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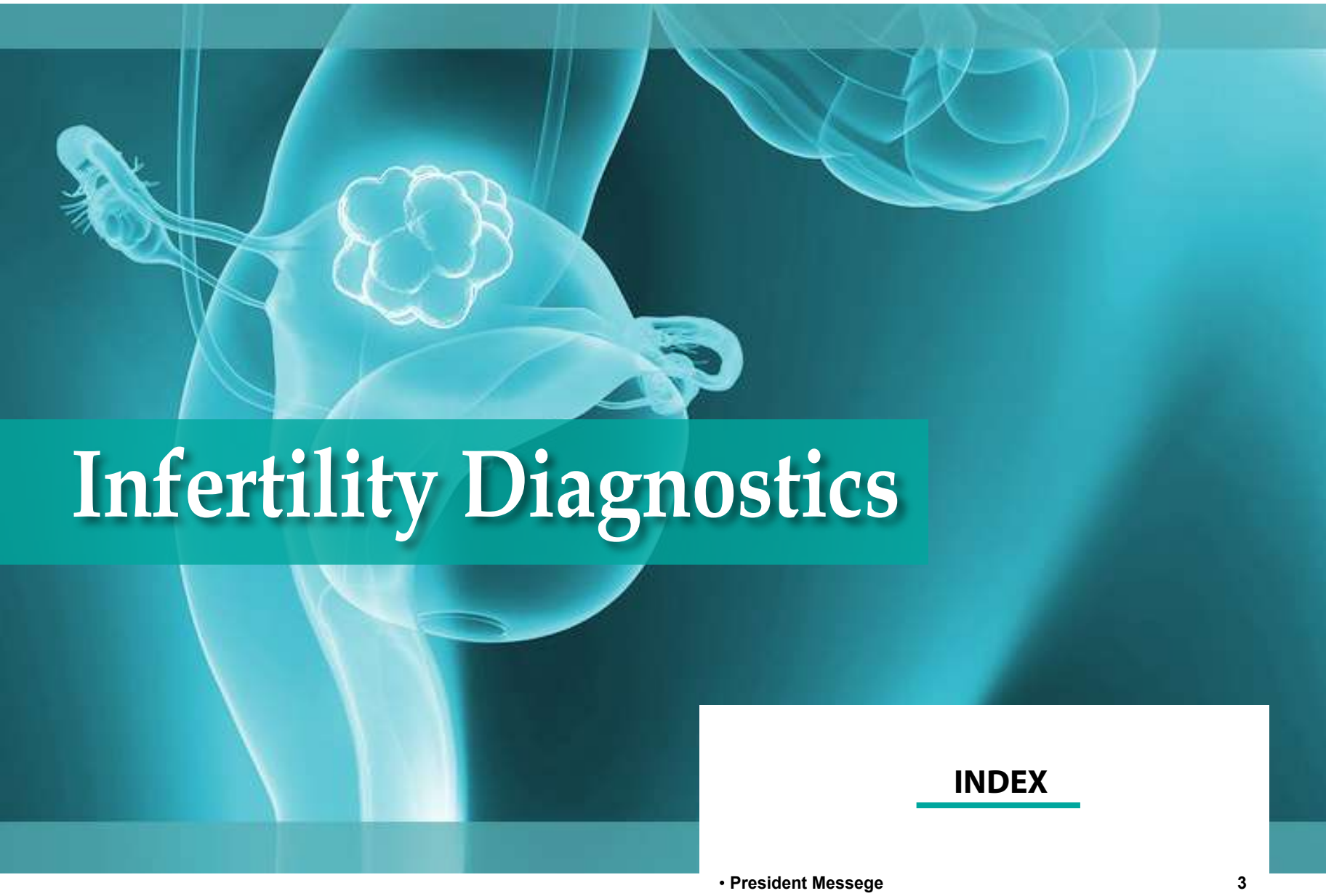

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MESSAGE FROM THE PRESIDENT DESK

DR GOURI DEVI
President - IFS

Dear Friends,

This issue of IFS Conversations focuses on diagnostics in infertility. What test to use when and false positive and false negative of a test wherever relevant is discussed. The "IFS Conversation" also showcases the various recent academic activities conducted by our extremely enthusiastic and committed members spread over 27 chapters across India.

Several of our members have also made IFS very proud through their remarkable achievements at the recent ESHRE Annual Meeting held at Vienna on 23rd to 26th June. My heartiest congratulations to all of you!

Fertivision 2019 is around the corner and is being held in Delhi. Those who have not registered yet, are gently reminded to visit the website and register immediately! We look forward to meeting each one of you at this annual academic event at Delhi.

Please do visit our website www.indianfertilitysociety.org for regular updates on our forthcoming courses, CMEs and conferences.

With best wishes,

Dr M. Gouri Devi

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MESSAGE FROM THE SECRETARY'S DESK

DR (PROF) PANKAJ TALWAR
Secretary General - IFS

Dear Friends,

Greetings from IFS Secretariat
It gives me immense pleasure to keep you updated on IFS activities through the IFS conversations, our newsletter. This issue is on Infertility diagnostics and focuses on testing of hormones and their relevance to an infertile couple. It also focuses on imaging and genetic testing.

We are proud to share that we have inaugurated our 27th chapter at Andhra Pradesh. As IFS expands throughout India we continue to train through our focused meetings, CMEs and fellowship program. An update of all has been included in this newsletter.

We are hosting the next fertivision 2019 at Delhi from 6th to 8th December at Leela ambience, Gurugram. There are 10 precongress workshops and a 2 day conference which includes a large international faculty. We hope you have registered. Online registration is available at the website Hope to see you there!

Dr (Prof) Pankaj Talwar

MESSAGE FROM THE EDITOR'S DESK

DR SURVEEN GHUMMAN
Editor - IFS

DR SHWETA GUPTA
Jt. Editor - IFS

Dear Friends,

Greeting from team IFS!

We have come out with yet another issue for IFS conversations, our newsletter which updates members on all activities of the society.

This issue discusses the test done infertility – Infertility Diagnostics. It starts with day 2 hormones and assessment of ovarian reserve with AMH. A review of the prolactin hormone is given.Methods to detect and interpret LH surge which is an important step to identify ovulation are discussed. Diagnosis of hyperandrogenemia and insulin resistance are outlined clearly. Further it discusses the genetic test required in both male and female partner. The role of ultrasound and HSG in finding causes of infertility is extensively discussed. The advantages of surgical diagnostic interventions like diagnostic hysteroscopy and laparoscopy and the endometrial biopsy are deliberated. Lastly diagnostic test for azoospermia are chalked out.

We have also included chapter activities and activities of special interest groups

Fertivision is approaching and we hope to see each one of our members there. Please do register from the website for this academic bonanza.

Any suggestions for the newsletter are welcome

Warm Regards!

Dr Surveen Ghumman

Dr Shweta Mittal Gupta

INDIAN FERTILITY SOCIETY INITIATIVES

INVITED ARTICLES

Investigations on Day 2 of an IVF Cycle

DR RENU MAKKAR

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Day 2 investigations are conducted on day 2 of period and includes Baseline ultrasound scan, Antral follicle count (AFC), serum estradiol (E2), follicle stimulating hormone (FSH), Luteinizing hormone (LH)and progesterone.These help to predict ovarian response. In the process of controlled ovarian hyperstimulation (COH), the principle steps are to evaluate ovarian reserve function, predict ovarian response, and develop an optimal individualized COH protocol.

Baseline scan should be trans-vaginal for higher accuracy. This scan is done on 2nd or 3rd day of the cycle. At this time of the cycle, the ovaries have no active follicles; estrogen and progesterone are both at lowest levels, endometrium is thin like a single line, as it has shed off during menstruation. It is done to classify the ovaries in one of the three categories:1. Polycystic ovaries,2. Normal ovaries,3. Low reserve ovaries. A normal ovary has the following reference values:

Diameter	2-3.5 cm
Volume	3-6.6 cc
Stromal RI	0.6-0.7
Stromal PSV	5-10 cm/sec
Follicle no per ovary (FNPO)	5-12

AFC (Antral Follicle Count)

The AFC indicates the number of follicles with diameters of 2mm to 9mm. They begin to develop after recruitment in the luteal phase of the previous cycle and reflect the number of follicles that will continue to mature during the ovulation treatment cycle.[1-3]

Counting ovarian antral follicles by ultrasound: AFC should be performed with the help of a transvaginal ultrasound (US) probe with frequency ≥7MHz. AFC is assessed by using real-time two-dimensional (2D) US, stored 2D-US cine-loops and stored three-dimensional (3D) US datasets. Real-time 2D-US is advantageous as it permits additional maneuvers to determine whether an anechoic structure is a follicle, but requires a longer scanning time, especially when there are large number of a follicles, resulting in more discomfort to the patient. 2D-US cine-loops have reduced scanning time and the possibility for other observers to perform the count.[4]

The antral follicles become identifiable by US more easily when they reach 2mm in diameter, coinciding with higher sensitivity to FSH. Antral follicles measuring between 2 and 10mm are 'recruitable', while antral follicles >10mm are usually referred to as 'dominant' follicles. It is believed by some that the number of follicles measuring only up to 5-6mm represents the best cohort of recruitable follicles and correlates better with the true ovarian reservehowever, distinguishing follicles that measure 5-6mm from those measuring 7-9mm may increase the time needed to count without any clinical benefit.[5]

Discordance between antral follicle counts and anti-Müllerian hormone levels in women undergoing in vitro fertilization: It has been observed that anti-Müllerian hormone (AMH) is positively associated with antral follicle count (AFC). But sometimes, there is often discordance between the AMH level and AFC in clinical practice. It is seen that approximately one in five patients in clinical practice showed discordance between AFCs and AMH levels. In cases when AMH and AFC are discordant, the higher AMH within the same AFC quartile have the higher number of retrieved oocytes, the cumulative live birth rate and the ovarian responsiveness are intermediate between that when both are concordant on either end. Both AMH and AFC would be recommended to be utilized for individualization of stimulation regimen; when they fall into discordant categories, it would be sensible to adopt an intermediate dose of gonadotrophin.[6]

Follicle Stimulating Hormone

Early follicular phase serum FSH is the commonly used endocrine test for determining the ovarian reserve. It is based on the feedback inhibition of FSH secretion by ovarian hormones and is an indirect marker of the ovarian reserve. At the beginning of the menstrual cycle, the estradiol (E2) and inhibin B levels inhibit FSH secretion from the pituitary. In women with diminished ovarian reserve, the production of ovarian hormones is insufficient, and this leads to elevated pituitary FSH secretion. Levels of FSH higher than 9 IU show a decreased ovarian reserve.[7]

Luteinizing hormone

Day 2 LH should ideally be below 5IU/l. The levels show down regulation when less than 2 IU/l. There may be raised levels in case of PCOS8.

Serum Estradiol

For IVF cycle E2 is done on day 2. If E2 > 80 pg/ml, it indicates poor IVF outcome as it indicates early follicular recruitment due to high FSH and poor ovarian reserve. Raised E2 on day 2 may also be present in case of a basal cyst and stimulation should be shifted to next cycle.

E2 levels are a reflection of the ovarian response. Early elevations in basal serum E2 are due to the advanced follicular development and the early selection of a dominant follicle, as seen in older women, due to rising FSH levels.It has been observed that women with E2 levels <20 pg/mL or ≥80 pg/mL have a higher artificial reproductive techniques (ART) cycle cancellation rate. Combining E2 with FSH on cycle day 3 is shown to have reduced the incidence of false-negative tests obtained when FSH alone was used. The elevation of both indicates poor ovarian response. E2, however, has low predictive accuracy and lacks high sensitivity and specificity cut-off levels.It may be used as a guide for starting stimulation with gonadotropins; however, it should not be used to exclude couples from ART program.[9]

Progesterone (P4)

Progesterone levels refer to the measurement of ovarian function. Progesterone levels are low during the follicular phase (<1 ng/mL), rise on the day luteinizing hormone (LH) surges (1–2 ng/mL), and increase until they peak approximately 1 week after ovulation. The levels <3 ng/mL imply anovulation, except when evaluated after a woman ovulates or prior to menses when progesterone levels are at a physiological low. Raised early follicular phase progesterone levels in menstrual cycle indicates an inefficient luteolysis. During early follicular phase adrenal gland contributes for progesterone secretion but late follicular phase progesterone is secreted mainly from ovary. As ovarian age advances, it causes shorter follicular phase and abnormalities in luteal phase function which may cause elevated p4 level due to insufficient luteolysis. If progesterone is more than 1.5 ng/ml stimulation should be postponed as it is indicative of a basal cyst or an active corpus luteum.

Inhibin B

The dynamic test evaluates the response of the hypothalamic–pituitary–ovarian axis to stimulation. Early follicular phase serum FSH is the commonly used endocrine test for determining the ovarian reserve. It is based on the feedback inhibition of FSH secretion by ovarian hormones and, hence, is an indirect marker of the ovarian reserve. At the beginning of the menstrual cycle, the E2 and inhibin B levels inhibit FSH secretion from the pituitary. In women with diminished ovarian reserve, the production of ovarian hormones is insufficient, and this leads to elevated pituitary FSH secretion. Inhibins are glycoproteins secreted by the granulosa and theca cells. It plays a major role in the selection of the dominant follicle and has a regulatory effect on the secretion of FSH. However, few studies have shown that inhibin B alone is not a very useful marker of the ovarian reserve. The routine use of inhibin B is, hence, not recommended in infertile couples.

Proposed protocol for Ovarian Reserve (OR) screening:

- Ovarian reserve screening should be provided to all women at 30 years of age who potentially seek future fertility and should be voluntary.
- Pre-screening counseling regarding the decline in fertility with age and merits/de-merits of ovarian reserve screening must be performed before the test is ordered.
- AMH is an ideal screening test of ovarian reserve as it is the least expensive and intrusive, has the least inter-observer variability and can be taken at any stage in the menstrual cycle.
- A serum AMH result below the 10th percentile for age indicates that the individual has poor ovarian reserve. A repeat confirmatory AMH and FSH test (Days 3–5, off-hormonal contraception for 2 months) should be performed with an AFC scan.
- Abnormal results must be discussed with a reproductive medicine physician with an understanding of the relative merits of the test and the available treatment options.
- Patients with borderline low ovarian reserve screening results may elect to have follow-up ovarian reserve testing 12 months later to assess the rate of decline in ovarian reserve before acting on the result

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LH Surge – Detection and Clinical Implications

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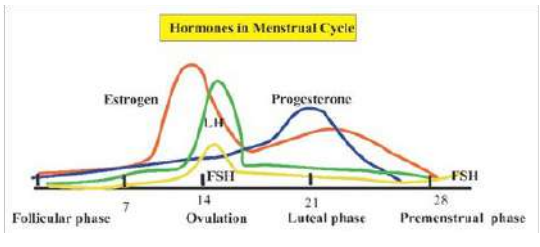
Luteinizing Hormone (LH) is a gonadotropin which is synthesized and secreted by the anterior pituitary gland in response to GnRH. LH surge is the significant peak and important milestone in ovarian cycle as it defines the end of follicular phase and marks the beginning of luteal phase. LH surge is a relatively precise predictor of ovulation and reflects the peak effect of interplay of complex autocrine and paracrine factors of pituitary and gonadal hormones. LH surge stimulates resumption of meiosis and the completion of reduction division in the oocyte with the release of the first polar body. Hence LH surge is the cardinal event for conception and fertility.

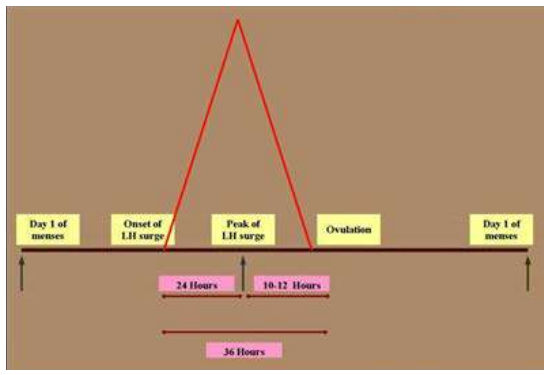
Physiology

LH is a glycoprotein with α subunit similar to HCG and biospecific β subunit, both share the same receptors. Follicular recruitment starts with late luteal phase rise of FSH. These recruited follicles secrete estrogen, which subsequently inhibits FSH secretion in a negative feedback loop involving the pituitary gland, the hypothalamus, and inhibin B. The follicle, most sensitive to lowered FSH levels continue to grow, while others become atretic. This dominant follicle continues to secrete estrogen. Serum estradiol concentration at a threshold level of greater than 200 pg/ml for approximately 50 hours exerts a positive feedback mechanism on the hypothalamus and anterior pituitary gland and results in an abrupt secretion of LH into bloodstream named as LH surge.[1] Positive feedback of estrogen in the pituitary region is by increase in sensitivity to GnRH (due to increase in GnRH receptors on gonadotropic cells) and at the hypothalamic level, is by increasing GnRH pulse frequency, and through the neuropeptide kisspeptin. Kissepeptin has an essential role in receiving stimulatory estrogen signals and generating full positive LH surge.[2]

The rapid increase in LH levels at mid-cycle (LH surge) causes a suspension of further granulosa cell mitosis and permits final oocyte maturation to begin and luteinization of the cumulus-oophorus to occur. The high levels of LH prevent further growth of the non-dominant follicles. This concept has led to the proposal of the 'LH ceiling' hypothesis wherein each follicle has an upper limit of responsiveness to LH beyond which follicle maturation ceases and degeneration occurs. Thus, the dominant follicle would have a much higher ceiling than the non-dominant ones, leading to their regression at the time of the LH surge(deselection).[3]



The LH surges that result in ovulation are extremely variable in configuration, amplitude, and duration. The onset of urinary LH surge can be categorized into rapid-onset type (within one day, 42.9%) and gradual onset type (over 2–6 days, 57.1%). Its onset occurs abruptly and characterized by three phases, an ascending phase of 14 hrs, peak plateau of 14 hrs and long descending phase of 20 hrs. The peak of LH surge is more acute than of FSH. Serum E2 peaking at the rapid ascent of LH surge and P levels increase approximately 12 hrs before onset of LH surge and continue to plateau till 36 hrs after LH surge. Increase in P levels terminates LH surge. The onset of the LH surge precedes ovulation by 35–44 hr, and the peak serum level of LH precedes ovulation by 10–12 hr.[4] Onset of the LH surge primarily occurs between midnight and early morning (37% between 00:00 and 04:00, 48% between 04:00 and 08:00).



