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From the Desk of Editor

Prof. Baidyanth Chakravarty — A Great Human Being

Prof. Dr. Gita Ganguly Mukherjee

Dr Baidyanath Chakravarty a living legend in the field of gynecology and reproductive medicine, was born on 2nd August 1928 at Faridpur, now in Bangladesh.

He had a brilliant academic carrier – since his school days at Chakradharpur where his father was a Station Master. He passed Matriculation examination from Patna University securing highest marks in the district. After that he came to Calcutta and after passing ISC examination he was admitted in Calcutta Medical College. He passed MBBS in 1952. During his college life he received Goodeve scholarship, Green armytage prize stood first in OBS GYN in MBBS exam. He passed Master & Obstetric (MO examination) from Calcutta University in 1959 and MRCOG examination from England in1961 & awarded FRCOG in 1977. North Bengal University honored him with DSC degree in 1990.

Dr B N Chakravarty was a bright clinician, and distinguished surgeon. Initially his interest was surgery for genital cancer and VVF. His colleagues used to come to OT to see his operation. Later he became interested in **reconstructive surgery** for congenital anomalies of reproductive tract in female. He became the pioneer of reconstructive surgery in cervico-vaginal atresia and got international fame for his work on congenital genital defect. The delivery of a baby in 1998 following correction of cervico-vaginal atresia has been ranked as the fifth viable baby recorded so far in the world literature.

His interest in infertility and ART- after meeting Dr Subhas Mukherjee at NRS Medical College, Dr Chakravarty became more interested in the management of infertility and ART. Dr Subhash Mukherjee's IVF baby Durga was born in Kolkata only 67 days after the first IVF baby Louie Brown. Though he is now recognized as the creator of first Indian IVF baby and second in the world, but initially he was not recognized. He committed suicide. The sad death of Dr Subhas Mukherjee acted as a trigger to devote the life of Dr Chakravarty for the treatment of infertile couple. Dr Chakravarty's initial work in Assisted Reproductive Technology (ART) was conducted at his clinic with a make shift arrangement of ART laboratory and operation theatre (OT). His first IVF baby was born in 1986. We became so proud of him. I still remember the overwhelming felicitation given to him with his team by BOGS - that time President of the organization was Dr Amiya Kumar Mukherjee and Secretary Dr Gita Ganguly Mukherjee. Huge numbers of BOGS members and also non BOGS people attended the program to show their respect to Dr. Chakravarty.

Setting up IRM – after birth of the first IVF baby, Dr Chakravarty realized the importance of setting up a separate institute for treating, teaching, and expanding knowledge in reproductive medicine along with collaborative research activity. Ultimately the Institute of Reproductive Medicine (IRM) was constructed at Salt Lake in the year 1989 and the second IRM in 1999.

Activities of the IRM – Activities of ART work and OPD, research and academic activity are done in new IRM and indoor treatment are provided in old building. Animal lab was also setup in old building in 2002.

Under the leadership of Dr. B N Chakravarty Clinicians, Embryologists and Scientists worked together to make ART safer and effective. There has been significant achievement in the clinical, scientific, and academic performance of the institute.

Every year more than 3000 couples were being treated and about 20 to 30 students from different parts of India and neighboring countries used to be trained at IRM under Dr B N Chakravarty. IRM has established itself as one of the pioneer Institute of Reproductive Medicine in India. In-recognition of its clinical, academic, and scientific activities, IRM has received affiliation with:

a) Calcutta University for PhD course in Biochemistry
b) West Bengal University of Health Sciences for PhD course in Reproductive Medicine.
c) National Board of Examinations, National Academy of Medical Science, Ministry of Health & Family Welfare, New Delhi for Fellowship Course in Reproductive Medicine.

Dr Chakravarty guided 8 PhD students, who are now scientists in their respective fields. He has also supervised more than 35 FNB students, who are practicing ART in different corners of India and abroad.

Bi-monthly CMEs- IRM organizes bi-monthly CMEs which are very effective.

Medical Bulletin published quarterly is the mirror of activities of IRM. This also contains important academic articles from the in-house consultants and also from outside stalwart. During covid situation most of the activities came to a grinding halt except the work of Dr. Chakravarty who continued writing and completed the volume 4 of his book "Clinics in Reproductive Medicine and Assisted Reproductive Technology." After covid situation gradually I.R.M. was back to normal again.

Book- "Clinics in Reproductive Medicine and Assisted Reproductive Technology" – Dr Chakravarty wrote four volumes. He always had a desire to intergrate clinical medicine with basic science. These we can find in his lecture and in his writings. Residents, fellows and practitioners interested in reproductive endocrinology and infertility will find these 4 volumes very valuable.

Eminent Teacher- Dr Chakravarty was an eminent teacher in Gynecology, Reproductive medicine, and ART in the country. He always desired to integrate clinical medicine with basic science. Since many of the teachers in our country and abroad, have been his students, he is most appropriately called the "Teacher of the Teachers." He also got 'Eminent Teacher' award from Calcutta University. He delivered several guest lectures, oration in our country and abroad. Every where during his lecture the hall used to be absolutely full and audience were listening.

Dr Chakravarty was pioneer in the field of ART in India and his devotion, dedication and research was appreciated not only nationally but internationally. He received many lifetime achievements and many awards from India and abroad.

Dr Chakravarty was very social and he had a helping attitude. If asked for help he used to help the doctor for any problem in patient's treatment. Inspite of his busy schedule he always used to attend the social invitation.

R N Ganguly Foundation was a philanthropic organization established in memory of my father. Dr Chakravarty never failed to attend any CMEs or Annual Conferences organized by the foundation. He delivered first R N Ganguly Memorial oration in 2010. This year we are going to miss his presence.

Dr Chakravarty was really a ***living legend.*** He never stopped work even with illness and during covid times. Till 16th March, 2022 the day of his hospitalization, he was busy with clinical checkup of the patients and writing the scientific article.

He was very big figure in the field of Gynecology and Reproductive Medicine. But above all he was a great human being — calm & quiet, always with smiling face, never got angry and had helping attitude to all who approached him for any help.

He left for his heavenly abode on 14th April, 2022. But he will always be remembered by his students, patients, colleagues, friends, and in the academic world.

However, it is also a fact that we always feel the vacuum created by his absence.

Dr Chakravarty's birth day is on 2nd August. We should celebrate his birth day with academic activities.

I hope his brain child and dream Institute IRM will work according to his ideal and will prosper more and more.

