Fertivision 2015 - Abstract

1. OHSS: Nuances of stimulation in PCO

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It is time to redefine success in ART. 'Achievement of pregnancy with no OHSS, leading to a singleton live term birth of a healthy child' is what we should all aim at in modern ART. Ovarian stimulation in PCOS patients is very tricky. The ovaries are like explosives ready to explode when they are exposed to gonadotrophins. This happens because of higher sensitivity of polycystic ovaries to gonadotrophin stimulation. Polycystic ovaries contain 6 times more number of follicles compared to normal ovaries.

So it is extremely important to be very careful when stimulating polycystic ovaries and grasp the subtle nuances of stimulation in these patients.

High levels of androgens in PCO patients can further have stimulatory role in early follicular growth by augmenting follicular FSH receptor expression and therefore amplifying FSH effects.

The Aim of ovarian stimulation should be obtaining optimum number of oocytes and prevention of OHSS.OHSS is one of the most dreaded complications of ovarian stimulation in PCOS patients. Report by ESHRE (de Mouzon et al., 2012) reveals OHSS is still one of the major complications of IVF. But it can be avoided by taking proper measures prior to stimulation and also selecting the right protocol.

Various studies have confirmed that use of metformin pretreatment has been associated with reduction in the incidence of hyperstimulation.(Tang, Bart and Balen, 2005). Metformin acts by reducing insulin resistance and intraovarian hyper-androgen status.

Use of oral contraceptive pills as pretreatment is controversial.

Use of mild stimulation protocols can reduce the chances of OHSS. Calculation of gonadotrophin dose depending on parameters like patients age, BMI, Sr AMH levels and previous response can help in titrating the right dose of gonadotrophins for the patient.

Advent of antagonist treatment protocols has given more freedom to the clinicians in regards to adjusting dose of gonadotrophins and coasting when required.

Also one major advantage of antagonist protocol is use of agonist trigger for final oocyte maturation. GnRHa trigger offers important advantages, including virtually complete prevention of OHSS.

With the advent of better vitrification techniques, segmental IVF can be offered to patients with OHSS.

Though IVM (In vitro maturation) appeared a promising option in earlier studies, there are no randomized controlled trials which compare IVM and conventional IVF in PCOS patients. Also recent studies have shown that IVM has deleterious effects on the spindle organization and chromosomal configuaration of oocytes from PCOS patients. (Yuan Li, Huai-Liang Feng *et al*, 2006)

To summarize, identifying patients who are at high risk of OHSS, pretreatment with Metformin, antagonist protocol with rightly titrated dose of gonadotrophins, agonist trigger for final oocyte maturation and segmental IVF, are the nuances of stimulation which can be considered in PCOS patient to prevent OHSS.

2. Surgical management of adenomyosis- When and how?"

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Adenomyosis is a benign uterine disease characterized by ectopic endometrial glands and stroma within the myometrium. It usually affects premenopausal women, mainly with symptoms of menorrhagia, dysmenorrhea, and infertility. Adenomyosis can either be diffuse or localized (focal), depending on the extent of myometrial invasion. Earlier the management of adenomyosis was used to be hysterectomy but with the trend of delayed childbearing, adenomyosis is diagnosed more frequently in fertility clinics.So minimal access surgery techniques and organ preserving surgery is a parallel trend that characterizes modern gynecology. It is of outmost important to preoperatively ensure the definite diagnosis of adenomyosis, and assess the location and the size of each adenomyotic focus. Magnetic resonance imaging assists in the achievement of both of these preoperative goals and helps the surgeon to remove each focus of adenomyosis completely.

Many uterus-sparing surgical techniques have been developed to treat adenomyosis. Adenomyomectomy has been considered as the first-line approach to treat adenomyosis, particularly focal adenomyosis while partial adenomyomectomy including wedge resection of the uterine wall, transverse H incision technique, and asymmetric dissection of uterus to treat diffuse adenomyosis have been described in literature with variable rates of recurrence. The complete excision of adenomyosis by employing several techniques, such as overlapping flaps and triple-flap method to treat diffuse adenomyosis have also been described but the data about the long term outcome of these techniques is still suboptimal and there is no strong evidence to indicate a technique that secures the best clinical and reproductive performance.

3. Uterine transplantation

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Uterine transplant is a surgical procedure whereby healthy uterus is transplanted into a female organism of which the uterus is absent or diseased. Diseased uterus does not allow normal implantation. This is described as absolute uterine factor infertility (AUFI). For these kind of patients till now there were not many avenues available in the management. This is typically so, in a case of Meyer Rokitansky Kuster Hauser syndrome (MRKHS) patients, or those who have had diseased uterus like severe Asherman syndrome, severe hypoplasia of uterus or hysterectomised due to development of cancer e.g. cervical cancer. The incidence of MRKHS is 1 in 5000.

Several attempts were made to correct this infertility starting from experiments in animals to treatment in human beings. In 1896 ovarian transplantation in a rabbit was documented by an austrian, Emil Knauer. In 1964, Eraslan Hamernik and Hardy et al from Jackson University, Missisipi were successful in performing uterine transplantation in animals and having a successful pregnancy. However, partly because of development of In Vitro fertilization and assisted reproductive techniques in seventies onwards, the attempts to develop uterine transplantation in humans took a backseat.

Two thousand onwards there were again attempts to correct AUFI. Dr Wafa Fagee *et al* in Saudi Arabia, in the year 2000, transplanted a uterus from 46 years old hysterectomy patient to a 26 years old patient who had a haemorrhage and hysterectomy earlier. It lasted for 99 days. She had two successful menstrual cycles but uterus had to be removed because of necrosis which was confirmed on laparotomy. In turkey in 2011, in Antalya, a woman named Derya Sert, who did not have a uterus, was given transplant from a deceased donor in Akdeniz University. This resulted in six menstrual cycles and ?aborted pregnancy at six and half weeks.

First successful pregnancy - In Sweden in 2012 at Gothenburg University, Dr Mats Brannstrom and his team performed total nine uterine transplantations. In October 2014, a healthy baby boy was born by caesarean to a woman who developed pre-eclampsia at 32 weeks of gestation. The Swedish woman who was 36 years old had received a uterus in 2013 from a live 61 years old donor, who was her friend. Prior to uterine transplantation, she had healthy ovaries and was diagnosed as Rokitansky syndrome. The patient underwent ovarian stimulation and IVF. The embryos thus obtained were cryopreserved. After transplantation patient was on immunosuppression, the medicines being initially anti-thymocyte globulin followed by oral tacrolimus, mycophenolate mofetil and later on oral azathioprine and prednisolone and 1 year later, the embryos were transferred to the uterus. Out of these nine transplants four were from mothers of the patients. Four pregnancies have been reported by Dr Mats Brannstrom and team.

Uterine transplantation evokes many issues. It's a team work involving many specialities mainly reproductive medicine specialist, transplant surgeons, anaesthetist, radiologist, immunologist / pathologist, geneticist and psychologist. It is unique organ transplantation which is not an emergency or lifesaving, thus equivocally acceptable. There are other options available like surrogacy and adoption. The patient has to undergo first transplant surgery then caesareans for one or two children followed by hysterectomy 3-4 years later. For many this is too complicated and unacceptable. There are other issues like live or deceased donor, the rejection and morbidity due to it in both donor and recipient. It is extravagantly expensive procedure with no insurance cover. There is an issue of transgender and male recipient.

There have been Montreal criteria published by Mcgill University in 2002. The issues discussed are autonomy versus nonmalificence, equivocal with regard to beneficence and justice. Criteria for recipient donor and health care team have been discussed.

With this interesting and wonderful invention the issue also is pertinent to developing countries regarding extravagantly high cost for the procedure and the feasibility for all strata of the society. Until we address these issues and find the solutions, this remains experimental. However, there are reports that countries like USA, UK and even India may soon follow the path.

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4. Surprises during controlled ovarian stimulation

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Controlled ovarian stimulation forms a important aspect of infertility management. Ovulation disorder account for 20% of all female infertility. The success of an IUI or an ART cycle depends on the ovulation stimulation protocol. Hence it is very important to perfect the art of ovulation induction.

But it has its short falls. The problems that can be encountered are as follows-

1. Failure of follicles to mature (Anovulation)
2. Luteinizing unruptured follicles (LUF)
3. Premature luetinization (PL)
4. Hyper secretion of LH
5. Failure of ovulation inspite of ovulation trigger

6. Ovarian hyperstimulation syndrome.
7. Persistant follicular cyst on D2/D3.
8. Poor endometrial response inspite of ovulation.

Ovulation induction with clomiphene

Ovulatory response to clomiphe citrate (CC) is dependent on

1. Body mass index
2. Free androgen index
3. Type of ovulatory dysfunction

Cumulative ovulation rate of 40% with a 50mg daily dose of CC, another 21% with a 100mg dose. 8% with 150mg daily dose. A cumulative conception rate of 45-75% is reached within 3-9 cycles. Thereafter the cycle fecund ability fails rapidly.

Discrepancy between ovulation and pregnancy rates may be explained by the peripheral antiestrogenic effects of CC on quality of cervical mucus & the Endometrium.

The principal risk of CC is a increased incidence of multiple gestations (<10%).

In Clomiphene failure cases ie. Inability to achieve a pregnancy inspite of ovulation, investigation should be expanded to exclude other coexisting infertility factors.

Clomiphene resistance describes, woman who do not ovulate in response to clomiphene, one should look for adjuvant treatment like-

1. Metformine in obese PCOS.
2. Downregulation with OCP prior to intiating COH.
3. Glucocorticoids if DHEA'S is high.
4. Use of hCG as ovulation trigger.
5. Combining with gonadotrophins if anovulatory with CC.

Luteinized unruptured follicle (LUF)

Described as failure of the follicle to ovulate inspite of midcycle LH surge though the follicle undergoes luteinization. It is also called trapped egg syndrome. More commonly seen with endometriosis & PCOD.

Treatment – Ovulation trigger by giving hCG in a dose of 10,000IU IM when the follicle reaches maturity.

Treating for endometriosis & PCOD surgically.

Premature luteinization (PL) Refers to a premature rise in serum progesterone (P) levels on the day of hCG administration.

Most studies use an cut off of 0.8 – 2 ng/ml on the day of hCG but in an ART cycle 0.3-1.2 ng/ml is considered as ideal. Some author defined PL as a P/E2 ratio >1.

In an ART cycle downregulation with the long protocol or the antagonist help in circumventing the problem of premature luteinization.

Hypersecretion of luteining hormone

Hypersecretion of luteining hormone is a significant cause of ovulatory dysfunction. Especially in women with polycystic ovarian syndrome.

D2/D3 LH level should ideally be < 5mIU/ml

A disordered ovarian pituitary feedback is central to the problem-

Downregulation with oral contraceptive pills prior to an IUI cycle helps in reducing the LH levels.

Ovulation trigger

Ovulation trigger is given as a surrogate for the LH surge seen in spontaneous menstrual cycle to control the timing of ovulation & the timing of sexual intercourse.

Untimely ovulation trigger given prior to follicular maturity leads to a premature luetinization of oocytes & a failed cycle.

In a hyperstimulated cycle, ovulation trigger can be given by giving GnRH agonist instead of hCG. The dose can be repeated if there is no ovulation with single dose.

In a normal cycle ovulation trigger can be given by giving inj. hCG 10,000IU if the 5000IU has failed in a previous cycle. This is especially true if the number of follicles are more than 5.

Poor endometrial response

Poor endometrial thickness <7cms on the day of ovulation trigger suggest poor pregnancy outcome.

This could be due to chronic endomeritis due to various organisms like Chlamydia, mycoplasma or tuberculosis or bacterial infection & repeated D&C's.

Tubercular endometritis is a very important reason for poor endometrial thickness inspite of a good ovarian response.

It could lead to formation of intrauterine synechaie or even Ashermann's syndrome.

Treatment of chronic endometritis prior to embarking on ovarian stimulation is the key to success.

Persistent ovarian cyst on D2/D3 of cycle

Most ovarian cysts in women of reproductive age are functional & usually undergo spontaneous resolution within four to eight weeks.

Cause of these functional cyst is unknown though aberrant FSH & LH secretion is incriminated. It is not known whether the susceptibility to cyst formation lies with the hypothalamus or pituitary or peripherally in the ovaries.

Though inhibing ovulation combined oral contraceptive pills are used to decrease their incidence. There is no clinical evidence to prove that.

Therefore expectant management is the preferred treatment of choice for those wishing to conceive.

5. Defending controversies- Uterine septum: Should we operate in all or only after pregnancy failure

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Septate uterus occurs when there is incomplete resorption of the paramesonephric Mullerian ducts during the first trimester of pregnancy. The resorption of the septum normally initiates at the level of the cervix and continues cephalad in the direction of the uterine fundus.

Septate uterus is one of the most common forms of congenital uterine malformations with an incidence as high as 3-4% in the general female population. However, it is significantly higher in patients with infertility and recurrent pregnancy loss. A uterine septum is known to affect female reproductive health in three ways: (i) obstetric complications like preterm birth (ii) recurrent miscarriages and (iii) infertility. In women with septate uterus there is inadequate uterine vascularisation leading to subsequent abnormal placentation. Clinical studies in women with septate uterus have demonstrated an increased content of muscle tissue and also increased uncoordinated contractility of the uterine septum. Septate uterus is most amenable to hysteroscopic resection.

Several retrospective studies have assessed the efficacy of the hysteroscopic removal of the uterine septum and restoration of uterine cavity. These studies do report improvements in miscarriage rates from before to after hysteroscopic resection (88% miscarriages before and 5.9% after surgery). Grade A evidence is lacking on the efficacy of hysteroscopic septal resection in women with recurrent miscarriage. The available evidence is biased because women with pregnancy loss are being used as their own controls, a methodological design that favors the intervention in these women. Surgical treatment of these women should be discouraged unless it is performed as part of a RCT. Women with pregnancy losses do have a good chance of conceiving and continuing a successful pregnancy to term without any surgical intervention. Any surgical correction can cause complications like perforation of the uterus, cervical lacerations or cervical incompetence.

Women with uterine septum and otherwise unexplained infertility might benefit from hysteroscopic septal resection. The Randomized Uterine Septum Transection Trial (TRUST), a large multi-centered Dutch study, states that removal of septum produced better pregnancy outcome. This study included women with two or more miscarriage before 20 weeks of pregnancy with a septate uterus with a septum length atleast 1/4th the uterine cavity. The study was underpowered and some surgeons were rather inexperienced. A properly conducted and powered RCT should be welcome to provide more robust, Grade A evidence to us clinicians.

6. Advent of latest techniques in assessing sperm function in men with idiopathic male infertility- Is their use justified for diagnostic workup?

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The conventional semen analysis is a critical first step in the evaluation of male fertility, but provides limited information about sperm function. Several semen parameters are used to discriminate the fertile male from the subfertile male. The most widely used parameters are sperm concentration, motility, progressive motility, and sperm morphology. Specialized semen tests (example: hypo-osmotic swelling, acrosome reaction, sperm DNA fragmentation, oxidative stress status, etc.) are needed in patients with idiopathic male infertility to elucidate the etiology of subfertility. These tests are useful for determining specific defects of human sperm physiology. Newer sperm function tests may eventually be useful clinically, but more information is needed to determine if these tests will truly predict fertility potential. However, the prediction of male fertility potential is probably an elusive goal owing to the multifactorial nature of conception. The implementation of ICSI has basically eliminated the need for such tests. It is felt by a number of reproductive specialists that this does not represent a right approach. The sperm function tests have the potential to assist the clinician in the decision-making process. If patients fail sperm functional testing, then that would eliminate the time, effort, and expense of couples undergoing lower complexity therapies such as IUI and direct them to ICSI without delay.

7. Have advances in imaging techniques improved infertility management

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Ultrasound is now an indispensable tool not only in the diagnostic evaluation of the infertile couple but in the treatment of infertility as well. This treatise illustrates the role of technological advances in ultrasound in the current management scenario of the infertile patient.

The four broad areas where technologically advanced ultrasound improves patient outcomes include

- Diagnosis of uterine, ovarian and other adnexal factors in the infertile patient,
- Prediction of outcomes in ART cycles,
- Ovulation Monitoring, and,
- Interventional Procedures

Delineation of Pelvic Pathology

The imaging of pelvic pathology and its consequent appropriate medical or surgical management is central to enhancing patient outcomes in infertility. The aim of the exercise is twofold: to identify lesions that impair fertilization or implantation and to exclude or confirm lesions that result in recurrent pregnancy loss.

Anomalous Mullerian development is estimated to occur in 3-4 % of women1 and about half of these are associated with clinical disease2,3,4. The clinical significance includes infertility and implantation failures5. Correction of these anomalies by hysteroscopy and other minimally invasive surgical procedures greatly improves reproductive outcomes6,7. Precise delineation of the type and extent of the anomaly is important to assess the need for surgical correction and the technique of correction.

For several decades, X-ray contrast hysterosalpingography has been the technique for evaluation of uterine malformations. Although the technique is reasonably safe, it involves radiation exposure and lacks specificity in detailing lateral fusion anomalies8. For some years now, saline infusion sonohysterography has evolved as an adjunct to transvaginal 2D sonography9,10,11. The procedure, however, fails to differentiate the septate uterus from the bicornuate uterus. In fact, a hysteroscopy alone also fails to make this distinction and a concurrent laparoscopy needs to be carried out to make a complete diagnosis, especially since the repair is so distinctively different in the two diagnoses. Magnetic Resonance Imaging can also delineate these malformations but the method is cumbersome, expensive and coarse12,13. 3D Ultrasound is emerging as a sensitive and remarkably specific modality for mapping the anomalous uterus14,15,16. The unique ability of 3D Ultrasound to display the coronal plane and the immense possibilities of multiplanar display consequent to interactive manipulation of acquired data sets, has placed this modality as the optimal method for evaluating the structurally anomalous uterus.

In a septate uterus the septum may be shallow (subseptate) or extensive (down to the external os), fibrous, muscular or mixed. Coronal plane analysis is necessary for assessing this condition and differentiating it from a bicornuate uterus. Recognition of this condition is imperative because obstetric outcomes greatly improve with hysteroscopic metroplasty in appropriately selected cases. With bicornuate and didelphys uteri, the approach has to be an abdominal one. In recent years, 3D criteria have helped greatly in patient selection17,18. The muscular septum lacks supporting connective tissue19, and shows poor preovulatory changes and poor decidualisation with consequently poor placentation. Additionally, a vascular septum shows enhanced irritability20 and this predisposes to spontaneous abortions. Most septa show poor vascularisation responses in the pregnant state. Although the incidence of septate uteri is similar in patients with recurrent pregnancy losses compared to women with normal obstetric outcomes, there is no doubt that the primary prognostic factor is the height of the residual undivided uterine cavity and not the height of the septum. It has been traditional obstetric practice not to treat a subseptate uterus until two pregnancy losses have occurred. Poor obstetric outcomes and the accurate information obtained by 3D now justify septal resection by a hysteroscopic method as soon as they are diagnosed.