With LH surge, there is maturation of oocyte, expansion of cumulus, disruption of granulosa cells, activation of proteolytic enzymes, production of prostaglandins. LH surge leads to luteinization of theca and granulosa cells, initiation of meiosis in ovum and finally ovulation and formation of corpus luteum. Till the LH surge the primary oocyte is arrested in prophase of the meiosis as cAMP and calcium influx activate protein kinase A(pkA) to inhibit oocyte maturation. LH blocks cAMP transport to oocytes, along with several autocrine and paracrine factors, primary oocyte resumes meiosis 1 and completion of division. Elevated LH is also responsible for increased stromal vascularization due to neoangiogenesis, catecholaminergic stimulation, leukocyte and cytokine activation.[1]

The threshold amplitude to define an LH surge is still a matter of debate, but levels more than 10 IU/L are commonly reported although a doubling from basal level could be a more appropriate definition, particularly for patients with high basal LH levels.

Detecting LH surge

Highly sensitive urinary LH kits detect concentrations as low as 22 mIU/ml, while natural LH surge concentration in urine ranges from 20 to 100 mIU/ml. Detecting LH surge in urine is cost effective, sensitive, effective and natural method to plan pregnancy but not ideal for contraception as sperm ejaculated before a woman's LH surge may survive long enough to fertilize the ovum.

The mean time interval after a positive urinary LH test to follicular rupture detected by sonography was reported to be 20+-3 hr (95% CI 14 –26), and in a study focused on infertile women, sensitivity, specificity, and accuracy of the urinary LH test to detect ovulation reached 1.00, 0.25, and 0.97, respectively.

Despite positive correlations, LH surge may not always signify true ovulation. "Luteinized unruptured follicle syndrome" can occur in 10.7% of menstrual cycles in normally fertile women. Women with this syndrome have a normal LH surge, functioning corpus luteum, and menstruation, but no oocyte is released. In infertile women, premature LH surge that did not trigger ovulation was detected in 46.8% of cycles.[5]

Clinical Implications

Physiologic LH surge is mimicked by pharmacological LH surge by agonist trigger or dual trigger in antagonist cycle and HCG trigger in agonist cycle. LH surge by use of GnRHa as a trigger in antagonist cycle is quite physiological with simultaneous induction of FSH surge. This FSH surge induces LH receptor formation in luteinizing granulosa cells, promotes oocyte nuclear maturation and cumulus expansion, opens the gap junctions between the oocyte and cumulus cells which are important in signaling pathways, allowing the oocyte–cumulus cell mass to detach from the follicular wall before ovulation. This might explain retrieval of more mature oocytes after GnRHa trigger compared with hCG trigger.[6] No precise threshold of circulating post-trigger LH- and progesterone-circulating levels has been defined .some investigators have indicated an LH circulating level of 15 IU/l as the cut-off value (Kummer et al., 2013).

LH is pulsatile with short biological half life of 60-120 minutes compared to HCG which is non-pulsatile, more angiogenic and persistent, with half-life of 24 hrs. GnRH-agonist is used to trigger ovulation instead of HCG, often to prevent severe OHSS in high-risk patients.[7]

Premature LH surge occurs in PCOS cases and in stimulated cycles (induced multifollicular growth) where exogenous FSH allows more follicles to grow and secrete higher serum estradiol concentrations which triggers pre-ovulatory LH surge prematurely. Premature luteinization may have an unfavorable impact on oocyte quality, fertilization, and implantation as LH surge is reached even before follicle is fully developed. Without intervention, premature luteinization occurs in about 25% of ovarian stimulation cycles, leading to compromised treatment outcomes. In high responders or stimulation with high doses of FSH, antagonist should be started on D5 of ovarian stimulation rather than on D6 to prevent premature LH rises.(flexible than fixed). For successful assisted reproduction treatment, it is essential to prevent premature luteinization and ovulation.[8]

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Anti Mullerian Hormone (AMH) evaluation and its clinical use

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AMH-anti Mullerian hormone also known as Mullerian inhibiting hormone (MIH) was discovered in 1947 by a French researcher Alfred jost. He observed that AMH is responsible for Mullerian duct regression during fetal development in rabbits. AMH was initially known known for its role in male sexual differentiation but in 1990's it was identified and reported in females. It was first isolated and purified 1984. In 1986 and 1994 respectively, genes for AMH and its receptor were sequenced and cloned.

AMH is a dimeric glycoprotein from transforming growth factor-β(TGF-β) family and has its gene on chromosome 19 p13.3. The hormone binds to anti-Mullerian hormone receptor (AMHR), which is a single transmembrane protein with serine-threonine kinase activity. The receptors are expressed-on target organs like Mullerian ducts, Sertoli and Leydig cells of testis, and granulosa cells of ovary. AMH is also regulated by a number of genes such as SF1, GATA1, WT1, DAX1, and SOX9.

Role of AMH in ovarian physiology

AMH was predominantly known for its role in inhibiting the development of Mullerian duct structures in males but newer studies have shown its relation to female reproductive physiology as well .The dormant follicles do not secrete AMH but as soon as they are recruited for maturation(preantral and small antral follicles) AMH expression has been observed. Follicle measuring 2 to 8 mm have been seen to express highest amount of AMH , thus making it the earliest marker of ovarian follicular growth. When follicles reach in FSH dependent phase of maturation(8-10mm) secretion of AMH by granulosa cells diminishes.AMH has a potential role in conservation of ovarian reserve .this is done by exerting dual actions.

1. It stops the primary recruitment of follicle for maturation (primordial to primary follicle transition) by hindering several growth factors like KIT ligand and basic fibroblast growth factor.
2. AMH also decreases the primordian follicle sensitivity to FSH since puberty, therefore reducing the possibility of their cyclic recruitment.

After the follicle matures to a size of 8mm it is selected for dominance, following which AMH production decreases.These observations reinforce the role of AMH as major regulator of initial and cyclic recruitment of follicles by maintaining their threshold for FSH sensitivity.This has been further upheld by the studies in AMH null mice. Increased number of follicle uptake leads to burnout of follicles at a much younger age in AMH null mice . Thus, AMH has negative effect on early follicular recruitment therefore limiting the entry of primordian follicles into the growing pool and preventing them from exhaustion at early age.AMH also has an inhibitory effect on cyclic follicular recruitment in vivo by reducing the follicle sensitivity to FSH.

AMH assays

AMH has great scope in clinical application but it is limited in its use due to its numerous biological features like -

1. Molecular heterogenicity of circulating AMH level with a non-cleaved biologically inactive form and a cleaved biologically active form.
2. Variable sensitivity of the immunoassays to interference by complement C1q and C3.
3. Stability of AMH sample during storage is not well known.
4. High interlaboratory variability chiefly for low value of serum AMH.

Because of all these variables ,measurement of AMH has not shown consistency . Also, because there are different ELISA immunoassays used all over the world.

a. Gen II (Beckman coulter)
b. EIA AMH/MHS kits (IOT or "immunotech" Beckman coulter)
c. AL-105-i(Anshlabs),

These all use different monoclonal antibody with different standards which leads to interlaboratory differences thus causing absence of consensus of reference values.

Hence to bypass these problems in serum AMH estimation various developments have taken place like –

a. Development of an ultrasensitive essay (pico AMH kit, Anshlabs)
b. Automation on immune-analysers (Access Dxi auomatic analyser, Beckman coulter. Cobas e instrument, Roche Diagnostics).

These newer advances have made it possible to achieve near identical values and hopefully an international standard of serum AMH values would be soon developed.

Ovarian reserve may also vary due to ethnicity as shown by some studies. Hence we need to establish a baseline level of AMH among the different populations of the world. As of now literature states that AMH levels in women between the ages of 25-40 years should be between 1.0 to 3.0ng/ml to be normal, 0.7 to 0.9 ng/ml as low normal and 0.3 to 0.6 ng/ml as low and below 0.3 is considered as very low.AMH is quite stable throughout the menstrual cycle since dominant follicle and corpus luteum do not secrete AMH (Tsepelidis S,2007) however long term hormonal contraception (Dólleman M)and pregnancy has been shown to significantly decrease AMH levels , hence AMH is not accurate as a predictor of ovarian reserve in women using OCP's.

Interpretation of AMH values and its clinical uses

1. **Relationship with age at menopause:** It is at present unclear whether AMH measurements can successfully predict the age at menopause.
2. **Detecting the chemotherapy , radiotherapy induced damage :** AMH appears to have greater sensitivity than FSH or Inhibin B in detecting the ovarian damage following chemotherapy, radiotherapy. Pre-treatment AMH level is also able to predict on-going ovarian activity following such therapy as pre-treatment AMH levels were significantly higher in women who continued to have menses . (Anderson et.al.,2013)
3. **Detecting the surgically induced damage:** Significant decline in AMH levels have been confirmed following endometrioma surgeries indicating removal of substantial part of ovarian reserve. (Somigliana et.al. 2012). This knowledge helps us in decision making process for ovarian surgery in women desirous of future fertility.
4. **Prediction of ovarian response following ovarian stimulation in ART :**That AMH can predict ovarian response following ovarian stimulation accurately, has been demonstrated in many studies (La Marca et.al. 2010 , Broer et.al.2011). This has enabled clinicians to develop individualized ovarian stimulation strategies for patients at the extremes of ovarian reserve like PCOS patients and poor ovarian reserve patients .AMH is also useful as a counselling tool for couples for realistic prediction of number of oocytes. Age as a surrogate marker for oocyte quality and AMH for oocyte quantity have been helpful in counselling couples.
5. **Prediction of clinical pregnancy following ART :** The significance of serum AMH levels in predicting clinical pregnancy during ART treatment is lower in patients with a low risk of DOR. Hence it is not advisable to refuse ART treatment based solely on AMH levels .

By utilizing AMH , it is now possible to measure the intrinsic or acyclic part of ovarian cycle serving as a very useful clinical biomarker of ovarian function . There is a need for improved assay validity and international standard for AMH for its better clinical use in a variety of clinical situations.

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Prolactin And Infertility

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Human prolactin (PRL) is a hormone secreted by the lactotropic cells of the anterior pituitary gland. It has a variety of biological functions in reproduction apart from playing an important role in lactation. Secretion of prolactin is under hypothalamic control and does not depend on any negative feedback mechanism by the peripheral hormones. Counter current flow in the hypophyseal pituitary portal system initiates secretion of dopamine from the hypothalamus which is believed to be the principle prolactin inhibiting factor(PIF). γ-aminobutyric acid (GABA), somatostatin, acetylcholine, and norepinephrine can also inhibit PRL release, but thyroid releasing hormone (TRH), vasoactive intestinal peptide (VIP), epidermal growth factor (EGF), serotonin, histamine and dopamine receptor antagonists tends to stimulate its secretion. Thus the primary control of prolactin is inhibitory rather than stimulatory . Its secretion is pulsatile and increases with sleep, stress, food ingestion, pregnancy, chest wall stimulation, and trauma.

Human PRL is found to be present in different molecular forms on which the bioactivity of the hormone depends. PRL is synthesized as a prehormone with a molecular weight of 26 kDa. When a preprolactin molecule is cleaved, the resulting PRL polypeptide has a molecular weight of 23 kDa (198 amino acid). This monomeric form of PRL is the major circulatory form and is also known as little PRL and it is known to be both biologically and immunologically active. The other forms mainly include the big PRL with a molecular weight of approximately 50 kDa and the tetrameric big-big form with a molecular weight greater than 150 kDa . These latter two forms are known to have low biological activity but high immunogenic properties. The first case of hyperprolactinemia with predominant big-big PRL on gel chromatography was described by Whittaker et al.[1] The patient was asymptomatic but despite high PRL levels, spontaneous pregnancy was possible . The absence of in vivo bioactivity of this big big prolactin is due to the high molecular mass of the complex which does not allow its passage through the capillary endothelium and finally to its target cells.[2] Jackson et al. in 1985[3] first used the term "macroprolactinemia" for such patients with marked hyperprolactinemia whose PRL mainly consisted of big-big PRL. Thus, macroprolactinemia is a condition in which high levels of the circulating 'big prolactin' molecules are present and this is identified as hyperprolactinemia in commonly done assays. Even though the test detects hyperprolactinemia , the biological active prolactin is normal and the patient is also asymptomatic , though a small subset of patients with macroprolactinemia, may be symptomatic. Thus to avoid unnecessary treatment of hyperprolactinemia on the basis of blood tests alone , routine screening of macroprolactinemia may be of help in asymptomatic hyperprolactinemia patients. Many commercial assays do not detect macroprolactin.

Etiology

Hyperprolactinemia refers to an increase in serum PRL concentrations in blood and it could be physiological or pathological.

Physiological causes include pregnancy,lactation/nipple stimulation, stress and sleep.

Pathological Causes

Table 1 : Main causes of pathologic hyperprolactinemia[4]

DYSFUNCTION/ DISEASE	MECHANISM
Idiopathic	Impaired hypothalamic dopamine secretion
Pituitary tumors : micro- or macroprolactinoma, adenoma	Disruption of dopamine delivery and/or secretion of prolactin
Acromegaly	Prolactin secretion from a GH adenoma
Empty Sella Syndrome	Damage of the pituitary
Primary hypothyroidism	Increased hypothalamic TRH
Polycystic ovary syndrome	Raised estrogen concentration
Renal failure	Reduced PRL clearance
Drugs --- Antidopaminergics, Antipshychotics, Antiemetics, Tricyclic antidepressants,	Inhibition of dopamine release
Drugs -- Opiates	Stimulation of opioid hypothalamic receptors
Drugs -- Estrogens	Stimulation of lactotrops

Diagnosis

Lack of awareness among clinicians together with lack of proper diagnostic methods is a major cause for unnecessary expensive investigations, treatments and follow ups regarding hyperprolactinemia. A cause of concern while diagnosing hyperprolactinemia is that macroprolactinemia is often overlooked. False high prolactin values, labelled as hyperprolactinemia are obtained as macroprolactin interferes with most commercially available immunoassays used for measuring prolactin. The values depend on the assay method used.No laboratory method was available to diagnose simple macroprolactinemia in the past.But now, screening of macroprolactinemia can be done by polyethylene glycol precipitation method (PEG) though the gold standard or the reference method remains the gel filtration chromatography (GFC). The other qualitative methods include protein A/G column and 125 I PRL binding sites.

1. Polyethylene glycol precipitation method : It is a simple, inexpensive , rapid and a reliable method for detection of macroprolactin. 50 microlitres of serum sample is mixed with 50 microlitres of cold PEG and centrifuged at 9100 x g for 10 minutes . This is to remove macroprolactin and determine free prolactin concentration. To determine total prolactin concentration serum samples are treated in the same manner but with water. Amount of macroprolactin is calculated as follows :

$$\frac{\text{Total PRL} - \text{Free PRL}}{\text{Total PRL}} \times 100$$

PEG precipitation ratio greater than 60% (recovery less than 40%) is used as a cut off for diagnosis of macroprlactinemia. Recovery value between 40 – 50 % should ideally be taken for gel chromatography . Values > 50 % rule out macro prolactinemia. Increased amount of serum globulin concentrations can increase the amount of monomeric PRL precipitated by PEG. This leads to a false presence of macroprolactin. Hence results of PEG test should be interpreted with great caution in patients with IgG myeloma and polyclonal hypergammaglobulinaemia as in HIV patients.[5]

2. Gel filtration chromatography :This method has been used to separate the three molecular forms of prolactin : the little prolactin , the big prolactin and the big big prolactin. A diagnosis of macroprolactinemia is made when more than 30 – 60 % is in big big prolactin form in gel filtration chromatography. Though this method is considered to be a gold standard , it is expensive , time consuming and labourious method discouraging the clinicians to make use of it.
3. Protein A/G column : Protein A binds to the Fc portion of immunoglobulin molecules and protein G binds only to IgG and its subclasses . This separates IgA, IgM , IgD , and albumin.
4. 125 I – PRL binding study : It helps in identifying anti prolactin autoantibodies which are a common cause of IgG bound PRL. But it is time consuming and hazardous as it requires radio isotope facilities.

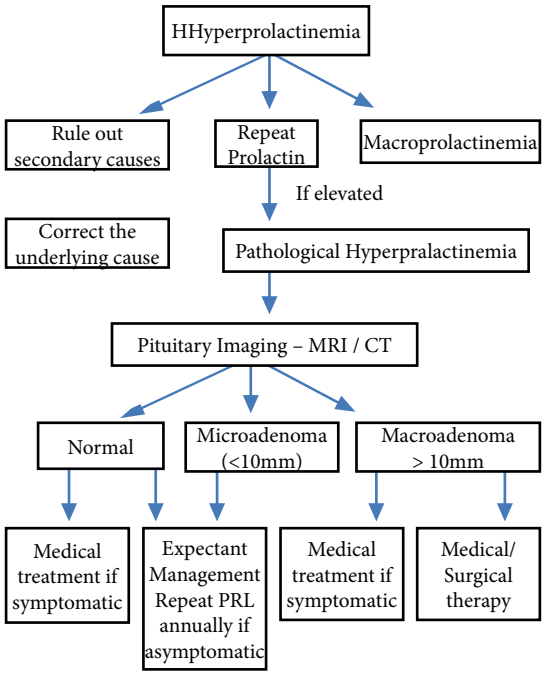
Table 2 : Comparison of different methods for the diagnosis of hyperprolactinemia

	Advantage	Disadvantage
Polyethylene glycol (PEG)	Simple, inexpensive screening test	Not highly specific
Gel Chro-matography	Accurate confir-matory test	Time consuming and expensive
Protein A/G	Identifies IgG bound prolactin	Expensive
125 I PRL	Identifies anti prolactin auto antibodies	Time consuming, hazardous and needs radioisotope facilities

Radiological Evaluation

Prolactin levels lower than 100ng/ml may be observed with all causes of hyperprolactinemia , while higher levels are usually indicative of a prolactin secreting tumour. Pituitary imaging should be performed in all patients with persistently high levels of prolactin levels. Magnetic resonance imaging is preferred over Computerized axial tomography (CT) are usually negative in macroprolactin to assess sellar area though both of them are uusually negative in macroprolactinemia cases. In few cases abnormalities do appear though they are infrequent as compared to those seen in patients of hyperprolactinemia due to other causes.