— My homage to this great soul.

From the Desk of Secretary

Dr. Ratna Chattopadhyay

We are extremely happy to publish another bi-monthly bulletin from IRM in 2023. On behalf of all the members of IRM, I would like to convey our heartfelt thanks to all who worked hard with sincerity to publish the bulletin full of scientific information related to assisted reproductive technology. We will be honored and grateful if these information are helpful and beneficial for the readers in their practice.

Sir's blessings, advices and teaching are helping us to proceed forward and to overcome the hurdles in his physical absence. We, the members of IRM need your support and good wishes to carry forward Sir's legacy.

Thanking you
Dr. Ratna Chattopadhyay

Obesity and Reproductive Health — Molecular and Clinical Aspect

Dr. B N Chakravarty

Introduction

During past few decades, incidence of obesity (defined as BMI over 30 kg/m2) is increasing rapidly both in the developed as well as in developing areas of the world.[1] In 2014, the number had reached to 641 million adults (266 million in men and 375 million in women) as against 105 million adults in 1975 (34 million men and 71 million women).[2] Moreover, if this trend continues, worldwide obesity prevalence will rise to 18% in men and surpass 21% in women by 2025.[2] To highlight the importance, world health organization (WHO) has declared obesity is a global epidemic; and simultaneously stressed the idea that it still remains under-recognized world health problem.[3]

It is now well recognized that depending on the degree and duration of weight gain, obesity can cause exacerbation of a number of co-morbidities including cardiovascular diseases, diabetes, musculoskeletal disorders, sleep apnoea, increased rise of certain malignancies,- such as breast, endometrial and colon cancer.[4]

Obesity also has an increased impact on reproductive health. Obese women have increased risk of menstrual dysfunction, anovulatory infertility and pregnancy related complications.[5]

All these adverse reproductive impacts of obesity are mediated through endocrinological and molecular pathways. Endocrinological link through GnRH pulse generator, androgen, insulin, IGF-1 have already been discussed in detail in earlier paragraphs of this chapter and previous volume of this book (Definition and etiology-PCOS and Infertility).

The following paragraphs will briefly outline a few molecular links between reproductive outcome and obesity during child bearing period. The clinical impact will be discussed in the final section of this chapter.

Molecular link between obesity and reproductive health

As far as molecular pathways are concerned, the adverse impact of obesity is mediated through certain inflammatory products. The important bioactive molecules in the adipose tissue are 'adipokines'. Adipokines interact through multiple molecular pathways of insulin resistance, inflammation, hypertension, cardio-vascular risk, oocyte development, maturation, and embryonic implantation. Other associated adverse reproductive outcomes of obesity are related to delayed conception, increased miscarriage rates and poor success rate following assisted reproduction.

The major role for these adverse impacts is played by adipokines which exist as 'major fat tissue soluble products'.

What are adipokines?

Adipokines are constituents of adipose tissue. Adipose tissue is now considered as an endocrine organ which will perform significant roles in coordinating many physiological events such as reproduction, immune response, glucose and lipid metabolism through release of a number of bioactive cytokines collectively known as adipokines- which commonly control metabolic regulation and inflammatory process.

Very recently adipokines, the important constituents of adipose tissue have been found to be intimately linked with pathophysiology of reproductive health of obese infertile women seeking treatment. The informations gathered about adipokines in obese women and their impact on reproductive outcomes are briefly outlined below:

The family of adipokines include two groups of molecules:

- Adipose specific cytokines
- Non adipose specific cytokines

Adipose specific cytokines include; - leptin, adiponectin (APN), resistin, visfatin and omentin.

Non adipose specific cytokines are; - retinol binding protein (RBP4), lipocalin2 (LCN2), chemerin, interleukin6 (IL6), interleukin 1β (IL1 β) and tumour necrosis factor α (TNF α). These molecules representing family of adipokines have both harmful and helpful effects of reproductive health of obese women. For example abnormal serum levels of most of these molecules have been shown to be strongly associated with both insulin resistance (IR), type-2 diabetes mellitus (T2DM) and PCOS. Severe dysfunction of adipose tissue molecules may lead to production of certain harmful cytokines TNF- α (non-adipose specific cytokines) and at the same time overproduction of some cytokines such as adipose specific cytokines (APN) may also be beneficial (Table 1).

Table 1: Beneficial and harmful effects of family of adipokines on reproductive outcome in obese women.

Name of Adipokines	Serum levels	Beneficial or harmful effect on reproduction in obese women
Leptin	Elevated	Reduce insulin induced ovarian sterioidogenesis and LH induced E2 production in granulosa cells
Adiponectin	Suppressed	Plasma insulin increases
Resistin	Elevated	Insulin resistance +
Visfatin	Elevated	Increases insulin sensitivity
Omentin	Suppressed	Increases insulin sensitivity
Chemerin	Elevated	Increases insulin sensitivity

Apart from generating molecules directly involved with reproduction, activated adipocytes also generates molecules which may accelerate comorbidity associated with reproduction in obese women. The possible comorbidities are presented in Fig. 1.

Take home message:

- It is already known that obesity has adverse impact on cardiovascular disease, diabetes, musculoskeletal disorder, sleep apnea, increased risk of certain malignancy such as breast, endometrial and colon cancer.
- In addition, obesity has an increased adverse impact on reproductive health.
- All these adverse impacts of obesity are mediated through endocrinological and molecular pathways.
- Regarding molecular link, the adverse impacts are the consequences of production of certain inflammatory products.
- The important bioactive molecules in the adipose tissue are known as 'adipokines'.
- The major endocrinological and clinical impacts of obesity are mediated through 'adipokines' which exist as 'major fat tissue soluble products'.
- Adipose tissue is now considered as an endocrine organ.
- Adipose tissue through adipokines perform many significant functions for coordinating various physiological events such as reproduction, immune response, glucose and lipid metabolism through release of a number of bioactive cytokines,- the different component of adipokines which commonly control metabolic regulation and inflammatory processes.
- Family of adipokines consist of two main groups; adipose specific cytokines and non-adipose specific cytokines.
- Adipose specific cytokines are leptin, adiponectin (APN), resistin, Visfatin and omentin.
- Non adipose cytokines are: retinal binding protein (RBP4), lipocalin 2 (LCN2), chemerin,

