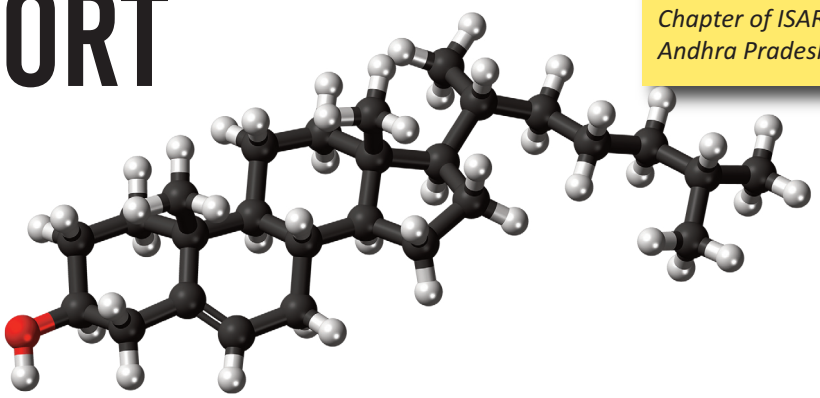
Concluding remark: Irrespective of the custodial settlement of issues between the biological and non-biological parent, the Fertility clinic can be held liable for negligence and compensation to both set of parents.

Take Home Message:
Maintaining medical records is critical. The lab protocols should be well in place to avoid the embryos and gametes mix up. The embryologist and lab technicians can be fixed up liability or indemnity for such mischief or mix up by executing proper documentation between clinic and embryologist. Execution of Consent forms for safeguarding interests of parties including clinic and doctor are very essential and critical part of medical record.

Please note that the above explanation is given as per author's understanding and knowledge of laws in the event of any case or litigation, kindly consult the lawyer in person.

PROGESTERONE IN LUTEAL PHASE SUPPORT

Dr. V. Padmaja, MD(AIIMS), FCGP, *FMAS, FICOG*
Chairperson Chapter of ISAR – Andhra Pradesh



Progesterone is the oldest steroid: formed by side-chain cleavage of cholesterol to pregnenolone
with formation about 423 million years before present, demonstrated only in vertebrates. It originated with CYP11A, vertebrate-specific gene duplication about 500 million years before present in the evolutionary scale along with evolution of basal vertebrate.[1]
During natural human reproduction rise in serum progesterone level in luteal phase is exquisitely timed to embryo development. The surge in luteinizing hormone surge leads to induction of oocyte maturation, ovulation, and formation of corpus luteum.
Corpus luteum is the temporary gland formed from the remnants of ruptured follicle, secretes upto to 40 mg progesterone every day from ovulation till the establishment of pregnancy or resumption of next menstrual cycle.
Progesterone induces endometrial changes in gene expression, histologic appearance, and structural arrangements which lead to an endometrium receptive for implantation five to six days after ovulation [2]. Pulsatile pituitary LH and later hCG from the implanted pregnancy stimulate corpus luteal progesterone [2,3]) which is necessary maintenance of pregnancy until placental progesterone production is adequate.

NEED FOR LUTEAL PHASE PROGESTERONE SUPPLEMENTATION:
Controlled ovarian stimulation, pituitary down-regulation by GnRH analogues, use of antagonists, use of hcg and analogues to trigger ovulation can suppress the lh activity, high e2 due to stimulation can also suppress lh activity, and removal of granulosa cells at oocyte retrieval in Assisted Reproductive Technologies (ART) can all result in a dysfunctional luteal phase. Exogenous progesterone administration has been used successfully in IVF to overcome this deficiency. Failure to use luteal phase progesterone results in low pregnancy rates between 0–18% [4].

TIMING OF PROGESTERONE SUPPLEMENTATION:
There has been significant debate and research regarding timing, dose, and routes of progesterone administration [5,6,7]). Endogenous progesterone production from the corpus luteum after hCG triggering persists until 5–6 days post oocyte retrieval [4,8]. So progesterone supplementation should be initiated prior to day 5–6, before the fall of endogenous progesterone. Early progesterone administration may be of benefit for embryo transfer via the smooth muscle relaxing effect of progesterone on the uterus [9].
ART cycles may be associated with advancement of the endometrium leading to embryo-endometrial asynchrony and implantation failure [10] and too early administration of progesterone may further expand this asynchrony [11].
Therefore, there is a window of progesterone initiation in ART cycles in which embryo, endometrial synchrony and exogenous luteal phase support can be optimized.
A systematic review was performed to summarize the available published randomized controlled trial data regarding timing of starting progesterone supplementation during the luteal phase of patients undergoing ART by Connell et al. P [12-17] where a total of 713 abstracts were identified, 14 full text articles were reviewed, and from these 5 trials met full inclusion criteria which comprised 872 patients undergoing 1,010 cycles, with only one study allowing multiple cycles per patient.
Although the studies had a high degree of clinical heterogeneity in regards to the timing, dose, and route of

progesterone the results suggest a window between the evening of oocyte retrieval and day 3 after retrieval as the ideal time for initiation of progesterone.

TYPE, DOSE AND ROUTES OF PROGESTERONE SUPPLEMENTATION:
Progesterone may be supplied as micronized progesterone or synthetic progestins.
Given reports of teratogenicity associated with synthetic progestins (that have since been disproven), natural micronized progesterone has been the treatment of choice.
Micronized progesterone can be potentially supplied orally, sublingually, rectally, as an oil-based vaginal suppository, an aqueous vaginal cream, or intramuscularly.

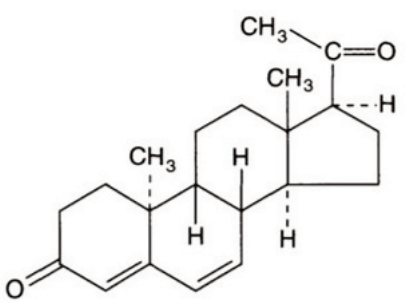
Oral micronized progesterone was the luteal support progesterone of choice in the 1980s; Although convenient it has poor and inconsistent bioavailability, after ingestion, it is absorbed 'by the intestines, undergoes a first-pass metabolism by the liver, and is excreted by the kidneys, resulting in a bioavailability that is only 10% of intramuscular preparations[18]. Serum levels reach maximum in 2 to 4 hours and remain significantly elevated for only 6 to 7 hours,[19] requiring more frequent dosing.
Metabolites produce a sedative & hypnotic effect and the adverse side are fatigue, dizziness, headache, constipation, urinary frequency.
In a randomized controlled trial, users of oral micronized progesterone had a significantly decreased implantation rate compared with users of intramuscular progesterone (18.1 vs 40.9%, P =.004).[20] In a second trial, when compared against vaginal micronized progesterone, the oral route once again resulted in a lower implantation rate (10.7 vs 30.7%, P≤.01).(21).