Common conditions which raise prolactin levels like vigorous exercise, drug intake, trauma, renal impairnment, cirrhosis and a non- fasting sample should be excluded while diagnosing hyper prolactinemia. Prolactin secretion follows a circadian rhythm with higher concentration during the night and lower during the day. Normal prolactin levels are typically 10 – 25 ng/ml in women. Levels should be measured preferably in the morning two hours after waking up . If the prolactin levels are markedly increased, repeat the levels. Even one normal value should be considered as normal and an isolated raised one should be discarded as spurious.[6] In a patient with suspected medication induced hyperprolactinemia , the drug should be discontinued for at least three days after physician consultation and then the levels should be repeated.



Hyperprolactinemia is a common finding and is associated with subfertility / infertility in young females but one must be cautious and suspect macroprolactinemia in the differential diagnosis before commencing any treatment for all cases of hyperprolactinemia.. The actual cases of true hyperprolactinemia are symptomatic and can be treated with dopamine agonists with restoration of fertility.

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Genetic Testing in Infertility

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Infertility is considered a major public health issue, and approximately 1 out of 6 people worldwide suffer from infertility during their reproductive lifespans. Genetic tests are becoming increasingly relevant in reproductive medicine. More genetic tests are required to identify the cause of male and/or female infertility, identify carriers of inherited diseases and plan antenatal testing. In particular, genetic tests are carried out for three main purposes in reproductive medicine: the identification of the infertility causes, identification of genetic diseases transmissible to offspring, and optimization of the assisted reproductive technology.

Normally, gametes with genetic or chromosomal alterations have reduced reproductive potential. Thanks to ART, many of these difficulties can be overcome, and therefore, genetic tests (carrier screening, preimplantation and prenatal diagnosis) have the crucial impact of monitoring the possible transmission of these genetic alterations to the offspring. To date, the diagnostic options for couples at risk of transmitting a specific inherited disorder to their offspring are preimplantation genetic testing (PGT) and prenatal diagnosis (PND). These two diagnostic procedures share the same purpose but differ in diagnostic time, type of sampling, and laboratory procedures.

To optimize the application of genetic tests in clinical practice, we need to discuss (1) the genetic conditions related to infertility, including the common and rare ones that are case appropriate;(2) the diagnostic strategies in families at risk of known monogenic disease transmission; and (3) the impact of PGT in the optimization of ART techniques.

Genetic tests in the identification of the causes of infertility

It has been estimated that every healthy subject is a carrier of 5/8 genetic alterations associated with recessive genetic disorders; therefore, even in the absence of specific symptoms, family planning and reproduction can be risky. Moreover, it has been reported that almost 50% of infertility cases are related to genetic disorders.

Male genetic infertility

Genetic factors have been found in all the etiological categories of male fertility (pre-testicular, testicular and post-testicular): OMIM (Online Mendelian Inheritance in Man) reports more than 200 genetic conditions related to male infertility, ranging from the most common clinical presentations of infertility to the rarest complex syndromes in which signs and symptoms are beyond the reproductive problems. In most cases, infertility is only one of the clinical signs of a complex syndrome; on the contrary, in some genetic conditions, infertility is the main phenotypic feature.

Today, the presence of alterations in the semen analysis is the first indication for genetic tests, particularly in cases of severe oligospermia (< 5 million/ml) (further parameters are hormonal levels, malformations, recurrent abortions, and family history).Interestingly, a recent study by Oud et al. highlighted how the number of genes that are definitively linked to the more common phenotypes of oligozoospermia or azoospermia remains limited (50%); the other half are genes involved in teratozoospermia, although the monomorphic forms of teratozoospermia are extremely rare (1). Genetic disorders related to male infertility include whole chromosomal aberrations (structural or numerical), partial chromosomal aberrations (i.e., microdeletions of the Y chromosome) and monogenic diseases. In particular, abnormalities in sex chromosomes have a greater impact on spermatogenesis, while mutations affecting autosomes are more related, for example, to hypogonadism, teratospermia or asthenozoospermia and to familial forms of obstructive azoospermia.

Currently, the main genetic tests routinely used for the diagnosis of male infertility are the karyotype, the study of chromosome Y microdeletions, and the analysis of the CFTR gene. It must also be considered that the role of de novo mutations should be further investigated, especially in light of what happens for Klinefelter syndrome and AZF deletions that occur almost exclusively de novo (1).Therefore, to improve and personalize the entire diagnostic–therapeutic pathway of male infertility, targeted genetic tests should be performed in the presence of specific clinical pictures, always after appropriate genetic counselling: (a) for diagnostic purposes, (b) during clinical decision-making to establish the most appropriate ART strategy (for example, in the presence of deletions of the AZFa and AZFb regions, the possibility of sperm recovery using testicular biopsy is extremely low), and (c) for prognostic purposes (to establish the risk of transmitting the pathology and plan a prenatal or preimplantation diagnostic procedures).

Whole chromosomal aberrations: The prevalence of chromosomal alterations varies from 1.05 to 17%, but is 0.84% in newborns [109]. Structural chromosomal rearrangements are more common with respect to numerical abnormalities; this does not apply to sex chromosomes whose abnormalities, accounting for approximately 4.2% of all whole chromosomal aberrations, are represented by sex chromosome aneuploidies in 84% of cases and by structural rearrangements of chromosome Y in the remaining 16% of cases. Klinefelter syndrome (karyotype 47, XXY) is the most frequent type of sex chromosome aneuploidy detected in infertile men (2,3), the second most frequent gonosomal abnormality is Double Y syndrome or Jacobs syndrome, characterized by the presence of Y chromosome disomy (4,5). In addition to reduced reproductive potential, carriers of chromosomal abnormalities have an increased risk of abortion or generate a child with an abnormal karyotype.

Partial chromosomal aberrations: Microdeletions in the long arm of the Y chromosome (Yq), named the AZF (Azoospermia Factor) region, have been found in 8–12% of azoospermic men and 3–7% of oligozoospermic men, resulting in the most common molecular genetic cause of male infertility. The AZF region includes three groups of genes (AZFa, AZFb and AZFc) that are most responsible for spermatogenesis, so partial or complete deletions in this area may impair reproductive capacity. Indications for AZF deletion screening are based on sperm count ($< 5 \times 106$ spermatozoa/ml) associated with primary testiculopathy, and ICSI is required to overcome infertility.Male offspring will carry the same father's Yq microdeletions or even a worse one; therefore, genetic counselling is mandatory (6). Parents should be aware of the risk of having a child affected by Turner's syndrome (45, X0) or other phenotypic anomalies associated with sex chromosome mosaicism.

The rearrangement of the AZFc zone is responsible for 60% of all Yq microdeletions (6). The AZFc region (3.5 Mb) contains several copies of five repeats (b1, b2, b3, b4, and gr), whose similarity and large size predispose an individual to a relatively high incidence of de novo

deletions via homologous recombination. The most common is the loss of the whole b2/b4 region, which includes the DAZ family (Deleted in Azoospermia) and leads to spermatogenesis deterioration.

Single gene mutations: Although thousands of genes are involved in male infertility, today, only a handful of genetic diseases are routinely investigated (e.g., cystic fibrosis).

Female genetic infertility

In contrast to male infertility, little is known about the genetic bases of female infertility. Accordingly, fewer specific tests are routinely recommended to infertile females to investigate the presence of chromosomal disorders or single-gene defects related to their clinical phenotypes. To date, genetic tests are mainly used for patients with POI, limited to chromosomal aberrations and FMR1 premutations.

Whole chromosomal aberrations: Considering that chromosomal disorders significantly impact fertility and the miscarriage risk, karyotype analysis is always advisable (7). The most clinically important structural disorders in infertile females are translocations, both reciprocal (exchange of two terminal segments from different chromosomes) or Robertsonian (centric fusion of two acrocentric chromosomes) responsible for blocks of meiosis and structural alterations of the X chromosome. Patients with reciprocal translocations are at a significantly increased risk of infertility, including hypogonadotropic hypogonadism with primary or secondary amenorrhea or oligomenorrhea. The balanced rearrangements can give rise to gametes in which the genetic information is unbalanced and can thus become a cause of infertility or multiple miscarriage.

Women with a normal karyotype produce a variable percentage of oocytes with chromosomal abnormalities due to errors occurring during crossing-over and/or meiotic nondisjunction. The three main classes of abnormalities are 45X, trisomy and polyploidy. It is well known that these events increase with maternal age. It is possible to analyze gametes or embryos while undergoing ART thanks to PGT.

Fragile X syndrome: Fragile X syndrome is an autosomal dominant genetic disorder caused by the presence of over 200 repetitions of the CGG triplet sequence in the FMR1(Fragile X Mental Retardation 1) gene or by a deletion affecting the FMR2 (Fragile X Mental Retardation 2) gene. Carriers of the female FMR1 premutation (when the number of CGG repeats falls between 55 and 200) or FMR2 microdeletion show menstrual dysfunction, diminished ovarian reserve, and premature ovarian failure. The most common genetic contributors to POI are X-chromosome-linked defects.
Molecular approaches in the identification of genetic diseases that are transmissible to offspring:The ACOG has issued standard recommendations for ethnic and general population genetic screening in couples based on reproductive age. Testing is available for more than 2000 genetic disorders, including common diseases, such as sickle-cell anaemia, cystic fibrosis, and spinal muscular atrophy, or more complex conditions, such as mental retardation and congenital heart disease

Molecular approaches for the optimization of art techniques:Human embryos that are developed in vitro show a great deal of acquired chromosomal abnormalities; for this reason, PGT for aneuploidy (PGT-A) has been developed to select euploid embryos that are suitable for transfer. PGT-A is primarily indicated for couples with advanced maternal age, recurrent implantation failure, recurrent abortions, or severe male infertility. Randomized studies and meta-analyses have shown that the PGT-A technique does not increase the live birth rate but decreases the miscarriage rate and increases the efficiency of IVF techniques.

Currently, the most commonly used technique is NGS. Literature data confirm that NGS can be successfully applied to the diagnosis of a variety of genetic abnormalities, even in single cells isolated from human embryos following WGA, and has numerous advantages over the technologies traditionally used for PGT-A.
Currently, both the entire diagnostic pathway and the effectiveness of genetic analysis for infertility suffer from an approach that is ineffective: only a few genetic variables are studied, each through a specific molecular diagnostic procedure. This makes the process of genetic investigation fragmented and cumbersome, with a negative impact on the couple, in addition to the psychological distress caused by infertility. However, recent developments in new sequencing technologies have made it possible to compact one or more tests into a single NGS-based analysis, thus reducing diagnostic costs and time. The European Society of Human Genetics (ESHG) and the ESHRE have recently issued a recommendation for the development and introduction of extended carrier screening (8).

Reproductive specialists have the task of evaluating infertile couples by considering both their general and reproductive health, since the relative conditions of comorbidity can influence their reproduction.

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Ultrasound To Detect Uterine, Tubal And Ovarian Pathology

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Ultrasound is the backbone of modern obstetrics and gynaecology practice. Recent technological breakthrough in diagnostic ultrasound, including the advent of color Doppler, power Doppler, three dimensional imaging have led ultrasound to surpass all expectations. Gynecologic sonography is a viable, well developing entity. It is able to accept new challenges and incorporate them into the diagnostic process. Novel sonographic methods enable us to perform gynecologic diagnosis more exactly.

Trans-vaginal Sonography is fundamental part of pelvic ultrasound examinations because of the use of higher-frequency transducers with better resolution, examination of patients who are unable to fill their bladder, examination of obese patients, better distinction between adnexal masses and bowel loops and better characterization of the internal characteristics of a pelvic mass.

However, **Trans-abdominal Sonography** continues to be the mainstay when structures are high in the pelvis, out of the field of the TVS probe, in pediatric patients, and in those who have difficulty tolerating TVS.

Color and spectral **Doppler Sonography** allows for the assessment of normal and pathologic blood flow. Doppler ultrasound can distinguish vascular structures from non vascular structures, such as dilated fallopian tubes or fluid filled bowel loops. Color Doppler flow imaging (CDFI) expands conventional duplex sonography by providing additional capability. Power Doppler provides further increased sensitivity for flow detection.

Three-Dimensional Multiplanar Sonography, performed using trans-vaginal transducer has been shown to be extremely useful in evaluating the mullerian abnormalities of uterus, abnormal position of intrauterine device, relationship of uterine lesions to the endometrium. 3-D /4-D USG has improved its functions with high definition live (HD live) technology and furthermore, great advances of ultrasound technology have produced new applications and HD live videos.

Sonohysterography provides more detailed evaluation of the endometrium or submucosal myometrium lesions by instillation of sterile saline or gel infusion into the endometrial cavity under ultrasound guidance. This distends the cavity, separating the walls of the endometrium. The most common indication for SHG is

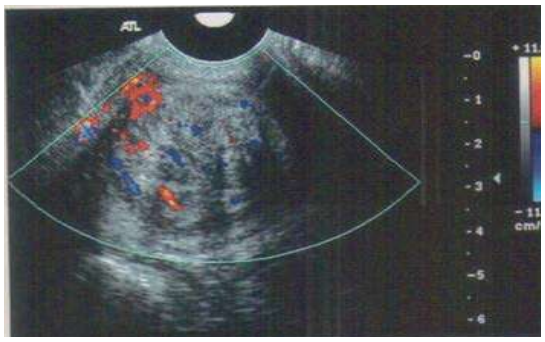
abnormal uterine bleeding in both premenopausal and postmenopausal women.

Leiomyoma

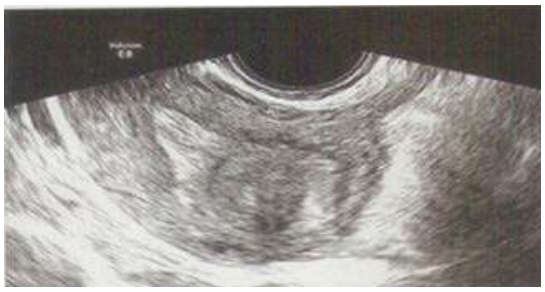
Diagnostic Features On USG

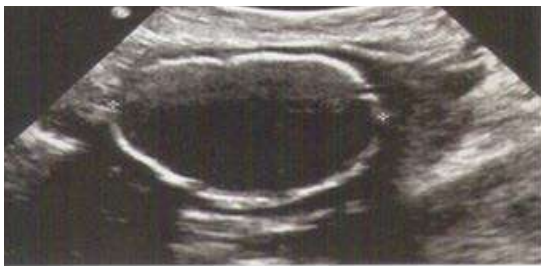
- Heterogenously enlarged uterus with lobular contour.
- Typically focal, well defined, round, sharply marginated, hypoechoic lesion within the myometrium or attached to it, often showing shadows at the edge of the lesion and/or internal fan- shaped shadowing.
- Leiomyoma can be hypo, iso or hyperechoic but majority arehypoechoic. Small leiomyomas are usually homogenous whereas those larger than 3 cm in diameter are often heterogenous.
- Surrounding myometrium can become compressed and form a pseudocapsule.
- Edge refraction at the interface of the leiomyoma with the normal surrounding myometrium may help to identify an isoechoic leiomyoma.
- The Venetian blind artifact (shadows) - a sonographic finding typically associated with adenomyosis can also occur in uterine fibroids. The posterior shadowing may be dense or striated (comb-like). This is believed to be caused by the transitional zone between apposed tissues of different acoustic properties such as fibrous tissue and smooth muscle.
- Degeneration may result in edema with cystic spaces, echogenic hemorrhagic areas, and dystrophic calcification. The calcifications can be curvilinear and peripheral or clump like and will demonstrate dense posterior shadowing.
- When one encounters a hyperechoic leiomyoma, lipoleiomyoma should be considered.

In order to assess the vascularization of the fibroids, color Doppler is used.



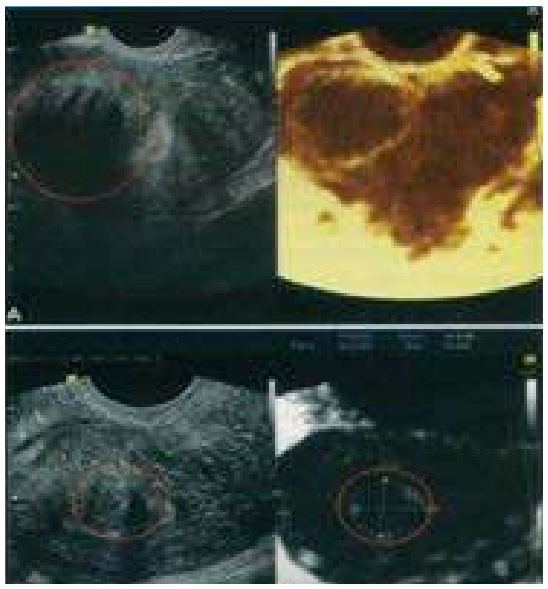
Colour Doppler scan of an intramural fibroid





Transvaginal USG image of a fibroid with discrete posterior shadowing. Using native scan it is impossible to differentiate between submucosal and intracavitatory fibroid.

Trans-vaginal color Doppler: -demonstrates vascularization on the periphery of the leiomyoma of uterine origin with the RI of 0.54±0.08. Uterine arteries present lower impedence to blood flow in patients with myomas compared to normal. Endometrial and subendometrial blood flow measured by 3D power Doppler USGin patient with small intramural uterine fibroid during IVF can be a predictor of treatment success. Saline Infusion Sonography (SIS) by 3D USG is valuable in obtaining submucosal fibroid.



Transvaginal USG of a calcified fibroid

Elastography: it is a non invasive method in which stiffness or strain images of soft tissues are used to detect or classify tumors. Real time elastography produces an instantaneous color map that precisely delineates the fibroids, thus overcoming the limitations of conventional ultrasound.

FIBROID ELASTOSCANS

Adenomyosis

It may be useful to categorize USG findings into these groups that mirror the histological findings:

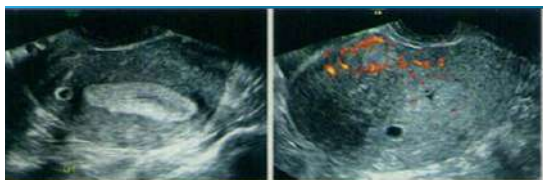
"Adeno": ectopic endometrial glands

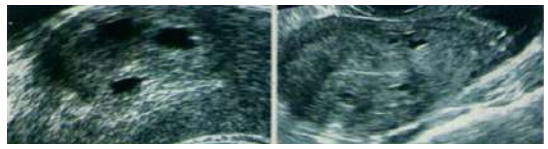
> Subendometrial echogenic linear striations and/or nodules, extending from endometrium and into inner myometrium.
> Hypoechoic islands (venetian blind and rain shower appearance).
> Irregular endometrial- myometrial junction.
> Tiny (1-5 mm) subendometrial cysts reflecting glands filled with fluids.
> Cystic striations.