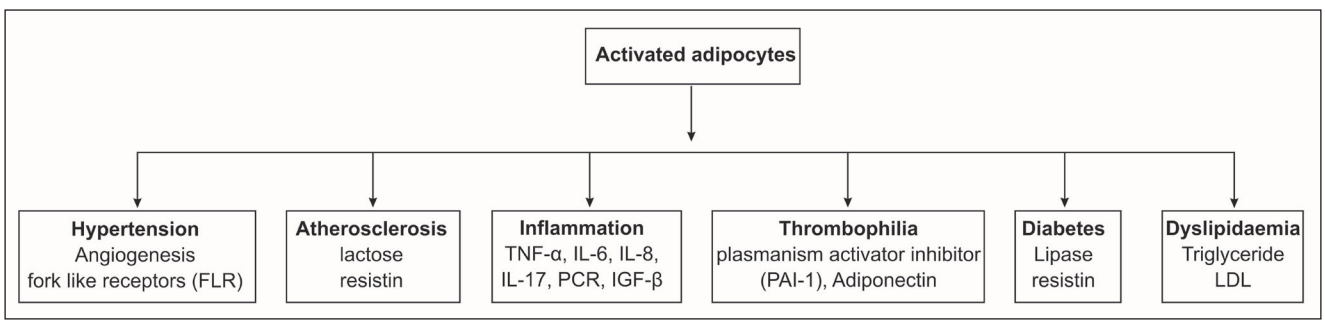

Fig. 1: The possible comorbidities of activated adipocytes which may accelerate comorbidity associated with reproduction in obese women.

interleukin 6 (IL6), interleukin 1β (IL1 β) and tumour necrosis factor α (TNF α).

- These molecules may have both harmful and helpful reproductive impact in obese women.
- For example abnormal serum levels of most of the molecules are strongly associated with both insulin resistance (IR), type-2 diabetes mellitus (T2DM).
- Similarly severe dysfunction of some adipose tissue molecules may lead to production of some harmful cytokines like TNF α (non-adipose specific cytokines).
- But at the same time, overproduction of some other cytokines such as APN may also be beneficial (adipose specific cytokines).
- Apart from generating molecules directly involved with reproduction, activated adipocytes also generate molecules which may increase co-morbidity associated with reproduction.
- The specific co-morbidities likely to be accelerated through activated adipocytes are, hypertension, atherosclerosis, thrombophilia, diabetes and dyslipidemia.

Clinical impact of obesity on fertility:

It is not a fact that obese women do not become pregnant. But certainly obesity reduces chances of fecundity.[6,7] Both hormonal and mechanical factors play their role.

The related hormonal factors associated with obesity and reproduction are well known. Interplay of these hormones namely viz, insulin resistance with consequent hyperinsulinemia, low sex hormone binding globulin (SHBG), elevated androgen, increased peripheral conversion of androgen to estrogen by aromatase, increased insulin like growth factor 1 and high leptin levels[8,9] lead to both menstrual and ovulatory irregularities. Because combined effects of these hormonal abnormalities induce hypothalamic dysfunction, abnormal gonadotropin secretion, reduced folliculogenesis, dysovulatory or anovulatory menstrual cycles and lower progesterone levels in the luteal phase,[10–12] are the other consequences.

It is accepted that anovulatory infertility is more common in obese than in non-obese women.[13,14] But it is also interesting to note that even in obese women the incidence of infertility is related to distribution of location of body fat. It has been shown that women with higher waist circumference (excess abdominal fat) are more likely to suffer from anovulatory infertility than similar obese women with same BMI who has less abdominal fat.[15,16]

On the other hand, even ovulatory women with obesity have less fecundity and take prolonged period for conception.[17–20]

The discrepancy may be due to decreased frequency of intercourse. In male obesity one of the important factors for decreased frequency of intercourse is erectile dysfunction and decreased desire. However, in obese women waist circumference, cycle irregularities and decreased frequency of intercourse increases the time to pregnancy (cycle fecundity) both in nulliparous and parous woman when BMI increases from 25 to 30 to more than 35.[21]

Obesity and male infertility:

If both partners are obese, sexual relationship may be affected. Scrotal temperature may be high because of closer contact with surrounding tissues.[22,23]

Male with obesity have decreased testosterone level, which correlate negatively with both their fasting insulin and leptin levels,[24,25] In spite of these hormonal aberrations, there is a great deal of controversy regarding abnormalities of semen profile in obese men. Although there is no doubt that obese couples suffer from higher incidence of infertility, but it is not very clear that to what extent the sperm quality contributes to this association.

Though infertility in obese man could not be precisely associated with sperm quality or function, but literature survey shows a distinct relationship between male obesity and erectile dysfunction.[26–28] Improvement of erectile dysfunction through any method e.g. change of life style, diet and exercise, pharmacological interventions or even through bariatric surgery[29,30] has helped to improve the situation.

Response of obese women to fertility treatment:

Though obesity as such is not an independent indication for fertility enhancing treatment but incidentally obesity may be an intermediate co-factor which may modify the drug dose, protocol and outcome of treatment.

Three common types of infertility treatments are ovulation induction, intrauterine insemination and IVF. Similarly three commonly used drugs used in these treatment protocols are –clomiphene citrate, letrozole and gonadotropins.

Between clomiphene and letrozole, letrozole is preferred in obese woman. Because fat cells are rich in aromatase. Aromatase helps in conversion of testosterone to oestrogen. Excessive "static" level of oestrogen, (not fluctuating levels) cannot produce a positive feedback effect on hypothalamus to induce LH surge hence leading to anovulation. In these cases overall suppression of static oestrogen with aromatase inhibitors (letrozole) will be better alternative than clomiphene.

With regard to gonadotropin, which is more frequently used with IUI and IVF rather than with simple ovulation induction, in patients with BMI more than 30, the dose requirement will be higher and duration of administration prolonged with lower peak estradiol level and less number of larger and medium sized follicles.[31] The lower response and larger dose requirement are possibly due to increased volume of distribution. Regarding hCG, absorption has been reported to be more with intramuscular rather than with subcutaneous injection.

With regard to pregnancy outcome, IUI-gonadotropin regime is more favorable than with simple ovulation induction.[31–34] The rational explanations are correction of anovulation, compensation for erectile dysfunction and decreased frequency of intercourse. So far the impact of obesity on IVF outcome is concerned, the results reported by society for Assisted Reproductive Technology (SART) registry[35] is given below in tabular form (Table 2).