IM progesterone: 50/100mg /Per day
Provides effective, physiological serum levels but is painful with occasional sterile abscess ,occasional allergic reaction (oil vehicle),and needs to be administered by nurse or husband, rarely has the adverse effect of acute eosinophilic pneumonia and may be associated with slight endometrial maturation delay (glands).

Vaginal Progesteorne:
Vaginal Formulations: Gel *(8%, 90 mg/day)*,Capsules *(200/400 mg twice daily)*,Suppositories *(400 mg daily)*.
Leads to progressive diffusion of P4 from the cervix to the fundus of the uterus by Uterine first pass effect effect, and assures high uterine progesterone concentration with low peripheral serum levels.It has the disadvantages of irritation, vaginal discharge, dyspareunia and is contraindicated in vaginal bleeding, recurrent cystitis. Vaginal pessary needs to be used in divided doses.

Rectal Progesteorne: 400 mg twice a day
Efficacy is similar when administered either by vaginal route or by rectal routes. But has difficulty in use and there is significantly increased prevalence of tenesmus (35.1 vs. 21.1 %) and rectal itching (26.7 vs. 2.8 %)
Randomised controlled trials (RCTs) of luteal phase support using progesterone, hCG or GnRH agonist supplementation in ART cycles were reviewed in cochrane review [6] in which ninety-four RCTs (26,198 women) were included.
Most studies had unclear or high risk of bias in most domains with the main limitations in evidence being poor reporting of study methods and imprecision due to small sample sizes.
In the Progesterone regimens (45 RCTs, 13,814 women) with nine different comparisons between progesterone regimens. The **conclusion** drawn was that Progesterone given during the luteal phase maybe associated with higher rates of live birth or ongoing pregnancy than placebo or no treatment, but the evidence is not conclusive. One particular route of progesterone administration does not appear to be associated with an improvement in outcome compared to other.

RECENT CHANGES:
Dydrogesterone is a retroprogesterone that has been used since the 1960s for the treatment of conditions associated with progesterone deficiency (Mirza et al., 2016).
Dydrogesterone is a more selective progesterone receptor agonist than progesterone, with lower affinity for androgen and glucocorticoid receptors (Rižner et al., 2011).



Importantly, oral administration of dydrogesterone circumvents the inconvenience and side effects related to intravaginal or intramuscular administration (Tavaniotou et al., 2000; Beltsos et al., 2014; Lockwood et al., 2014).
Numerous small-scale clinical trials and meta-analyses have indicated that dydrogesterone is at least as efficacious as MVP for luteal phase support (Chakravarty et al., 2005; Patki and Pawar, 2007; Ganesh et al., 2011; van

der Linden et al., 2011, 2015; Salehpour et al., 2013; Tomic et al., 2015; van der Linden et al., 2015; Barbosa et al., 2016; Saharkhiz et al., 2016; Zargar et al., 2016).
Recently, two new clinical studies (Lotus I and Lotus II) have been conducted for luteal phase support. The randomized, double-blind, double-dummy, Phase III study (Lotus I), conducted in 1031 women, demonstrated that oral dydrogesterone was non-inferior to MVP capsules in terms of pregnancy rates at 12 weeks of gestation following luteal phase support [22].
Since the publication of Lotus I and the completion of Lotus II, dydrogesterone has been approved for use in luteal support as part of assisted reproductive technology treatment in several countries.

LUTEAL PHASE PROGESTERONE DURING FROZEN EMBRYO TRANSFER AND DONOR OR RECIPIENT CYCLES

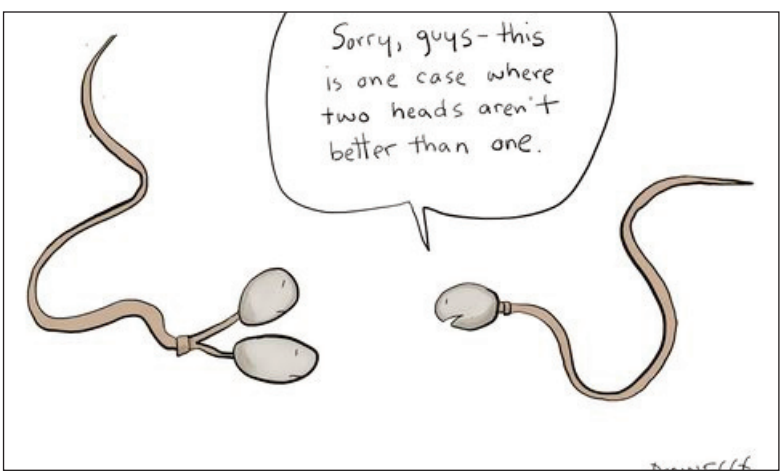
Unlike IVF cycles, there is no formed CL & no endogenous source of progesterone. In cycles of frozen embryo transfer and donor or recipient cycles where exogenous estradiol is typically used to proliferate the endometrium, then exogenous progesterone is added to induce secretory changes in preparation for implantation. Intramuscular progesterone is typically used for this purpose in the United States, whereas in Europe vaginal progesterone is preferred.(23).Due to paucity of data on this topic treatment decisions are based on limited information. Two small, prospective, randomized trials showed no difference in ongoing pregnancy rate when comparing intramuscular progesterone to vaginal progesterone in recipients of donor oocytes[24,25]
A retrospective study of donor oocyte recipients (26) and another retrospective study of subjects receiving donor and autologous frozen blastocysts[27] showed no difference in implantation, clinical pregnancy, or live birth rates.
However, 2 other retrospective studies of women undergoing FET illustrated a deceased live birth rate in subjects receiving vaginal progesterone (24.4 vs 39.1%)[28] and (22.8 vs 34.5%).[29] The timing of progesterone exposure in FET and donor cycles has not been fully studied; however, a review of luteal progesterone during FET cycles was performed by Nawroth and Ludwig[30] and concluded cleavage-stage embryos should not be transferred before 3 to 4 days of progesterone treatment.

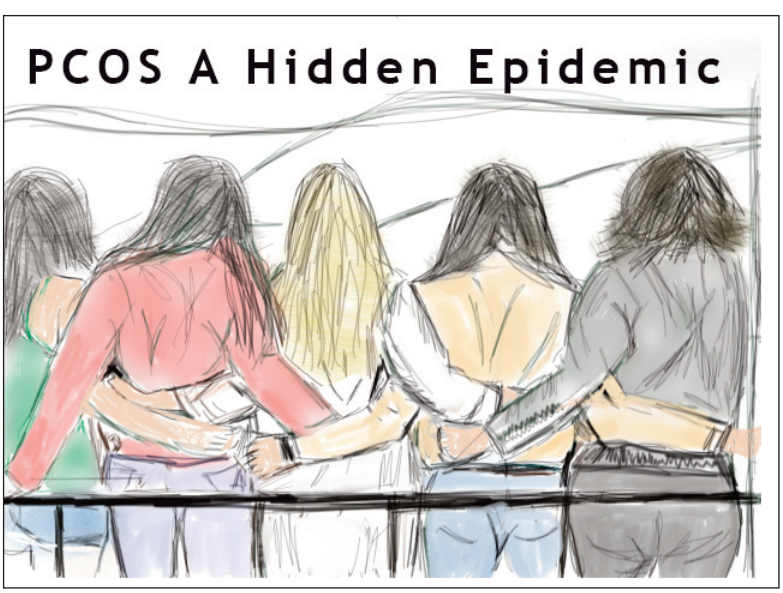
DURATION OF PROGESTERONE USE:

A recent meta-analysis of 6 eligible studies and 1201 randomized subjects concluded there may be no additional benefit of progesterone supplementation beyond the first positive HCG value, showing no difference in live birth (risk ratio [RR]: 0.95, CI 0.86–1.05), ongoing pregnancy (RR: 0.97, CI 0.90–1.05), or miscarriage (RR: 1.01, CI 0.74–1.38.(31)

CONCLUSION:

Progesterone is the key hormone for luteal phase support that is absolutely necessary for successful decidualization of the endometrium preceding implantation and the establishment of early pregnancy to improve clinical pregnancy rates and live births.
The most frequently used are natural progesterone vaginal and im progesterone, which are used based on doctors preferences and patient friendliness.
With studies on dydrogesterone showing its safety and tolerability, non inferiority to natural progesterone, patient-friendly oral administration route, it has the potential to induce a paradigm shift for luteal phase support in the estimated 1.5 million women undergoing IVF each year (Chambers et al., 2012)
Future research should concentrate on establishing thresholds of progesterone dose and timing for fertile and infertile women, as well as on a precise and accurate diagnostic test.





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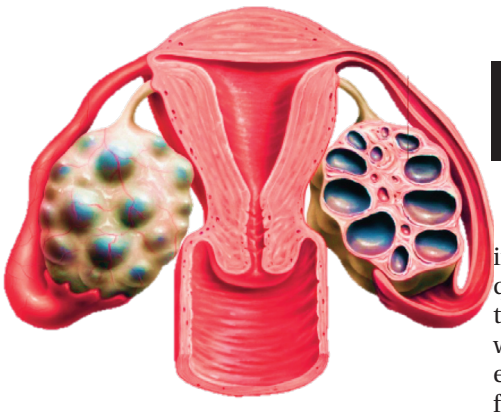
OVULATION INDUCTION IN PCOS



PCOS is one of the most common causes of infertility, Indian prevalence being quoted as anywhere between 3.7 to 22.5%. This is most likely on the raise due to Increasing obesity and other lifestyle factors.
Sub fertility in PCOS is mostly due to anovulation secondary to hyperinsulinism and hyperandrogenism1. Induction of ovulation in PCOS is often more challenging because most of them are also hyper responders.

OVULATION INDUCTION IN PCOS
In women with PCOS, effective ovulation induction serves as an important first-line treatment for anovulatory infertility . Ovulation induction using oral ovulogense is the preferred first choice for fertility management.
Clomiphene citrate has been the drug of choice for the past 50 years However, in a recent Cochrane review and a large randomized controlled trial, Letrozole, an aromatase inhibitor, was shown to lead to superior ovulation rates and live birth rates in women with PCOS when compared to CC . Legro et al reported in NEJM 2014 in a multicentre RCT with 700 women that letrozole was superior to CC for several outcomes – cumulative live birth rates (27.5% vs 19.1%) and cumulative ovulation rates. (62% vs 48%)
Other potential advantages of letrozole over CC were
• Higher rate of mono follicular development with reduction in the risk of multiple pregnancy
• Shorter half-life (48 hrs vs 2 weeks for CC) with lower risk of teratogenicity.
• No adverse anti estrogenic effect on endometrium
At present most professional bodies including ASRM recommend Letrozole as drug of choice in PCOS
However, with the current recommended protocol of letrozole a significant proportion of PCOS women do not respond. Another disadvantage of traditional protocol is the time taken to ultimately determine that a patient is nonresponsive. Due to these above disadvantages several modifications have been proposed to the existing protocols. We will be discussing couple of them.

STAIR STEP PROTOCOL
The "stair step" protocol eliminates the use of progestin to induce a withdrawal bleed between sequential treatments. The time to ovulation is decreased because the progestin withdrawal step is eliminated, and an effective dose of the ovulation agent is found more quickly2. In traditional protocol each cycle stimulates de novo cohort of follicles, starting from scratch. In Stair-Step protocol there is a cumulative effect of successive treatment cycles on the same follicles – "a single follicle step up effect" Hurst and colleagues found the time to ovulation using stair step protocol was significantly shorter, 32–53 days with the stair-step protocol compared to 35 – 88 days in the traditional approach. The reported successful ovulation rate by Hurst et al was 74.1 % as opposed to 35.5% in the traditional approach. A proof of concept study for letrozole – stair step (L-SS) protocol by Conant, et.al in 25 women showed that L-SS induced ovulation safely and effectively with no incident of ovarian hyperstimulation or multiple pregnancy.
Another study by Thomas et.al2, compared ovulation rates between Letrozole (n – 49) and Clomiphene Citrate (CC) (n – 43) using a stair-step protocol to achieve ovulation induction in women with Polycystic Ovarian Syndrome (PCOS). This was a retrospective cohort study where the medication was prescribed in stair step protocol as shown in the figure 1 below.

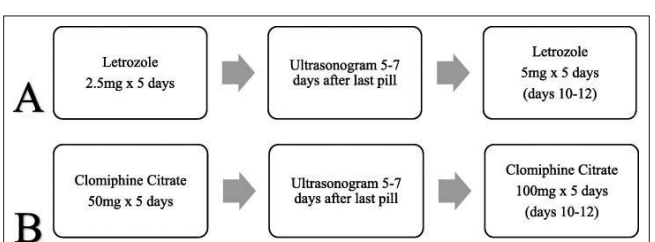


Figure 1: Flow diagrams for stair step protocols for (a) Letrozole and (b) Clomiphene Citrate for ovulation induction.