"Myosis": muscular hyperplasia +/- hypertrophy

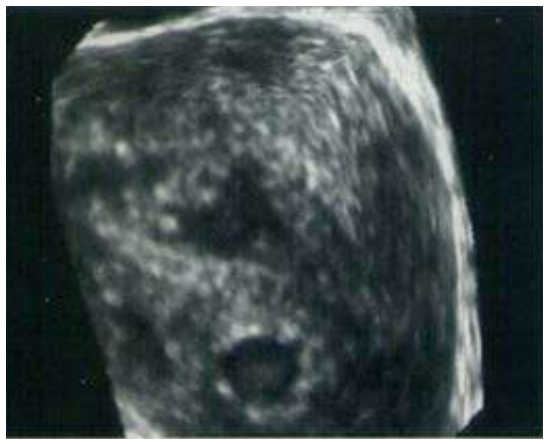
> Focal or diffuse myometrial bulkiness which may be asymmetric typically of fundal region and posterior wall
> Focal lesions have relatively indistinct borders
> Asymmetrical myometrial thickening
> Thickening of the transition one can sometimes be visualized as a hypoechoic halo surrounding surrounding the endometrial layer of >= 12 mm thickness
> Swiss cheese appearance due to area of fracture.

Vascularity: Flow on color Doppler is generally increased with increased number of tortuous vessels penetrating myometriumBecause of its USG image (myometrial heterogenecity and subendometrial echogenoic nodular and linear striation gives an appearance similar to chronic liver parenchymal disease. Hence, also known as "cirrhosis of the uterus".

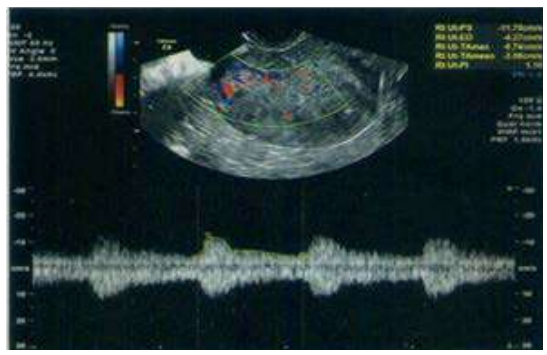




Transvaginal sonography of adenomyosis. Solitary focus of adenomyosis localized close to endometrium in upper left image, while multiple cystic structures within all three layers of the myometrium typical of severe adenomyosis



"Swiss cheese" appearance of the myometrium on 3D USG id typical of deep adenomyosis. Cystic lesions are visualized within the myometrial layer of uterus.



Moderate to high impedence blood flow signals are detected at the periphery of adenomyotic lesions.

Mullerian Duct Anomalies

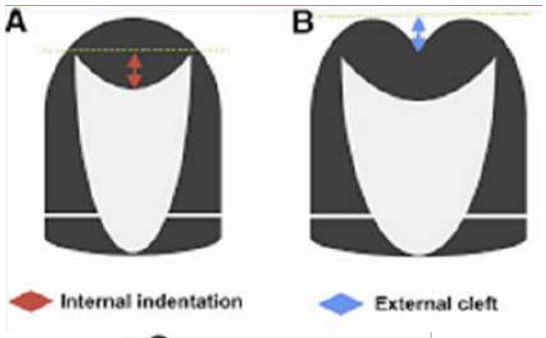
¬The prevalence of congenital anomalies of the reproductive tract is estimated to be as high as 7% in the female population. The fused caudal end of two mullerian duct (paramesonephric) form uterus, cervix and upper two thirds of vagina. Arrested development of mullerian ducts and/ or failure of fusion or resorption of median septum results in various forms of mullerian duct anomalies.

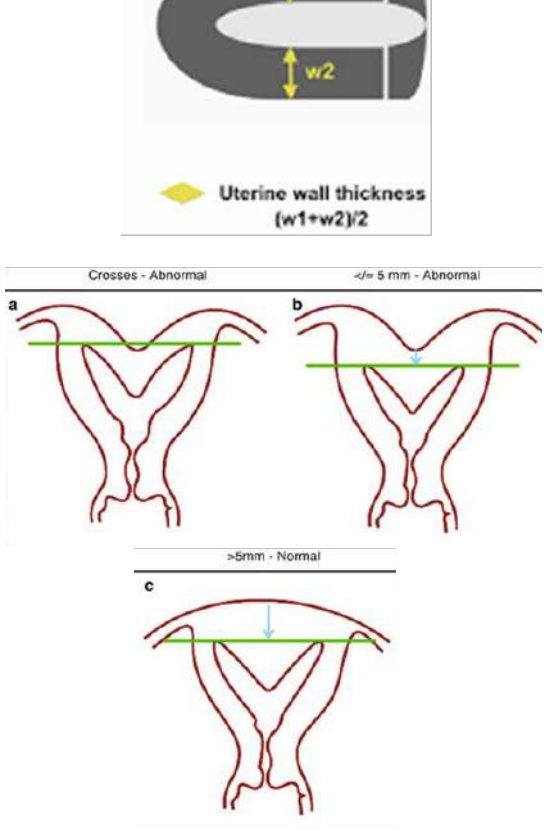
Three-dimensional (3D) techniques, USG may provide diagnostic accuracy similar to MRI. Imaging uterus in coronal plane provides information about fundus which is vital in characterizing various sub types of abnormalities. Better to carry it on during **secretory phase** when endometrium is thick.

The coronal view of uterus is important for many mullerian anomalies particularly septate uterus. Using the 3-D TVS reconstructed coronal view of the uterus, one should evaluate the external uterine fundal contour. If fundal myometrium is outwardly convex or has an inwardly concave indentation of less than 1 cm, the distinction narrows to septate verses arcuate uterus. Suggested criteria for diagnosis of an arcuate uterus are an angle greater than 90 degrees at the end point of the fundal indentation or less than 1cm indentation of myometrium into endometrium. If indentation is greater than 1 cm, then distinguish between bicornuate uterus and uterus didelphys. In bicornuate uterus, the two endometrial cavities join at the same point usually just above the cervix. There can be either one cervix (bicornis unicollis) or two cervices (bicornis bicollis). In uterus didelphys, there are two separate uterine horns and two cervices. Arrested development of one mullerian duct

results in unicornuate uterus that is easily diagnosed by 3 D ultrasound as banana shaped configuration.

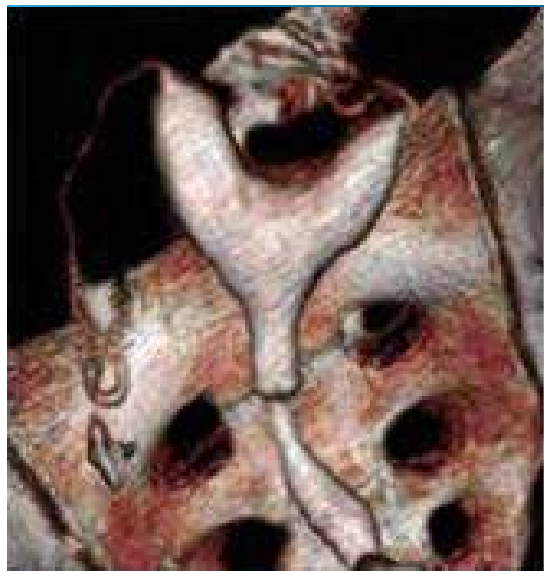
Characterstics	Bicornuate	Septate/ Subseptate	Arcuate
External countour	Concave	Flat/ Convex	Flat and broad/ Convex
External Fundal Cleft	>1 cm	Absent/<1cm	Absent/ <1cm
Intercornual angle	>105 0	>750	>900
Intercornual Distance	>4cm	<4cm	Not applicable
Medial Endometrial Shape	Convex	Flat/Acute	Flat/ obtuse





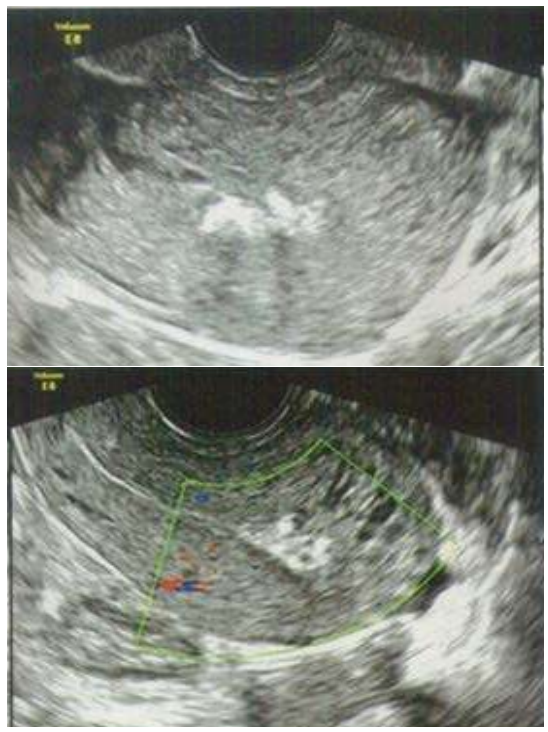
In cases of uterine hypoplasia, one may not be able to perform TVS and so MRI may be needed.

Virtual Hysterosalpingography: - Recently virtual HSG has appeared. The technique consists of a traditional HSG obtained by using a multidetector CT technology and making millimeter cuts of the area of interest. After doing this, all the information is processed by a software that performs three-dimensional virtual reconstructions. Besides showing uterine cavity and fallopian tubes contours, it also allows to see intrauterine cavity and intrauterine tubes as if a hysteroscopy has been done. Virtual HSG produces low radiation exposure and it is done in short time. It has been applied in the diagnosis of these malformations, particularly in the differential diagnosis of bicornuate uterus and subseptate one.



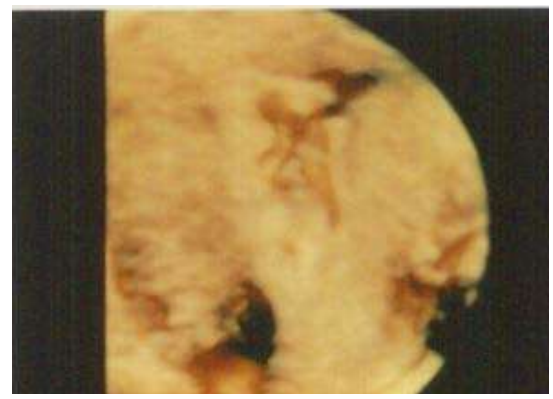
Intrauterine Adhesions

The sonographic diagnosis is difficult unless fluid is distending the endometrial cavity. The endometrium usually appears normal on trans-abdominal and trans-vaginal sonograms. Infection with tuberculosis may also cause uterine adhesions.



Irregular hyperechogenic bridges visualized within the central part of the uterine cavity in a patient with secondary amenorrhoea following dilatation and curettage. Intrauterine adhesions do not display increased vascularity on color Doppler examination.

> A mixed picture of uterine endometrium is seen. No endometrium is seen in some part whereas normal endometrium in other part.
>Integrity of the endometrial layer can be assessed including disruptions to the endometrial- myometrial junction.
>Adhesions may be seen trans-vaginally as irregularities or a hypoechoic bridge like band within the endometrium.
>Adhesions are seen as bands of myometrial tissue traversing the endometrial cavity and adjoining the opposing uterine walls.
>Mild adhesions appear typically as mobile, thin echogenic bands bridging a normally distensible endometrial cavity with pockets of fluid trapped between them.
>As severity of adhesions increases they appear as thick, broad bridging bands.
>Intrauterine adhesions do not display increased vascularity on colour Doppler.



3D ultrasound acquired HD live rendered coronal plane image of the endometrial cavity shows a thick echogenic fibrotic band (intrauterine synechia) extending from fundus downwards

They are better visualized during mensturation when intracavitatory fluid outlines them or following sonography.

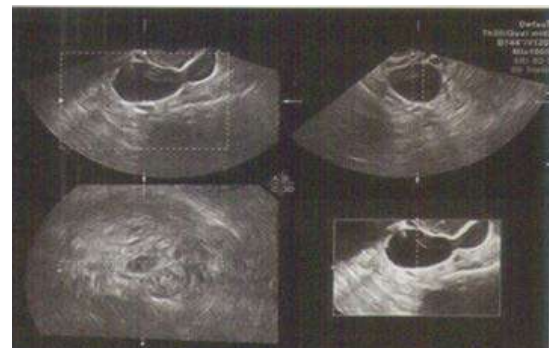
SIS with 3-D USG gives more accurate information for preoperative assessment and distinguishing pathologies. Use of echogenic contrast media will further increase the accuracy. Surface anatomy can confirm their presence, appearance, actual size, volume and relationship to surrounding structures.

Abnormalities Of Fallopian Tubes

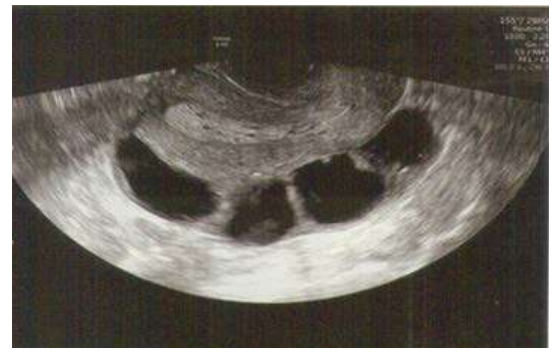
With careful TVS examination the normal fallopian tube is an undulating echogenic structure approximately8 to 10 mm in width, running posterolaterally from the uterus to lie within the posterior cul-de-sac near the ovary. The lumen is not seen unless it is fluid filled.

Fallopian tube pathology is discerned by evaluating the wall of the tube, the luminal content, the tubal motility, as well as its relation with the surrounding pelvic structures.

Anechoic fluid within the tube indicates hydrosalpinx.



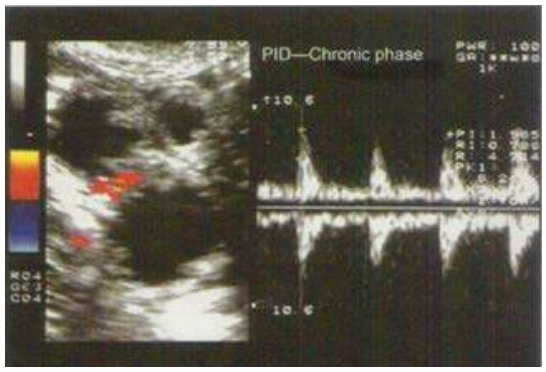
Multiplanar imaging of a thick walled elongated tubular fluid- filled structure, distinct from the uterus and ovaries



B- mode image of hydrosalpinx obtained by 2D ultrasound imitating a multilocular cyst

A thickened tubal wall (≥5 mm) is indicative of acute disease. In assessing 14 acute and 60 chronic cases of PID, Timor-Tritsch et al. described three appearances of tubal wall structure

> Cogwheel sign, an anechoic "cogwheel-shaped" structure visible in the cross section of the tube with thick walls, seen mainly in acute disease;



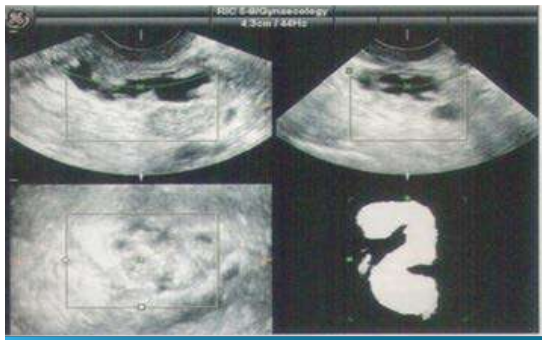
Cogwheel sign produced by hyperechoic knots and pseudopapillomatous structures is typical of chronic phase of PID. Color doppler helps to differentiate suspicious morphology.

> "beads on a string" sign, hyperechoic mural nodules measuring 2 to 3 mm on cross section of the fluid-filled distended tube, caused by degenerated and flattened endosalpingeal fold remnants and seen only in chronic disease; and

> Incomplete septa, hyperechoic septa that originate as a triangular protrusion from one of the walls, but do not reach the opposite wall, seen frequently in both acute and chronic disease and not discriminatory.

Patel et al. found that the presence of a tubular fluid filled mass with diametrically opposed indentations in the wall ("waist sign") had the highest likelihood ratio in discriminating hydrosalpinx from other adnexal masses. Sonograpic findings of fallopian tubes are the most specific and conspicuous indicators of PID.

Colour Doppler can depict movement of the liquid component when tubal content moves and changes the position compressed by vaginal probe. When colour is turned on, hydrosalpinx remains black and white, with specs of colour only on deliberate probe movement.



When 3D USG is applied, true, spatial position and shape of hydrosalpinx is clearly visible. By using 3D volume sections it is possible to visualize the torturous structure and contiguous spread of hydrosalpinx.

Endometrioma

Endometriomas have a variety of appearances, from an anechoic cyst to a solid-appearing mass caused by the degradation of blood products over time.

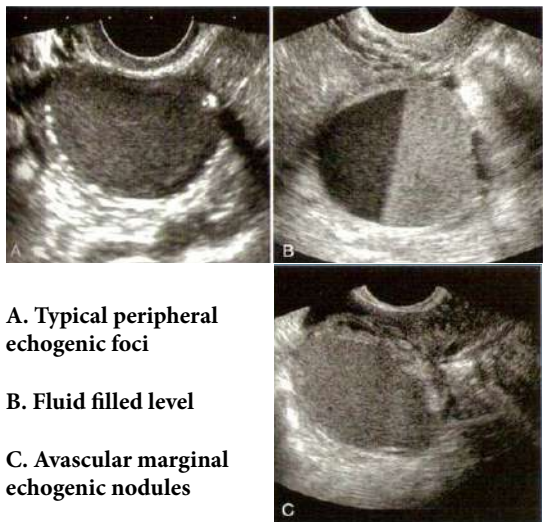
> The characteristic sonographic appearance is that of a well-defined, unilocular or multilocular, predominantly cystic mass containing diffuse, homogeneous, low-level internal echoes (ground glass appearance).

> The low-level internal echoes may be seen diffusely throughout the mass or in the dependent portion.

> Occasionally, a fluid-fluid level may be seen.

> Small, linear, hyperechoic foci may be present in the wall of the cyst, likely caused by cholesterol deposits accumulating in the cyst wall.