A true unicornuate uterus is the result of complete unilateral arrest of Mullerian development. If the arrest of development is partial, as is more usual, a rudimentary horn with or without functioning endometrium is present. 2D ultrasound is frequently unable to identify the subtle findings of a unicornuate uterus. 3D rendered images reveal a banana shaped relatively narrow uterus with or without a rudimentary horn. 3D Multiplanar imaging helps to confirm a single cornual angle21.

Hysteroscopic polypectomy improves pregnancy rates in patients with endometrial polyps22,23. 3D depiction of the endometrium improves the sensitivity of identifying focal lesions and accurately identifies location of the lesion24. Endometrial polyps are usually echo-rich and fibroid polyps are echo poor. Degenerated fibroid polyps may be echo rich. 3D rotations of ultrasound images enhances the identification of multiple polyps. 3D Power Doppler studies help not only in assessing vascularity of a polyp but in identifying the exact point of origin of the polyp.

Hysteroscopic myomectomy is currently the method of choice to improve the cumulative pregnancy rate as well as the live birth rate in selected women with submucous myomas and a history of reproductive failure25,26. Transvaginal scans with 7.5 MHz or higher frequency transducers offer excellent identification of submucous myomas. 3D Multiplanar views and the rendered coronal view offer an unsurpassed and exquisite delineation of fibroids in relation to the endometrium27,28,29,30. This demonstration of submucous, interstitial (intramural) and subserous extent of the fibroid, assists in planning an optimal surgical approach: hysteroscopic resection, laparoscopic myomectomy or conventional laparotomy. The location, number and size of fibroids can be more accurately evaluated by 3D rather than 2D. Infertile patients with endometriosis who also have focal or diffuse adenomyosis show hyperperistaltic and dysperistaltic uterotubal transport capacity31. Identification of adenomyosis and subsequent consideration for its attempted suppression with a GnRH analog prior to treatment cycles, therefore merits attention. For many years, workers believed that ultrasound has a poor sensitivity for identifying focal and diffuse adenomyosis. This is a myth. Adenomyosis can be delineated on transvaginal ultrasound and the sensitivity compares well with MRI. Findings include thickening of the posterior wall or entire myometrium, increased transverse diameter of the uterus relative to the length of the uterus, focal or diffuse speckling of the myometrium, thin walled, clear, myometrial cysts, a bosselated contour of the uterus and an obliteration of the subendometrial hypoechoic stripe.

Hydrosalpinx fluid is associated with impaired implantation rates32,33,34 in IVF. Laparoscopic procedures may enhance treatment outcomes in selected patients. In every infertile patient, therefore, it is worth analyzing the image morphology of any extra-ovarian adnexal fluid loculation in order to identify a fluid laden hydrosalpinx. This may have a spectrum of ultrasound morphology including a unilocular collection, multiloculations or a classical retort shape. The walls may be thick or thin and the contained fluid may be clear or echogenic. 3D software that enhances the computer aided recognition of fluid spaces enhances the ability of recognizing and characterizing tubal fluid collections.

Prediction of ART Outcomes

Ultrasound technology is making a major impact in the prediction of ART outcomes and in the understanding of endometrial receptivity.

The Antral Follicle Count35,36,37,38,39 done on any day between cycle day 2-5 yields a count of less than 4-5 in poor responders. The count correlates well with number of follicles

later in the cycle, E2 levels on the day of HCG administration, number of oocytes, fertilization rates and pregnancy rates. This can be done by 2D manual methods or a 3D computer assisted model35,40. Smaller ovarian diameter and ovarian volumes also correlate fairly well with poor response to ovarian stimulation and poor pregnancy rates. In patients with lower antral follicle counts the ovarian response to gonadotrophins can be modified by using an increased dose at the commencement of the cycle and by instituting medication that enhances ovarian flow35. Although results of studies correlating ovarian stromal blood flow as studied by 2D power Doppler and ovarian response during IVF were disappointing41, subsequent shifts to 3D power Doppler studies and reliance upon 3D flow indices rather than Peak Systolic Velocities have shown a reliable correlation.

The parameters that have been studied over the past two decades, moreso the last few years, include endometrial thickness, endometrial volume, endometrial ultrasound morphology, subendometrial peristalsis, endometrial and subendometrial vascularisation, subendometrial vascularisation, myometrial echogenecity, myometrial power Doppler, spectral analysis of uterine artery flow velocity waveforms and perifollicular vascularisation.

Endometrial thickness below 6-8 mm is rarely associated with conception42,43,44,45. Increase in endometrial thickness above this level, however, does not enhance implantation rates and there is no difference in the mean endometrial thickness in patients who become pregnant and those who do not become pregnant in ART cycles46. The minimum endometrial volume associated with pregnancy is 1.59 ml as calculated by three-dimensional ultrasound44. This is calculated using a manual or semi-automated planimetry and automated software called VOCAL, Virtual Organ Computer Aided Analysis.

Endometrial layering into a triple layer is associated with good implantation rates. Conversely, a homogeneous or a heterogeneous endometrium in the proliferative and midcycle phases is associated with poor outcomes42,44. Premature echogenic transformation of the endometrium, a consequence of progesterone action, if observed on the day of HCG administration or on the day of ovum pick-up, is associated with poor implantation rates47.

Less than three peristaltic contractions of the subendometrial myometrium over a 2 minute interval on the day of hCG administration are associated with poor implantation rates43. An inhomogeneous, poorly vascular myometrium is usually associated with poor implantation although the statistical significance of this preliminary observation needs further proof43.

By far the best correlation with implantation rates are being observed with power Doppler43 and three dimensional (3D) power Doppler studies48,49,50. Ultrasound delineation and quantification of endometrial and subendometrial angiogenesis is emerging as a reliable and reproducible indicator of endometrial receptivity51,52. Basic static 3D and Virtual Organ Computer Aided Analysis (VOCAL) and 3D shell imaging have been used to assess and quantify endometrial and subendometrial vascularisation48,49,50. The Vascularisation Index (VI), Flow Index (FI) and Vascularisation Flow Index of endometrial and subendometrial vessels increases during the proliferative phase, peaks 3 days prior to ovulation and decreases to a nadir 5 days post ovulation50. Endometrial and subendometrial VI/ FI/VFI is significantly lower in stimulated cycles than in natural cycles49. Smoking is associated with significantly lower VI and VFI. Patients who become pregnant have a significantly lower Resistive Index (RI) of subendometrial vessels: 0.53 compared to 0.64 +/- 0.04 in pregnant and non pregnant patients respectively48. Nondetectable subendometrial artery flow is not associated with a lower implantation rate44.

Uterine artery flow velocity waveforms show a remarkably good correlation with endometrial receptivity and implantation rates. Pregnant patients show considerably lower RI and pulsatility index (PI) values compared with the nonpregnant group, 5-6 days after ET46. The chance for pregnancy is almost zero if the PI is more than 3.019 on the day of hCG administration.

Inner zone vascularisation of the endometrium observed on the day of hCG administration or on the day of embryo transfer is associated with higher pregnancy rates. Quantification can be done with VOCAL which involves a 3D acquisition followed by semi-automated planimetry. Preretrieval hCG does not enhance endometrial PI although more embryos are generated53. Interestingly, impedance in the uterine and spiral arteries does not show any significant difference between normal pregnancies, missed abortions and anembryonic pregnancies54.

Vascularisation of maturing follicles has been graded on the percentage of follicular circumference seen to be vascularised using power Doppler techniques (Grade 1 < 25%, Grade 2 < 50%, Grade 3 < 75% & Grade 4 > 75%). Mean follicular diameter, oocyte retrieval rate, number of mature oocytes recovered & fertilization rates are all higher and triploidy rates significantly lower from follicles with > 50% vascularity. Pulsatility Index values of perifollicular flow do not correlate with pregnancy outcome. However, the Peak Systolic Velocity (PSV) is an excellent parameter to assess the chances of obtaining mature oocytes & high grade preimplantation embryos. The chances of producing a Grade I or II embryo is 75% if the PSV is > 10 cm/second. Interestingly, during the ovulatory process there are prominent changes in the regional blood flow of the follicle with a marked increase in flow to the base of the follicle & a concommitant decrease of blood flow to the apex. These changes may be essential for the release of a mature oocyte.

Patients with a PSV of > 10 cm/second in ovarian stromal arteries after pituitary suppression yield significantly higher mature oocytes & achieve a higher clinical pregnancy rate. Ovarian stromal Pulsatility Index does not correlate with these parameters. Interestingly Pulsatility Index, Resistive Index & Systolic/ Diastolic ratio of ovarian stromal arteries during ovulation induction correlates with the chances of developing ovarian hyperstimulation syndrome. If the Resistive Index < 0.48, more than two thirds of patients will develop a pleural

effusion. Over one half of patients with a Pulsatility Index < 0.75 & a Systolic/ Diastolic ratio < 1.92 develop pleural effusions.

Thin walled clear cysts discovered in baseline scans on the day of commencement of induction of ovulation have traditionally been aspirated. Evidence is building up, however, that apart from a small increase in the amount of gonadotrophin required and a minimal increase in the number of days of stimulation no other detrimental effects have been established55.

Ovulation Monitoring

The sequence of events that occurs in the natural menstrual cycle has been well characterised. The initiation of follicular growth is a continuous process that is independent of gonadotrophin stimulation. Gonadotrophin-independent growth proceeds until the follicle reaches 5 mm. High frequency transducers will always demonstrate follicles of this size in all women in the reproductive age group. Further growth of the follicle occurs only in an appropriate gonadotrophin environment. A decline in follicle stimulation hormone (FSH) occurs by day 6 to 9 in spontaneous cycles and is responsible for the selection of the single most mature follicle. Follicles with fewer FSH receptors on their surface will become atretic. Once the leading follicle reaches a diameter of 14 mm (Figure 29), it shows a daily growth of 1.5 to 2.3 mm per day until just before ovulation. Estradiol (E2) is produced by the granulosa cells and ovulation usually occurs after the serum E2 reaches 150 to 400 pg/ml. Extrusion takes place consequent to a surge of luteinising hormone (LH) at 18-24 mm size. In this size range, follicular growth is exponential and stands at 2.6 to 6.0 mm in 24 hours. The cumulus oophorus can be identified within a mature follicle frequently if high frequency transducers are used. It is seen as a mural echogenic focus 1 to 3 mm in maximum diameter. Following follicular extrusion, the corpus luteum can be visualised in the ovary.

A baseline evaluation, as deduced from the above events, is necessary on Day 1, 2 or 3 of the cycle with a follow up on Day 5 if a cystic area is seen. Follow up visits can then be scheduled depending on spontaneous cycle length & medication. Typically, for a 28 day cycle, this would mean daily monitoring from Day 10 upto extrusion. Luteal phase studies should be done on Day 07, 11 and 14 postextrusion.

In stimulated cycles the essential difference is the number of follicles that undergo maturation and the rate of follicular growth. Follicular growth is generally accelerated to 1.8-2.9 mm per day. Since endometrial disease is more evident in a thicker endometrium, the appearance of polyps or submucous fibroids, should be looked for at each sitting for follicular monitoring.

Ovarian Hyperstimulation correlates best with the observed number of smaller (10-14 mm) follicles in the late proliferative phase and not to the total number of follicles and with stromal flow.

Conclusion

Ultrasound has emerged as an indispensable component of treatment cycles and its role in ART is continuously expanding.

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8. Role of tubal surgery in the era of art

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The tubal disease accounts for 25% - 35% of female factor infertility, with more than half of the case due to salpingitis. In addition, large studies report that up to 20%-30% of women regret having a tubal ligation. Thus, there is a need to determine the optimal treatment methods for patients with tubal factor infertility. There are several surgical options for achieving patency in obstructed fallopian tubes, depending on the location of the blockage. This presentation reviews these procedures and the factors that must be considered when deciding between surgical repair and in vitro fertilization (IVF).

Many variables need to be taken into consideration when counseling patients with tubal infertility regarding corrective surgery or IVF. The age of the patient, ovarian reserve, prior fertility, number of children desired, site and extent of the tubal disease, religious beliefs, cost and insurance reimbursement also figure into the equation. In addition, a semen analysis should be performed early in the infertility investigation as the results may influence the management decision between tubal surgery and IVF.

The advantages and disadvantages of IVF and tubal surgery need to be reviewed with the patient to provide assistance in

her decision making. The main advantages of IVF are good per-cycle success rates and the fact that it is less surgically invasive. Its disadvantages include cost (especially if more than one cycle is required), the need for frequent injections and monitoring for several weeks, and ovarian hyperstimulation syndrome. Although perhaps not directly applicable to tubal factor infertility. IVF alone has been associated with a higher incidence of adverse perinatal outcomes in singleton infants, such as perinatal mortality, preterm delivery, low and very low birth weights, intrauterine growth restriction and congenital malformations.

The advantages of tubal surgery are that it is a one-time, usually minimally invasive outpatient procedure, and patients may attempt conception every month without further intervention and may conceive more than once. They also avoid the risks associated with IVF. The disadvantages are generalizable for surgeons with less skill and experience and include the risks for surgical complications, such as bleeding, infection, organ damage, and reaction to anesthesia. There is also postoperative discomfort during the short recovery phase. Although the risk of ectopic pregnancy is increased in patients having IVF for tubal disease, it is higher after tubal surgery. In addition, for some patients the success following tubal surgery may be significantly lower than for IVF. All of these factors need to be considered when choosing the appropriate treatment strategy. To optimize pregnancy rates and reduce the risks, only those surgeons facile and experienced in laparoscopic and/or microsurgical techniques should attempt to perform corrective tubal surgery. The ideal patient candidate for tubal surgery is young, has no other significant infertility factors, and has tubal anatomy that is amenable to repair.

Proximal tubal blockage accounts for 10% - 25% of tubal disease. Unless the proximal blockage on HSG is clearly due to SIN, selective salpingography or tubal cannulation can be attempted. Tubal cannulation is accomplished using a coaxial catheter system under fluoroscopic guidance of via hystertoscopy with laparoscopic confirmation. Although tubal patency rates are similar with both fluoroscopic and hysteroscopic techniques, a meta-analysis found that ongoing pregnancy rates are higher with hysteroscopic cannulation.

The decision to repair or remove fallopian tubes with distal tubal diseases is usually based on intraoperative findings. Distal tubal diseases are mainly hydrosalpinges and fimbrial phimosis caused mostly by PID. A good prognosis is associated with patients who have no more than limited flimsy adnexal adhesions, mildly dilated tubes < 3 cm with thin and pliable walls and a lush endosalpinx with preservation of mucosal folds. Laparoscopic neosalpingostomy and fimbrioplasty are carried out by opening a hydrosalpinx or by increasing the opening for fimbrial phimosis, respectively. Fimbria are then everted and made secure by Bruhat's procedure. Neosalpingostomy and fimbrioplasty should be performed by laparoscopy only.

Patients having distal tubal blockage with poor prognosis may have extensive dense peritubal adhesions, massively dilated tubes with thick fibrotic walls, sparse or absent tubal mucosa. Laparosacopic salpingectomy is indicated when fallopian tube is damaged beyond repair by infection, endometriosis or ectopic pregnancy. Many studies have shown that hydrosalpinges have a detrimental effect in IVF success rates. Cochrane analysis concluded that laparoscopic salpingectomy or occlusion should be considered before IVF for women with communicating hydrosalpinges, both for unilateral or bilateral cases. However, tubal sugeries have been found to have no effect on ovarian stimulation on subsequent cycles.

In patients with prior tubal ligation the decision regarding whether to undergo tubal anastomosis or IVF should take into consideration the pros and cons of each treatment option for that individual couple. These patients are fertile and have better success rates than patients of tubal pathology. Tubal reanastomosis can be performed by either laparotomy or laparoscopy (only experienced surgeon with expertise should perform). Studies have found that tubal anastomosis had a significantly higher cumulative pregnancy rates for women younger than 37 years of age, but there was no significant difference in women aged 37 years of age or older. In addition the average cost per delivery for tubal anastomosis was almost half that for IVF. The decision regarding whether to have tubal anastomosis or IVF is left up to the patient, after reviewing the pros and cons of each treatment option.

Conclusion

The evidence is fair to recommend

a) Tubal cannulation for proximal tubal obstruction in young women with no other significant infertility factors,
b) Laparoscopic fimbrioplasty or neosalpingostomy for the treatment of mild hydrosalpinges in young women with no other significant infertility factors.

There is good evidence for recommending

a) Laparoscopic salpingectomy or proximal tubal occlusion in cases of surgically irreparable hydrosalpinges to improve IVF pregnancy rates.
b) Microsurgical anastomosis for tubal ligation reversal.

9. Sperm selection in art and the role of paternal genome

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It has been well established that increasing maternal age leads to a higher risk of infertility, miscarriage, and chromosomal defects in offspring. The well documented increase in age, at which women conceive their first child, has detracted from a similar change observed in males. As both males and females decide to conceive later, the question of whether this may impact their fertility individually and as a couple becomes even more crucial. While paternal age of over 40 years at the time of conception is a frequently quoted male age threshold, currently

there is no clearly accepted definition of advanced paternal age or even a consensus on the implications of advancing male age.

In this lecture I will review some of the potential risks to the offspring of advancing male age and the data available regarding pregnancy outcomes based on paternal age in both the fertile and infertile populations. Finally, I will discuss the various mechanisms by which male age may impact sperm and fertility potential, including sperm DNA damage and discuss techniques that may allay the impact of a faulty paternal genome, including current and future sperm selection technologies.

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10. What is the aim of IVF treatment?

Dhiraj Gada

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We do not harm women undergoing IVF treatment, regardless they get pregnant, regardless they have babies and we consider long-term health of the babies. Apart from the mother and child, we have an obligation to the society. It is our responsibility to make IVF accessible to the whole country and affordable to low-income group. We must avoid unnecessary investigations and interventions. We have to learn minimum use of drugs. BUT we live in a Competitive, Commercial Environment where being in private sector, we are under the pressure to achieve high pregnancy rate… but at what cost?

We must accept MILD APPROACH or THE LITE IVF PROGRAMME. We use it for patients who are poor responders but it should not be so. These programmes are best for larger populations who have good prognosis. Ideal cases are patients below the age of 38 years, with normal ovarian reserve and no other auto-immune problems. Here we get better quality oocytes, embryos and endometrium at an affordable cost with single embryo transfer.

Apart from treating the patient we must take care of the psychological aspect of infertility. Worldwide only 55% infertile couples take treatment, 22% terminate treatment before completing desired course of success. The scenario in India is still worse. Most of IVF centre conversion rate is not more than 20%. There is paradigm shift for mental health profession and we need better insight. Has anybody thought about the psychological care of our laboratory and paramedical staff? We need to take a U- turn before starting infertility treatment. Let the patient's general health be optimum, control the weight, improve the nutritional status, eliminate addictions, treat pre-existing conditions and give good psycho-therapy. This U-turn will ultimately give you much better results.