Established doses of both medications were used in the respective stair-step protocols starting at the lowest dose: CC 50 mg increasing to 150 mg and up to 250 mg as needed; Letrozole 2.5 mg increasing to 5 mg and up to 7.5

mg as needed. The protocol was continued until a max of 7.5 mg for Letrozole or 250 mg for CC. When an 18 mm dominant follicle was noted on ultrasound, patients were triggered with 10,000 IU HCG.
Results showed that mean time (days) to ovulation was

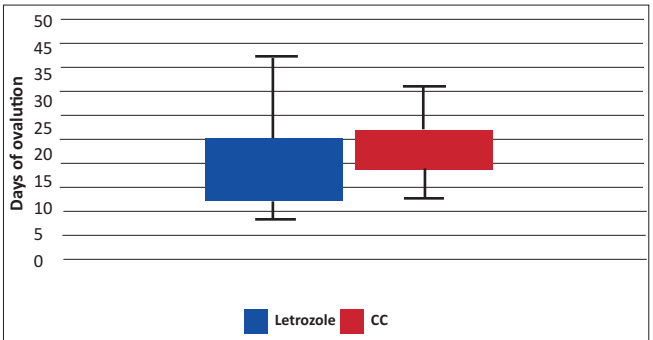


Figure 2: Comparison of mean time to ovulation between the CC and letrozole stair-step protocols. The letrozole shows overall lower mean time to ovulation (19.5 vs 23.1 days, p = 0.027)

shorter in the Letrozole group (19.5 vs 23.1, p = 0.027) (figure 2). Majority of patients ovulated under both Letrozole and CC stair step protocols, (96% vs 88%, p = 0.17)
Being the first study to compare that has compared the efficacy of the stair-step protocol using Letrozole and CC it revealed that the Letrozole stair-step protocol is as effective in inducing ovulation in PCOS patients as CC.
Given the superiority of Letrozole to CC in inducing ovulation and higher live birth rates in PCOS patients, providers using Letrozole for ovulation induction in PCOS patients can consider utilizing the stair step protocol which is associated with shorter time to ovulation and minimal side effects.

DOWN REGULATION WITH COMBINED OC PILLS
15-20% of patients don't ovulate with clomiphene citrate induction, i.e clomiphene citrate resistance "CCR". Aromatase inhibitors and gonadotropins are the most common alternative therapies to induce ovulation in CCR. The drawbacks of gonadotropin therapy are – expense involved, risk of high-order multiple gestations and ovarian hyperstimulation syndrome .
Previous studies showed that the use of oral contraceptive pills (OCP) for 2 months in CCR before repeating CC induction of ovulation produced effective ovulation and pregnancy rates. It was suggested that this approach may avoid the high cost and risks of gonadotropin therapy. Several studies showed that drospirenone-containing OCPs may be more effective in the treatment of PCOS than the traditional OCPs.
Salama M and Hazma H studied whether pre-treatment with drosperinone containing COC before letrozole induction of ovulation could improve the response in clomiphene (CC)-resistance women with polycystic ovarian syndrome. This was a prospective randomized study of 125 infertile women (227 cycles) with CC resistant who received either group (A) (61 patients, 116 cycles), drospirenone-containing oral contraceptive pills (OCP) for 42 days then letrozole 2.5 mg twice daily starting from the 2nd day of menstruation for 5 days or group (B) patients received letrozole in the same manner but without OCP pre-treatment (64 patients,111 cycles). 10000 IU hCG administered when mature follicles reached ≥ 18 mm in diameter.
Results showed that Ovulation was significantly higher in group (A) than in group (B) (p=0.003), Pregnancy rate per patients and cumulative pregnancy rate per cycles were significantly higher in group (A) than in group (B) (p=0.003 and 0.02 respectively). Below table shows comparison between group (A) and (B) regarding cycle outcomes.

	Group A: OCP/Letrozole (n= 116 Cycle)	Group B: Letrozole (n= 111 Cycles)	P Value
Ovulatory cycles	98/116 (84.5%)	74/111 (66.7%)	0.003*
Endometrium in ovulatory cycles	9.7 ± 0.28	9.6 ± 0.38	0.06
Pregnancy rate / Patients	32/61 (52.5%)	16/64 (25%)	0.003*
Pregnancy rate / Cycles	32/116 (27.6 %)	16/111 (14.4 %)	0.02*

*Table 1: Comparison between group (A) and group (B) regarding cycle outcomes. * Significant. OCP: Oral Contraceptive Pills.*

Authors concluded that, pre-treatment with OCPs was associated with higher rates of ovulation, no change in endometrial thickness and higher clinical pregnancy rates in CC resistant patients compared to patients who didn't receive pre-treatment. In patients who received pre-treatment, mean changes in levels of LH, Testosterone and FAI from baseline were the best predictors of pregnancy.

CONCLUSION:
Modifications to the traditional oral ovulogen regimen can bring about higher pregnancy rates with out compromising safety and efficacy. This may be beneficial in reducing costs and avoiding potential complications when the traditional second line strategies such as gonadotropins or surgical management was considered after the first line treatment failed.

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ADVANCED SPERM SELECTION TECHNIQUES FOR ART

INTRODUCTION

In conventional IVF, the zona pellucida functions as biological barrier against abnormal sperm, so that in most cases only "normal" sperm is able to fertilise an oocyte. Since ICSI bypasses the natural sperm selection process, there is an increased risk of genetic abnormalities being transmitted to the offspring. Hence, several advanced sperm selection methods have been developed to select the most "normal" spermatozoa. These include IMSI, PICSI, MACS and MICROFLUIDICS.

Table 1: Sperm selection methods.

SPERM SELECTION METHODS		FEATURES
IMSI	Intracytoplasmic Morphologically Selected Sperm Injection	Real time high magnification selection of morphologically normal spermatozoa.
PICSI	Physiological ICSI	Real time selection of mature spermatozoa.
MACS	Magnetic Activated Cell Sorting	Separation of apoptotic and non apoptotic spermatozoa.
MICROFLUIDICS	Microfluidics	Separation of spermatozoa with DNA fragmentation.

INTRACYTOPLASMIC MORPHOLOGICALLY SELECTED SPERM INJECTION

Table 2: ICSI v/s IMSI

PARAMETERS	ICSI	IMSI
Magnification	200 – 400 x	6600 x
Sperm abnormalities visualized	Head (round, large, tapering etc) Mid piece (thick, bent neck, etc) Tail (short, coiled, double)	Same as ICSI
Vacuoles	Not Visualized	Visualized
Sperm selection criteria	Kruger	MSOME
Sperm Measurements	Not possible	Head and tail dimensions can be accurately measured.
Dish	Polystyrene	Glass bottom
Optics	Hoffman modulation contrast	High power nomarski optics enhanced by digital imaging.