The results published by SART study is briefly outlined below:

- There is slight decline in the number of oocytes retrieved and also in the number of high quality embryos produced as the BMI rises over 40.
- Implantation, clinical pregnancy and live birth rates decline gradually with increasing severity of obesity.
- However, the absolute decline in pregnancy rate is small.
- Based on the data presented, implantation rate declines by 0.3 to 0.4% for each 1kg/m2 over 25 BMI.
- Thus the overall possibility of a live birth per each start cycle decline from 31.4% with a normal BMI to 28% in women with BMI 30-34.9.
- Further decline to 24.3% was observed in women with BMI between 40- 44.5.
- And declined birth rate was 21.2% in women with BMI more than 50.
- The cause of low success rate may be due to poor embryo quality or alternatively with defective endometrial receptivity.[36]
- Miscarriage rate is higher possibly, again due to poor endometrial receptivity.
- Even with donor egg, live birth rate per cycle start is lower compared to non-obese recipient again

Table 2: The impact of female obesity of IVF outcomes.

The impact of female obesity on IVF Outcomes

	BMI 18.5–24.9	BMI 25–29.9	BMI 30–34.9	BMI 35–39.9	BMI 40–44.9	BMI 45–49.9	BMI >50
Number of IVF cycles	134 588	54 822	24 922	11 747	4084	1292	463
Oocytes retrieved	12.4	12.3	12.3	12.1	11.6	11.2	10.5
Cancellation rate	10.3%	11.3%	11.3%	12.2%	13.3%	14.2%	11.7%
Embryos transferred	2.4	2.4	2.4	2.4	2.4	2.5	2.3
Implantation rate	29.5%	28.3%	26.9%	25.8%	23.6%	22.9%	20.3%
Clinical pregnancy rate	37.9%	36.8%	35.7%	33.7%	32%	30.6%	30%
Pregnancy loss rate	11.3%	12.7%	14.6%	15.3%	14.8%	17.6%	20.3%
Live birth rate	31.4%	29.8%	28%	26.3%	24.3%	22.8%	21.2%

Adapted from Provost et al.[101]

BMI: body mass index.

suggesting the cause being poor endometrial receptivity.

- Obesity may create problem in the technologies of ART. Following COH;-the ovaries may shift to higher position in the pelvis,-making them more difficult to visualize with trans vaginal scanning.
- Obesity Increases the risk of complications of oocyte retrieval, such as bleeding, infection and injury to surrounding structures. Anaesthesia may create problem.

Impact of obesity on maternal risks of pregnancy:

Miscarriage:

Miscarriage rate increases with increasing level of obesity. The incidence is similar for all types of pregnancies; either spontaneous, induced or IVF pregnancies. The hazard ratio is 1.23 in obese women compared to non-obese control.[37] Miscarriage rate in these women is higher both in sporadic as well as in recurrent miscarriage groups. In women with history of recurrent miscarriage, the risk of subsequent miscarriage is elevated 3-4 fold in obese compared to non-obese control.[38,39]

Apart from miscarriage, the risk of gestational diabetes, pre-eclamptic (PE), preterm labour (PTL), premature rupture of membranes (PROM), risk of macrosomia, shoulder dystocia etc. are all increased.

Incidence of increased risk of PROM and PTL in obese patients may be due to increased circulating adipokines and inflammatory proteins. Increased risk of sleep apnoea and ante natal depression has also been reported.[40–43] Additionally, following operative delivery, there is increased incidence of wound infection, wound dehiscence, post-partum haemorrhage and deep venous thrombosis.

Fetal and neonatal risks:

Incidence of fetal macrosomia in patients with obesity is not directly related to her fat volume but indirectly associated through association of gestational diabetes. The incidence of macrosomia has been reported higher 7.7% in obese non-diabetic compared to obese diabetic woman.[44] Maternal obesity associated with fetal macrosomia increases the incidence of obstetric intervention. In addition, child born of obese mother has higher risk of obesity, diabetes and cardiovascular diseases in later life.[44]

The risk of congenital anomaly in the offspring is higher suggesting that maternal obesity alters fetal development during the embryonic period.[45] Neural tube, oral & gastro intestinal defects are also common. Other commonly observed defects consist of spina bifida, hydrocephaly, anorectal atresia and oral clefts etc. One of the causes of these congenital anomalies is possibly poor glycaemic control. Another problem in relation to fetal anomaly may be a relative difficulty in the detection of the anomaly because maternal obesity may obstruct visualisation of the defect which may be responsible for error of prenatal detection. A retrospective study has reported 20% less detection of anomalous fetus in women with high BMI compared to normal BMI.

Screening and advice to obese woman seeking fertility care:

Comorbidities with obesity are well known. It has already been discussed in the earlier part of this chapter that obesity increases insulin resistance and type 2 diabetes mellitus. In addition obesity induces an inflammatory state which also accelerates the risk of hypertension, dyslipidaemia and cardiovascular diseases[45–47] central obesity in particular is associated with greater risk of cardiovascular diseases.[48–50] In addition to these well-known metabolic disorders, obesity is also associated with two other co-morbidities which may affect a woman in her reproductive years. One is a risk of endometrial hyperplasia and endometrial carcinoma in premenopausal years.[50–53] The other one is obstructive sleep apnoea.[54]

Plenty of literature demonstrate that increased risk of endometrial carcinoma has a positive correlation with increasing level of BMI. It has been reported that compared to non-obese controls, women with BMI 40 or more has a relative risk of 2.1 dying from breast cancer and relative risk of 6.3 dyeing from endometrial cancer.

Sleep apnoea also in the long run will lead to cardio vascular dysfunction. About 35% of woman more than 35 report problem of sleep apnoea.[55] Sleep apnoea may be associated with oxygen desaturation, episodic hypercapnia, negative intrathoracic pressure,-all of which will cause repeated arousal from sleep. Sleep apnoea itself may be an independent prognostic marker for aggravating cardiovascular risk in obese woman during pregnancy.