Let us look at the rationale of investigations. Investigations are necessary for three reasons. 1) To reach a diagnosis, 2) To monitor the response of treatment, 3) To avoid medico-legal litigations. Unfortunately, health care costs have sky-rocketed because we follow western standards without logical reasons to advice investigations. Can't we reduce the cost of unnecessary investigations?

To make IVF an accessible programme, we must teach our colleagues from rural area at an affordable cost. We can start a teaching institute with boarding facilities and a cost effective programme. I am sure all the experts seated in the audience would like to contribute to this programme.

Do you think that we must do some basic and advance research and come out with our own guidelines? All this can be done if we look into our souls and unite with to form an institute.

It will not be MY Institute, It will not be YOUR Institute….. it will be OUR INSTITUTE.

11. The endometrium in recurrent implantation failure – Strategies for improving implantation potential

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Recurrent implantation failure refers to failure to achieve a pregnancy after transfer of at least four good-quality embryos in a minimum of three fresh or frozen cycles in a woman under the age of 40 years. The failure to implant could be due to embryo or uterine factors.

Uterine receptivity is defined as a restricted time-related period when the uterus is receptive to blastocyst attachment and implantation. The implantation window is a period during which the endometrium is optimally receptive to implanting the blastocyst. The window occurs between LH+7 and LH+11 and has many phases – signaling, apposition, adhesion & invasion.

The key factors involved are luminal epithelial pinopodes, expression of adhesion molecules and novel cytokine profile. Assessing the endometrial receptivity is by histological assessment of the endometrium, morphological assessment, functional assessment of the endometrium by ultrasound, hysteroscopy biomarkers and omics.

The endometrial receptivity array for diagnosis and personalized embryo transfer as a treatment for patients with repeated implantation failure is now coming up.

Ultrasonography is a time tested modality which helps to assess the endometrial thickness, volume, pattern, pulsatile and resistance index of the uterine vessels, vascularisation index (subendometrial and endometrial flow) as well as identify patients with poor implantation prognosis but is of low predictive value.

Biomarkers are in the research setting where during the window of implantation, screening could be performed for some of the known 'likely'players, including pinopodes, mucins, OL$3, trophinin/tastin EGF, HB-EGF, amphiregulin, CSF-1,LIF, IL-lp, calcitonin, Hoxa-10, and COX-2.

Diagnostic and therapeutic hysterolaparoscopy should be done to tackle intra uterine problems and manage hydrosalpinges effectively. The presence of a thin/ hyperechogenic endometrium and / or persistent endometrial fluid are challenges in the management of recurrent implantation failure.

Low dose aspirin and vaginal sildenafil have a role in improving uterine blood flow. Intra uterine cavitory administration of G CSF and vaginal administration of micronized estradial and progesterone help in improving the implantation site. Site specific endometrial injury also helps in improving the endometrium. Low dose HCG, immunotherapy, corticosteroids and intra lipids have been introduced empirically in the IVF programme.

A multi-modal approach should be adopted in the management of Recurrent Implantation Failure.

12. Newer molecules in the treatment of endometriosis. Help or harm?

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Endometriosis is a disease with chronic debilitating disease associated with chronic pelvic pain, dysmenorrhoea, dyspareunia and infertility. Often they have to undergo multiple surgery as recurrence is very common. The medical treatment of endometriosis has long centered upon producing a hypoestrogenic environment by producing pituitary suppression or a progestin-dominant environment. However, as more is uncovered regarding the pathogenesis and pathophysiology of this disease, more targeted therapies can be developed.

Uptil now the treatment was

1. Combined Oral contraceptive pills
2. Progestins
3. GnRh analogues
4. Androgenic agents –Danazole
5. Surgery.

The current available treatment does not ensure freedom from pain, recurrence is common, weight gain is a problem with some agents, Danazole has gone out of vogue and GnRh agonists can affect the bone mineral density if given for longer periods, though till today for ART patients we use it as pretreatment in endometriosis.

There are newer molecules are available and their effectiveness has been studied.To enumerate them we have:

Newer progestin: Dionogest: Pharmacologically, dienogest combines the advantages of the 19-norprogestin and the progesterone derivative classes. When given continuously, induces a hypoestrogenic, hypergestagenic local endocrine environment, causing a decidualization of endometrial tissue followed by atrophy of the endometriotic lesions (Sasagawa:2008). Studies show a prompt return to fertility (eg, mean about 30 days) and include cases of successful pregnancy in women with endometriosis following the cessation of dienogest treatment 2 mg daily for durations up to one year. (Petraglia:2011)

GnRh Antagonists: we have studies to show that GnRh antagonists given as 3mgm/week for 8 weeks reduces the lesions and then one can take them up for ART. It does not affect the bone mineral density either and there is no need for estrogen add back therapy.

Aromatase inhibitors: Letrazole, Anastrazole target Aromatase enzyme P450 to decrease local estrogen synthesis and thus to inhibit the growth of endometriotic implant. (Bulun et al., 2004).They are effective in reducing the pelvic pain scores (Ailawadi:2004).

Progesterone receptor modulators. Mifiprestone In doses of 50mg/day is found to reduce the endometriotic lesions considerably.More RCTs are needed

TNF alpha inhibitors, Angiogenesis inhibitors, Matrix metalloproteinase Inhibitors

Pentoxifylline (and ither general immune modulators) have all been tried in endometriosis but more RCts are needed.

13. PCOS - GnRH agonist trigger: Current concepts

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Human Chorionic Gonadotrophin has been the gold standard to trigger the final oocyte maturation and induction of ovulation.

This however, is associated with the high risk of OHSS in hyperresponders by:

1) Mediating the release of vascular endothelial growth factor-A which promotes angiogenesis and vascular hyperpermeability.
2) prolonged luteotropic effect resulting in multiple corpora lutea and supraphysiological levels of oestradiol and progesterone in ART cycles.

To reduce the incidence of OHSS in PCOS patients, GnRH antagonist is the protocol of choice as it gives the opportunity to trigger ovulation with GnRHa and possibly freeze all embryos (Kol et al. 2012). A midcycle single bolus of GnRH agonist may be injected sub-cutaneously 0.2 to 0.5 mg of triptorelin, leuprorelin or busere-lin. It is associated with a simultaneous induction of FSH surge comparable to the surge of a natural cycle.

The results of using GnRH agonist and HCG were comparable regarding the number of oocytes capable of being fertilized and undergoing embryonic cleavage. The incidence of empty follicle syndrome was also found to be similar.

An inferior pregnancy rate in fresh autologous transfers is a drawback, this is not due to an effect on the embryo quality

but due to inadequate corpus luteum formation and a defective luteal phase causing poor implantation.It is also associated with a high rate of early pregnancy loss.

Newer stratagies to correct the luteal phase insufficiency includes :

Dual Trigger - Supplementing the GnRH analogue with low dose of HCG (1500 iu) to trigger ovulation (Humaidin et al. 2006).

Repeated low doses of HCG during the luteal phase normalizes the reproductive outcome in a high risk group of IVF/ICSI patients.

Good Estrogen and Progesterone support in these group of patients.

With this modified lutel phase support, pregnancy rate is comparable as with HCG group and it also lowers the incidence of early pregnancy loss.

Oocyte maturation triggering with GnRH agonists may provide several advantages over that achieved with HCG.

- First, GnRH agonists reduce the risk of OHSS due to quick an irreversible luteolysis (Kol 2004).
- Second, a more physiological LH and FSH surge is induced by the agonists, which may result in better oocyte and embryo quality (Humaidan 2005).
- Third, GnRH agonists may improve endometrial quality as a result of the lower luteal phase steroid levels (Forman 1998; Simon 1998).

14. The role of sperm in achieving the best embryo in-vitro

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The importance of paternal contribution to the development of an implantable embryo in vitro has received extensive debate. A mature sperm delivers a haploid set of chromosomes, an oocyte activation stimulus, functional centrosomes. Centrosomes are essential for the maintenance of sperm euplody, formation of microtubules- organising centre and facilitate proper segregation of chromosomes during cell division. Sperm also contributes a competent genome with proper packaging and coding of the genome through imprinting and chromatin modifications. The importance of mRNAs as a regulator of transcription can not be ignored. It also compliments maternal transcriptome within the cleavage stage embryo. A lack of sperm-specific activating protein and defects in the centrosome of the sperm can compromise early cell division in human embryo (the so-called early paternal effect).

In recent years, the intactness of sperm DNA- the nuclear sperm deficiencies and what effect a strand breaks would have on the development of the resulting embryo and also on implantation has been revisited. In addition to the routine semen analysis, a number of leading andrology laboratories now advocate the introduction of Sperm DNA integrity testing as a routine assessment and to use it as a predictor of blastocyst development and implantation. It is now well established that the activation of the embryonic genome (EGA) on day 2/3 (8 to 10 cells stage) of human embryos will influence the morphokinetics of the resulting blastocyst. The switch from a transcriptionally quiescent to an active embryonic genome displays chromosomal or genetic errors of paternal genes - 'late paternal effect'. The late paternal effect is often suggested to be associated with DNA fragmentation and disorganisation of the sperm chromatin. It is known that individuals with balanced translocations often produce gametes with chromosomal aberrations that may in turn result in various forms of reproductive failure, ranging from defective gametogenesis to recurrent spontaneous miscarriage.

The introduction of motile sperm organelle morphology examination (MSOME), has enabled the evaluation of the very fine subtle nuclear morphology changes of motile spermatozoa in real time at high magnification. These observations have not only helped embryologist select normal spermatozoa, defined by the shape and size but also to select spermatozoa devoid of nuclear vacuoles located in sperm head.

There is a growing catalogue of evidence suggestive of the fact that large nuclear vacuoles are a sign of nuclear dysfunction, reflecting a failure of chromatin condensation and packaging. Furthermore, it has been suggested that the negative impact of large vacuoles is evident after the onset of the EGA leading to reduced blastocyst formation. Also, ongoing pregnancy rates, miscarriage rates and malformation in offspring have been attributed to the large nuclear vacuoles.

In the absence of an acceptable universal algorithm that takes the importance of the sperm factor into account, any criteria used for embryo selections have a potentially high risk of embryo wastage.

15. G-CSF is the answer for the obstinate endometrium?

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Quality of Embryo and Endometrial receptivity are the most important factors for the success of IVF. Endometrial thickness has been accepted as one of the indicator of Endometrial receptivity and an assessment of endometrial <7mm is widely considered suboptimal for transfer with significant reduction in implantation rate and pregnancy rate. Thin endometrium can be due to iatrogenic response like repeated D&Cs but most of times the causative agent remain unknown.

Various remedies have been proposed, including extended estrogen administration if time allows (Chen et al., 2006), low-dose aspirin (Weckstein et al., 1997) and treatment with pentoxifylline (800 mg/d) and tocopherol (Vitamin E 100 IU/d)

(Le´de´e-Bataille et al., 2002) and with vaginal sildenafil citrate (ViagraTM) (Sher and Fisch, 2002). However, even utilizing these remedies,a small number of women remain unresponsive. The prevalence of patients who remain unresponsive to such standard treatment have estimated it to be <1%. such patients and for their treating physicians, such a chronically thin endometrium offers considerable treatment challenges,i) resulting in cycle cancellations, ii) unplanned cryopreservation of embryos and, in the most extreme cases,iii) in the utilization of gestational carriers.

Decidua exerts control over trophoblast invasion via secretion of cytokines. That local injury can induce endometrial decidualization and improve implantation has been known since 1907. Barash et al. applied planned endometrial injury to human IVF, increasing pregnancy rates. Massive release of cytokines and growth factors from injured endometrium has been suggested as an underlying process. Granulocyte colony-stimulating factor thus demonstrates divergent roles in reproduction, having distinct effects on endometrium and implantation. A potentially growth-expanding effect on endometrium may be suspected from its role in establishing early endometriotic lesions.

Granulocyte colony-stimulating factor (GCSF) is a recently discovered cytokine. In the female reproductive tract, G-CSF is synthesized under the regulatory influence of estrogen in uterus. Fertility declines in G-CSF deficient mice. Few data exists about G-CSF use for IVF patients with thin endometrium and recurrent aborters with promising results. However, exact way of administration, timing and dosing are still debatable...

F. Scarpellini and M. Sbracia in 2009 reported that G-CSF may be effective in the treatment of unexplained RM (1 mg/kg/day S/C) starting on the sixth day after ovulation.

In 2011 Gleicher et al described, for the first time, the use of rG-CSF for improvement of the endometrium in cases of women undergoing IVF with thin endometrium. At that time, four patients between 33 and 45 years of age were treated with intrauterine infusions of rG-CSF [30 mU (300 µg/1 mL)]. All patients became pregnant and had ongoing pregnancies, except for one patient who experienced an ectopic pregnancy.

Again in 2013, Gleicher et al. reported uncontrolled cohort study involving 21 patients in whom rG-CSF was administered by an intrauterine route to improve endometrial thickness Patients assigned to receive 30 mU (300 µg/mL) of rG-CSF approximately 6-12 hrs before hCG trigger had an endometrial thickness of 7 mm. A growth spurt in endometrial thickness can be observed in 48 hrs. If ET still <7mm a second infusion of G-CSF performed following oocyte retrieval.

Michał Kunicki et al in 2014. presented a study on 37 patients to assess the granulocyte colony-stimulating factor (G-CSF) effects on unresponsive (< 7mm) endometrium in women undergoing in vitro fertilization (IVF) The endometrium significantly increased after infusion of GCSF. The increase of endometrium thickness was greater in group of women who conceived but the difference between groups was not statistically significant.

David H. Barad et al. Fertili Steril 2014, presented 141 consecutive, unselected, consenting women with no history of renal disease, sickle cell disease, or malignancy who were undergoing IVF. In normal IVF patients, G-CSF does not affect endometrial thickness, implantation rates, or clinical pregnancy rates. Because these results were obtained in an older patient population, they may not necessarily apply to younger women.

Uterine perfusion with G-CSF represents a promising new tool for the currently mostly intractable problem of inadequate, thin endometrium. This treatment also deserves further investigation for its potential to improve implantation chances in association with IVF and, therefore, pregnancy rates

16. Tips and tricks in semen preparation

Kersi Avari

The most dynamic and motile cell of the body with its phenomenal power and punch exhibiting linearity and excellent yawing is albeit instrumental in achieving fertilization -the phenomenon which till date remains most mysterious!!. The rapid strides achieved in ART and boisterous support provided by innovated gadgets and tools help us achieve the "once upon a time thought unachievable phenomenon" by the unfortunate OAT male. The priming or the activation of the sperm achieved by the terminology "sperm wash" is an optimization achieved for the subsequent functional capacity. An insight into various fields and aspects need to be deeply looked into for its fruition, which unfortunately are bypassed since the advent of ICSI. So the easy, cheap yet effective modes of ART with their optimal indications can still be effectively implemented with a favourable outcome. In depth reading of the semen sample, optimal culturing, harvesting and an effective recovery without compromising the structural and functional capacity and integrity of the sperm can easily elevate the infertile couple from the physical, mental and financial trauma which they usually face. The current presentation highlights such points with explanations and scientific principles.

17. Endometrial scratch-Have we accepted it as a practice in RIF?

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Successful ART treatment cycles depend mainly on two factors – good quality embryos transferred and a receptive endometrium. Implantation is a key event in the establishment of pregnancy. Recurrent implantation failure (RIF) is an iatrogenic condition, being the result of repetitive unsuccessful cycles of IVF or intracytoplasmic sperm injection (ICSI) treatment. There is lack of uniformity of the definition of RIF. It should be defined as the absence of implantation after two consecutive cycles of IVF, ICSI or frozen embryo replacement cycles where the cumulative number of transferred embryos was no less than four for cleavage-stage

embryos and no less than two for blastocysts, with all embryos being of good quality and of appropriate developmental stage. (Polanski et al 2014). It is estimated that approximately 10% of women seeking IVF treatment will experience RIF. It may be attributed to poor endometrial receptivity. Endometrial scratch improves endometrial receptivity by inducing decidualisation and promoting local secretion of cytokines, NK cells and interleukins, all beneficial for embryo implantation. In controlled ovarian stimulation, corrects asynchrony between endometrium and the conceptus and thereby helps in improving implantation rate. (Cochrane review 2012)

A systematic review and meta-analysis of studies was conducted by Potdar et al 2012 comparing the efficacy of endometrial injury versus no intervention in women with RIF undergoing IVF. Seven controlled studies were pooled (four randomized and three non-randomized), with 2062 participants. It was shown that local endometrial injury induced in the cycle preceding ovarian

stimulation is 70% more likely to result in a clinical pregnancy as opposed to no intervention. Clinical pregnancy rates were twice as high with biopsy/scratch (RR 2.32, 95% CI 1.72–3.13) as opposed to hysteroscopy (RR 1.51, 95% CI 1.30–1.75).

RCT by Baum et al (2012) did not find any benefit from local injury to the endometrium in women with a high number of RIFs. The sample size was small (36) in his study.

In an observational prospective cohort experimental study by Nossair (2014), 30 patients with previous failed IVF despite transfer of good-quality embryos were allocated to endometrial scratch-suction from day 6 to day 7 of same ICSI cycle, using insertion tube of (IUCD) and infant feeding tube 8F. Implantation occurred in 24/30 (80%) and clinical pregnancy occurred in 20/30 patients (66.66%).40% cases small endometrial polyp during scratch suction.

Although the literature supports the role of endometrial scratching, it is still not clear at what day or phase of menstrual cycle endometrial scratching may be more beneficial and the number and degree of endometrial injuries and the time between injury & ET cycle.

Endometrial scratching is easy and cost effective practise which may be useful in clinical practice to improve ART success rate.

However, large randomized studies are required before iatrogenic induction of local endometrial injury can be warranted in routine clinical practice.

18. Stimulation protocols for cancer patients

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The patients referred for fertility preservation owing to a malignant disease do not represent the typical population of subfertile patients treated in IVF units. Cancer may affect multiple tissues throughout the body and can result in a variety of complications during controlled ovarian stimulation. Determination of the controlled ovarian stimulation protocol and gonadotropin dose for oocyte/embryo cryopreservation requires an individualized assessment.

When stimulating women with cancer for IVF it is of utmost importance to look at time and safety of the drugs used. Ovarian stimulation, oocyte retrieval and IVF cause a delay in the initiation of chemotherapy or radiotherapy that may not be acceptable in some cases. Moreover high estrogen concentrations associated with ovarian stimulation may be contraindicated in women with estrogen sensitive malignancies. Hence it becomes mandatory to select protocols with minimal risk. As there is urgency in staring the cycle with limited time in hand, one needs to use a short protocol.