Fig 1: ICSI v/s IMSI

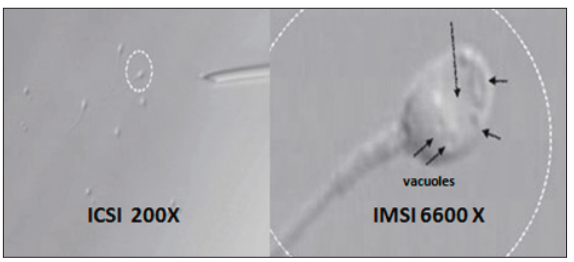
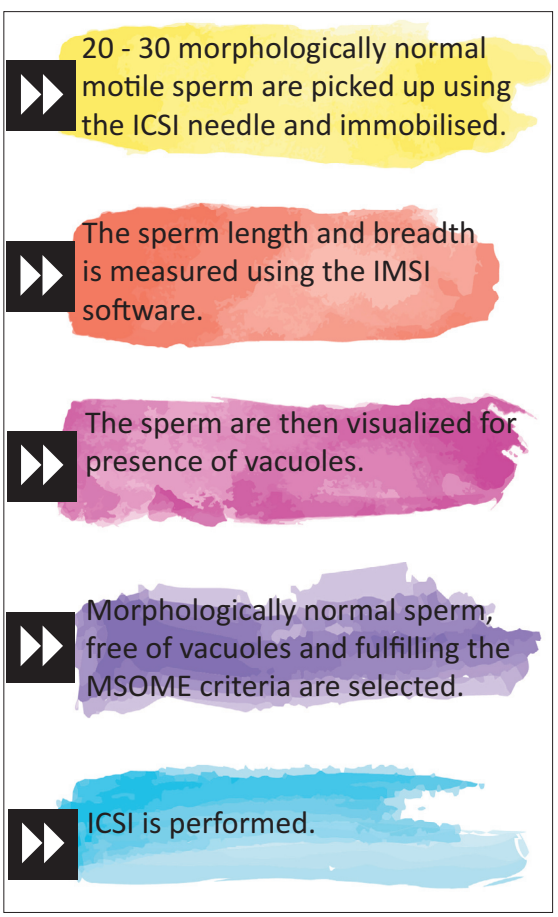


Table 3: IMSI PROTOCOL



Several studies have shown that high magnification selection of morphologically normal motile sperma-

tozoa, free of head vacuoles, is positively associated with increased pregnancy rates in couples with previous and repeated implantation failures. In one study 1,891 IVF-ICSI cycles were compared to 577 IVF-IMSI. In first IVF cycles, either technique was equally effective in producing pregnancies and life births so first cycles saw no difference. However, in second cycles after the first ICSI cycle failed, using IMSI to identify normal sperm showed a better pregnancy rate (56% vs. 38% PRs and 28% vs. 18% delivery rates, respectively). In our own study of patients with recurrent implantation failure, the implantation and pregnancy rates were significantly higher in the IMSI group.

Key Point

IMSI is a highly effective method for selecting morphologically normal sperm in patients with severe OAT, high DNA fragmentation and recurrent implantation failure cases.

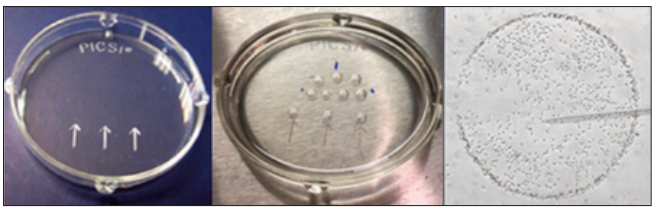
PHYSIOLOGICAL ICSI

PICSI is a variation of the ICSI technique where the 'P' stands for 'Physiological'. This variation of ICSI is carried out using a similar molecule i.e. Hyaluronic acid, that surrounds the oocyte naturally. The head of a mature sperm carries a hyaluronan specific receptor that enables mature sperm to bind to hyaluronan. In contrast, immature sperm lack the receptor and do not bind to hyaluron..

Table 4 PICSI protocol

A PICSI dish with small dots of hyaluronan on the bottom of the dish is used.
A drop of prepared sperm is added to the hyaluronan drop at room temperature.
The embryologist selects a hyaluronan bound sperm within the droplet with injection pipette within 20 minutes of sample addition.
Mature sperms are selected for injection.
ICSI is performed.

Fig. 2. PICSI Dishes and Sperms bound to Hyaluronan drop



Many studies have shown that sperm bound to hyaluronan are more likely to have less DNA damage and a normal chromosome complement. A prospective randomized study showed a significant better embryo quality after ICSI done using HA bound spermatozoa versus PVP –ICSI. A recent systematic review that compared PICSI v/s ICSI in couples with male factor reported no significant difference in any of the outcome measures like fertilization rate, embryo quality, clinical pregnancy and live birth rate. Neither any difference was seen in miscarriage rates between both the techniques.

Key Point

PICSI allows selection of functionally competent mature sperm, free of DNA fragmentation, indicated by its ability to bind to hyaluron.

MAGNETIC ACTIVATED CELL SORTING

MACS system is an efficient method of sperm selection. In most eukaryotic cells, the negatively charged phospholipid phosphatidylserine (PS) is located in the cytosolic leaflet of the plasma membrane lipid bilayer. PS redistribution from the inner to outer leaflet is an early and widespread event during apoptosis. Annexin V has high affinity to PS in the presence of physiological concentrations of calcium and has been used to isolate cells with exposed PS using MACS micro beads. PS-exposing cells are attracted by magnetic enrichment using Annexin V micro beads. The sperms are magnetically labelled with Annexin V micro beads and passed through a MACS column which is placed in the magnetic field of a MACS Separator.

Fig.3. MACS setup

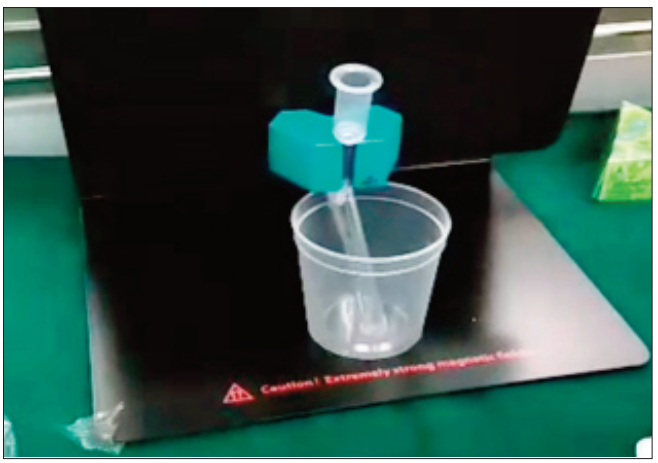


Table 5 MACS Protocol

Prepare the semen sample to be used with density gradient
Post wash allow the 100µl of washed sperm suspension to mix with 100µl Annexin V beads and allow them to conjugate for 15 mins at room temperature.
The sperm - microbead suspension is loaded in the separation column which is placed in the magnetic field.
The sperms labeled with micro beads are retained in the column while unlabeled sperms pass through.
The unlabeled sperms that are collected in a test tube are then washed with sperm wash medium using swim up technique and can be used for ICSI.

Key Points

• MACS technique removes morphologically indistinguishable, apoptotic spermatozoa and selects the functionally healthy sperm, thus increasing the possibility of pregnancy.

• It has enhanced the percentage of spermatozoa with intact mitochondria and mitochondrial survival rates post cryopreservation.