> On Doppler USG, colour score between 1 and 3 (i.e. no vascularization to moderate vascularization) with no flow inside the papillary projection.



A. Typical peripheral echogenic foci

B. Fluid filled level

C. Avascular marginal echogenic nodules

The appearance of an endometrioma may be similar to a hemorrhagic ovarian cyst because both are cystic masses that contain blood of variable age. However, a hemorrhagic cyst more frequently demonstrates a reticular internal pattern and is more frequently associated with free fluid in the cul-de-sac. A hemorrhagic cyst will resolve or show a significant decrease in size over the next few menstrual cycles, whereas endometriomas tend to show little change in size and internal echo pattern. Calcification is occasionally present in an endometrioma and misdiagnosed as a dermoid.

Ultrasound, is the preferred imaging modality in the study of the female pelvis, and provides information of basic importance in detecting and characterizing pelvic masses of uterine, ovarian, or adnexal origin, providing also criteria useful in predicting their benign vs malignant nature **Its use has decreased the need for more invasive procedures in women and allowed significant advances in there management.**

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Diagnosing Hyperandrogenism and Hyperinsulinemia

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Diagnosis of Hyperandrogenism

Hyperandrogenismis a medical condition characterized by high levels of androgens in females. It is established that androgens incoming from both the ovary and the adrenal are the underlying sources of hyperandrogenemia in PCOSwomen Thecause in about 70% of cases is polycystic ovary syndrome (PCOS). Othercauses include adrenal hyperplasia, Cushing's disease, certain types of cancers, and certain medications.

Hyperandrogenismis most often diagnosed by checking for signs of hirsutism according to a standardized method that scores the range of excess hair growth.

- Checkingmedical history and a physical examination of symptoms are used for an initial diagnosis.
- Patienthistory assessed includes age at thelarche, adrenarche, and menarche; patterns of menstruation; obesity; reproductive history; and the start and advancement of hyperandrogenism symptoms.
- Patternsof menstruation are examined since irregular patterns may appear withhirsutism.
- A laboratory test can also be done on the patient to evaluate levels ofFSH, LH, DHEAS, prolactin, 17OHP, and total and free testosterone in the patient's blood. Abnormally high levels of any of these hormones help in diagnosing hyperandrogenism.

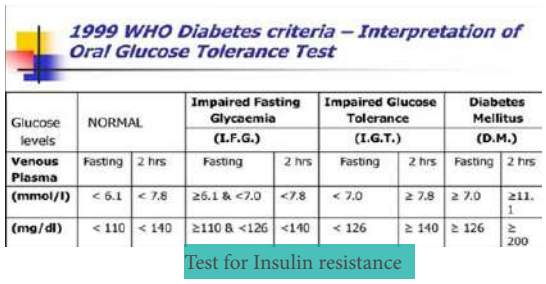
Diagnosis of Hyperinsulinemia,

Hyperinsulinemia, is a condition in which there are excess levels of insulin circulating in the blood relative to the level of glucose. While it is often mistaken for diabetes or hyperglycaemia, hyperinsulinemia can result from a variety of metabolic diseasesand conditions

1. The cut off value of HBA1C>6, in high risk group of infertility patient – like PCOS, obese, advancematernal age or known case of DM
2. ORAL GTT – different organization recommend 75 to 100 g loaded dose of glucose followed by 1 hr, ,2 hr, 3 hr –glucose estimation and following table suggesting the cut off value to be considered as pre diabetic or diabetic condition.

This test should be recommended to infertile couple where RBS / average blood sugar > 140 mg/dl (HBA1C – 5.8) or FBS > 100 mg/dl

	Plasma glucose concentration (mg/dl)				
rganization	OGTT glucose load	Fasting	1 hour	2 hour	3 hour
ADA*	100g	95	180	155	140
ACOG*	100g	105	190	165	145
NCE[9]	75g	100.8		140	
IADPSG[8]	75g	92	189	153	
DIPSI[8]	75g			140	



Homa 2 calculator

The Homeostasis Model Assessment (HOMA) estimates steady state beta cell function (%B) and insulin sensitivity (%S), as percentages of a normal reference population. These measures correspond well, but are not necessarily equivalent, to non-steady state estimates of beta cell function and insulin sensitivity derived from stimulatory models such as the hyperinsulinaemic clamp, the hyperglycaemic clamp, the intravenous glucose tolerance test (acute insulin response, minimal model), and the oral glucose tolerance.

- Thenormal HOMA–IR value of healthy human ranges from 0.5–1.4
- <1.0meansyouareinsulin–sensitivewhichisoptimal
- >1.9indicatesearlyinsulinresistance

In 2004, the HOMA2 Calculator was released. This provides quick and easy access to the HOMA2 model for researchers who wish to use model-derived estimates of %B and %S, rather than linear approximations.

Hysterosalpingography And Saline-Infusion-Sonography

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The problem of infertility (subfertility) is increasing day by day and according to WHO reports [1] approximately 10% of women are suffering from infertility globally. Etiology of infertility is multifactorial, and fallopian tube abnormality being one of the most important causes accounts for up to 40% of cases.[2,3] Hence, screening for tubal occlusion is one of the first important steps in fertility assessment of subfertile couples. Till date, HSG has been the preferred choice of investigation followed by laparoscopy with chromotubation.[4]

Hysterosalpingography (HSG)

HSG is basically the radiographic evaluation of uterine cavity and fallopian tubes by administering radio-opaque contrast medium (water or lipid soluble). It is a safe, relatively inexpensive, easily available, simple and rapid method of imaging. It is still the most commonly used diagnostic test to evaluate tubal patency and tubal integrity. By HSG, along with tubal patency and pathology, uterine pathologies like submucous fibroid, endometrial polyp, intrauterine synechiae, congenital malformations of uterus and cervical stenosis can also be detected. It can document proximal and distal tubal occlusion, demonstrate salpingitis isthmica nodosa, reveal tubal architectural details of potential prognostic value and suggest the presence of fimbrial phimosis or peritubular adhesions when escape of contrast is delayed or becomes loculated, respectively.

Drawbacks and limitations: Iodinated contrast and X-rays are used in HSG which are potentially hazardous. The procedure is painful and uncomfortable for the patient. Moreover, because of its low sensitivity, HSG is of limited use in detecting adhesions and mild to moderate endometriosis.[2]

Characteristics of normal HSG – Patency with free spill, preserved distal tubal folds, normal proximal, mid, distal tubal dimensions and appearance, no fimbrial end clumping, no detected peritubal disease, normal tubal pressures with free flow, lack of sharp pain on forceful flushing.

Characteristics of moderate/ severe tubal disease – Patent or blocked tubes, loss of distal tubal folds, altered proximal, mid, distal tubal dimensions and appearance with dilatation/narrowing/scarring/tubal rigidity, fimbrial end dilatation/narrowing with clumping present, peritubal disease may or may not be seen, usually elevated tubal pressures, but can be normal.

Hysterosalpingo-contrast-sonography (HYCOSY)

It is a transvaginal ultrasound technique in which a solution of galactose and 1% palmitic acid (Echovist) or a mixture of air and saline is infused into the uterine cavity and observed to flow along the fallopian tubes to assess tubal patency. The bright echoes generated by the Hysterosalpingo-contrast-sonography (HyCoSy) solution make tubal visualization possible on ultrasonography. Results can be further improved by the use of Color Doppler imaging or 3D/4D ultrasonography.

HSG Compared With Laparoscopy & Chromopertubation

HSG and laparoscopy with dye are the two most widely used methods to test for tubal pathology. For tubal evaluation, laparoscopy remains the gold standard, but in an era where cost-effectiveness becomes more important, it is debatable whether laparoscopy should always be a mandatory step in the subfertility work-up after HSG. HSG and laparoscopy are both invasive procedures but HSG is the first step in uncomplicated, young women. As compared to laparoscopy, HSG does not require anaesthesia and is faster and cheaper with no need to admit the patient.

A word of caution: Among women whose tubes were found to be patent using HSG, 18% were found to have tubal obstruction or peritubal adhesions using laparoscopy and a further 34% were found to have endometriosis and/or fibroids. However, the detection and treatment of pathology missed by HSG did not increase live birth rates.

HYCOSY Compared with Laparoscopy and Chromopertubationor HSG

Evaluative studies of HyCoSy showed good statistical comparability and concordance with HSG and laparoscopy combined with dye. HyCoSy is well tolerated and can be a suitable alternative outpatient procedure. HyCoSy using contrast agent Infoson® appears to be more efficient than saline solution in detecting tubal obstruction.

Saline Infusion Sonohysterography (SIS)

Saline infusion sonohysterography (SIS) or saline ultrasound uterine scan uses a small amount of saline inserted into the uterus that allows the endometrium to be clearly seen on an ultrasound scan. Saline infusion sonohysterography (SIS or SHG) is a procedure to evaluate the uterus and the shape of the uterine cavity using ultrasound and sterile fluid. The ovaries are also seen at the time of SHG. The purpose is to detect any abnormalities.

SHG can be done to investigate conditions such as abnormal uterine bleeding, infertility, and recurrent miscarriage. SHG can also be performed to see the structure of the uterus. This may be done in women with congenital abnormalities of the uterus, before and after surgery on the uterus, or to detect problems thatappear later in life such as polyps or suspected scar tissue inside the uterus. SHG may also help check uterine abnormalities found during a routine ultrasound.

Method of SIS

SIS is usually done postmenstrually. The procedure begins with a TVS and then a narrow catheter is placed through the cervix into the uterine cavity. The ultrasound examination is continued while sterile saline is put into the uterus. The saline solution fills the uterus, helping to outline the uterine walls and cavity. This shows abnormalities such as fibroids, polyps, or scar tissue inside the uterus.

Results

SIS had sensitivity of 91%, specificity of 76% positive predictive value of 95%, negative predictive value of 66%, and an accuracy of 89% in evaluating tubal patency. Further, SIS showed sensitivity of 83.3%, specificity of 60%, PPV of 75%, NPV of 75%, and accuracy of 72% in detecting pelvic pathology.

In a low-resource country like India with a huge burden of subfertile women population, SIS can prove to be a useful tool in the initial workup of infertility patients with better compliance, low cost, and better results in a single visit.

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Diagnostic Hysteroscopy In Infertility

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Hysteroscopy is a commonly performed procedure to diagnose and treat the pathologies in the uterine cavity. The initial work-up of the infertile couple include investigations for general well being, endocrine profile, documentation of ovulation, tubal patency, as well as semen analysis. Transvaginal sonography (TVS), hysterosalpingography (HSG) and saline infusion sonography (SIS) are the first line investigations widely used to assess uterine cavity and its pathologies. Hysteroscopy, since it enables direct visualization of the uterine cavity and its relevant pathological disorders as well as the treatment of any detected abnormality at the same time, is the gold standard technique for uterine factor evaluation. There is no doubt in benefits of operative hysteroscopy in case of known intrauterine pathologies e.g. Intrauterine adhesion, endometrial polyp, leiomyomas bulging into uterine cavity, infertility associated with abnormal uterine bleeding ,abnormal endometrial thickening, suspicion of endometrial hyperplasia or malignancy(1). Other widely accepted indications for hysteroscopy in infertile women are suspicion of congenital anomaly like uterine septum, unicornuate/ bicornuate uterus or uterus didelphys. Second-look for hysteroscopy post septal incision for septate uterus,and adhesiolysis are also some of the other indications.

But the use of hysteroscopy as a routine procedure in the infertility work-up is still under debate and there is no consensus on its efficacy and role in improving the prognosis of infertile couples without any obvious uterine factor directly contributing to infertility. It is still considered a second-line procedure in women with unexplained infertility. This is mainly related to its invasiveness and cost. Similarly in the group of infertile women with multiple unsuccessful IUI and failed one or two IVF attempts, the role of diagnostic hysteroscopy is also a subject of debate (2).

A systematic review and meta-analysis of 9 studies with a total of 2976 participants, was published in 2016 to assess the efficacy of hysteroscopy in improving reproductive outcomes of infertile couples (3).Three studies comparing LBR between hysteroscopy versus no hysteroscopy in the routine infertility work-up with 1088 participants found a significantly higher LBR in the hysteroscopy group (RR1.48, 95% CI 1.20–1.81, I 2= 0%, P = 0.82). One study compared the outcome of IVF for women with previous implantation failure and women undergoing the first IVF/ICSI attempt. In the subgroup analysis of 972 women with implantation failure (one or more) after IVF/ICSI authors found a higher LBR (RR 1.48, 95% CI 1.19–1.85,I 2 = 0%, P = 0.64), but not in the subgroup of 116 participants where hysteroscopy was performed before the first IVF/ICSI attempt (RR 1.44, 95% CI 0.83–2.48). Quality of evidence was judged as very low (3).
PR was assessed by seven studies. Only a moderate quality of evidence of a beneficial effect of hysteroscopy for women experiencing one or more implantation failures after IVF/ICSI and also for women undergoing their first IVF/ICSI was found (RR 1.45, 95% CI 1.26–1.67, I 2 = 38%, P= 0.12). (3) There could be two reasons to explain such results. First, hysteroscopy may reveal an unsuspected intrauterine abnormality in patients with a normal US, HSG or SIS which may potentially compromise the implantation rate after IVF/ICSI. The treatment of these 'hidden' abnormalities may have contributed to the improved reproductive outcome in patients with failed treatment of unexplained infertility. USG was reported to have 84.5% sensitivity, 98.7% specificity, 98% positive predictive value and 89.2% negative predictive value to detect the uterine and endometrial anomalies contributing to infertility (4). However, USG might not diagnose all the abnormalities of endometrium e.g submucosal fibroids in the presence of other multiple intramural fibroids or sometimes it may be difficult to differentiate between a large polyp from the hyperplasic endometrium (5). USG may also not differentiate between congenital uterine malformations. Therefore, it appears that there will be abnormalities in approximately one-third of the patients where the HSG and/ or USG is interpreted as normal, which may cause a false reassurance and will actually lead to failure of conception. Recent papers have reported that hysteroscopy allows the diagnosis of unsuspected intrauterine abnormalities in infertile women for IVF in almost 50% of cases (6)

Second reason for improved reproductive outcome after hysteroscopy could be irrigation of the cavity with saline may have a beneficial effect on implantation and PR, since saline mechanically removes harmful anti-adhesive glycoprotein molecules on the endometrial surface involved in endometrial receptivity. Furthermore, the hysteroscopic diagnostic act itself may allow easier embryo transfer in subsequent cycles due to lysis of cervical adhesions and dilatation of cervical canal(7). Indeed, mechanical manipulation of the endometrium may enhance receptivity by modulating the expression of gene encoding factors required for implantation, such as glycodelin A , laminin alpha-4, integrin alpha-6 and matrix metalloproteinase-1 (8).

Cochrane Database Systematic Review (9) on screening hysteroscopy in subfertile women and women undergoing assisted reproduction including 11 studies was published in April 2019. Authors evaluated ten trials that included 1836 women who had a screening hysteroscopy and 1914 women who had no hysteroscopy prior to IVF. They found that performing a screening hysteroscopy before IVF may increase LBR (RR 1.26, 95% CI 1.11 to 1.43; 6 RCTs; participants = 2745; I^2 = 69 %; low-quality evidence). For a typical clinic with a 22% LBR, performing a screening hysteroscopy would be expected to result in live birth rates between 25% and 32%. However, sensitivity analysis done by pooling results from trials at low risk of bias showed no increase in LBR following a screening hysteroscopy (RR 0.99, 95% CI 0.82 to 1.18; 2 RCTs; participants = 1452; I^2 = 0%). Performing a screening hysteroscopy before IVF may increase clinical pregnancy rate (RR 1.32, 95% CI 1.20 to 1.45; 10 RCTs; participants = 3750; I^2 = 49%; low-quality evidence). For a typical clinic with a 28% clinical pregnancy rate, performing a screening hysteroscopy would be expected to result in clinical pregnancy rates between 33% and 40%. There may be little or no difference in miscarriage rate following screening hysteroscopy (RR 1.01, 95% CI 0.67 to 1.50; 3 RCTs; Participants = 1669; I^2 = 0%; low-quality evidence). Authors found no trials that compared a screening hysteroscopy versus no hysteroscopy before IUI.

In summary the systematic reviews didn't find strong evidence about the role of hysteroscopy as a basic infertility evaluation tool. Robust and high-quality RCTs are still needed before hysteroscopy can be regarded as a first-line procedure in all infertile women, especially during the initial clinical assessment of a couple where it could reduce the time-to-pregnancy and the need for ART.

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Diagnostic Laparoscopy In Reproductive Medicine

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It has been more than 4 decades since the first IVF baby, reproductive medicine has witnessed advancements in not only investigations, imaging but also embryology procedures still there is small yet significant room for diagnostic laparoscopy in infertility.

Diagnostic laparoscopy once performed as a mandatory initial workup for all infertile woman as recommended by American Fertility Society in 1992 and by the World Health Organization guidelines (Rowe et al., 1993)[1], has taken a backseat with advances in non invasive techniques like HSG and trans vaginal ultrasonography and is only offered when either other tests are inconclusive or when there is a treatment failure. But some authors debate in its favour as it is the only tool that can accurately diagnose and correct tubal (adhesions, blocks) and pelvic pathologies like minimal to mild endometriosis in same sitting.

Need of laparoscopy: Pitfalls with other screening tests: the two main factors that require assessment apart from ovulation and semen analysis are Tubal patency and pelvic pathology.

Tubal Patency

It is usually tested by Hysterosalpingography or HyCoSY: It provides a morphological view of the uterine cavity, the Fallopian tubes and their patency. According to a meta-analysis, HSG has a reasonable specificity (83%) but a low sensitivity (65%) to document patency of the Fallopian tubes.[2] Also, it has been shown that HSG is insufficient for predicting tubal patency for some patients with risk of pelvic adhesions, with a sensitivity between 0.01% and 83% and specificity between 50% and 90%.[3] The prognostic significance of HSG and laparoscopy for fertility outcome was studied and published in a large prospective cohort study which showed that laparoscopy can be delayed after normal HSG for at least 10 months because of the very low probability of only 5% that bilateral tubal occlusion may be found.[4]

Pelvic pathologies

Ultrasound: A simple transvaginal 2D ultrasound is advised for evaluation of pelvic pathologies as initial work up. Endomterioma, leiomyomas, hydrosalpinges can be picked up with good sensitivity by TVS, however it is difficult to diagnose pelvic adhesions and minimal to mild endometriosis by TVS. At the same time diagnosing and treating such pathologies may not increase the pregnancy rate.