Stimulation Protocols

Conventional

Conventionally, ovarian stimulation for oocyte/embryo cryopreservation with GnRH antagonist is initiated at the beginning of the follicular phase. The usual protocol used for controlled ovarian stimulation in cancer patients is use of letrazole or tamoxifen along with gonadotropins in a GnRH antagonist cycle with GnRH agonist for trigger. The Letrazole is continued after oocyte retrieval especially in cases of estrogen sensitive tumors to keep the estradiol levels low. The gonadotropin dose should be kept low to avoid ovarian hyperstimulation syndrome.

Although aromatase inhibitors are primarily used as adjuvant treatment of hormone-positive breast cancers, they can act as ovarian stimulants yet suppress estrogen levels. As a result, letrozole has been used for ovulation induction in infertility patients and, in the last 10 years, for the purpose of ovarian stimulation for fertility preservation via oocyte or embryo cryopreservation in women with estrogen-sensitive cancer. When combined with standard fertility drugs, letrozole enhances ovarian stimulation while keeping estrogen levels near physiologic levels. Use of Letrozole resulted in similar embryo yield as tubal disease but with 44% lower FSH dose requirement. Thus this approach results in similar numbers of eggs and embryos and similar pregnancy outcomes. Short-term follow-up indicated no impact on cancer-free survival.

Random-Start Controlled Ovarian Stimulation Protocols

Conventional stimulation protocol may require 2–6 weeks, depending on the patient's menstrual cycle day. Due to the urgent need of medical or surgical intervention in patients with known malignancies, antagonist protocols with random start COS have been used. This approach is designed to provide the shortest time for oocyte collection and claimed to be as effective as conventional-start COS in cancer patients.

Random-start COS protocols minimize delays in cancer therapy. Two different approaches, late follicular and luteal phase, have been suggested for random-start COS protocols.

Late follicular phase

Late follicular phase protocols begin after menstrual cycle day 7 with emergence of a dominant follicle (>13 mm). The GnRH antagonist is administered for 4–5 days, and then stimulation is

begun. The objective of the antagonists of GnRH is to achieve estradiol levels below 60 pg/ml and not to delay treatment until the start of a new physiological cycle.

Luteal phase

In some cases, the administration of gonadotropins had been attempted during the secretory phase to simulate the appearance of a second wave of endogenous gonadotropins that occur in a physiological way. However, ovarian stimulation, which is started during the secretory phase is a strategy which has variable results

Similar to conventional-start COS-GnRH-antagonist protocols, these luteal phase start protocols utilize a GnRH-antagonist administered later in the cycle to prevent a premature LH surge, and continue until the hCG or GnRH-a triggers final oocyte maturation.

Conclusion

Oncology patients present as a unique treatment challenge as the physician must balance the urgency of fertility preservation with the risks of delaying cancer therapy. Controlled ovarian stimulation is routinely applied in assisted reproductive technology but can be contraindicated in women with estrogen-receptor-positive tumors. Therefore ovarian stimulation protocols must be individualized based on time available prior to cancer treatment and fertility status of the patient. Fertility preservation for oncology patients should be carried out with a multi-disciplinary approach, including oncologists and fertility specialists.

19. Time-lapse imaging and aneuploidy screening

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1. Introduction

Time-lapse imaging systems are becoming increasingly used in the human IVF laboratory. Besides undisturbed culture, the exact assessment of the timing of the occurrence of cleavage or any other morphological event has attracted embryologists and researchers likewise. The combination of morphology and kinetics, referred to as morphokinetics, was used to investigate patterns that may be used to assess the implantation potential of embryos compared to each other (Meseguer et al., 2011; Basile et al., 2015). Further studies were aiming at identifying morphokinetic characteristics of aneuploid versus euploid embryos. Some studies indicated that there might be a correlation that parameters assessed by time-lapse imaging are linked to the chromosomal status of an embryo.

2. Aneuploidy prediction by time-lapse imaging

The first study that described a correlation between morphokinetics and aneuploidy detected in trophectoderm biopsies by array-comparative genetic hybridization (aCGH) was published in 2013 (Campbell et al., 2013a). This study used the time of the start of the formation of the blastocoel cavity (tSB) and the time of blastocyst (tB). Based on time values from 98 embryos, the authors defined cut-off points for tSB and tB for low, medium or high aneuploidy risk. In a follow up study these cut-off points were validated for a viable pregnancy based on fetal heartbeat and/or live birth (Campbell et al., 2013b). Others performed similar evaluations on a larger number of day 5 biopsied embryos (Ottolini et al., 2014), but failed to confirm the initial findings.

For day 3 biopsies an algorithm was presented (Basile et al., 2014) that was based on three morphokinetic variables (t5, t5-t3, t5-t2) and classified four categories that showed a different degree of aneuploid embryos. A drawback of this study was the concept of day 3 biopsy, where one would expect a certain number of mosaic embryos.

Aneuploidy prediction is prone to very controversial discussions. Although it is an interesting thought to be able to assess aneuploidy by a non-invasive time-lapse technology, the common understanding is that aneuploidy risk assessment is not acceptable for patients who opt for PGS and do want a 100% accurate diagnosis of their embryos. Still, there may be a niche for aneuploidy risk assessment for patients who reject any invasive biopsy technology out of ethical or religious reasons and are attracted by having a possibility to reducing the overall risk.

3. Combining time-lapse imaging and PGS

Aneuploidy risk assessment cannot only be used for patients who definitely do not want PGS, on the contrary, it may eventually be used to convince patients to opt for PGS. This may apply to patients who present with embryos that have a high aneuploidy risk or low implantation potential based on morphokinetic assessment. In such a situation, PGS may be indicated in order to exclude major chromosomal anomalies and eventually identify the one embryo that may be euploid.

The ultimate way of combining both technologies rather than favoring one or the other is in cases, where after PGS diagnosis more than one euploid embryo is available. In that situation additional information by time-lapse imaging may be helpful to decide which embryo to choose for transfer. Such a study has already been performed and it was reported, that euploid embryos developing to blastocyst before a given cut-off time had a 4 fold higher chance for implantation than those embryos that did not meet that criteria (Kofinas et al., 2015).

Another benefit of time-lapse imaging in a PGS program was highlighted in a study that investigated compaction and de-compaction at morula stage (Lagalla et al., 2015), which can only be seen by time-lapse. It was found that during de-compaction occasionally blastomeres or large cellular fragments are extruded and that these remain separate from the reforming morula and eventually degenerate during the formation and expansion of the blastocyst. Biopsy and diagnosis of such blastomeres or fragments by aCGH revealed a high chance for complex aneuploidies. Interestingly, aneuploidy screening from trophectoderm biopsies taken from corresponding embryos did not always match the result of the extruded blastomeres / fragments.

These findings have several implications. First it shows, that aneuploidy diagnosis of trophectoderm biopsy material can be influenced by these blastomeres / fragments. If remnants of these are biopsied together with "normal" trophectoderm cells, the diagnosis can be mosaicism. If only the remnant material is tested, the PGS result could eventually give a wrong diagnosis for the embryo as the biopsy material was not representative. As such fragments / blastomeres eventually degenerate, it may also lead to the release of DNA, which may be of importance for other investigations such as blastocoel fluid biopsy.

4. Additional benefits

Besides the pure PGS aspect, time-lapse offers other benefits to the embryology laboratory. Some laboratories report a higher blastocyst formation rate in an integrated time-lapse imaging system (Wale & Edgar, 2014). In any program that applies blastocyst biopsy, be for the detection of a genetic disease or for screening purposes, even a single additional blastocyst for diagnosis is considered to increase the final chance in finding an embryo that is disease-free.

In addition, the continuous observation possibility offered by time-lapse allows for choosing the best time point for blastocyst biopsy. As embryos are already cultured individually, there is no need for an additional culture dish allowing separate culture of the biopsied blastocysts until further processing.

Conclusions

Time-lapse imaging will never replace PGS, as it cannot give an absolute diagnosis and because morphokinetic parameters that are used in time-lapse algorithms may vary between centers, thus making an accurate diagnosis even more difficult to achieve. However, time-lapse imaging is a perfect complement for PGD and PGS programs. It may identify among euploid embryos those with a higher implantation potential based on morphokinetics. In addition, it will help to optimize the workflow in the laboratory and thus reduce risks that may occur during handling of embryos for PGD/PGS.

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20. Aqueous progesterone- Is it call for change?

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Luteal phase support has been clearly demonstrated to improve pregnancy rates in women undergoing IVF. Because of increased risk of OHSS associated with use of hCG, progesterone has become treatment of choice for luteal support. Progesterone is typically administered by intramuscular(I/M) injection or vaginal inserts. These, most commonly used, I/M injections are oil based and are extremely painful. As progesterone supplementation is required for first three months of IVF conceptions, its daily & prolonged administration can lead to significant skin inflammation, extreme tenderness in local area and sometime can cause sterile abscess too. Expertise is required to administer this injection which becomes an added burden on patient. Vaginal progesterone(gel or capsule) provides a well accepted and effective form of luteal phase support with adequate endometrial secretory transformation notwithstanding low circulating progesterone levels. This is due to direct transport across vagina to uterus. Sometime it can cause discomfort in administration, vaginal discharge, and severe itching, which can limit its use.

For these reasons, a water soluble formulation of progesterone was developed that can be administered by subcutaneous injection. This formulation was possible by coating lipid soluble progesterone with water soluble starch(hydroxy propyl beta cyclodextrin). Adequate decidualization of endometrium can be achieved with aqueous progesterone at 25mg or 50 mg dose as demonstrated by dose finding study. Within one hour of administration it reaches peak serum levels. It is metabolized mainly in liver. Contraindications for its use are similar to that of progesterone. Various RCT's have compared it with I/M, vaginal gel and pessaries and found to be non inferior to them. Aqueous progesterone appears to be safe, well tolerated and non inferior to other well accepted progesterone preparations.

21. Intra-uterine administration of human chorionic gonadotrophin (hcg) before embryo transfer in recurrent implantation failure(RIF) patients improves implantation and pregnancy rates in IVF-ICSI cycles

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Introduction

Does intrauterine administration of human chorionic gonadotropin (hcg) before embryo transfer in recurrent implantation failure (RIF) patients improve pregnancy rates in IVF-ICSI cycles?

Intrauterine injection of hCG before embryo transfer in IVF/ICSI cycles may increase endometrial regulatory T cells and improve the implantation and pregnancy rates.

Human chorionic gonadotrophin (hCG) was found to secreted immediately after fertilization by the embryo. It plays an important role in implantation and in attracting regulatory T cells to the endometrium.

Material & Methods

Study design, size, duration 286 Infertile recurrent implantation failure (RIF) patients younger than 42 years from 2006 to 2014. were included in this study.Patients were randomly divided into two groups using computer generated list.The study group received intrauterine administration of 500 IU of HCG and control group received nothing before ET. Primary Outcome Measures: implantation and pregnancy rates

Secondary Outcome Measures:miscarriage and delivery rates

The study group (n = 143) received intrauterine injection of 500 IU of hCG, and the control group (n = 143) underwent ET without hCG.

RESULTS

The IR and PR were statistically significantly higher in the group received intrauterine injection of 500 hCG (25% and 40%, respectively) as compared with the control group (14% and 21%, respectively)

Limitations, reason for caution A relatively new concept in recurrent implantation failure, requiring more multicentric trials worldwide.

22. Does physical exercise matter in PCOS? emerging evidence

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Polycystic Ovarian Syndrome (PCOS) is the most common endocrine disorder observed in pre-menopausal women, with a prevalence of up to 20%. Management of PCOS is complex and involves strategies including diet modification, physical activity, and medication. The increasing evidence of an association between PCOS and cardiovascular disease (CVD) and stroke stimulated an interest in the possible role that PCOS plays in the development of endothelial dysfunction. Endothelial dysfunction signifies an early stage point in the development of atherosclerosis, preceding that of plaque formation. Endothelial dysfunction is also a marker indicative of future CVD or cardiovascular events

Comparing obese women with PCOS patients revealed that this endothelial dysfunction is an intrinsic defect, not explained by either obesity or ectopic fat deposition. There is now evidence that microvascular endothelial function is impaired in PCOS due to a reduced bioavailability of nitric oxide. Exercise training in these patients has been shown to improve conduit artery and microvascular endothelial function in PCOS through up-regulating NO bioavailability. In the light of this exercise training in PCOS may be viewed as first line defence in reducing CVD risk.

23. Ethical, social and clinical aspects of oocyte freezing

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The presentation will cover the ethical and clinical aspects of Social and Medical egg freezing both of which are being widely promoted by ART specialists. Social egg freezing or egg freeing for non-medical reasons is also being extensively covered in the media and companies are offering financial help to women to freeze their eggs and postpone motherhood so that they can continue to be productive at work. Both indications for oocyte freezing have raised an intense ethical debate.

Social/ 'non-medical' reasons for oocyte freezing

- Shifts in socioeconomic & reproductive behaviour
- Both women & men are postponing parenthood
- Extended educational and career aspirations (especially women)
- Later marriage – commitment phobia
- More frequent marital & relationship breakdown,
- Idealized parenthood – couples feel they should be able to provide all material comforts to the child.
- Parenthood not essential for 'personal fulfillment'. Material assets can achieve personal fulfillment.

Why it is being encouraged/accepted by nations and fertility societies

- Developed & now even in developing nations are seeing a delay in the age of first pregnancy, This delay translates in fewer children born to women less than 35 years thereby decreasing the growth rate. A higher risk of being childless with > maternal age. 6% risk of permanent childlessness when women delay pregnancy attempts until age 30, 14% when those attempts begin at 35 and 35% when they begin at 40 (Brown, 2010).
- Demand for ART in women is increasing in women over 40. A 40 – 41% increase has been reported. (ASRM).PR in older women with their own eggs are lower and obstetric and neonatal risks are higher. Women go through repeated cycles to achieve pregnancy. Maternal and neonatal risks are also higher in older women.

- This has also increased the demand for donor eggs which may not be so easily available.

Social Freezing of oocytes is said to offer reproductive freedom to women. They are liberated from the shackles of their biological clock. The picture however may not be as rosy as it is made out to be and there may be a false sense of security.

Research data has shown that social freezing should ideally be performed on women around 25 years of age when the oocytes are healthier / have less aneuploidy. It is however mostly performed after the age of 35. Six to 10 oocytes may be required to achieve one live birth, so a woman may need more than one stimulation to store enough eggs. Technical expertise is essential for a good outcome. Advanced maternal age is associated with increased obstetric and neonatal complications.

Social freezing is not a solution for the underlying societal problems and just delays the existing problems. There is never a good enough time to have a baby. The debate between the right to Reproductive autonomy versus the Well-being of the child needs to put into proper perspective.

Medical reasons for oocyte freezing

Until recently there was little to offer young women with cancer facing chemotherapy, radiotherapy or surgery and the probability of premature menopause and sterility. Oocyte freezing has given these young women a chance at motherhood. The ethical debate here relates to the dilemma of offering fertility preservation to a patient facing a life threatening illness, invasive nature of the procedure, current success rates and technical expertise required. Posthumous use of gametes can also pose a problem.

It has been proposed by that all applications, regardless of the reason, should be evaluated with the same criteria: efficiency, safety and justice (Pennigs et al 2013).

24. Monitoring follicles in COH by TVS

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The outcome of a pregnancy depends on the quality of the embryo.

This, in turn, depends in part on the quality of the oocyte contained in the dominant follicle, therefore, the quality of the follicle itself, which supports oocyte growth and maturation.

Not all dominant follicles ovulate and of those that do, not all are of sufficiently high quality to result in pregnancy.

The term "monitoring" means "close continuous observation", so when we refer to monitoring an in vitro fertilization and embryo transfer (IVF-ET) cycle we mean close observation not only of a patient's initial parameters and her own ovarian response to ovulation induction, but also events after completion of the therapy.

Follicular Monitoring is the process of serial ultrasonic monitoring of the ovarian follicles used to identify maturation status of eggs. It is useful for assessing the size of the follicle, Antral follicle count and for determining the endometrial thickness and also assessing any abnormalities in the uterus. (fibroids/ adenomyosis/ovarian cysts etc.)

In monitoring of follicles in COH we need to see the following:

Follicular recruitment

Selection of dominant follicle

Follicular growth & Estradiol synthesis

Endometrial proliferation

Follicular rupture

Luteal phase changes

Follicular monitoring/tracking

Is done from day 2/day 6 and thenday 9 onwards till the documentation of ovulation is dome and then once in the luteal phase to document secretary endometrium

Day 2 - Baseline scan for

Persistent corpus luteal cysts

Follicular cysts

PCOD

Chocolate cysts

Endometrial shedding

Antral follicle count

DAY 6 SCAN

To assess follicular response to COHS

Ideal > 6 follicles > 10-12 mm in mean diameter

Endometrial triple line pattern

Mean endometrial thickness > 6 mm

Dosage adjustments of Gn

DAY 9 SCAN

Follicular sizes

Total number of follicles

Mean diameter @ 16 - 17 mm in mean dia

Endometrium > 8 - 10 mm in thickness

Good blood flow in Zone III and IV

Low resistance pattern in uterine arteries

SONO AVC

Sonography-based Automated Volume Count

Automatically calculates the number and volume of hypoechoic structures in a volume dataset.

Can significantly reduce time for assessment and reporting.

From the calculated volume an average diameter can be calculated.

It also lists the objects according to their size.

SOET

= Self Operated Endovaginal Telemonitoring

Today with modern technology it is possible to self monitor on computerised internet connections,so the patients visits are reduced

Also we today have SONO AVC for automatic counting and accurate volume assessment of all the follicles, specially in cases where there are many follicles as in cases of COH and hyperstimulated ovaries

Perifollicular perfusion and ovarian stromal blood flow are useful markers for optimal evaluation of follicles, with the former having a direct relationship with follicular oxygenation and oocyte maturation.

In order to increase the chance of pregnancy in ART cycles, better synchronization between ovarian follicles and the endometrium is required; with a suitable oocyte (metaphase II), endometrial receptivity must be around a specific time (implantation window). Therefore, it would be more helpful if utero-ovarian vascularity was assessed at the same time, especially in cases with monofollicular cycles or in patients which need natural cycle, such as those with specific medical conditions

Doppler will be a useful tool for evaluating IVF cylces and will definately increase the pregnancy outcomes.

25. Single v/s sequential media

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The success of in vitro fertilization techniques is defined by multiple factors including embryo culture conditions, related to the composition of the culture medium. Currently, there is discussion about the ideal composition of culture media with two opposing views "back to nature" and "embryo free choice" (Macklon et al 2001).

According to the "back to nature/need for sequential medium" principle, embryo culture media mimic in vivo conditions when the zygote moves from the fallopian tube to the uterus during early development. In order to fulfill the needs of the embryo at each moment in development and to optimize the embryo quality, the sequential medium contains a different composition during different days of culture. These "ideal" compositions are based on studies on animal models reporting a change of energy requirements as the in vitro culture of pre-implantation embryos evolves over time. Moreover, molecules like EDTA, glutamine and some amino acids have been reported to have a variable effect on the embryo during development.