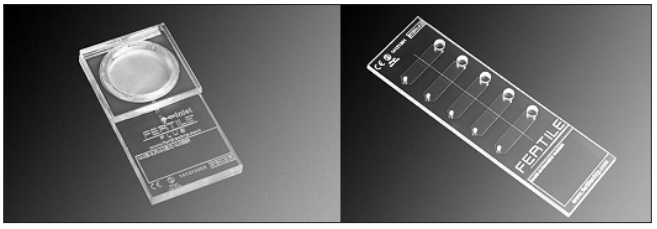
MICROFLUIDICS

Microfluidic Sperm Sorting Chip provides an alternative to procedures requiring extensive centrifugation and vortex mixing that cause irreplaceable damage to sperm. Two types of microfluidics device are available in the market, one which works on membrane permeability principle and the other on laminar flow principle. Only motile sperm can traverse the border that separates the parallel streams of diluted semen and fresh medium. Thus, the laminar flow properties exhibited by media in micro channels allows motile sperm to swim away from non- motile sperm, debris and seminal plasma and collect in a separate outlet reservoir. Follow-up experiments demonstrated this microfluidic device design was not only biocompatible with human sperm, but that it could isolate motile, morphologically normal sperm. This novel approach appears to offer a feasible alternative to, isolate sperm from oligozoospermic patients for use in ICSI only.

Table 6: Microfluidics microporous membrane chip protocol

Microfluidic Sperm Sorting Chip is a flow-free, dual chambered, single use device.
The lower chamber has a sample inlet and fluid channel separated from the upper collection chamber by a microporous membrane.
A liquefied semen sample is injected via the inlet.
The sorted sperms are collected from the upper chamber.
Sperms are sorted by the separation of healthy motile sperms from the many compromised poorly motile sperms present in the semen sample.

Fig. 4 Microfluidics Sperm Sorting Chip

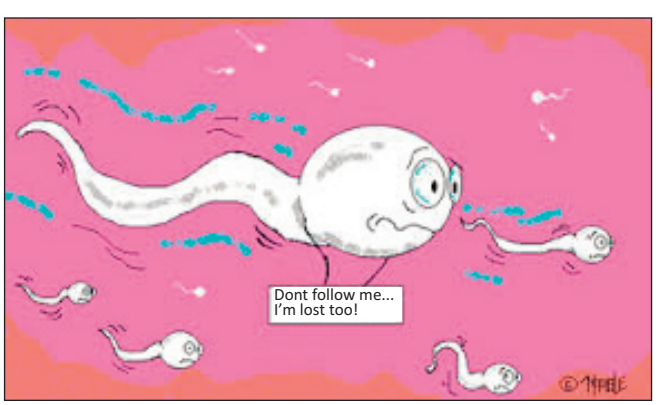


Key points

Microfluidics sorted sperms exhibit better motility and morphology, less DNA damage and lower levels of Reactive oxygen species (ROS).

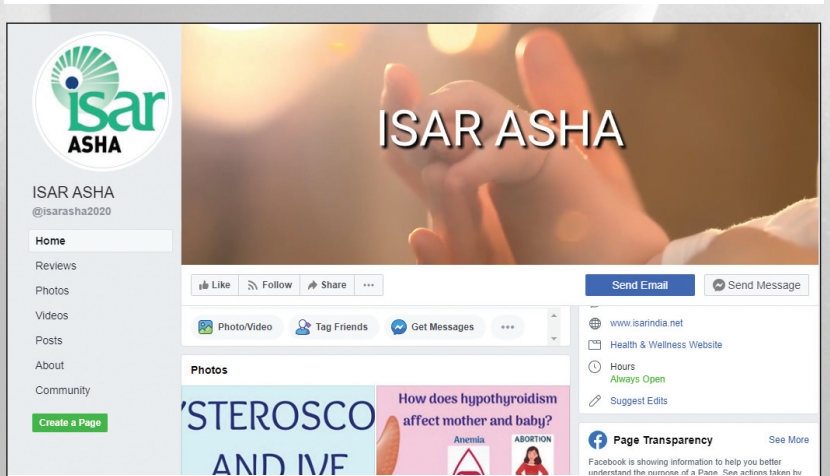
Table 7 Other sperm selection techniques

Sperm VD	Single sperms from the micro tese tissue are picked up by ICSI needle, transferred to the sperm VD device containing sperm freezing media and kept in liquid N_2 for use in subsequent cycles.[11]
MTT (Mechanical touch technique)	MTT allows identification of immotile but viable spermatozoa because their tail is flexible when applying a lateral force to the flagella with the ICSI micropipette. On the other hand, flagella of non-viable spermatozoa remain rigid to the same force.[12]
HOS (Hypo-osmotic swelling) test	The tail of viable spermatozoa swell or curl under hypo-osmotic conditions due to normal membrane function, thus allowing their identification and recovery under the microscope, for their use in ICSI.[13]
Polarized light microscopy	The head of viable spermatozoa is birefringent. On this basis it was proposed to use this property as a parameter for sperm selection[14]
Chemical inducers	Theophylline has been shown to activate the motility in a fraction of immotile testicular sperm.[15]
Laser-assisted immotile sperm selection (LAISS)	Motility of immotile but vital spermatozoa can also be induced with a single laser shot to the tip of the flagellum.[16]



"He's trying to find proof that drinking beer will increase his sperm count."

ISAR ASHA



ISAR ASHA is a patient support initiative by the Indian Society of Assisted Reproduction, dedicated to all fertility patients with the purpose of posting positive information and health care advices that are useful during their fertility journey.
We invite members to send their contributions to Dr Sulbha Arora: drsulbha.arora@gmail.com

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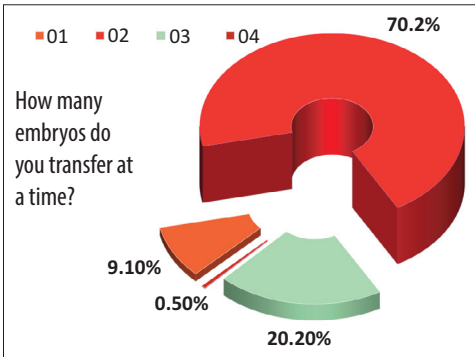
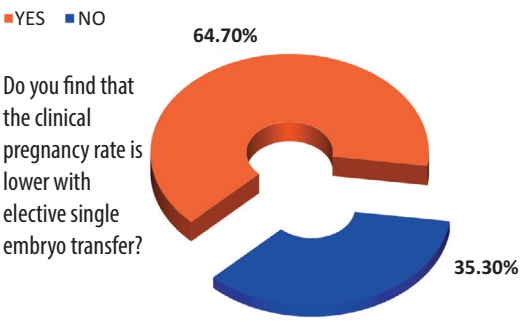
ISAR SURVEY ON EMBRYO TRANSFER BY 252 PARTICIPANTS



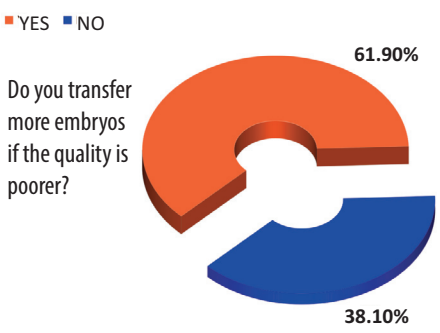
What does evidence say?