HSG can detect peritubal, specially distal adhesions, but the extent of tubal disease can only be seen by

laparoscopy.Therefore, role of laparoscopy, especially in women whose normal screening tests suggest that pelvic pathology seem to be unlikely.[5] However in following situations, laparoscopy may have a role

- Ovulation Induction: Before initiating ovulation induction there seems no role of laparoscopy if all screening tests are normal.
- There is Ovulation failure with oral ovulogens: Diagnostic laparoscopy can be considered in PCOS women drilling can be considered depending upon case based scenarios.[6]
- There has been Lutenised enraptured follicle: To diagnose and treat peri tubal adhesions usually in PID and endomteriosis.[7]

2. Intrauterine insemination
A. Before IUI: Diagnostic laparoscopy can be advised to couples with suspected minimal tubal abnormalities, unilateral tubal block with contralateral ovulation resulting in no pregnancy, Endometriomas with patent tubes to downstage the disease and improvise the chances of pregnancy with ovulation induction and IUI combined.[8]

B. After failed intrauterine insemination: To diagnose and treat mild endometriosis, peri tubal adhesions, pelvic adhesions in otherwise unexplained infertility couples, who have failed cycles of IUI, yet do not want or can't afford to go for in vitro fertilisation (IVF)

3. In-Vitro Fertilisation: Pre IVF tubal delinking in women with hydrosalpinges has been useful to improve success rates. Ultrasound usually can pick up hydrosalpinx, but when in doubt, in woman with history of IVF failure, diagnostic laparoscopy with hysteroscopy can be offered.

Advantages
A gold standard test to evaluate the pelvis, tubes and uterine cavity, simultaneously perform necessary corrective procedures in day care admission setting, diagnostic laparoscopy is the most informative test beyond doubt.

Disadvantages
It is invasive, skill dependent, costly procedure with risk of surgical and anaesthetic complications. The utility of the information and its effect on success rate of infertility treatment is doubtful. A cost benefit analysis of IVF versus diagnostic laparoscopy favours IVF.

Pre-procedure counseling: Should include
1. Detailed explanation of the procedure to be performed.
2. Intended benefits
3. Likelihood of finding a pathology and improvement in pregnancy rates with corrective procedure.
4. Alternative treatment options
5. Cost- benefit analysis compared to ART

Conclusion
Diagnostic laparoscopy is no more a test to which all infertile women should be subjected to, for screening tubal or pelvic factors causing infertility. Rather it should be offered to women with suspected disease for confirmation and treatment or in otherwise unexplained cases where ART has failed.

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Endometrial Biopsy – As A Diagnostic Modality In Infertility

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Endometrium is the mirror of hypothalamus, pituitary and ovarian function. It is the soil for fertilised ovum to be implanted. Almost all functional disturbances involved in infertility result in morphological changes in the endometrium since hormone level fluctuate depending upon various biorhythms, the histological examination of endometrial biopsy is the most reliable parameter for evaluating the cause of infertility and thus endometrial biopsy is one of the cardinal investigations in infertility. Endometrial biopsy is an office procedure that serves as a helpful tool in diagnosing various other uterine abnormalities also.

Indications for Endometrial Biopsy
- Abnormal uterine bleeding
- Postmenopausal bleeding
- Cancer screening (e.g., hereditary nonpolyposiscolorectal cancer)
- Detection of precancerous hyperplasia and atypiaEndometrial dating
- Follow-up of previously diagnosed endometrialhyperplasia
- Evaluation of uterine response to hormone therapy
- Evaluation of patient with one year of amenorrhea
- Evaluation of infertility
- Abnormal Papanicolaou smear with atypical cells favoring endometrial origin.

Contraindications & Relative Contraindications for Endometrial Biopsy
Contraindications
-Pregnancy
-Acute pelvic inflammatory disease
-Clotting disorders (coagulopathy)
-Acute cervical or vaginal infections
-Cervical cancer

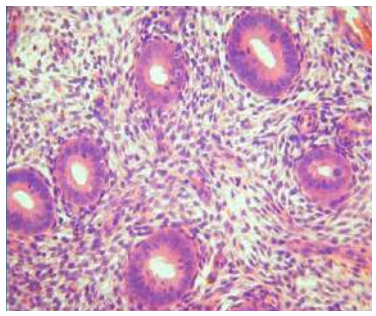
Relative Contraindications
-Morbid obesity
-Severe cervical stenosis

Endomerial biopsy a simple and convenient procedure gives important information regarding :
- It documents the secretory endometrium which is indirectly evidence that ovulation has occurred.
- To evaluate whether the maturity of the secretory endometrium is in phase (i.e consistent with menstrual cycle date) or out of phase (i.e luteal phase defect).
- As an adjunct to the monitoring of the efficacy of treatment for ovutatory failure and in the confirmation and typing of endometrial hyperplasia in women with persistent anovulatory cycle.
- For the diagnosis of genital tuberculosis and as a means of culturing the mycobacterium.

Procedure Pitfalls/Complications
- Inability of catheter to pass through cervix
- Cramping associated with the procedure.
- Infection may occur following the procedure.
- The pathologist reports that the specimens have insufficient sample for diagnosis.
- The Tenaculum causes discomfort when applied to the cervix.

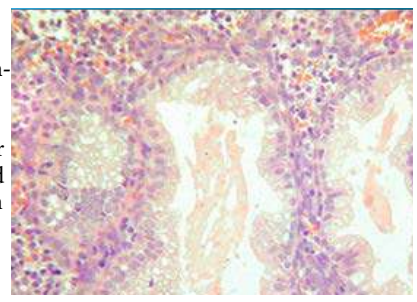
Infertility
Female infertility may occur due to disturbance involving any part or parts of genital system or due to the involvement of the central nervous system that control the ovaries hormonally.Endometrial biopsy in infertility is not only the simplest , quickest , cheapest and useful method of determining the occurrence of ovulation , also yields valuable supplementary information about the utero-ovarian endocrine relation. The biopsy should be taken within 2 to 3 days of the expected menses, to allow full endometrial development; the tissue then reflects the entire progesterone output in that cycle, and is a bioassay of progesterone output.

Female infertility can be categorised into who fail to ovulate (anovulatory infertility) because of some defect at hypothalamic-pituitary-ovarian axis and those who ovulate (ovulatory infertility), but are infertile because of some lesion present in genital tract. The significance of detection of ovulation is therefore immense.Histological study of endometrium can be an effective screening test in infertility if done in premenstrual phase . Hormonal disturbances if present in the patients are reflected in the endometrium in the form of anovulatory cycle, inadequate proliferative /secretary phase, endometrium.



Proliferative endometrium - Widely spaced tubular-glands which exhibit mitotic activity

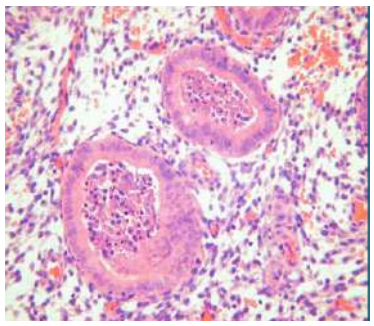
Secretory ndometrium- Glands contain supranuclear vacuoles and secretions in the lumen



In clinical practice, the luteal phase defect(LPD) has been associated with infertility, recurrent miscarriage, and occult miscarriage. The defect occurs in approximately 3% of the infertile population, but women with certain clinical entities seem to have a higher incidence of luteal phase inadequacy. Although controversy surrounds the method of diagnosis, the endometrial biopsy has been a reproducible and adequate means of providing histologic evidence of normal endometrial development and bioassay evidence of adequate progesterone output. A single progesterone measurement provides little information about luteal adequacy, and serial or even frequent plasma progesterone levels are difficult to justify in patients in whom only a 3% incidence of a defect can be documented.

Endometritis
Endometritis is a known cause of abnormal uterine bleeding, recurrent abortions and infertility. It is a subtle condition and difficult to diagnose. The diagnosis

is ultimately based on presence of plasma cells in the endometrial stroma on histopathological examination after taking endometrial biopsy. Agreeing a precise histopathological definition of endometritis is difficult since different features are seen – the inflammatory infiltrate may be confined to the surface epithelium or spread deeply into stroma, inflammatory infiltrate may comprise of neutrophil and plasma cells, lymphoid aggregates or subepithelial haemorrhage have also been reported.



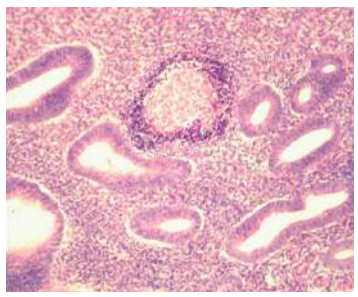
Acute endometritis - Neutrophils are present, sometimes forming microabscesses within glandular lumina

Genital tuberculosis

Genital tuberculosis is one of the major causes for severe tubal disease leading to infertility. Its magnitude is underreported because the diagnosis is difficult and require invasive techniques.

Unlike pulmonary tuberculosis, clinical diagnosis of genital tuberculosis is difficult because in majority of cases the disease is either asymptomatic or has varied clinical presentation. Mycobacterium tuberculosis is the etiological agent for tuberculosis . Fallopian tubes are involved in 90-100% cases, endometrium in 50-80%, ovaries in 20-30%. Tuberculosis of vulva and vagina is rare .In addition to the subtle presentation of the disease, the low sensitivity and specificity of routine diagnostic methods and the paucity of organism in clinical samples are the main factors for lower detection rate of genital TB. The diagnosis is made by detection of acid fast bacilli on microscopy or culture on endometrial biopsy or histopathological detection of epitheloid granuloma on biopsy. Polymerase chain reaction may be false positive and alone is not sufficient to make the diagnosis Laparoscopy and hysteroscopy can diagnose genital tuberculosis by various finding. The diagnostic dilemma arises due to varied clinical presentation.

Granulomatous endometritis-. Single granuloma is presentwithin the endometrial stroma



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Basic Semen Analysis

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Its one of the most basic laboratory test for clinical assessment of infertile couple. Semen evaluation provide information about sperm production by testis, patency and function of male reproductive tract, as well as activity of accessory gland and capability of ejaculation, thus helps in proper categorization of semen sample, biochemical assay and immunological assay.

Normal semen is a mixture of spermatozoa suspended in secretion from the testis and epidydmis, which at the time of ejaculation are combined with secretion with prostate, seminal vesicles and bulbo-urethral gland,the final composition is a viscous fluid that compromises of ejaculate.

- 60% of semen volume is derived from seminal vesicle with is a major source of fructose.
- 20% of volume of semen is contributed by prostate gland (milky in appearance and also rich in proteolytic enzyme)
- 10-15% of semen volume is contributed by epidydmis,vasdeferens, cooper's gland and urethral gland.
- Less than 5% of semen volumeis contributed by spermatozoa.

Sample collection

Ideally the sample should be collected in a private room near the laboratory, obtained by masturbation and ejaculated in a clean,wide mouth plastic disposable container, history is to be taken of fever, drug abuse, alcohol intake.

Assessment of semen according to WHO criterion is done

Standard Test

1. Volume
2. pH
3. Sperm concentration
4. Total Sperm Count
5. Motility
6. Morphology
7. Vitality
8. White blood cells
9. Immunological test
10. MAR test

Macroscopic Examination

Semen sample should be stored at room temperature (24-270C),if not possible at 370C in incubator.

Volume- Itis measured by transferring the entire content to conical bottom test tube. Normal volume is 2-5ml/ejaculate.WHO regards 1.5ml as the lower reference limit.[1]

Low Volume (<1.5ml)
- Incomplete collection
- Bilateral congenital vasal aplasia
- Bilateral Ejaculatory duct obstruction

High volume (>10ml)
- Dilution oligozoospermia

Aspermia
- Absence of ejaculate
- Retrograde ejaculate

Colour

Semen normally is grayish white opalescent, it tends to get a yellowish tint as a man ages.
- Translucent color is associated with low or absent sperm count
- Deep yellow indicates pyospermia
- pink or red dioscoluration indicates blood
- Yellow after taking high sulpher(garlic).
- Brown semen is a result of infection / inflammation of prostate gland.
- Any abnormal smell of purification or urine should be noted

Viscosity(Assessing liquefication)
- After ejaculation semen sample is coagulated and needs to be liquefied.Semen normally liquefies between 20 min from thick gel to liquid, NICE guidelines has considered 60 min within normal range.[2]
- When semen sample is very viscous it may indicate a prostatic dysfunction(prostatic enzyme).
- To liquefy chromotrypsin, bromelin or plasmin may be added.

PH

pHmeasured by using Ph meter or PH paper
- WHO criteria specify normal as 7.2-7.8, semen is the strongest buffer of body[3]
- Seminal vesicle and vas deferens secretion is alkaline
- Prostatic secretion is acidic due to citric acid,proteolytic enzyme
- Acidic ejaculate indicate blockage of one or both seminal vesicles
- Basic ph may indicate infection.

Fructose level

Fructose level in semen may be analyzed to determine the amount of energy available to the semen for moving.
- WHO specifies a normal level of 13 micro mol/sample.
- Absence of fructose may indicate a problem with seminal vesicle.

Testing for Fructose: Pipette 5ml of resorcinol reagent in a test tube.0.5ml of semen is added, mixed and placed in boiling water bath for 5 min, red color ppt. in 30 sec indicate presence.

Microscopic Examination

Sperm count

Sperm count is the concentration of sperm in man's ejaculate. Total sperm count is the sperm count multiplied with volume. According to WHO 2010, 15 million sperm per milliliter is considered normal. Oligospermia less than 15 milliom/ ml

Causes
- Mumps orchitis
- Prostatis
- Hypopituratism
- Hypogonadotrpic hypogonadism
- Estrogen producing tumors
- drugs

Prepare a wet preparation for assessing microscopic appearance and sperm motility. Dilution required for assessing number

Motility

WHO has a value of 40% and this must be measured within 60 min. of collection. The progressive motility value should be over 32% or it might indicate Asthenozoopermia a more specified measure is motility grade, where the motility of sperm are divided into four different grades—
- Grade A –Rapid progressive motility (grade 4)
- Grade B-slow sluggish progressive motility, travel in a curved /crooked motion (grade 3)
- Grade C- Non progressive motility do not move forward.(grade2)
- Grade d immotile (grade 1)

Asthenozoospermia may be due to-
- Cold
- Radiation

- Spermicide pesticide
- Prolonged heat exposure
- Prolonged abstinence
- autoimmunity

Sperm morphology
Assessment of morphological character is important for complete evaluation of semen sample. For that, air dried smear is made from fresh semen sample and they are fixed and stained with suitable stainslike Papanicolaou, Giemsa, Leishmann.WHO –sample more than 4% (5 percentile) is considered as normal

According to WHO
- normal sperm head is considered to be 3-5 micron in length
- 2-3 micron in width with perfect oval shape
- mid piece is about 1 micron in diameter with straight and regular outline, it must be aligned to longitudinal axis of head and should be 7-8 micron in length.
- The tail must be slender, uncoiled and at least 45 micron in length.
- Any sperm not meeting these criteria is considered abnormal.Percentage lower than 4% indicate teratozoospermia.

Normal stain of sperm takes-
- Sperm head cap-light blue
- Nuclear post—dark blue
- Body & tail-red pink

Total motile sperm count- is the combination of sperm count , motility and volume , measuring how many million sperm in an entire ejaculate are motile.

Sperm Viability (Sperm Membrane Function)
If motility is < 40% viability it should be performed. The sperm membrane structure and function can be determined by evaluating sperm vitalityand hypo-osmotic swelling test.

Sperm Vitality test- Sperm with intact membrane do not allow eosin stain to pass into sperm head and they appear white against dark background. When there is defect in the membrane they allow eosin to leak and they appear pink.

HOS test (Hypo-osmotic swelling test);-when the sperm are exposed to hypo-osmotic solution , the sperm with intact membrane result in imbibing water which leads to coiling of tail. Dead sperm have no coiling of tail. According to WHO vitality should must be more than 55%.
MAR (mixed antigloublin reaction)- Antisperm Antibodies test – The number of spermatozoa with adherent particles or cell is reflected. More then 50% spermatozoa clustered together suggests an immunological problem.Antibody are found to react with front of acrosome, post nuclear cap, tail piece. Agglutination points to immunological cause of infertility Antisperm antibody can occur in serum of male /female, seminal plasma, spermatozoa thus leading to decrease progressive motility, decrease ability to penetrate cervical mucus.Condition that lead to this are testicular disease, autoimmune following vasectomy, repeated infection cryptorchidism, trauma, torsion.

Between 30-60 mintues(Other microscopic evaluation)-Assessing peroxides positive cell if round cell is present
- Round cell—may be leukocyte or immature germ cell, if more than 1 round cell per high power field is seen it is necessary to differentiate between leukocyte and immature germ cell. While stained leucocytes show presence of brown granules, germ cell remain unstained.
- Epithelial cell from reproductive cell normally contaminate semen, but a high number is associated with infection.
- RBC is not the normal contaminant of semen, usually present in TB of seminal vesicles, rupture of blood vessels, infection of prostate, vitamin c deficiency.[3]

Lower Refrence Limit (LRL)- was established in the last manual of the WHO.If values are over the limit it does not guarantee a successful fertilization or an on-going pregnancy, but it does increase possibilities. The LRL has progressively been reduced due to social behaviors and new life habits as food, tobacco, environmental toxics etc. The reference values established in the 4th manual edition of the WHO compared with those in the 5th and last edition are shown in the board below.

	4th edition (1999)	5th edition (2010)
Liquefaction	Complete in 60min	Complete in 60min
Volume	2 ml	1.5ml
Color	Opalescent white	Opalescent white
pH	7.2-7.8	> 7.1
Concentration (ml)	20 million	15 million
Progressive motility	50%	32%
Vitality	75%	58%
Morphology	15%	4 %
Leukocytes(ml)	> 1 million	> 1 million
Mar test	< 50% sperm with bound Particles	<50% sperm Particles

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1. World Health Organization. WHO laboratory manual for the examination and processing of human semen. 5th ed. Geneva: World Health Organization. 2010.
2. Fertility assessment and treatment for people with fertility problems; National Institute for Health and Clinical Excellence: Guidance .London ;RCOG Press 2004 ISBN978-1-900364-97-3
3. Jequier AM Semen analysis: a new manual and its application to the understanding of semen and its pathology Asian J Androl. 2010 Jan; 12(1): 11–13.