EFFECTIVE TREATMENT FOR OBESE INFERTILE WOMAN FOR WEIGHT REDUCTION

Depending on degree of obesity, three types of treatment are advocated; in India morbidly obese patient seeking infertility treatment are not very frequently seen as they are in USA or in Europe. The recommended treatments are:

- Life style modification, diet and exercise
- Pharmacotherapy
- Bariatric surgery

In general, treatment outcome to some extent is negatively influenced due to two following limitations

a) It is to be realised that body weight is tightly regulated by a complex homeostatic system and a strong neuroendocrine mechanism which defend the body against weight loss accounting for 95% "weight regain" (RECIDIVISM).[56–57] This is more commonly associated with behaviour and obesity management. This means that unless behavioural management is continued for a longer period there is always a risk of weight gain. The same phenomenon is also common following discontinuation of pharmacologic treatment or if bariatric surgery is reversed.

b) The second problem is associated with poor body image and low self-esteem. These will lead to depression, anxiety and an inferiority complex. These may have an influence on binge eating (frequent snacking, — a craze for high glycaemic food).[58,59] These are the two challenges of the therapy advocated for the weight loss. Specific therapy will now be briefly outlined:

   A) Life style modification, diet and exercise: This is the first and acceptable line of treatment for the woman with low or modest obesity (BMI < 40). It has been observed that weight loss upto 4-6 kg will be achieved following an intensive programme of life style changes (counselling, education, support changes, exercise and diet) and that is enough for minimising the incidence of type 2 diabetes, metabolic syndrome and dyslipidaemia of obese women at risk. Average weight loss of only 3.1 kg has been reported with behavioural intervention.[60]

   B) Pharmacological intervention: As far as possible, pharmacotherapy for weight loss should be avoided. Because two drugs commonly used have specific side effects and more importantly the safety of these two drugs during pregnancy is still unknown. Pharmacotherapy is indicated when life style modification, diet and exercise are not sufficient to achieve and maintain clinically significant weight loss. Currently two drugs are commonly used;- orlistat and liraglutide.

   Orlistat (Xenical) is a selective fat absorption inhibitor.[61–63] The drug exerts its effect by preventing absorption of atleast 30% fat by binding to gastric and pancreatic lipases. However there are side effects:- flatulence, greasy stool, fecal urgency and abdominal cramps.

   The second drug liraglutide (Saxenda) is an analogue of human glucagon –like peptide 1. The action is mediated through decreased appetite. The common side effects are,-nausea, diarrhoea and hypoglycemia. Hypoglycemia is an effect of stimulation of insulin secretion and inhibition of glucagon action. The dose of liraglutide is 1.2 to 3 mg subcutaneously everyday and the dose of orlistat is 120 mg orally three times daily. The average weight loss is around 4.8-7.2 kg after 20 weeks using Liraglutide Whereas the weight loss is only 4.1 kg after 20 weeks following use of orlistat.[64]

   Metformin commonly used drug is also effective for weight loss of 1.9 kg as against orlistat which reduces weight on an average 3.1 kg. However the reports of case controlled studies are not yet available as large number of cases are usually lost on follow up.

   C) Bariatric surgery: The Objective of bariatric surgery is to reduce calorie intake. Indications of bariatric surgery (for fertility enhancement particularly) include (a) BMI over 40 (b) if BMI exceeds 35 with associated comorbidities (c) when other weight loss attempts of treatment have failed.[65]

Objective of bariatric surgery is to modify the architecture of the digestive tract in order to limit calorie intake.

To achieve these objectives, three types of bariatric surgery are performed:

- Restrictive procedure
- Mal absorption procedure
- Mixed,- combination of restrictive and malabsorption type

Restrictive procedure:-consists of vertical banded gastroplasty, laparoscopic adjustable gastric banding or sleeve gastrectomy. All these procedures are designed to accelerate the feeling of being full.

Malabsorption procedure:-such as biliopancreatic diversion with or without duodenal switch. The procedure reduces nutrient absorption.

The third one is the mixed variety;- combination of restrictive/ malabsorption procedure,-the example being "Roux –en –Y gastric bypass". In both malabsorption and mixed variety, the decrease of calorie intake will depend upon the extent of length of excluded intestinal portion.[66,67]

Benefits and risks of bariatric surgery:

In morbidly obese women apart from benefits of reproductive health, there are significant areas of advantages of the surgical procedure. The most important advantage is "guaranteed" health promoting weight loss after all types of bariatric surgery. Moreover, bariatric surgery improves metabolic profile and significantly reduces overall mortality.[55,68] In some countries bariatric surgeries is preferred to medical therapy or life style modification for prevention of type 2 diabetes and associated comorbidities when BMI is more than 40.[69]

However, there are risks as well. But with advancing technology and expanding surgical skill the risks have been reduced to a minimum level. Mortality following surgery is less than 1%. The commonest post-operative complication is pulmonary morbidity (6.1%). Long term complications include iron and vitamin deficiency (20-50%) of cases. Bowel herniation during pregnancy related to surgery has been reported in 1% of cases. Other far reaching consequences include anemia (iron, vit b12, folate deficiencies), osteopenia (calcium, vit. D deficiency), alopecia Zinc deficiency, muscle weakness (Magnesium deficiency), hemorrhagic disease (deficiency of vitamin A,E,C & K).[70,71] A long term follow up is essential in all cases who had bariatric surgery.

Delay in attempting conception after bariatric surgery—does it help?

Conventionally because of impact of rapid weight loss, vitamin deficiency of both mother and fetus, attempt for pregnancy usually is advised 1-2 years after surgery.[71–73] But this advice has been contradicted by other groups of workers.[74] Therefore, specially in the late reproductive years, the advantages of postponing pregnancies should be intelligently balanced against risk of low reproductive potential with advancing age.

Obstetric and neonatal outcome following bariatric surgery:

Bariatric surgery definitely reduces the risk of gestational diabetes, hypertension, macrosomia, higher risk of still birth, anesthetic problems and other risk of comorbidities associated with obesity. But two problems though not very significant, have been observed. One is maternal anemia[75] and the other affecting neonates for example small for gestational age (SGA).[76–78]

However uptil now, no difference in obstetric and neonatal outcome has been reported based on type of bariatric surgery performed.

Does weight loss with or without bariatric surgery improve chances of pregnancy either spontaneously or following IVF?

The answer is yes. Weight loss of 5-10% can induce ovulation and increases chances of pregnancy.[79,80] Striking improved results (live birth rate 71% vs 37%, n=52) have been reported for women with BMI more than 25 who succeeded in achieving significant weight loss.[81] Similar results have been reported following weight loss after bariatric surgery.