Correct Answer- "Single Embryo"
Single ET is recommended to avoid multiple pregnancies. The decision on the number of embryos to transfer should be based on

- embryo quality, stage of development
- female age
- Stimulation response

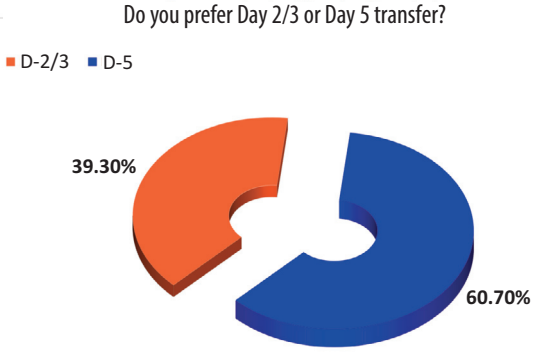


Correct Answer "Yes"
But transferring more than one embryo increases the chance of having a multiple pregnancy (twins, triplets, etc.).
ASRM



Correct Answer "Yes"
A poor quality embryo does not negatively affect a good quality embryo, when transferred together in a double embryo transfer.
So you can also choose to transfer one good and one bad quality embryo together.

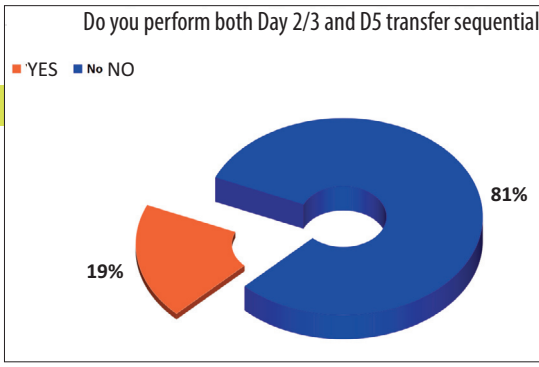
- Wintner et al. Journal of Ovarian Research (2017) 10:2



Correct Answer "Day 5 Transfer"
Evidence supports blastocyst transfer due to

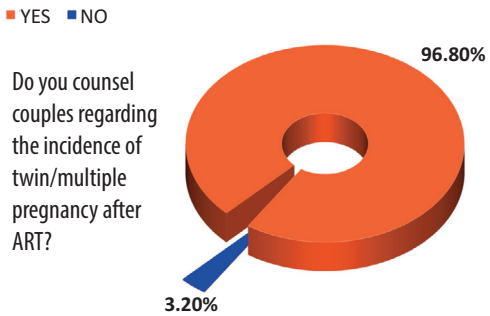
- Higher implantation rate & LBR
- Select most viable embryo for ET
- Better temporal synchronization between embryo & endometrium at the time of ET

ASRM Practice Guidelines



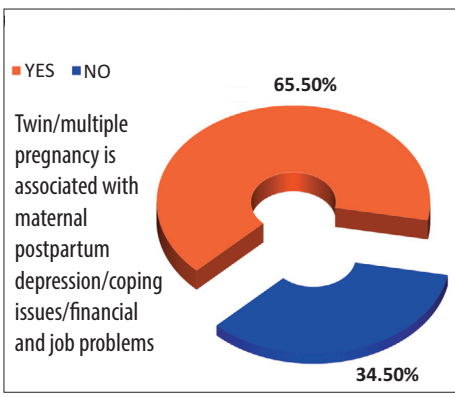
Correct Answer "NO"

For patients with repeated IVF–embryo transfer failures, sequential transfer on day 3 and day 5 may improve the clinical pregnancy rate in cases of repeated implantation failure as long as good-quality embryos are available
Middle East Fertility Society Journal
Volume 20, Issue 4, December 2015, Pages 255-261

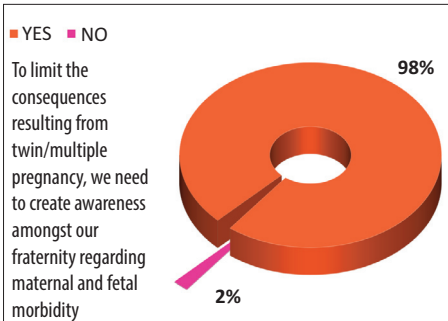


Correct Answer "Yes"

Its always a good practice to counsel the couples regarding outcome and its encouraging that >96 of us are doing the same.

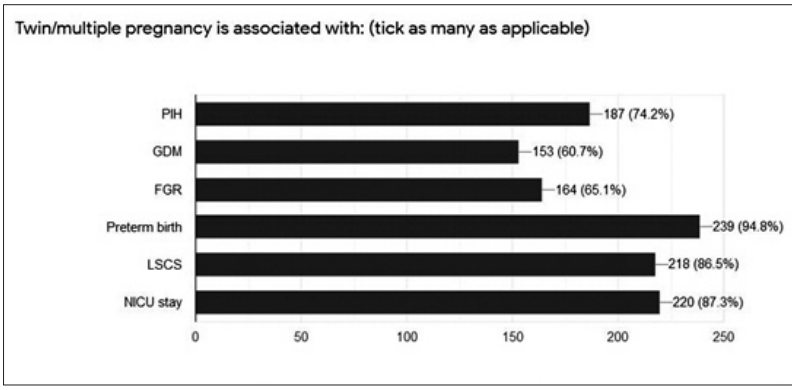


Correct Answer "Yes"



Correct Answer "Yes"

The best part is our fraternity is well aware about it.



Correct Answer "all of the above"

Women with Multiple gestations are prone to have PIH, Pre-term deliveries, GDM, FGR and all these complications along with mal presentation leads to increased cesarean section and NICU admissions.

Dilip Patil
Founder, Trivector Biomed and BabyQuest Cryobank

The Patil family

A Pioneering Service Provider's Perspective

IVF (R)EVOLUTION IN INDIA

Dr. MN Parekh Fellicitating Dilip Patil

It was the year 1993, as a Service Engineer I had been visiting IVF labs (very few in numbers) all over India for repairs of CO2 Incubators, programmable Freezers and other gadgets. The ICSI technology was yet to arrive in India. No readymade culture media (except for EBSS and Ham's F-10) was available, neither was 'Tissue Culture Grade' labware easily accessible. For the well-connected the culture media was 'hand-carried' by airline pilots or 'passengers'. For others, it was an adventure of home-cooking of media – borrowing ultra-pure water, filter papers, balance, osmometer from friendly institutes in the vicinity. An infant feeding tube acted as a transfer catheter; USG guided ovum pick-up with an aspiration pump was a luxury. Plain lab Incubators acted as culture Incubators and circulatory water baths were used to heat the test-tubes. With this back-drop, at the behest of a few harried customers, I decided to act as an 'enabler' or 'provider' of the day to day needs of the IVF labs fighting to survive at that time. Thus Trivector (Care, Commitment and Convenience) was born in a borrowed table space of a civil contractor's office in the bylanes of Dadar, Mumbai in April 1994.