Investigating Azoospermia

DR SUPARNA BANERJEE DGO, MD,MRCOG

Secretary, West Bengal Chapter, IFS
Clinical director of Ankur Fertility Clinic & Research Centre

Infertility or subfertility affects 15% of couples in India, with a male factor contributing to the fertility problem is close to 50% of these couples. Of the men presenting for fertility investigation, up to 20% of male infertility is due to azoospermia.

Causes of Azoospermia is categorized as follows
1. Pre-testicular azoospermia (2% of men with azoospermia, due to a hypothalamic or pituitary abnormality diagnosed with hypo-gonadotropic-hypogonadism).
2. Testicular failure or non-obstructive azoospermia (49% to 93%). It is not always a complete absence of spermatogenesis, some men with testicular failure have reduced spermatogenesis [hypospermatogenesis], maturation arrest or a complete failure of spermatogenesis noted with Sertoli-cell only syndrome).
3. Post-testicular obstruction or retrograde ejaculation:Some men have an ejaculation failure. They might have spinal cord injury, psychogenic failure to ejaculate or neurological damage.

Since discovery of ICSI a breakthroughs in the ARTs have allowed us to treat 98% of couples with male factor infertility. Using ICSI, it is now possible to produce a pregnancy with any live sperm (moving or not).

History and initial investigations for men with azoospermia
After at least 2 semen analyses have confirmed azoospermia, men should be investigated with a history, physical examination and laboratory tests and imaging studies. The history should include information about:
1. The infertility history, such as duration of infertility, whether the infertility is primary or secondary, any treatments to date, libido and sexual activity.
2. The general health of the man, specially check for the presence of diabetes, respiratory issues.
3. The history of proven / suspected genito-urinary infections.
4. Any exposure to agents which might have an adverse impact on spermatogenesis.
   - Medical agents like hormone /steroid therapy, antibiotics (sulphasalazine), alpha-blockers, 5 alpha-reductase inhibitors, chemotherapeutic agents.
   - Environmental factors like pesticides, excessive heat on the testicles;
   - Recreational drugs (marijuana, excessive alcohol);
5. The surgery of the reproductive tract (hydrocelectomies, varicocelectomies etc); and
6. The history of any genetic abnormalities in the patient or his family.

If the man has been exposed to any of the above agents, than he should be advised discontinue and the semen retested in 3 to 6 months. If the man has had a recent serious medical illness or injury or he has evidence of a recent reproductive tract infection, semen testing should be repeated at least 3 months following recovery from

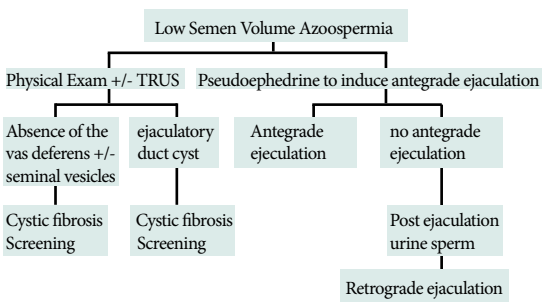
the illness. Physical examination of genital area should be done. We need to check (size and consistency of the testis, presence and grade of varicoceles and palpable vas deferens).

Reduced semen volume Azoospermia:
If the semen volume is low (<1.5 mL) and documented on repeat testing, careful questioning should elicit whether this is an artifact (missed the container, difficulty providing specimen, etc.) or truly a low semen volume. Low semen volume could be due to:

1. Absence/abnormalities of the vas deferens/seminal vesicles,
2. Retrograde ejaculation, or
3. Failure of emission.

Testing the post-ejaculate urine should help to determine if there is retrograde ejaculation.Occasionally, an alpha agonist will convert retrograde into ante grade ejaculation. Diabetic men often have retrograde ejaculation or failure of emission.Physical examination will help to determine if the vas deferens is present in the scrotum and a trans rectal ultrasound (TRUS) will determine if the seminal vesicles and vas deferens close to the prostate are normal. If there is absence of the vas deferens and/or the seminal vesicle, then the man has about an 80% chance to carry a genetic mutation associated with cystic fibrosis. Checking for CF for men with bilateral absent vas deferens is Grade A recommendation.

Fig.1



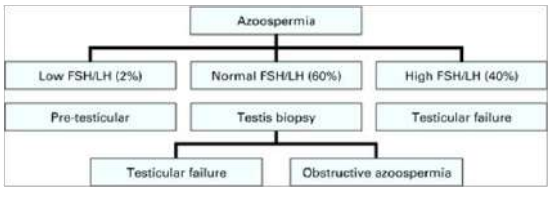
Algorithm for the investigation of azoospermic men with low semen volume.

Obstruction of the ejaculatory duct is detected by TRUS and is usually accompanied by dilation of the seminal vesicles. If an ejaculatory duct obstruction is identified, the man has about a 25% chance to carry a genetic alteration associated with cystic fibrosis. Cystic fibrosis testing should be performed on all men with ejaculatory duct cysts.

Differentiating the causes of normal volume azoospermia:
As mentioned above, the categories of the etiology of azoospermia are:

1. Pre-testicular azoospermia (2%: hypothalamic or pituitary etiology)
2. Testicular failure or non-obstructive azoospermia (49% to 93%)
3. Post-testicular obstruction (7% to 51%: normal spermatogenesis but obstructive azoospermia).

The diagnosis of pre-testicular azoospermia is relatively uncomplicated: LH and FSH levels will be low and the testosterone levels will be either low or normal. Men with elevated FSH and LH and small testis bilaterally have non-obstructive azoospermia. However, men with normal levels of FSH and LH could have either non-obstructive or obstructive azoospermia.Unfortunately, there is no non-invasive method to differentiate obstructive from non-obstructive azoospermia in this group of men. A testicular biopsy is usually required to provide a definitive diagnosis **(Fig 2)**



A number of authors report on the use of Inhibin B serum levels to determine testicular function. While Inhibin B levels are generally lower in those men with more severe testicular dysfunction and is undetectable in those with a Sertoli cell only pattern on testicular biopsy, Inhibin B levels in men with maturation arrest or hyopospermatogenesis patterns on testis biopsies may be identical to those found in men with full spermatogenesis. At present, serum inhibin B levels do not provide significant clinical benefit. (Level of evidence 3, Grade C recommendation).About 60% of men with azoospermia will require a testicular biopsy to provide a definitive diagnosis.

Failure to ejaculate: In men with a clear neurological cause no further investigations are required prior to treatment. Men with idiopathic failure to ejaculate sex therapist should be consulted.

Genetic investigations for men with azoospermia
All men with hypogonadotropic hypogonadism should be referred for genetic counseling as almost all of the congenital abnormalities of the hypothalamus are due to a genetic alteration.All men with absence or obstruction of the reproductive tract ductal structures are at an elevated risk cystic fibrosis. It is recommended that not only the man but his partner should be offered cystic fibrosis testing in this situation. If cystic fibrosis mutation present then genetic alteration is identified, then genetic counseling is suggested (Level of evidence 2, Grade B recommendation).All men with testicular failure should be offered karyotype and Y-micro-deletion testing then referred for genetic counseling if an abnormality is identified (Level of evidence 1, Grade A recommendation).

CHAPTER ACTIVITIES

A CME on "FertiMed" 2019 (20th October 2019) IFS Western UP Chapter

When you pick up your child you can feel the map of your own bones beneath your hands, or smell the scent of your skin in the nape of his neck.There is an instinct in every woman to be a mother.

A CME cum Workshop on "FertiMed" 2019 with the theme 'optimizing infertility management' was organized by Department of Obstetrics and Gynecology, Teerthanker Medical College, Moradabad on 20thOctober 2019 under the aegis of IFS Western UP Chapter.

This prestigious event was inaugurated by Dr. Neena Mohan, senior most President, Moradabad Obstetrics and Gynaecological Society. It was graced by renowned Obstetrician& Gynecologist from Aligarh, Meerut, Bareilly, Noida, Haldwani and Moradabad. Huge spectrum of topics were discussed like follicular monitoring, present status of IUI, setting up IUI lab, luteal phase support in IUI, optimizing its results, and when to stop IUI and think of IVF,ICSI, endometriosis &infertility, ovarian rejuvenation so that all can be benefitted starting from general practitioners, post graduates, practicing gynecologists and faculty in various medical colleges.

Dr. J.K.Goel enlightened the audience about the medicolegal& ethical aspects of ART. The event had panel discussions on Ovulation Induction Protocols and Male Subfertility. A hands on workshop on IUI-tips and trickswas conducted by Dr. Kanthi Bansal, Dr. Mukesh Bansal and team, renowned IVF specialist from Ahemdabad. The response was very heartening and overwhelming. Around 11o delegates from various places of Uttar Pradesh and Uttarakhand attended the CME. Feedback from delegates was very positive and complimentary on both professional and administrative arrangements.


16th ART update, 25th and 26th May 2019 at Deventure Hill Resort, Kandhaghat, HP joint efforts of IFS Chandigarh & Himachal Chapters

Morethan 150 delegates got benefitted and got opportunity to interact with renowned faculty from North India including Delhi. Two pre-conference workshops on IVF lab setup and hands-on hystero-trainer and endo-suturing under the guidance of Dr NeenaMalhotrawere conducted with participation of more than 50 candidates in each.

The international guidelines of PCOS were discussed. Newer Gonadotropin delivery systems in ART and bio-similar assays were addressed by senior production head from Intas Pharmaceuticals .Dr Shweta Mittal elaborated the modalities to get best IVF outcome in women with poor ovarian reserve.

There was a blend of controversial topics and basics in ART. Controversies like Blastocyst for all, uterine septum removal before ART were discussed very well. Panel discussion on basics of ovarian stimulation for IUI and IVF werehighly acclaimed by young ART specialist as well as experienced one.

A penal on medical disorders in ARTwas moderated by Dr RashmiBagga and Dr Bharti Joshi from PGI Chandigarh was highly appreciated. Challenging aspect of ART like PGT and genetic counseling was taken were enthusiastically. Updates on newer modalities in RIF,relevant issues pertaining to male infertility were also highlighted. Talented young researches also presented their papers and poster presentation competition was also held.

Academic sessions were followed by entertaining and rejuvenating cultural events prepared by organizing team and delegates.

Learning points–

1. Basics for ovarian stimulation in IUI and IVF cycles
2. Fine skills for endo-suturing
3. Hystero- trainer was a big attraction and experience was appreciated by nearly 50 delegates.

Comments from audience and faculty on how they felt-

1. Medical disorders complicating ART were highly appreciated.
2. Role and Judicious application of PGT in ART was thoroughly discussed.



CME on 'Male Infertility and DNA Fragmentation Index' Rajasthan Chapter (10th August, 2019)

IFS Rajasthan had organised a CME on 'Male Infertility and DNA Fragmentation Index' on 10th August, 2019. There were two talks. The first was on 'Overview on Male Infertility : from guidelines to clinical practice', given by Dr Suchika Mangal from Jaipur.

The Guest Speaker was Dr Sayali Kandari from Mumbai. She presented her original research paper, recently presented in ESHRE 2019, and spoke on DFI. Her talk was : 'First Clinical Study of India on the effect of Medical Therapy on Sperm DNA Fragmentation and Improved Clinical Pregnancy Rates.'

It was attended by about 30 Gynaecologists and Embryologists. The aim was to increase the awareness on Male Infertility, guidelines about its management and more specifically the role of sperm DNA Fragmentation in Reproductive outcomes.


IUI Workshop at Hotel Capitol Hill, Ranchi (12.08.2019) Jharkhand Chapter

List Of Organising Committee: Secretary: Dr. Archana Kumari, Joint Secretary: Dr. Sunita Jha, Treasurer : Dr Rupashree Purshottam

Guest Speaker : Dr(Col) Pankaj Talwar (Delhi), Dr Suparna Banerjee (Kolkata)

The pleasant cloudy weather in the holy month of Sawan on 12thAugust, 2019, Monday, witnessed the 1st annual conference of IFS Jharkhand Chapter, exactly one year after the formation of Jharkhand Chapter on 11thAugust 2018. The conference started with welcome address by Patron Jharkhand chapter, Dr. Karuna Jha and traditional lamp lighting by the dignitaries. It was followed by Secretary general IFS Dr Pankaj Talwar report where he presented the mission and vision of IFS, the outreach programme, the academic calendar of IFS for 2019-2020, various courses conducted by IFS and about FERTIVISION -2019.

Half day live workshop on intrauterine insemination was conducted byInfertility stalwart Dr Pankaj Talwar. Live demonstrations of semen analysis and various semen preparation methods was very informative as well as interactive. A detailed discussion on semen analysis (WHO 2010) and male factor infertility by Dr. Pankaj Talwar kept the audience mesmerized. Dr. Suparna Banerjee, secretary IFS Bengal chapter, discussed evidencebased practices in IUI and tricks to improve the success rate in IUI.Both the sessions were very interactive and held the utmost attention of the audience.A case based panel discussion on secondary subfertility was moderated by Dr. Archana Kumari, Dr. Sunita Jha being the co-moderator. Panelists were infertility specialists of Ranchi- Dr. Sashi Bala Singh, Dr, Nirmala Singh, Dr. Rupashree Puroshotam, Dr. Sakshi Singh.Expert inputs were made by Dr. Karuna Jha and Dr. Pankaj Talwar. The programme ended with vote of thanks proposed by Dr. Archana Kumari, Secretary Jharkhand Chapter.
Around 70 delegates attended the workshopwhich included the postgraduates students from Rajendra Institute of Medical Sciences, Ranchi

Learning Point:
1.Clinical understanding of Semen analysis(WHO-2010), male factor infertility.
2.Methods of semen preparation for IUI
3.Evidence based practice in IUI.

Comment from audience:
A very interesting and captivating workshop which gave everyone a chance to understand the very basis of IUI and semen analysis…..Dr. Suman Sinha
A new insight to old topic ….Dr.Soumya Sinha
Very helpful for newcomers in the field of infertility especially who wish to start IUI setup…. Dr. Reena Godsara
Comment from faculty :
A very enthusiastic audience and in such large number with active participation in all sessions, gives the reason to promote many such basic workshops in future and give Jharkhand more importance in academic activities of IFS in future….. Dr. Pankaj Talwar

IFS Punjab Chapter in association with POGS organized a conference on Infertility updates and ART Workshop on 22nd Sept. 2019 at Harpal Tiwana Hall at Patiala

IFS Punjab chapter in association with POGS organized a conference on infertility updates and art workshop on 22nd September 2019 at HarpalTiwana hall at Patiala. Organizing committee-chairperson: DrHarinderkaurOberoi and DrSaritaAgrawal Organizing secretary: Dr Monica Verma and DrRanjana

Joint organizing secretary: DrSarabjit, DrShalini and DrSarabpreet Singh Work shop coordinator :DrDeepaGoel, DrSukriti and DrJaslin Guest speakers: Dr K D Nayar, Dr Surveen Ghuman, DrUmesh Jindal, DrShweta Mittal, Dr Sarabpreet Singh, DrLakhbirDhaliwal, DrSarlaMalhotra, DrManjitMohi, DrShaliniGaindher, DrLovleenSodhi. 215 delegates and 35 faculty members attended the conference, 80 attended the Hysteroscopy work and 25 attended the OPU and ET workshop. This was wonderful gathering from all over North.

Topics and discussion were excellent with good interactions. Punjab medical council granted 4 CME hours. There was a good panel discussion which was appreciated by audiences and faculty members Topics- free papers, unexplained infertility, panels on fibroids and male infertility, PGD In aneuploidy, ovulation induction , first trimester treatments of IVF pregnancy, pregnancy in art and workshop on Hysteroscopy, OPU and embryo transfer, vitrification and cryobiology workshop were discussed in detail. Panels and Hysteroscopy workshop Appreciated by delegates

Whole conference was enjoyed and applauded by all the faculty and delegates

Shivani CME for Embryologist conducted on 29th September 2019 from 9 to 2pm at Hotel Ramada encore Jalandhar, 40 delegates participated including Embryologist.

Shivani meeting with IFS Punjab chapter conducted a CME on1st Navrata day at Jalandhar, Hotel Ramada encore. Attendance good with good discussion and exchange of views. Very good informative and interactive topics.


IFS - Representing India At Global Level

Dr Gouri Devi (President IFS) At ESHRE 2019 – IFS/ISAR Session

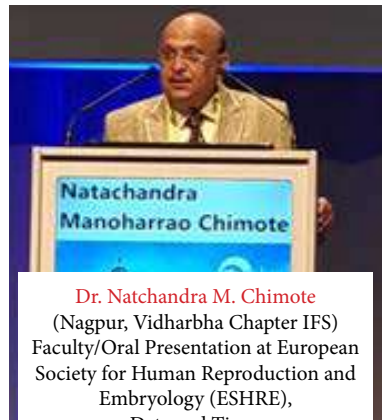
Dr Gouri Devi, Dr KD Nayar and Dr Neena Malhotrs at the IFS ISAR session in ESHRE 2019, Vienna

Dr KD Nayar
(Vice President, IFS)
Oral presention at ESHRE Vienna 2019

Faculty/Oral Presentation at European Society for Human Reproduction &Embryology (ESHRE), Date and Time: 26th June 2019, 14.30 hours.

O- 280 Topic:
"Evaluation of the hormone Dehydro-epiandrosterone sulphate (DHEAS) as a potentially compelling 'oocyte-related factor' in mammalian oocyte activation: A paradigm shift?"