Necessity of BMI cut off value for eligibility of availing infertility treatment:

Though it is not yet a serious concern for Indian infertile women, but some of the developed countries are really getting concerned and seriously thinking about setting an arbitrary cut off value of BMI for

accepting them for fertility promoting interventions. This is not primarily because of poor clinical outcome for available modalities of treatment, but because of some unavoidable maternal and fetal risk associated with pregnancies in woman with abnormally elevated BMI. So there should be a cut off value which at the moment has been increased to arbitrary level which vary from country to country. For example in Canada, IVF directors following a nationwide survey found that the median upper permitted BMI was 38 with an average between 35 to 40. National institute of clinical excellence (NICE) recommended desirable BMI to be below 30 and New Zealand also recommends woman with BMI less than 32 for accessing fertility treatment.[82] British fertility society (2007) recommends deferring to entering into fertility treatment with BMI more than 35. ASRM has however resisted for ceiling of a national BMI cutoff. But many clinics in America are in favour of having a cut off BMI level. Majority who are in favour having a cut off BMI value have cited anesthetic complications are the primary reason.

It must be accepted that there is no consensus in clinical practice. There are points in favour of those who do not want to have a cut off value. The points raised by them are

I. Any cut off value is arbitrary and for many patients weight loss is difficult to achieve.

II. Most weight loss interventions result in less than 7 kg of weight reduction.

III. Attempt of weight loss with life style changes, diet and exercise, woman of average height (163.5 cm) results in 3 kg or less change in BMI.

IV. This may improve their metabolic profile but this amount of weight loss will not substantially change maternal or fetal risk in pregnancy.

V. On the other hand, the number of obese patients are also increasing globally (even in India). From ethical point of view, the clinician's duty is to respect patient's decision making autonomy. But again this should be counter balanced by clinician's obligations to do no harm. The obligations in pregnancy are not only restricted to help complication of the mother as a consequence of pregnancy but also to the child not yet born, who may also be significantly compromised as a result of mothers obesity.

However it is always advisable to lose weight before fertility treatment starts rather than directly proceeding to fertility treatment.

It is also important to realize, that denying fertility treatment care may lead to stigmatisation, which will exacerbate feeling of low esteem, social isolation, anxiety and depression.[82,83] In addition denying fertility treatment to older obese woman with insufficient ovarian reserve until they have lost a specific amount of weight may lose valuable time for availing any chance of pregnancies.

So in general, it is difficult to advocate for a universal BMI cut off value; rather careful counselling, screening for metabolic abnormalities and informed consent are more rational alternatives.

TAKE HOME MESSAGE:

- Obesity is associated with increased adverse impact on reproductive health.
- Obese women are at higher risk of menstrual dysfunction, anovulatory infertility and pregnancy related complications.
- Even obese women with ovulatory cycle will have less fecundity and may take prolonged period for conception.
- The discrepancy may be due to decreased frequency of intercourse.
- This is more applicable for obese male infertility.
- Erectile dysfunction, sperm abnormalities are due to elevated scrotal temperature and decreased testosterone level are the probable contributory factors.
- Selection of drug, dose adjustment and response to conventional fertility enhancing treatment in the female partner are also modified by obesity and have to be adjusted with regard to patient's individual BMI.
- For induction of ovulation, Letrozole is more acceptable than clomiphene.
- This is because of possible association of hyperestrogenism in obese women compared to their non-obese counterpart.
- Fat cells contain more aromatase which will convert excess androgen (also a common association of obesity) into excess oestrogen.

- Excess static oestrogen (non-pulsatile) cannot induce ovulation.
- In obese women gonadotropin –IUI protocol is better than simple induction of ovulation.
- With regard to IVF outcome, clinical pregnancy and live birth rates decline gradually with increasing severity of obesity.
- The causes of low success rate are primarily related to low embryo quality and defective endometrial receptivity.
- Miscarriage rate is also higher for similar reason; poor endometrial receptivity.
- Obesity may cause problems in the technologies of ART;- during COH, shifting of ovaries to higher position in the pelvis making accessibility of ovaries for oocyte retrieval difficult.
- The other technical problems are related to anaesthesia and trauma to surrounding structures during oocyte retrieval.
- Apart from these problems, other pregnancy complications associated with obesity are preeclampsia, preterm labour, premature rupture of membranes and shoulder dystocia etc.
- Additionally following operative delivery, there is increased risk of wound infection, wound dehiscence, post-partum haemorrhage and deep venous thrombosis.
- Congenital fetal anomalies involving mostly neural tubes and gastrointestinal defects such as spina bifida or anorectal atresia, are not infrequently encountered.
- Congenital fetal anomaly in obese women is more commonly related to poor glycaemic control.
- Another practical problem is related to prenatal detection of these anomalies with ultrasound due to poor visualisation of fetal organs through excess maternal abdominal fat.
- Obese women seeking infertility treatment should be thoroughly counselled about the risk of co-morbidities and benefits of pre-treatment weight reduction.
- Depending on the degree of obesity. Three types of treatment are advocated.
- Specific suggestions are: life style modification, diet and exercise, pharmacotherapy and bariatric surgery.
- In general, except for bariatric surgery the treatment outcome is negatively influenced due to two limitations.
- The first limitation is physiological;- a complex homeostatic system which always tries to protect the body against weight loss.
- Hence there is always weight regain following specific treatment of pharmacotherapy, diet and exercise.
- The second constraint is psychological,-a sense of inferiority complex due to obesity which induces the habit of binge eating (frequent snacking) craze for high glycaemic food.
- Women with low and modest obesity will respond to diet, exercise, and life style changes.
- Reduction of approximately 4 to 6 kg (10%) will be helpful in avoiding morbidities and at the same time for onset of ovulatory menstrual cycle.
- For modest to severe obesity and for those who are not responding to simple diet and exercise protocol, pharmacotherapy is the next choice.
- Drugs commonly used are Orlistat (Xenical), a fat absorption inhibitor; and second one is Liraglutide, - an analogue human glucagon like peptide.
- Metformin is also effective, but not as effective as orlistat or liraglutide.
- Both these drugs have side effects and still there are not enough reports about safety of their use during pregnancy.
- Indications of bariatric surgery are;- (a) BMI over 40 (b) BMI over 35 with associated comorbidities (c) when other weight loss attempts in obese or morbidly obese patients have failed.
- Objective of bariatric surgery is to modify the architecture of digestive tract in order to restrict calorie intake.
- Three types of bariatric surgery are performed; (a) restrictive procedure (b) malabsorptive procedure (c) mixed, - combination of restrictive and malabsorptive type.
- The most important benefit of bariatric surgery is 'guaranteed' health promoting procedure.
- With increasing skill and experience in surgical procedure, the mortality rate has been reduced to less than 1%.
- The commonest operative problem is pulmonary complications (6%).
- Long term risks include vitamin and iron deficiency and associated occasionally bowel herniation during pregnancy has been reported.