According to the "embryo free choice/singe culture medium" paradigm, the embryo is cultured in a single medium which is constant and contains all the components needed during its development and the composition of this single medium does not change during embryo culture. Proponents of this paradigm argue that there is no direct experimental evidence that sequential media are required for optimal embryo development.

It is not clear which type of culture media (sequential or single) is associated with the best quality of embryos on day 2, day 3 or day 5 or, more importantly, with the highest implantation rate per embryo transferred. No differences in embryo quality on day 3 and day 5 were found between a single medium and a sequential medium. Optimal embryo quality has been reported after embryo culture in either a sequential or a single medium. However, the quality of these studies was limited due to lack of randomization or, in case of randomization, due to the lack of a specific hypothesis with a power calculation or the failure to include a sufficient number of embryos. It is not clear how the clinical implantation rate per embryo transferred is affected by embryo culture since only a few studies evaluated the difference in implantation rate with no significant advantage using one type of medium. The latter can be due to the fact that there are an insufficient number of embryos transferred.

We will be analyzing the and discussing the above said data over in this particular lecture

26. Oocyte vitrification are we confident enough?

Priya Kannan

Oocyte cryopreservation as Dr Roy Homburg, Dr Fulco van der Veen and Sherman J. Silber, famously declared in 2008, is Women's emancipation set in stone.

Over the past 3 decades, oocyte cryopreservation procedures have improved rapidly. In the era prior to 2006, slow freezing methods were used to cryopreserve oocytes which did not yield promising results. In a meta-analysis in 2006, the results were as follows:

Slow frozen oocytes	Fresh oocytes	
LBR/injected oocyte	3.9%	6.6%
LBR/ET	21.6%	60.4%

LBR – Live Birth Rate

Compared to women who underwent IVF after slow frozen oocytes, IVF with unfrozen oocytes resulted in significantly better rates of fertilization (odds ratio [95% confidence interval]); 2.22 (1.80, 2.74), of live birth per injected oocyte; 1.5 (1.26, 1.79), and of implantation; 4.66 (3.93, 5.52). Hence it was concluded that In vitro fertilization success rates with slow-frozen oocytes are significantly lower when compared with the case of IVF with unfrozen oocytes. The suggestion proposed was although oocyte cryopreservation with the slow freezing method appears to be justified for preserving fertility when a medical indication exists, its value for elective applications remains to be determined. (Otkay et al., 2006).

The ice was broken with the introduction of vitrification as the method of choice of cryopreservation of oocytes. Current literature and practise suggests that vitrified oocytes produce superior IVF results to slow-frozen oocytes and may yield comparable outcomes to IVF with fresh oocytes in certain patient populations. Some of the indications for oocyte cryopreservation presently are patients at risk of infertility due to disease or age-related decline or oocyte donation programs, couples who fail to produce semen when required for IVF,

and patients with legal or ethical reasons against embryo cryopreservation may access cryopreserved oocytes.

In a observational multicentre study by Rienzi et al., 2012, on 486 cycles performed in 450 couples, 2721 oocytes were warmed and 2304 of them survived cryopreservation (84.7%). Of the 2182 oocytes subjected to ICSI, the rates of fertilization and development to top-quality embryos were 75.2 and 48.1%, respectively. A total of 128 deliveries were obtained (26.3% per cycle and 29.4% per transfer) for 450 patients (28.4%) and 147 babies were live born from 929 embryos transferred (15.8%). The forward logistic regression analysis on a per patient basis showed that female age [odds ratio (OR): 0.93, 95% confidence interval (CI): 0.88-0.98], number of vitrified oocytes (OR: 1.08, 95% CI: 1.01-1.17) and the day of transfer (OR: 1.97, 95% CI: 1.14-3.42) influenced delivery rate. By recursive partitioning analysis, it was estimated that more than eight oocytes vitrified were required to improve the outcome (22.6 versus 46.4% delivery rate, respectively). When fewer oocytes were available in women aged >38 years, results dramatically reduced (12.6 versus 27.5% delivery rate, respectively).

In 2013, ASRM made a landmark declaration after a careful analysis of scientific publications on oocyte vitrification that this technique is no longer experimental.

Implantation rate (%)	Coba 2010 (donor oocytes)	Rienzi 2010 (Infertile Patients)	Parmegiani 2011 (Infertile patients)
Vitrified oocyte	39.9	20.4	17.1
Fresh oocytes	40.9	21.7	NA

Clinical pregnancy rate/ ET (%)	Coba 2010 (donor oocytes)	Rienzi 2010 (Infertile Patients)	Parmegiani 2011 (Infertile patients)
Vitrified oocyte	55.4	38.5	35.5
Fresh oocytes	55.6	43.5	13.3

Clinical pregnancy rate/ oocyte thawed (%)	Coba 2010 (donor oocytes)	Rienzi 2010 (Infertile Patients)	Parmegiani 2011 (Infertile patients)
Vitrified oocyte	4.5	12	6.5

Regarding the safety of vitrification of oocytes, a recent study by Stigliani et al., found that Comparison of gene expr ession profiles between surviving thawed oocytes after 3 and 6 years of storage in liquid nitrogen found no differently expressed genes. Thus confirming that length of storage of oocytes does not seem to alter the gene expression profiles.v

tNow, coming back to the question of are we confident about oocyte vitrification, Yes, we are definitely more confident of oocyte vitrification than slow freezing and it is time and probably the need of the hour for all ART programs to inculcate this one more armamentarium into their profiles!

27. Management of large and multiple myomas. The limit

Punita Bhardwaj

Uterine leiomyomas are benign tumors of the uterus with prevalence of 80%by age 50.They are associated with AUB,infertility,recurrent pregnancy loss and bulk symptoms due to size

Hysteroscopic removal of us mucus fibroids is not appropriate for all patients making evaluation and selection important feature of clinical care

Uterine occur in 5-10% of women with infertility.In 1-2.5%myomas only abnormal finding.

Post myomectomy pregnancy rates are 40-60%, spontaneous conception rates are 50%

Route of myomectomy depends on desire for pregnancy,size,number,location of myomas,type2 lesion,relationship to serosa.Presence of pelvic disease,trainin g,experience,surgical expertise,bias of surgeon and equipment availability may also affect the choice of route

28. How should fertility techniques be introduced in clinical practice?

Raj Mathur

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Assisted conception lies at the intersection of clinical medicine, basic science, ethics and the law. Perhaps more than most other fields, the rate of new devlopments in this area is rapid. The complexity of the field and the pace of innovation present challenges to clinicians, embryologists, regulators and health policy-makers. Nowhere is this more marked than when we consider how to introduce new techniques into clinical practice.

In this lecture, I concentrate on the role of clinicians in this process. It goes without saying that certain preconditions have to be met before we can begin to consider whether a technique can be considered for introduction. For instance, the technique must be considered legal in the jurisdiction. It is striking that even in these days of homogenised 'standard' practice, significant variations exist in what is allowed or not. An example is the ban on embryo cryopreservation in Germany. Further, the technique must be effective. This means that, at a minimum, the technique should have been demonstrated to do what it is meant to do. However, a more desirable standard is that the technique should be shown to be superior, or at least non-inferior, to the existing standard technique. An example of an innovation that meets both levels of efficacy criteria is oocyte vitrification. A further requirement is that the technique should have demonstrated safety. This is a particular difficulty in assisted conception, where the safety or otherwise of a technique may not be clear until future generations have been studied. However, a sensible scientific model based on the precautionary principle may be used for this purpose. Initial studies should be performed on animals, including large mammals, followed by studies on human embryos donated for research. When the technique is first introduced into clinical practice, it should be through the medium of well-

designed randomised controlled trials which allow follow-up of the children conceived through treatment and the patients being treated. A national or international register would give greater ongoing confidence into both safety and efficacy of the technique.

It is clear that the majority of technical innovations in assisted conception (ICSI being a prime example) were not introduced in this systematic way. Perhaps this is the likely consequence of the coming together of commercial interests, clinician's desire to help their patients and the urgent desire of subfertile persons to achieve parenthood. However, clinicians have an ethical duty to minimise harm in the course of trying to help patients, and a duty to adequately inform patients which can only be fully met if these matters are considered. The dilemma remains for which there is no easy answer – how can we not introduce a technique when patients wish it and it seems to 'make sense' and, importantly, other clinics may be willing to offer it? In this scenario, the minimum that clinics should ensure is that patients are aware of the experimental nature of the technique and are enrolled in well-designed studies that have a chance of addressing safety and efficacy. An example of this may be PGS, which many professional bodies recommend should not presently be offered for routine clinical use.

It is important not to minimise the financial imperative, and it is of course important for clinics, both state and private, to ensure their viability and progress. Hence, it is perfectly right to introduce treatments that are not clinically superior, but offer similar efficacy to standard at a lower cost, for instance the use of biosimilar recombinant FSH. Similarly, it is appropriate to consider the business environment and attempt to match competitors, without allowing patients to be exploited. This can be a difficult balance to strike in assisted conception. National and international professional bodies, such as the Indian Fertility Society may have role here, as indeed do local ethics committees.

The process of introducing a new technique brings a test of the quality management system, staff resource, commercial skill and communication ability of the clinic. A validated evidence-based guideline is an initial step and should be tailored to the particular requirements of each clinic. Training and certification of the relevant staff is critical, and this may again involve professional bodies. Patient information resources in paper and electronic form are key to a smooth introduction.

This process does not end when the clinic starts to use the new process. For each new technique or treatment, key performance indicators should be identified and monitored on a regular basis. The introduction of a new technique or treatment is therefore a process, rather than an event. It should be considered part of the continuous improvement strategy of a fertility clinic or service, requiring leadership, clinical judgment and scientific knowledge.

29. Evidence based medicine for luteal phase support

Rajan S. Vaidya

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What is luteal phase?

Luteal phase is defined as the period between ovulation and the onset of menses or the establishment of a pregnancy (Fatemi et al., 2007)

Corpus Luteum

1. A temporary endocrine gland
2. Develops after ovulation from the ruptured follicle during the luteal phase
3. Secretes progesterone, and is critical for the maintenance of early pregnancy
4. Luteal-phase dysfunction - premature regression of the gland, with a subsequent shift to an infertile cycle

Luteal phase defect in a natural cycle

In 1949: premature onset of menses: indication of luteal phase deficiency of progesterone production (correctable by exogenous progesterone administration) (Jones, 1979).

The prevalence of a luteal phase defect in natural cycles in normo-ovulatory patients with primary or secondary infertility = 8.1% (Rosenberg et al., 1980)

30. Controlling in-vitro environment in IVF lab

Randhir Singh

MD, MIAP,LLB (ESHRE Certified Embryologist

Supplying and maintaining appropriate culture conditions is critical to minimize stress imposed upon gametes and embryos and to optimize the in-vitro environment. One parameter that requires close scrutiny in this endeavour is pH. Though embryos have a limited ability to regulate their internal pH (pHi), oocytes lack robust mechanisms. Thus, careful attention to external pH (pHe) of culture media is imperative in IVF. Ability to withstand deviations in hydrogen ion concentration varies depending on culture conditions, as well as laboratory procedures. Cryopreserved–thaw–thawed embryos, as well as denuded oocytes, are especially susceptible to perturbations in pHe. Therefore, proper setting, monitoring and stabilizing of pHe during IVF laboratory procedures is a crucial component of a rigorous quality control programme. Here, importance of both pHi and pHe in respect to gamete and embryo quality are discussed. Furthermore, factors influencing selection of pHe, as well as emerging methods to stabilize pHe in the IVF laboratory are detailed

One of the primary goals of an embryologist is to improve the quality of embryos developing in the laboratory (which hopefully leads to more babies being taken home). A key factor in this endeavour is minimizing stresses imposed on gametes and embryos during their manipulations within the

in-vitro environment. It is readily apparent that improper set-points in growth conditions are stressors and are, therefore, detrimental to embryo development, whether it be improper media energy substrate composition, temperature or osmolality. However, periodic fluctuations in environmental conditions are also harmful stressors, as these are easily transduced into deleterious intracellular perturbations. One such environmental parameter, which not only requires strict attention to its set-point but which is also especially susceptible to these damaging oscillations, is pH.

Future directions Although likely media dependent, there remains a gap in knowledge regarding the ideal pHe in which to culture cells during IVF. There may be differential pHe conditions needed for optimal oocyte maturation, fertilization and various stages of developing embryos. Until properly controlled studies are performed, debate will remain as to the proper conditions. What is inarguable is that stabilization of environmental parameters, such as pH, are crucial for optimized culture conditions. Potential for mitigating these damaging oscillations lies in the use of various buffering systems. Use of combined buffers in handling media or within the incubator environment may prove beneficial over current approaches by allowing for selection of a specific pKa and optimal buffering at various temperatures, while lowering individual buffer concentration. Increasing buffering capacity of media, using combination buffers or some other novel approach may also be beneficial during fertilization or IVM, where high concentrations of spermatozoa or cumulus cells could acidify local pHe due to cellular metabolic processes. This would likely be more pronounced in small volumes of media and may be useful for emerging technology such as microfluidics. Finally, examination into the formulation of a temperature-independent buffer, possibly through use of combined buffers, to resist changes in pH due to temperature fluctuation offers the opportunity of improved pH stability and perhaps benefits during or after cryopreservation. Continued research in this field and examination of molecular endpoints will aid in this.

31. Kisspeptin

Rashmi Sharma

The molecule of puberty and sexual fertility

They are a family of neuro-peptide hormones which play a critical role in the regulation of the hypothalamic– pituitary–gonadal axis, thus in turn influencing fertility and reproduction

The kisspeptins (KP) were originally identified as a product of a metastasis suppressor gene, KiSS-1, in malignant melanomas by Lee et al (1996). As it was discovered in Hershey (Pennsylvania), the gene was named after Hershey's famous chocolate "Kisses". However, the nomenclature also has a scientific grounding as the inclusion of 'SS' in the name also indicates that the gene is a suppressor sequence. Three years later in 1999, a G protein coupled receptor was identified in rat, cloned, and termed GPR54. Two years later, this receptor's ortholog in humans was isolated.

The KiSS-1 gene is found on the long-arm of chromosome 1.The protein it makes is a peptide containing 145 amino-acids, which are then cleaved into smaller, 54-amino-acid chunks. These may also be further truncated down to 14, 13 or even 10 amino-acids fragments with carboxylic acid terminations. These N-terminally truncated peptides are known as the family of kisspeptins. Adult humans and mice with nonfunctional KISS1R (or GPR54) both exhibit low plasma levels of gonadotropins and sex steroids, have underdeveloped gonads and are infertile. Despite being prepubertal, these individuals are otherwise normal and apparently healthy.

Kisspeptin is expressed abundantly in the arcuate nucleus (Arc) and the anteroventral periventricular nucleus (AVPV) of the forebrain.

kisspeptins stimulate the secretion of gonadotropins from the pituitary by stimulating the release of GnRH from the forebrain after the activation of GPR54, which is expressed by GnRH neurons.

Kisspeptin neurons express the estrogen receptor and the androgen receptor, and these cells are direct targets for the action of gonadal steroids in both male and female animals, suggesting that kisspeptin signaling could mediate the neuroendocrine events that trigger the onset of puberty.

Kisspeptin signaling in the brain has been implicated in generating the preovulatory GnRH/LH surge, triggering and guiding the tempo of sexual maturation at puberty, controlling seasonal reproduction, and restraining reproductive activity during lactation.

Kisspeptin signaling may also serve diverse functions outside of the classical realm of reproductive neuroendocrinology, including the regulation of metastasis in certain cancers, vascular dynamics, placental physiology etc.

Many mammals only become fertile during the annual breeding season, and this is controlled by the duration of daylight per day. During short winter days, these anmials have a reduced amount of kisspeptin in certain parts of the brain, and sexual activity is switched off (or greatly reduced). But in the long summer days, kisspeptin amounts increase, stimulating the animals to breed. Artificially injecting animals (such as ewes) with kisspeptin during the winter months causes ovulation even in the non-breeding season [Dhilo 2008]. This has implications for farmers, who may soon be able to choose when their livestock reproduce rather than be restricted by the natural annual cycle.

Very recently, a group of Japanese scientists delivered an antibody directed against kisspeptin into the brain of female rats. This stopped the rats' reproductive cycle demonstrating that inhibiting the effect of

kisspeptin, even after puberty, still blocks reproductive function. Therefore, as well as being vital to initiate puberty, kisspeptin is necessary for reproductive function to continue later in life.

Spanish scientists discovered that administering kisspeptin in food-restricted rats still stimulated the release of gonadotropins.

This is a remarkable discovery, since normally when a mammal is facing starvation its reproductive system becomes dormant to conserve the body's stored food supply. Kisspeptin injections over-ride this defence mechanism, and can kick-start normally dormant reproductive systems.

Milton et al. suggested in 2012 that kisspeptin peptides are neuroprotective against Alzheimer disease (AD). The group reported that kisspeptin peptides inhibit the neurotoxicity of Aβ, IAPP, and PrP peptides via a receptor independent action involving direct binding to the amyloid peptides. This indicates that kisspeptin peptides may be useful for the treatment of Alzheimer and prion type diseases.

kisspeptin has been suggested as a possible treatment for some forms of cancer. In particular, when breast and prostate cancers develop, they are nurtured by the sex hormones oestrogen and testosterone. If the production of these hormones could be switched off the tumours should shrivel and die. One way to do this might be to block the kisspeptin-receptor in the brain.

Scientists are now actively looking for molecules that can block this receptor.

Kisspeptin's role in switching-on sexual hormones could be vital in controlling the timing of puberty when this goes wrong.

There are a number of conditions where children that reach puberty much too early (five or six years old) could possibly be treated by simply taking pills containing kispeptin derivatives.

Conversely, for young people in which puberty does not begin as normal in their teenage years, it may be possible to administer kisspeptin to kick-start the process.

Jayasena and colleagues in 2010 used single kisspeptin-54 (KP-54) injections to show that the peptide stimulates the release of reproductive hormones in women with hypothalamic amenorrhea (HA), a commonly occurring condition characterized by absence of menstruation. The same group compared the effects of kisspeptin-10 administration on gonadotropin release in healthy men and women in 2012. They administered IV bolus injections of kisspeptin-10 to men and women. The researchers collected blood samples at regular time periods for 4 hours after injection to measure plasma luteinizing hormone (LH) levels. The results showed that that Kisspeptin-10 stimulates gonadotropin release in men as well as women during the preovulatory phase of menstrual cycle, but failed to stimulate gonadotropin release in women during the follicular phase. This sexual dimorphism of the reaction of healthy men and women to kisspeptin-10 administration has important clinical implications for the potential use of kisspeptin-10 for the treatment of disorders of reproduction.

Orally active FSH

Currently, purified and recombinant human FSH are the only FSH receptor (FSH-R) agonists available for infertility treatment. Treatment typically involves multiple injections, which can have a negative impact on patient compliance so development of FSH oral mimetics has been highly sought after by several pharmaceutical companies.