It was a tight rope-walk to arrange IVF supplies from different sources across India. We got indigenous digital heating blocks, stage warmers, incubators and gas mixers designed and manufactured. There was that option of importing this stuff from abroad. But several hand-written fax communications with the companies abroad had gone un-answered. Those who answered, were sceptical about whether India really needed to produce more babies? Persistence paid and a couple of companies agreed to work with us on a case-to case basis. The economy was yet to open up, there were several restrictions on import of goods, the average customs duty rate was more than 200% and a heap of documentation and permissions were needed. It was especially a nightmare for a perishable item like culture media (the shelf-life of culture media in the initial days was about a week to ten days from manufacturing date). Quick customs clearance and maintenance of cold-chain was a huge challenge. Bigger challenge was to deliver this media in a perfect cold-chain to the clinics across India. Coolant packs, thermocol boxes were unheard of and no courier company was willing to touch perishable items . We would use thermal flasks with ice-cubes to deliver media personally to the clinic's door-step across India. I still remember spending several nights and days at cargo running around officers to get the consignment quickly. Half of the consignments would have to be abandoned due to break in cold-chain resulting in huge financial blows which would make us want to shut down the activity. It was support of our foreign partners and some good-hearted customers which inspired us to sustain and grow the activity. Over next few months with help from Danish Embassy, RBI and DGCI, we streamlined the import processes, cold-chain and logistics which is still being followed by several other companies in the field as a routine.

Trivector has collaborated with the Indian Society for Assisted Reproduction (ISAR) right from its inception under the leadership of Dr. M.N. Parekh in November 1994. Trivector has been proud participant of all ISAR meetings since this first meeting till date. Trivector also part of the first ever hands-on ICSI workshop in India held at Jaslok Hospital. As the interest in setting up IVF labs in India increased in following years, the requirement of training and hand-holding was also felt. To cater to this ever-growing demand for training, Trivector conducted several training road-shows across India, especially for IUI, ICSI, LAH and Spindle Imaging. In 2005, Trivector collaborated with Copenhagen University, Denmark to set-up 'MediCult Fertility School' in Mumbai at an investment of about Rs. 2 Crore. Hundreds of candidates have been trained on 'hands-on' techniques (including Laser, IMSI, Polscope, Time-lapse, CASA, PGD, Vitrification etc.) by the international experts in a well-equipped IVF lab. It is good to see that now every established IVF clinic and other institutes are offering training to the IVF-aspirants.

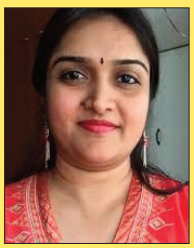
Soon we felt a need to establish a reliable Sperm-bank which would follow international standards in India. In 2008, we set-up 'Baby Quest Cryobank' in franchisee agreement with world's biggest sperm bank. Baby Quest has carved a niche for itself as a first choice for the quality sperm samples and has been setting standards for others to follow.

We are now in 2020, and Indian IVF is the biggest in the world which the world is looking at with great expectations. All the products, procedures and training modules are standardized and easily accessible to all. As one of the first companies in this field since 1993, I am proud to see coming of age of IVF in India. Trivector is committed towards continuing to play its role of offering innovative solutions to continually improve ART outcomes. We are also proud to see many of Trivector's erstwhile associates serving this community in their own way on the foundation and template created by Trivector. Let us celebrate this journey of evolution of IVF in India and enjoy more success together!

CONCEPT BY:

Dr Kedar N. Ganla
MD, DNB, DGO, DFP, FCPS
Hon Secretary, ISAR

Written By:



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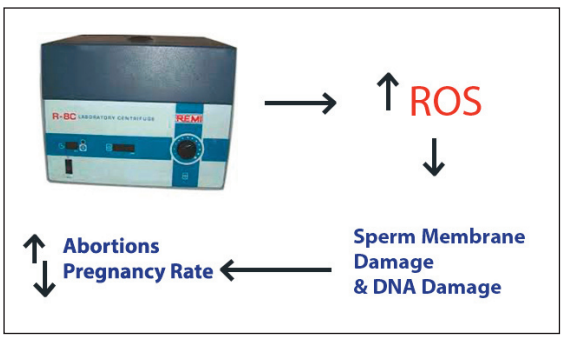
Food For Thought

IS THERE A NEED FOR NEWER SEMEN PROCESSING METHODS?

Semen washing is an integral part of the assisted reproduction techniques. The conventional technique for preparing human spermatozoa involves the use of repeated cycles of centrifugation and resuspension in a simple culture medium.



Available methods to process semen:	
Centrifugation methods	
A.	Swim up
a)	Classical
b)	Standard
B.	Gradient method

Urgent need to explore the non-centrifugation methods of semen processing

	Centrifugation methods	Non-centrifugation methods
Motile sperm recovery	Same	Same
Set Up requirements	Laminar flow, centrifuge, labware, media etc.	None. Only device and culture media needed
DNA fragmentation index	Higher	Lower
Sperm concentration	Higher	Lower
Pregnancy rates	Lower	Higher
Miscarriage rate	Higher	Lower
Cost (INR)	Initial set up cost – high Processing cost – low	Cost Rs 500 - 6000

Study	Sample size	Result	Significance
Blinded, controlled (2018). Quinn et al	70	Microfluidic chip - ? DFI Density-gradient centrifugation - ? DFI	P = 0.0029
Controlled randomised (Sep-D)	200	Semen ROS -? DFI (centrifugation)	P < 0.05
(2019). M. Muratori et al.	40	Sperm selection with DG centrifugation & swim up - ?DNA fragmentation by 60% in DG & 40% in swim up	P < 0.05

Available non – centrifugation kits: Zymot device, Zymot Fertile, Qualis, Sep-D kit, Seaforia

Non centrifugation methods of semen processing thus appear more attractive as a semen processing method with the promise of being a non-surgical option for men with high sperm DNA fragmentation.

We invite feedback/comments of the readers regarding their thoughts that this article may have generated. Please email your feedback to the authors on isar.elibrary@gmail.com

ISAR & FEQH

(Forum of Enhancement of Quality in Healthcare)

Launching the VIRTUAL AUDIT for Accreditation of IVF Clinics – ASIC2020

What is Virtual/ e-Audit?

- A remote audit using electronic means to obtain & evaluate audit evidence.
- Physical Presence of auditor not required.
- Documents shared by E-mail/ Google Drive/ Box & verified on live video conferencing.

Why Accreditation With ISAR?

- Uniquely designed for IVF clinics.
- 3rd party audit (No ISAR member involved in audit)
- Based on ISO-Principles.
- Prepares you for future audits like ICMR.
- Provides you with standard SOPs & quality policy.
- Makes your clinic more efficient & operator independent.

Benefits of Virtual Accreditation

- Time & cost efficient.
- Ease of carrying the audit for remote locations.
- Saves the expenditure on audit logistics.
- The best audit team involved.

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