- Because of rapid weight loss, iron and vitamin deficiency following surgery, it has been suggested that pregnancy should be delayed by 1 to 2 years.
- But the advantages of postponing pregnancy may be counterbalanced by decreasing pregnancy potential with advancing age.
- The decision should be intelligently balanced.
- Many developed countries are in favour of setting a 'cut-off' value for BMI of obese patients for their access to fertility treatment.
- However, at the moment there is no such cut off value.
- Careful counselling, screening for metabolic abnormalities and informed consent are more rational alternatives.

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Good Embryo Assessment – Path Towards Perfect Ten

Dr. Ratna Chattopadhyay, Dr. Sanghamitra Ghosh, Manisha Goswami, Gunja Bose, Dr. Pratip Chakraborty

Three major determinants for a successful IVF cycle are embryo quality, endometrium receptivity and embryo transfer techniques. So, evaluation and selection of good quality embryo is the most critical responsibility of an embryologist. A prolonged and invasive procedure for evaluation may turn a good quality embryo into a bad one. Till date in majority of ART centers, embryo quality are evaluated by conventional morphology. But, morphology alone may not always reveal the truth, sometimes a good-looking embryo may be chromosomally abnormal, and a bad-looking embryo may end up in a take home baby. Therefore, to strengthen the conventional morphological evaluation many unconventional embryo assessment techniques are gradually developed for accurate assessment during the last two decades. Now-a-days embryo selection is based on chromosomal integrity and expression of appropriate developmental genes. Moreover, metabolic activity of an embryo can assess it's developmental and implantation competence.

The goal of embryo assessment is to identify the best or better embryo to improve the implantation rates and to reduce multiple gestation rates by transferring a single, competent embryo following precise evaluation.

The learning objective of the present topic is to discuss about

1. Different techniques of embryo assessment
2. How effective and practical are these techniques

1. Different techniques of embryo assessment

   A. Morphological evaluation

      a. Conventional morphological evaluation
      b. Unconventional morphological evaluation

         1. Spindle view using birefringence
         2. Embryoscope using Time Lapse Videography
         3. Morphometry using measurement of volume and surface area of blastomere as well as whole embryo

   B. Embryo assessment by using omics technology

      a. Non-invasive
         Metabolomics
         Proteiomics
         Secretomics

      b. Invasive
         Transcriptomics
         Genomics

   C. Microfluidic with omics technology

   D. Recent innovation by using Artificial intelligence (AI)

A. a. Conventional morphological evaluation

Embryo assessment should begin from the follicles. Follicles having good perivascular blood flow yield good quality oocytes, intended to develop competent embryos. Embryos from highly vascularized follicles are associated with more pregnancies.[1,2]

About 80% of good quality oocytes are capable of developing good quality embryos. Good quality oocytes should have homogenous ooplasm with fine granules, thin zona pellucida (≤20 microns), and narrow perivitalline space and smooth, round first polar body.

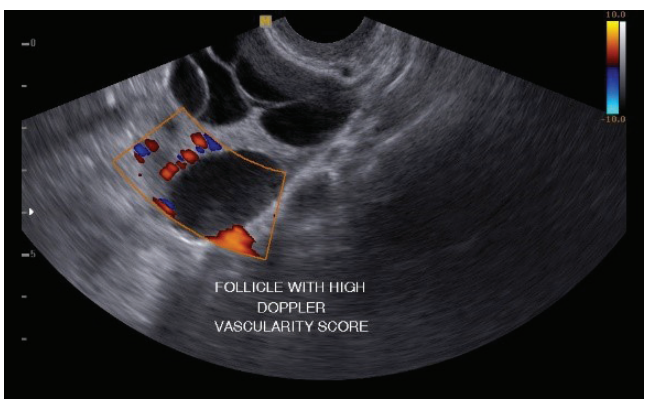


Fig 1: Excellent peri-follicular blood flow

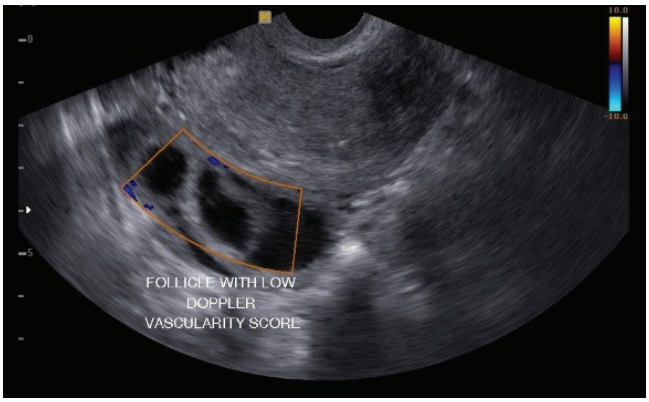


Fig 2: Follicles with low Doppler vascularity score

Pro-nuclear morphology (PN score), play an important role in embryo assessment. Pro-nuclear morphology should be checked 16-18 hrs. of post injection and 18-20 hrs. post conventional insemination. Two centrally located juxtaposed pro nuclei having equal number and size of nucleoli aligned at the PN junction or equally distributed within the pro-nuclei and a cytoplasmic hallow surrounding the pro-nuclei signify good quality zygote (Z1 and Z2 zygote according to Z-score) can develop about 72% good quality blastocyst.

Day 2 and day 3 embryos are morphologically evaluated by degree of fragmentation, stage specific cell size and number and the number of nucleus within the blastomeres.