2 important papers in this connection are presented here. First breakthrough was in 2006 in which class of thiazolidinone agonists were identified through unbiased combinatorial chemistry library screening. Additional optimization through analog library screening and parallel synthesis led to the development of a potent FSH-R-selective and -dependent agonist with full efficacy to FSH. The thiazolidinone agonists appeared to activate the FSH-R through an allosteric mechanism, which was determined by the use of

human FSH-R/TSH-R chimeras.

(Allosteric Activation of the Follicle-stimulating Hormone (FSH) Receptor by Selective, Nonpeptide Agonists. Stephen D. Yanofsky et.al. Journal of Biological Chemistry 2006)

Very recently, a paper was presented about interesting compound, TOP00004. TOP00004 stimulated cAMP in CHO cells expressing hFSHR. The compound was also tested in more relevant functional assays. In rat granulosa cells, in the presence of low dose of FSH, TOP00004 stimulated estradiol with an EC50 of 37nM, while in the absence of FSH, the potency was reduced (EC50 at 103nM). In a translation model of human granulosa cell, TOP00004 dose dependently increased estradiol secretion in the media with EC50 at 44nM. On the other hand, in rat Leydig cells, the compound increased testosterone only at very high concentrations (EC50-1980 nM). This result suggests the molecule is quite specific for activation of FSHR. To demonstrate the efficacy of the molecule in vivo, rat superovulation model was used. Immature female rats were administered with increasing doses of TOP00004 (po), as control low and high dose of hFSH were also tested. Oral administration of TOP00004 in the presence of low level of FSH showed dose dependent increase in the number of egg released following hCG administration. The compound was effective from 1 to 20 mg/Kg body weight. Maximal response as that of high dose FSH was reached at 5 mg/kg. Thus the authors have successfully demonstrated in preclinical model an orally active FSHR PAM capable of stimulating follicular development. This molecule is currently being evaluated in preclinical safety and toxicology studies to develop as clinical candidate to be used in OI and COH in IVF cycles.

(7th World Congress on Ovulation Induction, Bologna 2015. Development of an Orally Active Follicle Stimulating Hormone Receptor Agonist for Infertility Treatment. Nataraja S. et al.)

32. High dose FSH in DOR- Is it justified

Reeta Mahey

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IVF in poor ovarian reserve patients is a real challenge. To define poor ovarian response in IVF according to Bologna criteria, at least two of the following three features must be present: (i) advanced maternal age (more than 40 yrs) or any other risk factor for poor ovarian reserve; (ii) a previous poor ovarian response (≤3 oocytes) with a standard dose of medication; and (iii) an abnormal ovarian reserve test (AFC <5-7 follicles or AMH <0.5-1.1ng/ml).

The management of poor ovarian reserve is highly controversial and no ideal protocol has been defined till date for this group of patients. Though the reasonable option for poor ovarian responders is donor oocyte IVF, but sometimes patients want to try IVF with their own oocytes.

IVF with self oocytes in poor ovarian reserve patients is a real challenge. Traditionally, the options for poor responders is either high dose FSH(HD-FSH) with antagonist protocol or microdose protocol with high dose FSH (HDFSH). But high dose gonadotrophins leads to high E2 levels on day of hCG trigger thus altering ovarian endometrial receptivity. This ultimately causes poor embryo implantation thus decreasing the live birth rate.

There is always a debate on IVF protocols to be used in poor responders. In a randomised controlled trial, Morgia et al(2004) compared natural cycle IVF and flare up FSH protocol in poor ovarian responders. The study concluded that clinical pregnancy was similar between the two protocols (6.1% vs 6.9% respectively).

Laser T et al compared minimal stimulation protocol and high dose FSH antagonist protocol in terms of clinical pregnancy rate. The study concluded MS IVF is less expensive and resulted in higher pregnancy rate as compared to high dose FSH protocol.

Lainas et al.(2015) did a retrospective analysis to compare modified natural cycle (MNC) versus high dose FSH in poor responders. MNC-IVF was associated with 4 times higher probability of live birth as compared to HD-FSH antagonist protocol in patients with poor ovarian response according to Bologna criteria.

There is no prospective randomised trial directly comparing minimal stimulation IVF and HD FSH protocols. Though both MNC and HD-FSH cycles offer comparable overall live birth rate but minimal stimulation IVF cycle is a patient friendly approach with comparatively less cost and minimally effect on endometrial receptivity. The protocol can be used in poor responders who don't wish to go for oocyte donation or adoption. Further prospective large number trials are required to evaluate the efficacy of minimal stimulation as compared to HDFSH protocol in poor ovarian responders.

33. What is standard IVF in 2015? and what is the evidence for the increasing use of adjuvant therapy

Richard Kennedy

President IFFS

We are rapidly appraoching the 40th anniversary of the first successful IVF pregnancy and the Nobel Assembly at Karolinska Institute saw fit to recognise this scientifc triumph. It has been estimated that over 5 million children have been conceived by ART and approximately 1 million cycles of IVF/ICSI are carried out globally each year. From cutting science at the very frontiers of medicine, IVF has become normalised, standard, almost everyday clinical practice. But what exactly is standard IVF? In addition ART remains inaccessible to many who are in need of this treatment. Infertility knows no bounds whether social or ethnic but despite the meteoric rise of IVF clinics in middle income and low resource countries how accessible is this treatment to the majority of people?

Standardisation of ART has become the norm, yet it is increasingly common for a range of additional interventions of increasng complexity to be applied to the extent that it is appropriate to ask the question "what is standard IVF?" and what is there evidence for these additions. Proponents argue individualisatiion of approach to treatment, patient choice and maximising conception rates. Cynics argue that this is at best experimentation on an uncontrolled scale and worst manipulation of a vulnerable group of patients. Furthermore vulnerable patients whose faith in specialist advice may lead them to significant financial hardship in the perhaps misguided belief that the addition of a variet of investigation and intervention may lead them to succss. The truth is likely somewhere between the two. In this presentation we discuss the facts of the case.

We consider a range of interventions which to be advocated into standard practice must pose no risk to patient or offspring, offer a proven benefit in outcome and should be cost effective compared to "norm". As we shall see there is a paucity of well conducted, prospective randomised controlled trials the gold standard of evidence of effectiveness. For example Hysteroscopy is an investigation often routine advocated before ART. Not without its risks and adding significant cost it is pertinent to challenge its cost benefit. What of the range of adjuvants that are advocated for poor ovarian responders. There is limited evidence for the addition of androgens and growth hormone but to dat these need to be tested in properly powered RCTs before they become embedded in routine practice without evidence. Embryological practices over and above the norm such as morphometric analysis and PGS. The value of the former remains to be proven and its success in non randomised trials is more likely to be due to lack of interference of the delicate environmental environment of the embryos. PGS has waxed, then waned in its purported benefit but following the recent advent of whole genome array studies and the improvement in the techniques it is once more coming to the fore as a real advance. Still it requires a thorough cost benefit analysis and its benefit will only be evident in high quality laboratories. A range of interventions are also advocated to improve implantation including endometrial scratching, assisted hatching and parenteral drug therapies none with proven benefit but all adding to the cost of treatment.

It is clear that access to more patients will be achieved through the provision of low cost treatment. Low cost does not equate to low quality. Attention should be paid to basic laboratory techniques, embryo handling, avoidance of complications such as OHSS and skillful embryo transfer without adding numerous additional intervemtions without proof of benefit.

34. Thrombophilia diagnosis and management in RIF

Ritu Khanna

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Disturbance of the embryo-maternal interrelationship leads to repetitive implantation failure. This distruption can take place because of immunological factors, thrombophilia and alteration in prostaglandin synthesis or due to other endometrial molecules.

Women with inherited or acquired thrombophilia appear to have problems with trophoblast function, and abnormal placental development is more likely to result in implantation failure.

Inherited thrombophilia defects like activated protein C resistance, (due to Factor V Leiden gene mutation), deficiencies or protein C and S and antithrombin III, hyperhomocysteinemia and prothrombin gene mutation can cause thrombosis and lead to RIF. Poor pregnancy outcome associated with Factor V leiden (FVL) mutation, coupled with the maternal risk during pregnancy, may justify routine screening for FVL mutation and offering thromboprophylaxis for those with FVL mutation.

Amongst the acquired thrombophilias, the antiphospholipid syndrome (APL) with anticardiolipin antibodies and /or lupus anticoagulant and conditions associated with antiunclear (ANA) may lead to RIF.

At least one inherited or acquired thrombophilic factor was detected in 68.9% of women with repeated IVF failure compared with 25% in controlled group. Combined thrombophilia (two or more thrombophilic factors) was significantly role in IVF implantation failure.

Investigation for thrombophilia

- Coagulation profile
- Tests for presence of
  - Inherited factor V Leiden (FVL) mutation.
  - Prothrombin mutation,
  - Methylenetetrahydrofolate reductase (MTHFR) mutation
  - Deficiencies in proteins S and C and antithrombin III.

Treatment for thrombophilic defects antiplatelet agents (aspirin), anticoagulants (heparin) and immunosuppressive therapies (Prednisolone, intravenous immunolobulins).

Qublan et al. observed that women with recurrent IVF ET failure who received LMWH for thromboprophylaxis had a significant increase in the implantation and pregnancy rates compared with the placebo group(20.9%) vs. 6.1% and 31% vs. 9.6% respectively). A significant increase in the live birth rate was observed in the heparin treated group compared with placebo (23.8% vs. 2.8%).

The frequency of treatment complications did not differ between the two study groups thus making LMWH a safe and effective thromboprophylactic treatement for women with thrombophilia and recurrent IVF-ET failures.

A combined treatment regimen includes aspirin (75-85 mg/ day), low molecular weight heparin (LMWH, 40 mg /day) or enoxaparin started on the day of ET and continued until delivery.

35. Role of epigenetics in ART

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Epigenetics is the study, in the field of genetics, of heritable changes caused by environmental factors, in cellular function and phenotype. These changes in form and function are brought about by altering gene expression but without changes in DNA sequence.

Not only has epigenetics been of immense interest to evolutionary biologists, who have long been debating which of the two, nature or nurture, has a predominant bearing on human evolution, but also, it has sparked interests of reproductive physicians and embryologists in equal measures. The idea that culture environment can affect embryonic development by altering gene expression is not new. But these effects are largely recognized as temporally, a more immediate event like embryonic arrest, poor blastulation, poor morphology or poor implantation rates. What is not recognized is that some temporally distant effects, not normally attributed to culture conditions, such as abortion rates, fetal growth disorders, birth weights, congenital abnormality rates, childhood cancers, syndromes associated with imprinting errors, can also be linked to epigenetic errors introduced during embryogenesis in the laboratory.

Whether these abnormalities are a result of ART per se or factors intrinsic to the patient is a question that needs answering. And is there a way to minimize epigenetic errors resulting from ART procedures? What are the molecular mechanisms behind altered gene expression? This talk attempts to throw some light on this utterly complex maze that is the epigenome.

36. When to stop IVF treatment?

S. N. Basu

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The diagnosis of Infertility is very traumatic. But the harsh reality is that 1 in 6-7 couples will be faced with this diagnosis.

Changing lifestyles, education of women, their careers, and consequent delayed marriages and delayed childbearing have no doubt contributed to an increased incidence of infertility.

Assisted reproductive techniques have been a boon to millions across the globe.

Due to the progress made in the field of bio- technology, an increasing understanding and greater expertise in reproductive medicine, pregnancy rates by assisted reproductive treatment have surpassed the natural conception rates.

The decision to undergo the treatment of infertility and IVF is not an easy one. However, it is a decision that is taken with a lot of hope and positive expectation. Every couple is hopeful of achieving a pregnancy by ART.

The sobering thought is that despite all advances, not all couples will succeed in having a baby. These couples will go through tremendous physical, emotional, psychological and financial strain but will still not be able to conceive. The repeated failures will make them depressed and sad. There will come a time when they have to ask themselves; How much longer ? How many more attempts?

Theoretically, if the couple keeps on trying they do have a chance of getting pregnant. Statistics, however. show that the success is maximum with the first 3-4 cycles. There are cases reported where the patient has continued with IVF cycle after cycle and conceived but this is not true for most couples.

Barbara Luke et al of Michigan State University in Denver, based on the data of more than half million IVF procedures in US between 2004 and 2008. Among 300,000 women who underwent IVF there were 171327 first time deliveries. The live birth rate was 36% with the first IVF cycle 48 % after the 2nd attempt and 53%with the third attempt. After this the success did not increase much. Even women who tried for 7 cycles or more the chance of a live birth rate only increased to 56%. The study therefore concluded that if the couple has not conceived by the third attempt the chances are slim to continue.

Despite this research, there are couples who get addicted to the hope of having a child especially those who who have not entered this journey with a clear decision of when "enough is enough". They approach each cycle with a fresh hope and keep convincing themselves that the next IVF attempt will be their last attempt when actually this is not so.

According to Professor Samuel Lee, one of United Kingdom's leading experts on infertility who pioneered egg donation when he was the Chief Scientist at the IVF Unit of Wellington Hospital, London has concluded after his years in this field that "Some couples going through fertility treatment are driven by an urge "stronger than addiction and more powerful than obsession. Women are risking death and bankruptcy in their desperation to become mothers. The quest to have children can become a vortex that gets faster and faster and sucks people in. Women will sell everything and anything to have the treatment if they are short of funds. They will risk their lives, there's no doubt about it." Dr Lee has helped some couples through as many as 12 cycles of IVF. The maximum number of treatments provided on the National Health Service in UK is three (The Observer UK).

The decision to stop treatment can vary according to the socio demographic, cultural, emotional and psychological factors. Financial factors are also important in many areas of the developing world.

For some patients, being turned down by a doctor or restricted by regulations isn't a stopping point. For them it is just another hurdle to overcome. When it comes to having a child, many people will exhaust any and all avenues—financial or geographic—to find a way around "no."

How long would the couple like to try? Thorough counselling of the couple along with statistical information may help them in their decision making.

The couple with repeated IVF failures, need to address questions which, may seem unpleasant and depressing. They need to take stock about how their desire to have children has negatively affected their lifestyle and continuing can be potentially dangerous. The choice to end treatment can be devastating and we as clinicians have to be aware of this difficult psychological aspect and help the patients through this difficult journey.

After counseling, the couple may contemplate to discontinue any further treatment and lead a normal life without the vortex of clinic visits, injections, ultrasounds and planning a life around all the requirements which IVF entails. They may gradually get used to the idea of making alternative choices for parenthood.

There cannot be absolute guidelines by any society of reproductive medicine as to how many cycles of IVF should be offered to a couple before they are recommended to stop and consider other methods of parenthood. Doctors and clinics can only counsel patients about alternative options of parenthood. They cannot tell the patients to discontinue treatment because decisions cannot be forced.

ASRM has published an ethics committee report on the topic: "Fertility treatment when the prognosis is very poor or futile". The entire paper can be read on-line but here are the take home messages as published on the net.

1. Definitions: A "futile" treatment is one with less than or equal to a 1% chance of a live birth. "Very poor prognosis" means more than 1% but less than 5% chance of a live birth per cycle.
2. Clinicians can refuse to offer futile treatments, but should consider a referral to another provider, if appropriate.
3. Decisions to treat should be patient centered. Protecting your high success rates is not a good reason to deny treatment. Making money is not a good reason to offer treatment.
4. Clinics can offer futile treatments if the patient has been fully informed about the Risks, Benefits and Alternatives to treatment.
5. Thorough discussions are advisable. Decisions to treat or not to treat should be made in cooperation with the couple. The clinic should have evidence -based policies to uniformly offer treatments (or not offer treatments) to patients." (ASRM)

Can we, as clinicians continue to accede to the demands of the patients to continue with IVF knowing the potential risks involved? This is a difficult question. Patient autonomy is very important in medicine. Counseling can be done but decisions cannot be forced. The final decision to stop IVF can only rest with the couple.

37. Role of AMH in management of PCOS

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9.13 – 22.5% females in India are suffering from PCOS

Pathophysiology of PCOS:

- THREE MAJOR CULPRITS
- CENTRAL PLAYER : INSULIN RESISTANCE
- HYPERANDROGENISM &
- ALTERED GONADOTROPINS

Diagnostic Criteria :Rotterdam -2003:

- 2 out of 3 required
- Menstrual Irregularity
- Hyperandrogenism
- USG – Polycystic ovary
- Exclusion of other etiologies

AES 2006:

- Menstrual irregularity +/- USG - Polycystic ovary
- Hyperandrogenism
- Exclusion of other etiologies

AMH & PCOS:

Since AMH levels reflect the number of developing follicles, their measurement may be used as a marker of ovarian follicle impairment in polycystic ovary syndrome.Women with PCOS have a two to six-fold greater number of follicles (primary, secondary and antral) in their ovaries, possibly due to the hyperandrogenemia..(Norman RJ, Dewailly D, Legro RS, Hickley T, 2007 Polycystic ovary syndrome. Lancet 370: 685-697.)In anovulatory women with PCOS, the follicular development is halted when follicular diameter is 6-9mm, that is just before the selection of the dominant follicle.(Rey R, Belville C, Nihoul-Fekete C, et al, 1999 Evaluation of gonadal function in 107 intersex patients by means of serum anti-Müllerian hormone measurement. J Clin Endocrinol Metab 84: 627-631.)

In PCOS, serum and follicular AMH levels are higher than in healthy controls. Elevated levels of AMH were related to increased number of follicles with a diameter of 2-5mm in women with PCOS. Hence, AMH serum values could be a precise, subsidiary diagnostic marker of the syndrome. Additionally, these high AMH levels are probably related to the follicular arrest, during the selection process of the dominant follicle, through a negative interaction between AMH and FSH. AMH inhibits the production of aromatase which is activated by FSH action on Granulosa cells. AMH concentrations in women with PCOS were independently and positively correlated with :testosterone, androstendione and free androgen index (FAI) values. Several clinical studies have confirmed that serum AMH levels are two to three times higher in PCOS compared with levels in women with normal ovaries.

A great number of women with PCOS have insulin resistance and compensatory hyperinsulinemia. A correlation has been reported between AMH levels and HOMA-IR values in women with the syndrome.

Currently the values for AMH in diagnosis of PCOS vary widely. AMH level of 30 pmol/l in discriminating between women with and without PCOS, which is similar to an AMH cut-off reported in a recent systematic review and meta-analysis of 33•6 pmol/l. (Iliodromiti, S., *et al*. (2013)The Journal of Clinical Endocrinology and Metabolism, 98, 3332–3340.]

AMH measurement is the best prognostic marker of the ovarian response to controlled ovarian stimulation during IVF cycles, especially when a single marker is determined.(La Marca A, Sighinolfi G, Radi D, et al, 2010 Anti-Müllerian hormone (AMH) as a predictive marker in assisted reproductive technology (ART). Hum Reprod Update 16: 113-130). AMH levels have prognostic value for both the number of oocytes retrieved during follicular aspiration and the number of arrested cycles.(Al-Qahtami A, Groome N, 2006 Anti-Müllerian hormone: Cinderella finds new admirers. J Clin Endocrinol Metab 91: 3760-3762.) Compared to antral follicle count, AMH concentrations could reliably and equally predict poor response to ovarian stimulation in IVF cycles. AMH levels could recognise those women prone to express ovarian hyperstimulation syndrome (OHSS) during multiple ovulation induction with human gonadotropins.