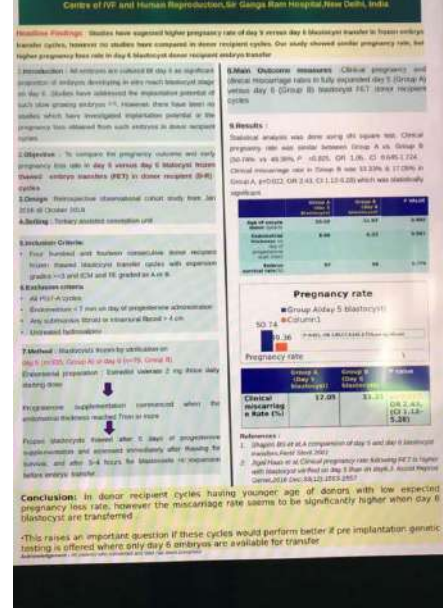
Dr. Bindu N. Chimote
(Nagpur, Vidharbha Chapter IFS)
Presented an Oral Presentation at ESHRE

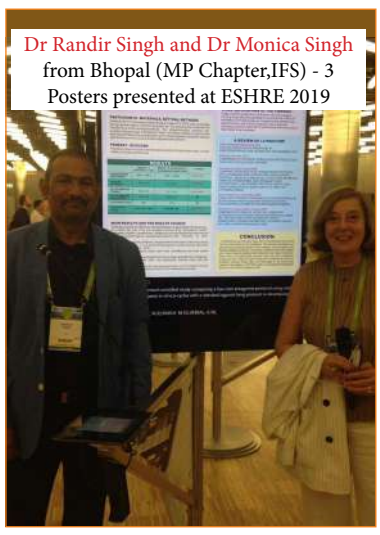


Dr. Natchandra M. Chimote
(Nagpur, Vidharbha Chapter IFS)
Faculty/Oral Presentation at European Society for Human Reproduction and Embryology (ESHRE),
Date and Time:
24th June 2019, 10.00 am

Dr Shweta Mittal
Presented a poster at ESHRE

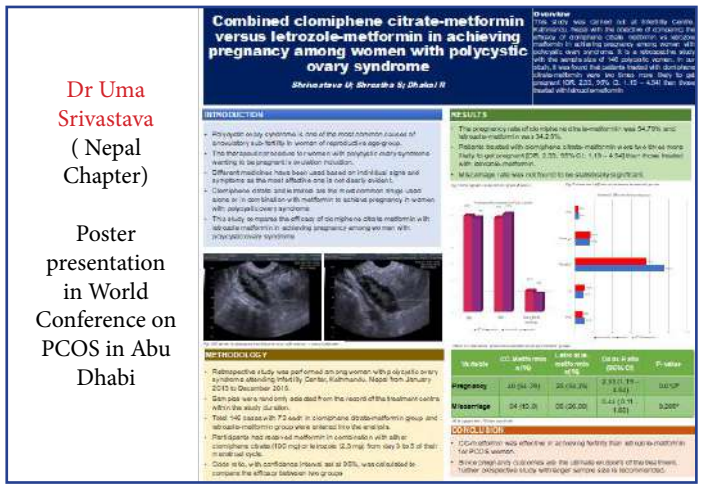
Poster: Impact of day 5 versus day 6 blastocyst on pregnancy outcome of frozen thawed donor recipient cycles, Poster presentation, ESHRE, VIenna, June 2019





Dr Randir Singh and Dr Monica Singh from Bhopal (MP Chapter,IFS) - 3 Posters presented at ESHRE 2019

O- 003 Topic: "Mapping the follicular fluid bio-molecular profile: Dynamic interactions set the algorithm for oocyte maturation, embryo development and successful outcomes in IVF cycles"



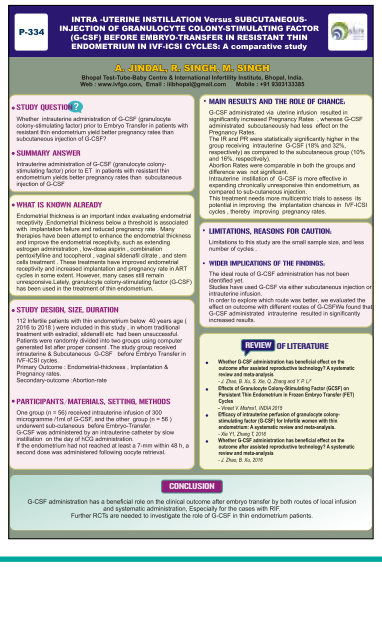
Dr Uma Srivastava
(Nepal Chapter)

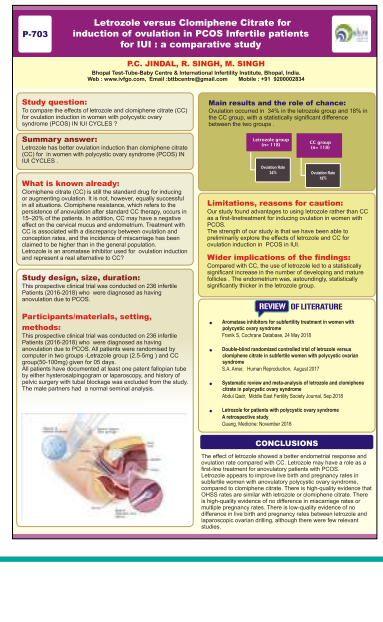
Poster presentation in World Conference on PCOS in Abu Dhabi

Poster-1
"Intra-Uterine Instillation Versus Subcutaneous - Injection Of Granulocyte Colony - Stimulating Factor (G-Csf) Before Embryo-Transfer In Resistant Thin Endometrium In Ivf-Icsi Cycles: A Comparative Study "

Poster-2
"Letrozole versus Clomiphene Citrate for induction of ovulation in PCOS Infertile patients for IUI : a comparative study "

Poster-3
"Comparative results of IUI performed 24 hours and 40 hours after HCG-Trigger"





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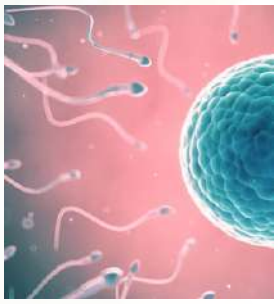

2ND, 3RD & 4TH OCTOBER 2020

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Kuldeep Jain

Rishma Dhillon Pai

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Sudha Prasad

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- First Joint conference of ESHRE, IFS & ISAR
- 30+ Leading Scientific Faculties from Europe.
- Experienced Scientific National Faculties from IFS & ISAR
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- Ten, Including few Live Workshops
- Free communication with E- Posters / oral presentations
- An opportunity to meet and interact with leading national and international faculties
- Fun filled evening cultural programmes.

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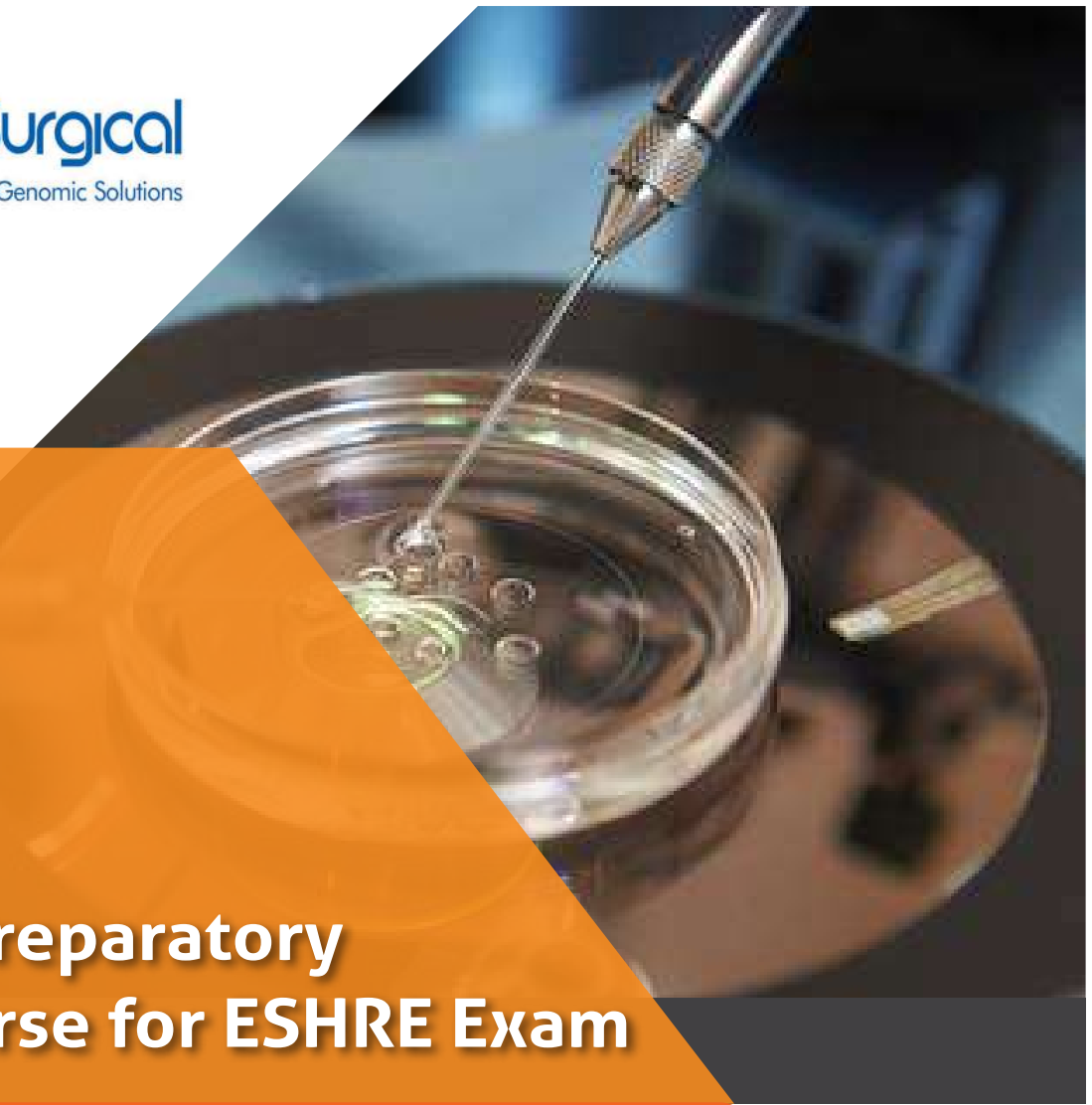

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&

DIPLOMA IN CLINICAL EMBRYOLOGY

1 YEAR DIPLOMA PROGRAMMES WITH UNIQUE ADVANTAGES

- Includes monthly teaching modules along with CME conducted in reputed IFS centers under eminent ART and reproductive medicine consultants
- The Clinical ART Fellowship includes hands-on training in clinical ART procedures, ultrasound and endoscopic surgeries
- Fellows can avail travel and academic grants
- Clinical Embryology includes intensive practical training in embryology lab procedures
- Completely merit based selection of candidates for a limited number of seats

EXPLORE EXCITING CAREER OPPORTUNITIES IN INDIA AND ABROAD

Career opportunities range from jobs in Fertility Clinics in India and abroad, opportunities in private healthcare organisations and in the field of infertility treatment, and research-centric roles like embryonic studies research in reputed universities and research in the reproductive sciences.

20 SEATS EACH IN CLINICAL ART AND CLINICAL EMBRYOLOGY ALL OVER INDIA

ELIGIBILITY & ENTRANCE EXAMINATION SYLLABUS

DIPLOMA IN CLINICAL ART

Eligibility: Postgraduate in OBGYN (MD/DNB). Registered with the MCI / State Medical Council. The candidate must be a life member of IFS.

Entrance Examination Syllabus: Clinical Reproductive Biology, Physiology, Anatomy, Endocrinology, Basic Embryology and Andrology. Clinical Genetics.

DIPLOMA IN CLINICAL EMBRYOLOGY

Eligibility: MBBS/Postgraduate in Medical Sciences or M.Sc./Ph.D in Life Sciences or Veterinary Sciences (Regular Course) from recognised institute in India.

Entrance Examination Syllabus: ICMR Guidelines, Basic Human Embryology, Human Cell culture, Genetics, TQM, Basic Semenology, Anatomy, Physiology & Pathalogy of Reproductive Biology.

Entrance test of 2020 - 2021 batch DCE and DCR Courses and interview have been finalized as June, 2020

Amity University, Sec-125, Noida (New Delhi NCR) | Tel: 98-160-26719, 98-105-66870 | www.amity.edu

VENUE

IFS SECRETARIAT
302, 3rd Floor, Kailash Building,
26, Kasturba Gandhi Marg, CP, New Delhi-110001

+91 9899308083
+91 11 40018184

+91 9667742015
indianfertilitysocietydelhi@gmail.com
www.indianfertilitysociety.org

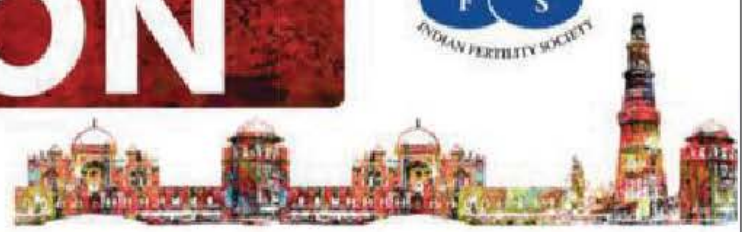
indianfertilitysociety indianfertilitysociety ifsdelhi

15th Annual Congress of Indian Fertility Society

FERTIVISION 2019

Organised by IFS Indian Fertility Society

6-8 December
The Leela Ambience Hotel,
Gurugram, New Delhi, NCR | India



THE BIGGEST ACADEMIC EVENT OF FERTILITY IN INDIA AT NEW DELHI

International Faculty

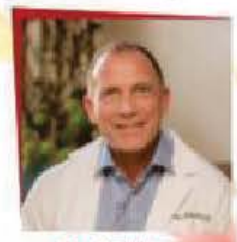

Dr. Arne Sunde
Norway


Dr. Ashok Agarwal
USA


Dr. E Balaji
Singapore


Dr. Edgar Mocanu
Ireland


Dr. Ephia Yasmin
UK


Dr. Gad Lavy
USA

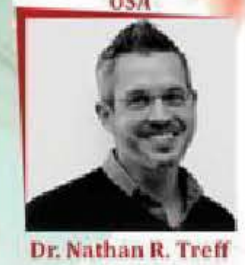

Dr. Gabor Vajta
Australia


Dr. Jane Stewart
UK


Dr. Jayant G. Mehta
UK


Dr. Joanne Carwardine
UK


Dr. Jyotsna Pundir
UK

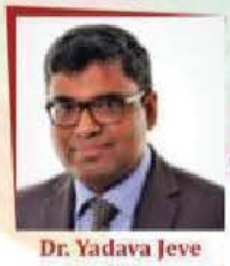

Dr. Nathan R. Treff
USA


Dr. Peter Humaidan
Denmark


Dr. Raj Mathur
UK


Dr. Richard Kennedy
UK


Dr. Sandra Bateman
UK


Dr. Yadava Jeve
UK

More Than 350 National Faculties

Limited Seats

Dr. M Gouri Devi
Organizing Chairperson
FERTIVISION 2019

Dr. Pankaj Talwar
Organizing Secretary
FERTIVISION 2019
Email-pankaj_1310@yahoo.co.in
Mob.: +91 98107 90063

Theme: Beyond Tomorrow

Deadline for **Abstract Submission** 15th Nov, 2019

Choose from 10 Pre Conference Workshops | 6 December (Choose Any 1)

1) *IFFS Workshop on Do's and Don'ts in Ovarian Stimulation(0900 - 1700)*	6) *Cryobiology (0900 - 1700)*
2) *Reproductive Surgery (0900 - 1700)*	7) *Total Quality Management (0900 - 1700)*
3) *Ultrasonography Imaging In Infertility (0900 - 1700)*	8) *Counselling and Holistic Medicine (0900 - 1300)*
4) *Andrology & Semenology (0900 - 1700)*	9) *Publish or Perish (0900 - 1300)*
5) *Ovum Pickup and Embryo Transfer (With Simulators) (0900 - 1700)*	10) *PGT and Genomics (0900 - 1700)*

Registration Fees

Category	Regular Fees Till 31st October		Onspot	
IFS Member	INR 12500		INR 14500	
Non IFS Member	INR 14500		INR 16500	
Conference Registration plus Life Time IFS Membership	Embryologist	INR 16500	Embryologist	INR 18500
	Gynaecologist	INR 17500	Gynaecologist	INR 19500
PG Students (No Dinner)	INR 7000		INR 8000	
Accompanying Person	INR 11500		INR 12500	
Foreign Delegates	$ 400		$ 500	

One Day Registration Fees

PG Students (7th Dec or 8th Dec)	INR 4000

Inclusive of 18% GST

Register at www.fertivision2019.com

Conference Secretariat

Indian Fertility Society
302, 3rd Floor, Kailash Building,
26, Kasturba Gandhi Marg
C.P, New Delhi - 110001
+91-9667742015, +91-9899308083
+91-11-40018184

Conference Manager

Conferences International
Mr. Sumeet Ghai
B-220/2, 2nd Floor,
Opposite Kali Masjid, Savitri Nagar
New Delhi – 110017
Mobile: +91 9560493999

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Treat infertility & other Gynaecology Complication

In male infertility

CoQ10 Available as **Trans CoQ10** form

- Improves sperm count, functional sperm concentration, motility & morphology

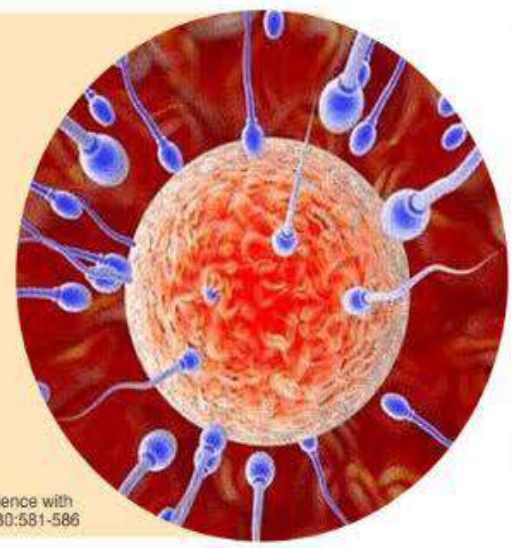
Indian J Urol 2001;18:57-61

Methylcobalamin administration increases

- Sperm concentration 38.4%
- Sperm count 53.8%
- Sperm motility 50.0%

Methylcobalamin enhances the testicular functions, resulting in a increased output of motile sperm.

Ref.: Isoyama R. KAWAI S. Shimixu Y et al. Clinical Experience with Methylcobalamin for male infertility. Hinyokika kiye 1984;30:581-586



CoQ10	100 mg
Lycopene 6%	10000 mcg
Methylcobalamine	500 mcg
Vit C	100 mg
Niacinamide	50 mg
Vit B1	10 mg
Vit B2	10 mg
Vitb 6	3 mg
Calcium Pantothoate	12.5 mg
Folic Acid	1 mg
Vit A	5000 I.U
Vit D3	500 I.U
Vit E	25 I.U
Zinc Oxide	15 mg
Cupric Oxide	2.5 mg
Sodium Selenate	60 mcg
Mangnese Chloride	1.4 mg
Chromium Chloride	65 mcg

In

- Infertile couple
- Male Infertility
- Prostate Cancer
- Pre-eclampsia & IUGR
- Uterine Fibroid Tumours
- Habitual & Spontaneous Abortion

Tackles complicated conditions .... naturally