AMH is used to individualise Ovarian Stimulation protocols: The gonadotrophin dose and possible protocol modifications should be tailored to each individual, and AMH could be a useful component in this. The gonadotrophin dose and possible protocol modifications should be tailored to each individual, and AMH could be a useful component in these algorithms [Arce J, La Marca A, Mirner Klein B, Nyboe Anderson A, Fleming R. Antimüllerian hormone in gonadotropin releasing-hormone antagonist cycles : prediction of ovarian response and cumulative treatment outcome in good-prognosis patients. Fertil Steril. 2013;99:1644–53].

Anti-Müllerian hormone-tailored stimulation protocols improve outcomes whilst reducing adverse effects and costs of IVF.A.P.Yates, O.Rustamov, Human Reproduction, 2011.

In this study patients whose AMH levels fell within the acceptable range were divided into three strata (optimal, satisfactory or low ovarian fertility), which determined their stimulation protocol. Essentially, those with higher levels received lower doses of gonadotrophins and vice versa. Women in the satisfactory ovarian fertility group received Long protocol with a GnRH agonist, whereas those in the optimal or low ovarian fertility groups underwent a GnRH antagonist cycle, There was a significant reduction in the incidence of OHSS as well as a non-significant reduction in hospital admissions from severe OHSS using the new protocol. General increase in the rate of ongoing pregnancy and live births in the AMH-tailored treatment group regardless of the type of gonadotrophin or ART used, or the number of embryos transferred, adding further weight to the proposal that AMH-tailored protocols optimize stimulation. High predictive values of AMH for

excessive and poor responses, and such predictive values are prerequisites of a reliable marker. Choosing an appropriate 'cut-off' level requires the assessment of the eventual benefits versus the harms of the possible misclassification of patients. Regarding excessive responses, the threshold of 3.07 ng/mL was shown to result in a sensitivity of 83.0 % and a specificity of 78.0 %. Patients with AMH levels above this threshold should be considered to be at high risk of developing OHSS, and more intense monitoring of ovarian stimulation is warranted. Moreover, the dose of gonadotrophins should be individualised regardless of the patients' age prior to initiating the first ovarian stimulation cycle.

38. Predicting success for individual patients undergoing COH

Sankalp Singh

Controlled ovarian stimulation for IVF is an integral part of present day fertility treatment.The success of COS is dependent on multitude of factors with no single factor having the decisive lead. Variability in the subfertile patient population excludes the possibility of a single approach to controlled ovarian stimulation. In fact, the patient is the main variable in ovarian stimulation response. There are multiple factors interacting mutually, such as demographics and anthropometrics, genetic profile, cause and duration of infertility, health and nutritional status. At the same time, the therapeutic armamentarium for ovarian stimulation has increased over the last years and clinicians face the problem of which drug regimen to use.

The predictors of a successful COS includes:

1) Age of the female
2) Type of infertility (primary or secondary)
3) Duration of infertility
4) Ovarian reserve tests mainly antral follicle count(AFC) and anti mullerian hormone(AMH)
5) BMI of the female
6) Smoking
7) Ethnicity
8) Previous ovarian response
9) Previous ovarian surgery

Females have maximum number of oocytes at 20 weeks of her gestation when approximately 6-7 million oogonia are there in her ovaries. This number decreases to approximately 2-3 million at birth and then drops off to 300,000 at the time of puberty. After that, during reproductive years, ovary starts to recruit at least 30-50 oocytes from ovarian reserve. They compete with each other to become the dominant follicle and ultimately ovulate to release an egg for fertilization.

There is no new production of oocytes after birth.With female age more than 30 the oocyte quantity starts declining.As the age increases further,especially beyond 35 years there is a steep decline in oocyte quantity,thus leading to reduced response to ovarian stimulation and low yield of oocytes.

Currently, the central paradigm of all ovarian stimulation protocols is to maximize the beneficial effects of treatment (relating to high-quality oocyte yield) while minimizing the potential risks associated with OHSS and multiple pregnancy. In a recent meta-analysis examining predictive factors for pregnancy in IVF, all significant factors were age related and reflected ovarian reserve, which was the major determinant of success. Although age and FSH levels remain the most commonly used single patient characteristics in clinical practice, these markers of ovarian reserve only provide a basic prognosis for success and indications for standard COS treatment. In contrast, anti-Müllerian hormone levels and the antral follicle count (AFC) appear to be accurate predictors of ovarian reserve and response to COS, and could be used successfully to guide tailored COS.

Both AFC and AMH are excellent markers of ovarian responsiveness.There are plenty of data which suggests that both of them have same predictive value for it.Both are used in various FSH normograms to decide the dose of FSH necessary for individual patients.

A combination of advanced age, high intra-menstrual FSH concentration, low AMH concentrations or severely reduced antral follicle count depicts a poor prognosis not only for ovarian response to stimulation but also for pregnancy. This should be taken seriously and consideration should be given to stop further investigations and treatment,especially if these have been accompanied by an earlier very poor or non-response to ovarian stimulation with maximum dose of gonadotrophins. Oocyte donation, if acceptable to the couple can be an excellent alternative in these situations. However, women with moderately high FSH concentrations and low AMH/antral follicle count in the younger age groups, have a better chance of conceiving compared to their counterparts having advanced age.

Obesity is one of the many undesirable product of modern society and maternal weight seems to have a substantial effect on fertility potential. Obese women are less fertile both in natural as well as ovulation induction cycles and have higher rates of miscarriage than their counterparts of normal weight. They also requires higher doses of ovulation inducing agents.

Obesity is having a close association with the signs and symptoms of insulin resistance in women with PCOS. Loss of weight by even few percentage can reverse this process, improve ovarian function and the associated hormonal abnormalities.

39. Sperm DNA fragmentation and pregnancy outcome

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Recently, the functional competence of spermatozoa evaluated in terms of nuclear DNA compaction and integrity has gained importance (Aitken & Krausz, 2001; Virro et al., 2004). It

influences embryo development and implantation rates as well.

The structure of spermatozoa renders it particularly susceptible to damage by oxidative stress leading to DNA fragmentation. The oocytes are bestowed with an inherent ability to repair low levels of sperm DNA fragmentation (Sakkas et al., 1996; Ahmadi and Ng, 1999b), but in case the DNA fragmentation is high, capacity to repair is exhausted.

The techniques commonly used to quantify DNA fragmentation are the TUNEL technique, the Comet technique, Sperm chromatin structure assay (SCSA) and sperm chromatin dispersion test.

It has been shown that sperm DNA fragmentation is an independent predictor of fertility in couples undergoing assisted reproduction treatment and studies have shown that ICSI should be the treatment of choice if DFI exceeds 30%. At a DFI level of 30-40%, there is reduction of natural fertility and TTP (time to pregnancy) is significantly longer (Evenson et al., 1999; Spano et al., 2000). DFI levels more than 60% are associated with higher risk of early pregnancy loss. DNA fragmentation has a role in recurrent pregnancy loss and is a contributing factor in half of the cases of unexplained infertility. These observations necessitate the use of DFI as a supplement to standard semen analysis for all infertile men.

40. LH administration in COH: Where are we today?

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LH is required for follicular steroidogenesis, stimulation of androgen synthesis by theca cells, follicular maturation. LH can support terminal stages of follicular maturation. During ovulation LH is required for resumption of meiosis, ovulation and luteinization. LH is also important for maintenance of luteal function

In assisted reproduction technologies (ART), the importance of LH is demonstrated clearly in hypogonadotropic hypogonadic patients. Patients with a profound lack of endogenous LH fail to undergo complete follicular maturation in the absence of exogenous LH. Such patients require the exogenous administration of both LH and FSH to optimize reproductive outcomes

1% of LH receptors need to be occupied for ovarian steroidogenesis. In ART cycles in normogonadotropic patients despite downregulation with GnRh agonist have sufficient endogenous LH. So the question arises do we need to add LH universally during ovarian stimulation in IVF?

For optimal follicular development, levels of LH should be above a certain 'threshold' but below the LH 'ceiling' i.e ≥1.2 IU/L and ≤5 IU/L. Majority of patients do not need LH supplementation. LH supplementation seems to be beneficial only in subgroups of the ART patients: Age > 35 years, abnormal ovarian reserve tests, previous poor response, deeply suppressed LH levels (< 0.5IU/L after agonist downregulation) and follicular stagnation/steady response (No follicle >10mm by D6, E2 < 200pg/ml on D6, slow growth<2mm/day).

No difference was observed in terms of MII oocytes, number of embryos or clinical pregnancy rates when LH was added in unselected normogonadotropic patients undergoing IVF. LH supplement seemed to be beneficial in women aged > 35 years in GnRH antagonist protocols.

LH can be supplemented either with help of recombinant LH or HMG preparations. A fixed dose of 75-150 IU rec LH seems to be adequate to restore androgen secretory capacity with ovarian aging

LH supplementation can start either from day 1 or day 6 of ovarian stimulation with maximum beneficial effect yet to be determined.

41. Presidential oration technology innovation and ethics, what rules ART?

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The human mind and brain are functioning constantly to improve life and living conditions on this planet. Reproductive medicine and assisted reproductive techniques are an example of man's endeavor to improve the lot of fellow living beings. From the time assisted reproduction was thought of, it has undergone radical improvements, innovations through technological advances bringing ART to its present position. For the person pursuing the subject of ART, it is important to understand a) how the subject has evolved b) have these technological innovations advanced science ethically or are there some grey areas that need to be addressed before we proceed further.

In 1978, while Steptoe and Edwards were successful in bringing the first IVF baby into the world, India witnessed the biggest tragedy – Dr Subhash Mukhhopadyay committed suicide when his own countrymen and the world refused to accept his IVF baby and his technique! It was a shameful expression of ethics to its extreme where our country was not ready to accept innovation and technology! (1)The debate therefore is – should ethics be overruling innovation and technology?

More than 4 million children have been born with IVF, mostly to couples who would otherwise not have been able to conceive. This would not have been possible but for constant innovation in the highly dynamic field of assisted reproduction. This makes assisted reproduction fundamentally an ethically and morally sound, not an ethically problematic practice.

This can also mean that there is a chance for further innovation: to make assisted reproduction more effective, less burdensome and cost effective for the women involved and more widely accessible to those who without this technology would not be able to have children or those who, because of a high risk of

having a child affected with a genetic disease, would otherwise not be able to reproduce with confidence.

IVF Advancements In the last Century

The birth of IVF virtually opened a wonderland for scientists and biologists. New developments took place in quick succession in order to make the process simple and effective. Innovations in folliculogenesis lead to COH and multifolliculogenesis. There was a constant development of new drugs and protocols in order to achieve this. However, this led to the risk of OHSS and multi order pregnancies!

In order to get better outcomes, there was a constant upgradation of IVF Labs and lab conditions. Better incubators, culture conditions improved results to a great degree but the problem of male infertility remained. A major break through albeit accidently, was ICSI in 1992. It seemed the panacea for male factor infertility. However, very soon it was realized that this was not true and the quest for solutions for male factor infertility began once again.

As we stepped into the 21st century, rapid advances are further taking place in the ART space. Following are some of the new advances

IVM : though not very popular presently, it has been initiated for the PCOS patients but is now also being used for fertility preservation. Its biggest problem is its not too good a success rate.

Cryopreservation : This technology has now evolved with the advent of vitrification. It enables the clinician to juggle with the IVF cycle in what ever way is beneficial for the patient. It has reduced OHSS to negligible, given better success rates and given an opportunity to couples to preserve their fertility. Hiowever, concerns regarding the long term safety of the procedure, effects on the offspring, technical expertise and increased cycle cost are reported.

Technologies Evaluating Embryos: What seems like a good embryo morphologically, may not be so. Various methods to assess abnormalities in embryos have been devised but not without risks and ethical questions. These are:

PGD;PGS.

SECRETOMICS, METABOLOMICS

TIME LAPSE IMAGING

Responsible Innovation : There are two forms of innovation in medicine (2): formal 'medical research' on the one hand and 'innovative treatment' or 'clinical innovation' on the other. The latter is what clinicians do when they try something new that has not yet been thoroughly tested in a research setting. For instance - a new surgical technique or the off-label use of certain drugs. This form of innovation circumvents the strict requirements and rules of formal research (3.). As a result, patients may not always be aware of the experimental nature of proposed innovative treatment, nor about possible risks. Furthermore, although innovative treatments often lead to publications, these mostly do not yield robust data about the efficacy and safety of the relevant procedures. As a consequence, insufficiently validated treatments may transform into regular practice. The flip side of the coin is that it allows innovations to become available for helping patients sooner than would have been possible if the lengthy and arduous route of regulated research were chosen. A good illustration is the off-label use of anti-retroviral and anti-infective drugs that turned out to be life-saving for thousands of patients who other- wise would have died of AIDS (4). In the light of such examples, there is an strong feeling among many practitioners that research stifles innovation.

On the other hand, illustrations can also be given of cases where procedures were introduced into clinical care without proper testing that later on turned out to be ineffective or harmful. In the context of medically assisted reproduction the premature introduction of preimplantation genetic screening (PGS) for aneuploidy is a good example. This was introduced as regular care in many clinics, but in subsequent trials turned out not to do what it was thought to do, with the possible implication of reducing rather than enhancing chances of successful pregnancy (Geraedts and De Wert, 2009). PGS is now in the process of being re-evaluated in a different form (polar body biopsy and analysis of all chromosomes) in a proper research settings.

According to authoritative documents, including the Helsinki declaration (World Medical Association, 2008) American Belmont report (National Commission, 1979), major innovative treatment ought to be made the object of research as soon as this is practically possible

CONTROVERSIAL INNOVATIONS:

Third party reproduction is a very good example of an innovation that is controversial and can have ethical ramifications! Unethical mixing of gametes has been seen in most countries. India is presently reeling under the stigma of " rent a womb" – surrogacy due to ethical dillemas. Technically, the procedures of third party reproduction are safe whether it is donor oocytes or sperms or embryos or even surrogacy.

Techno Ethics The term Technoethics (TE) is an interdisciplinary research area concerned with all moral and ethical aspects of technology in society. It focuses on discovering the ethical use of technology, protecting against the misuse of technology, and devising common principles to guide new advances in technological development and application to benefit society. Rapid advances in technology provoked a negative reaction from scholars who saw technology as a controlling force in society with the potential to destroy how people live.

Those practicing assisted reproduction are constantly driven with the need to better results in the form of live birth rates and in the Indian context, particularly keeping the costs down.

Our challenges in India are to work with

a) No formal ART training available the country
b) No research facilities
c) An unregulated environment where anyone can practice ART without training.
d) No national or insurance cover for infertility
e) Limited cost of treatment.

Hence, introduction of any new technology or innovation should understandably be cautious. Unfortunately it is not. The severe competition amongst IVF specialists drives them to offer what is new in the world without caring about research outcomes and utility. Many new innovations have disappeared from the field of ART because they proved to be of low utility but they have been introduced in the country without giving heed to the outcomes.

The field of ART is evolving in India. It is therefore important that we understand the ethics involved in the processes and view technological innovations rationally.

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42. Improving implantation fresh Vs frozen embryo transfer

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To achieve a high success rate in IVF-ET program is a challenge for the whole team involved in infertility management.

The COH exerts detrimental effect upon the implantation as a result, the endometrial receptivity may be compromised for the transferred embryos. Controlled ovarian hyper stimulation many a times is responsible for cancellation of the embryo transfer procedure in fresh cycle also. In such cases embryo cryopreservation is the only option for the utilization of the embryos for future cycles. Embryo cryopreservation followed by thawing and transfers into the uterus offers several advantages in assisted reproductive technology (ART) program.

The pregnancy rate after transfer of frozen-thawed embryos transfer cycles depends on various factors; like the freezing programme used and the stage of the embryo at freezing as the vitrification and warming steps are very crucial for the survival of embryos selected for cryopreservation. Also the quality of the frozen embryo and the survival rate after thawing1, 2 as well as the number of frozen-thawed embryos transferred also ascertain the pregnancy rate. It has been reported that pregnancy and birth rates per transfer of one or more frozen-thawed embryo as 25– 30% and 15– 20%, respectively3, 4.

In our centre at MAMC, in frozen embryo transfer group, women with extended culture up to blastocyst stage experienced highly improved pregnancy outcome. Pregnancy rate in extended culture group was significantly higher (32/65, 49.23% p=0.002) than conventional culture of 2-4 hours (19/104, 18.2%), 24 hours (28/87 32.2%) after warming. Embryo transfer in fresh cycle had significantly lower success rate (154/460 33.2%) as well.

Therefore, with improved techniques and good expertise in embryo cryopreservation procedures, more than 50% of clinical pregnancy rate can be achieved in the cryo-embryo transfer cycle which is higher than fresh cycles irrespective of the etiology.

Cryo-embryo transfer cycles were proven as unambiguously efficient aspect of ART modality with higher success rate as compared to fresh embryo transfer cycle. The cryo embryo transfer cycles combined with extended culture not only asses the morpho-kinetics but the viability also.

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43. Adjusting stimulation to individualize COH

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Introduction:

The most relevant measure of success to a couple suffering from infertility is the ability to have a single healthy child delivered per initiated cycle. One patient, one embryo, one baby without provoking ovarian hyper stimulation syndrome. Data shows that natural ovarian cycle leads to a significantly lower pregnancy rate than is achieved with stimulated cycles. Hence, ovarian stimulation is routinely used in ART to produce more oocytes and improve the chances of conception.

What is control ovarian hyper stimulation (COH)?

The regimens of infertility medications used to stimulate multiple follicles, the aim being to get 6-12 follicles.

Ovarian stimulation protocol should also avoid complication like ovarian hyper stimulation syndrome & multiple pregnancies.

OHSS can be severe in upto 5% cases & life threatening in 1% cases. Young, lean, low BMI, PCOS patients are more vulnerable. Multiple pregnancies can be in upto 30% of cases. Studies have shown four fold rise of perinatal mortality with twins & six fold rise in triplets & higher order pregnancy & apart from that long term consequences of neurological deficits.

It is of Paramount importance to individualize the ovarian hyperstimulation protocols to be able to produce the optimal quantity and quality of oocytes and embryos, and thereby to maximize the chances of overall success and minimize the complication rate.

One cannot adopt the policy of "one size shoes fit all" as the outcome of ovarian stimulation is determined by many interacting factors like

- Age
- BMI
- Ovarian reserve
- Polycystic ovarian disease
- Presence of Endometriosis
- Genetic factors
- Demographic characteristics of patients.

Given the factors mentioned above it is inaccurate to put forward one single protocol to all the patients even within one group of patients as there is a considerable genetic variations.

As a result, generalization of COH regimens in IVF is difficult due to the variability in chronological decline of the total follicular cohort between individuals (faddy, 2000) and the limited ability of tests of ovarian reserve to detect extremes of response to COH (Broekmans et al 2006, Fausal et al; 2008).

How to individualize /customize ovarian stimulation?

A key factor determining the outcome of COH and subsequent IVF outcome is the selection of the gonadotrophin starting dose. The correct individualization of treatment protocols should be based on the correct prediction of ovarian response. The predicting factors help to optimize individual stimulation protocols while minimizing potential complications. The most commonly used factors are

- Age
- BMI
- Baseline day 3 FSH Level
- Antimullerian hormone.
- Antral follicle count in early follicular phase.
- Previous history of OHSS or high response.
- Practicing guidelines of individual fertility clinics

The selection of a protocol appears to be much easier in women who have undergone previous IVF attempt than in a new entrant as the response to much extent is known in the first group and according to the over/ under response the dose can be adjusted in the current cycle.

To achieve optimal ovarian response patient's preparation starts before initiating ovarian stimulation. As obesity/ increased BMI is associated with poor ovarian response an advice on reduction of body weight by diet and exercise is required but not at the cost of increase in age of the patients.

Normogram to dictate the starting doses of gonadotrophins were decided by Popovic Todorovic et al 2003 & Hawler et al 2006. Both the studies used a combination of factors for predicting response such as antral follicle count & Antimullerian hormone, age etc.

An ideal regimen of stimulation is unfortunately still not achievable and is a subject of much current debate. At the present time COH is generally being utilized in an empirical way. Trial and error methodology is the most frequent path for infertile patients.

There are three groups of patients which can be identified as per ovarian response.

- Normal responders
- Hyper responders
- Poor responders

Normal responders

This is the best group of patients for COH. Easy to stimulate, with minimal complications & reasonable pregnancy rate. Depending upon the age, starting dose of FSH/HMG in the dose of 150-300 IU gives a low cancellation & OHSS rate with adequate live birth rate.

Long Agonist protocol is the most commonly used protocol worldwide with the disadvantages of it being time consuming with considerable patient's discomfort, higher cost, relatively high risk of ovarian hyper stimulation syndrome and high incidence of multiple pregnancies.

Antagonist protocol can give similar result and is less time consuming, lower risk of OHSS & lower total cost. No significant difference in outcome is found between the two protocols. In comparison to short of flare-up protocol, the long GnRh a protocol gives a better pregnancy rate. There is no difference in reproductive outcome whether urinary or recombinant HCG is used so continuation of use of uHCG is recommended. (Cochrane database April 2011)

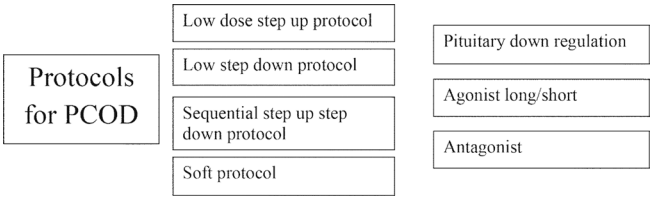
Hyper responders

The term hyper response refers to the retrieval of more than 15(La Marca et al 2010; Broer et al 2011) or more than 20(Nelson et al, 2007) oocytes following a standard protocol.

33-50% of IVF patients have polycystic ovaries (PCO) on baseline scan. Hyperinsulinaemia, high LH & high androgens increase the number of pre antral & small antral follicles before they are sensitive to gonadotrophins. Androgens have stimulatory role in early follicular growth by augmenting follicular FSH receptor expression and therefore amplifying FSH effects hence explosive follicular development takes place.

OHSS risk is 7 times more in PCOS & occurs in upto 25% of cases.

Although there is a low fertilization rate, high no. of immature oocytes, reduced implantation rate & high



miscarriage rate, at the end the pregnancy rate is equivalent to normal responders.

Low dose approach reduces complications of OHSS & multiple pregnancies.

The starting dose should be based on age, BMI, previous history of OHSS & high response.

GnRH antagonist protocol-This protocol gives a similar pregnancy rates per started cycle compared with standard GnRH agonist long protocol with lower chances of OHSS, lower cost, higher proportion of chromosomally normal embryos and lower psychological impact.Initial concern of High LH >10 in some patients at the start of antagonist administration does not influence the outcome.

No significant difference in ovulation induction in these women from FSH Vs hMG stimulation but a significant reduction in OHSS associated with FSH is observed.

Ovulation trigger in hyper responders:

HCG dose should be 5000 IU as there is no difference in pregnancy rate whether 10000 IU or 5000 IU of HCG is used.

In case of GnRH antagonist protocol, HCG can be replaced by GnRH agonist to trigger ovulation to reduce OHSS rate. Although pregnancy rate has been found to be lower when GnRh agonist is used for ovulation trigger in antagonist protocol.

No statistically significant difference between rhCG Vs uhCG regarding the incidence of OHSS has been found.

Luteal phase support in hyper responders:

The odds of OHSS were found to be more than 2 fold higher with treatments involving HCG than with progesterone alone (OR 3.06, 95 % CI 1.59 to 5.86) hence, HCG should never be used for luteal phase support in hyper responders.

Poor responders

No universal definition of poor responders.

The Poor responders include women in whom a previous cycle yielded 3 or fewer oocytes or was cancelled because of observation of 3 or fewer follicles 16 mm or greater or a peak serum estradiol less than 500 pg / ml on the day of HCG administration.

Prediction of a poor response can be done if

FSH ≥10 on D3 of the period

E2> 75 pg/ml on D2/3 of the period

AMH< 1 ng /ml on any day

AFC < 4 antral follicle count on baseline scan & poor ovarian volume

Multiple Options have been tried

Long Protocol with higher doses of gonadotrophin stimulation, decreasing the dose of GnRH agonist at the time of stimulation, a short follicular phase GnRH agonist treatment regimen using a standard or micro dose flare protocol, antagonist protocol, soft protocol, adding letrozole prior to gonadotrophin stimulation, low hCG dose in early stimulation or adding dexamethasone, growth hormone or other adjoined therapy such as nitric oxide, L- argenine, testosterone, bromocriptine, DHEA etc.

Optimistic data has been for IVF with high dose of gonadotrophin, antagonist protocol, flare up protocol, addition of LH, soft protocol, growth hormones etc.

Using a GnRH antagonist protocol instead of agonist –likelihood of pregnancy rate was found to be higher.(OR 1.28).

Result of meta-analysis demonstrated a statistically significant difference in both live birth rates & pregnancy rate favouring the use of adjuvant growth hormone in in-vitro fertilization protocol Or 5.39, 95% CI 1.89 to 5.35 and Or 3.28 95% CI to 1.74 to 6.20 respectively.

James MN duffy et al,Cochrane database systemic review CD 00099 pub 3

There is insufficient evidence to support routine use of adjoint therapy to improve the pregnancy rate in poor responders.

One can try sequential treatment with chomiphene citrate and exogenous gonadotrophin.

Data suggests that addition of dehydroepiandrosterone before assisted reproduction improves the pregnancy rates in selected group of patients.

Whatsoever used significant improvement in pregnancy rate has not been achieved in this group of patients and the ideal stimulation for these patients with diminished ovarian reserve remains a great challenge for the clinician.

AMH based strategic response- Adapted from recommendations from Nelson et al 2009-2011

Normal responders AMH 2-5	Antagonist or agonist + rFSH 150-300 Low cancellation, OHSS Adequate Live Birth Rate
High responders AMH >5	Antagonist + rFSH dose 75-150iu Normal oocytes yield Very low cancellation rate/OHSS Adequate LBR
Poor responders AMH < 1	Antagonist + rFSH 300-450IU (+ rLH) Moderate cancellation Low LBR

Endometriosis forms a special group

Cystectomy or drainage and fulguration of ovarian endomerioma should be done prior to ovarian stimulation in women with endometrioma4cm or more in size. Long agonist protocol is advisable instead of antagonist protocol. Downregulation with GnRH agonist for 3- 6 months before the assisted reproduction may lead to increase in the pregnancy rate.

Conclusion:

To optimize the ovarian stimulation

- Successful outcome following assisted reproduction are largely dependant on the patient's response to ovarian stimulation.
- As different group of patients respond differently to the same type of ovarian stimulation an individualized approach to COH is crucial for optimal result.
- OHSS & multiple pregnancies are unwanted & cost has to be taken in consideration specially in our scenario.
- Predictive factors for optimizing ovarian stimulation treatment are of value but not absolute in this regard.
- Weight reduction advice to both PCO or non PCO overweight patients
- For hyper responders, low stimulation dose of gonadotrophins in long protocol or antagonist protocol,

ovulation trigger with lower dose of HCG and use of progesterone for luteal phase support will reduce the risk of OHSS.

- High gonadotrophin dose, antagonist protocol or low dose agonist protocol and addition of LH early during simulation may work for poor responders. Growth hormone or DHEAS may be of some help to such patients.
- Down regulation with GnRH agonist for 3 months before assisted reproduction in endometriosis patients improves the pregnancy rate.

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44. Current concepts in the management of OHSS

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Ovarian Hyperstimulation Syndrome (OHSS) is a syndrome of multi-organ dysfunction and has an unpredictable response to ovarian stimulation thus making the prediction of OHSS difficult. Heightened clinical suspicion and early intervention are paramount to the reduction of morbidity and mortality.

Conservative management in the outpatient setting is appropriate for mild-to-moderate (OHSS) with review every 48hours until spontaneous resolution occurs.

However, management in the inpatient setting should be considered if :

- Inability to tolerate oral food or hydration
- Severe abdominal pain
- Hypotension
- Shortness of breath
- Tense ascites
- Peritoneal signs
- Hematocrit greater than 45%
- Creatinine greater than 1.2 mg/dL

Assessment

Assessment	Measurements
History and Examination	Pain
	Breathlessness
	Hydration
	Weight
	Cardiovascular
	Heart rate, blood pressure
	Abdominal girth, distension, ascites
	Intake and output chart
Investigations	Full bipod count
	Haemoglobin, haematocrit, white cell count
	Urea & electrolytes
	Liver function tests
	Baseline clotting studies
	Pelvic ultrasound (for ascites and ovarian size)
	Chest X-ray or ultrasonography (if respiratory symptoms)
	ECG and echocardiogram (if suspect pericardial effusion)

Pain Relief/Antiemetics

Pain relief is best provided with paracetamol and if necessary oral or parenteral opiates. Nonsteroidal anti-inflammatory agents are not recommended. Antiemetic drugs used should be those appropriate for the possibility of early pregnancy,

Moderate Hyperstimulation

The treatment of moderate OHSS consists of observation, bed rest, the provision of adequate fluids, and ultrasonographic monitoring of the size of the ovaries. Serum electrolyte concentrations, hematocrits,

and creatinine levels should also be evaluated.

Patients should be asked to keep track of their fluid intake and output. Discrepancy in fluid balance of greater than 1000 mL daily is a cause for concern.

Severe Hyperstimulation

Medical treatment of severe hyperstimulation is directed at maintaining intravascular blood volume along with correcting the disturbed fluid and electrolyte balance, relieving secondary complications of ascites and hydrothorax, and preventing thromboembolic phenomena.

Fluid Management

The main interventions are correction of hypovolemia and maintain urine output.

First 24h: 1500-3000ml

These measures consist of initial intravenous (IV) administration of 1 L Ringer Lactate/ Normal Saline over 1 hour then infused at a rate of 125-150mL/h, with 4-hour tabulations of urine production. If urine production is restored or improved, IV hydration at a maintenance rate of 125-150 mL/h is continued.

HES – 6% Hydroxyethyl Starch

Maximum daily dose: 33ml/kg in 250 - 500 cc per day, drop wise, slow administration to avoid lung congestion. HES has been reported to be associated with a higher mean daily urine output, fewer paracenteses and shorter hospital stay than human albumin.

Albumin Infusion

It should be kept for a later stage, once hypo-albuminemia is proven along with reduction in urine output because of risk of hepatitis, excessive albumin overload and renal function impairment.

Administration is mainly important during drainage of ascites

Daily dose : 25 - 75g (100 – 300 ml) of 25% human albumin every 6 hours according to the severity of hypoalbuminemia and the total volume of ascitic fluid drained.

The use of diuretics in patients with low urine production and intravascular volume depletion is counterproductive and should be avoided.

Heparin Thromboprophylaxis

Thromboprophylaxis should be provided for all women admitted to hospital with OHSS. This should be continued at least until discharge from hospital and possibly longer, depending on other risk factors.

Paracentesis

To manage ascites, ultrasonographically guided abdominal or vaginal paracentesis is indicated if the patient has severe discomfort or pain or if she has pulmonary or renal compromise.

Critical Hyperstimulation

Its management and treatment require intensive care in a critical care unit with invasive monitoring of circulatory indicators, including venous pressure and wedge pressure. The patient may need extra oxygenation (assisted ventilation).

If renal failure is present, an IV dopamine regimen should be started. To treat thromboembolism, therapeutic doses of anticoagulants should be administered. Thoracocentesis should be performed in cases of severe hydrothorax.

Finally, if a pregnancy is maintaining a life-threatening OHSS, therapeutic abortion must be considered.

Role of Surgery

Pelvic surgery should be restricted to cases with adnexal torsion or co-incident problems requiring surgery following careful assessment.

New Concepts

1. OHSS Free Clinic-Segmental approach
   - It consists of optimization of the ovarian stimulation, including GnRHa triggering in a GnRH antagonist cycle (segment A). Segment B then consists of optimum cryopreservation methods for oocyte or embryo vitrification. Segment C includes embryo replacement in a receptive, non-stimulated endometrium in a natural cycle or with artificial endometrial preparation.
2. Metformin Co-Administration
   - Metformin given 2 months before starting COS significantly reduces the risk of severe OHSS.
3. Dopamine Agonists-Cabergoline
   - Cabergoline (dopamine D2 receptor agonist) appears to reduce the risk of OHSS in high-risk women, especially for moderate OHSS.
4. GnRH antagonist in the Luteal phase
   - The luteolytic effect of the GnRH antagonist has been proposed as the main theory to explain the mode of action of these drugs
5. IV Calcium Gluconate
   - 10 ml of 10% calcium gluconate solution in 200 ml physiologic saline within 30 min of ovum pick up and continued thereafter on day 1, day 2 and day 3.
   - Calcium infusion reduces renin secretion and decreased angiotensin II and VEGF synthesis.It is comparable to cabergoline in OHSS prevention.

Key Recommendations

- Prevention is the best cure
- The addition of metformin should be considered in patients with polycystic ovarian syndrome who are undergoing in vitro fertilization, because it may reduce the incidence of OHSS
- Gonadotropin dosing should be carefully individualized, taking into account the patient's age, body mass, antral follicle count, and previous response to gonadotropins
- Coasting can be tried if Estradiol concentrations are > 4500pg/ml, however abandon if it takes more than 4 days
- A GnRH antagonist protocol with a GnRH agonist trigger for final oocyte maturation is recommended for donor oocyte and fertility preservation cycles
- Albumin or other plasma expanders at the time of egg retrieval are not recommended for the prevention of OHSS
- Elective single-embryo transfer is recommended in patients at high risk for OHSS
- Progesterone, rather than hCG, should be used for luteal-phase support

New Insights

1. Meloxican, a COX2 inhibitor, has been tried in rats, suggesting that the inhibition of COX2 could have a positive effect on OHSS.
2. Tetracyclines inhibit angiogenesis and prevent VEGF induced

VP in human and animal models. Researchers have found a reduction in peritoneal fluid accumulation in animals that received doxycycline.

45. Perinatal outcomes of IVF pregnancies what to tell parents

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All men with severe oligozoospermia or azoospermia (sperm count < 5 million/hpf) should be offered genetic/clinical counselling, karyotype assessment for chromosomal abnormalities, and Y-chromosome microdeletion testing prior to in vitro fertilization with intracytoplasmic sperm injection. (II-2A). All men with unexplained obstructive azoospermia should be offered genetic/clinical counselling and genetic testing for cystic fibrosis prior to in vitro fertilization with intracytoplasmic sperm injection. (II-2A). Multiple pregnancy is the most powerful predictive factor for adverse maternal, obstetrical, and perinatal outcomes. Couples should be thoroughly counselled about the significant risks of multiple pregnancies associated with all assisted human reproductive treatments. (II-2A). The benefits and cumulative pregnancy rates of elective single embryo transfer support a policy of using this protocol in couples with good prognosis for success, and elective single embryo transfer should be strongly encouraged in this population. (II- 2A). To reduce the incidence of multiple pregnancy, health care policies that support public funding for assisted human reproduction, with regulations promoting best practice regarding elective single embryo transfer, should be strongly encouraged. (II-2A). Among singleton pregnancies, assisted reproductive technology is associated with increased risks of preterm birth and low birth weight infants, and ovulation induction is associated with an increased risk of low birth weight infants. Until sufficient research has clarified the independent roles of infertility and treatment for infertility, couples should be counselled about the risks associated with treatment. (II-2B). There is a role for closer obstetric surveillance of women who conceive with assisted human reproduction. (III-L) There is growing evidence that pregnancy outcomes are better for cryopreserved embryos fertilized in vitro than for fresh embryo transfers. This finding supports a policy of elective single embryo transfer for women with a good prognosis (with subsequent use of cryopreserved embryos as necessary), and may reassure women who are considering in vitro fertilization. (II-2A). 0Women and couples considering assisted human reproduction and concerned about perinatal outcomes in singleton pregnancies should be advised that (1) intracytoplasmic sperm injection does not appear to confer increased adverse perinatal or maternal risk over standard in vitro fertilization, and (2) the use of donor oocytes increases successful pregnancy rates in selected women, but even when accounting for maternal age, can increase the risks of low birth weight and preeclampsia. (II-2B). Any assisted reproductive technology procedure should be prefaced by a discussion of fetal outcomes and the slight increase in the risk of congenital structural abnormalities, with emphasis on known confounding factors such as infertility and body mass index. (II-2B). In pregnancies achieved by artificial reproductive technology, routine anatomic ultrasound for congenital structural abnormalities is recommended between 18 and 22 weeks. (II-2A). Pregnancies conceived by intracytoplasmic sperm injection may be at increased risk of chromosomal aberrations, including sex chromosome abnormalities. Diagnostic testing should be offered after appropriate counselling. (II-2A). The possible increased risk for late onset cancer due to gene dysregulation for tumour suppression requires more long-term follow-up before the true risk can be determined. (III-A). The clinical application of preimplantation genetic testing in fertile couples must balance the benefits of avoiding disease transmission with the medical risks and financial burden of in vitro fertilization. (III-B). Preimplantation screening for aneuploidy is associated with inconsistent findings for improving pregnancy outcomes. Any discussion of preimplantation genetic screening with patients should clarify that there is no adequate information on the long term effect of embryo single cell biopsy. (I-C)
reference- SOGC guideline 2014