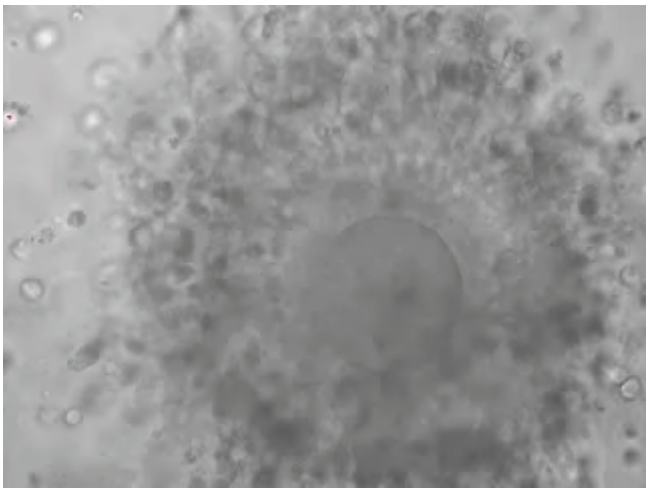


Fig 3: Before denudation

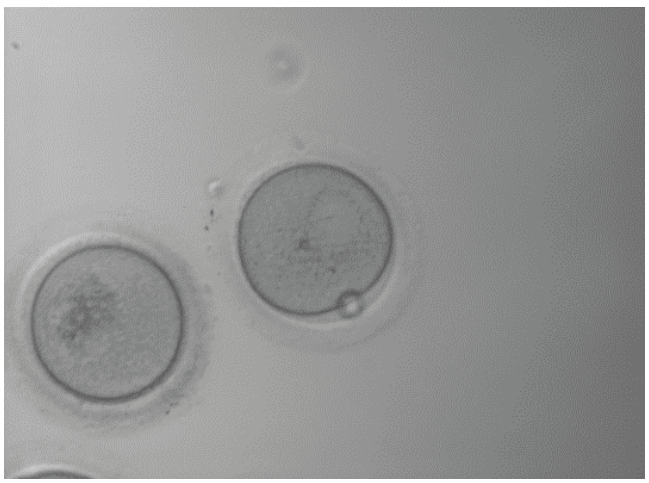
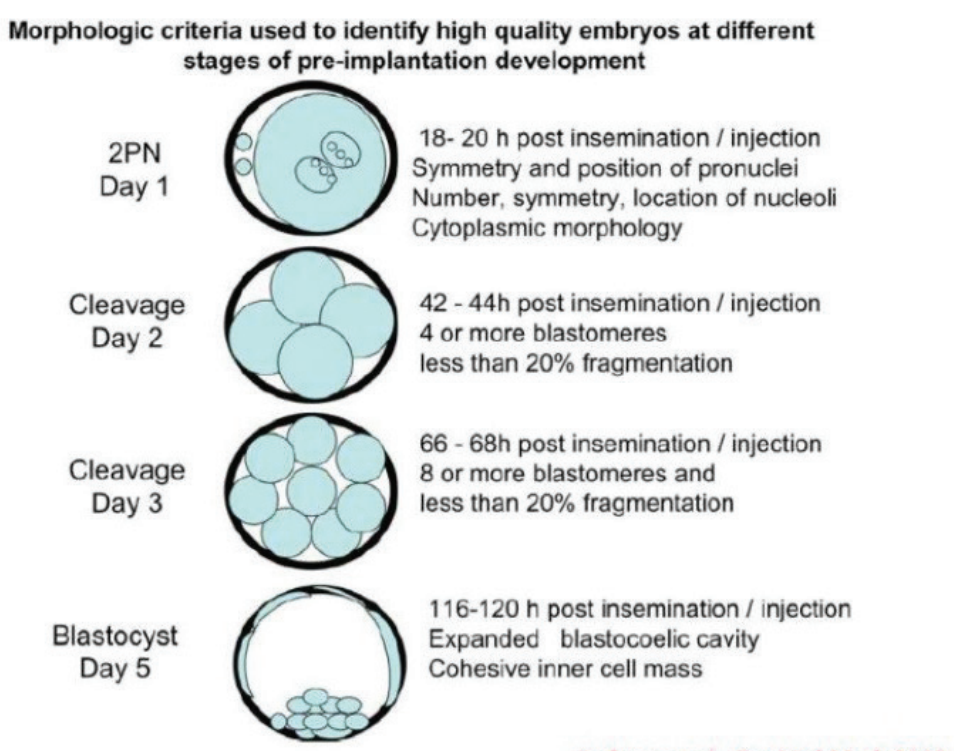
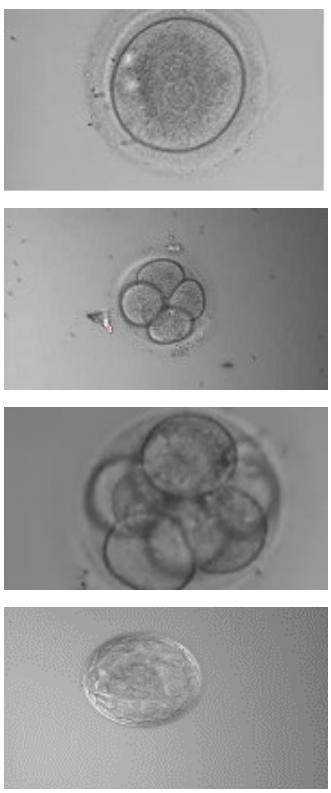


Fig 4: After denudation





1	Good	<10% fragmentation No multinucleation Stage specific cell size
2	Fair	10-25% fragmentation No multinucleation Stage specific cell size in majority of blastomeres
3	Poor	Severe fragmentation Cell size not stage specific Evidence of multinucleation seen

Fig 6: Alpha scientist group in reproductive medicine & ESHRE special interest group in embryology, Istanbul 2011

A. b. 1. Unconventional morphological evaluation by spindle view using birefringence

Visualization of MS in the oocyte is an important criterion of the good quality oocyte. A positive correlation between MS retardance and good embryo formation followed by higher implantation and pregnancy rate was observed.[3] For precise assessment, spindle imaging should be performed after hyaluronidase treatment and immediately prior to ICSI. Spindle retardance, length, and angle to PB are confounding parameters of oocytes that are able to develop good-quality embryos.[4,5]

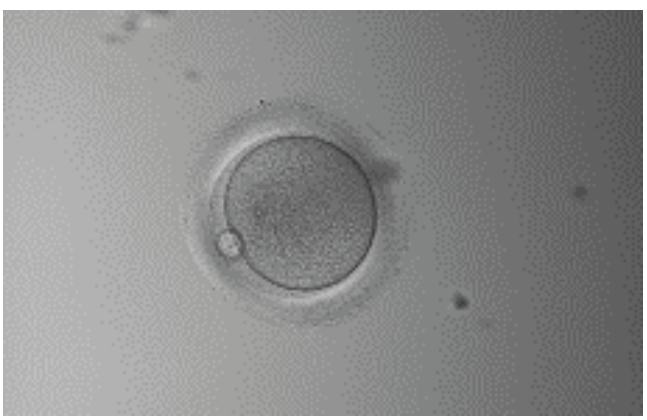


Fig 7: Good morphology oocyte

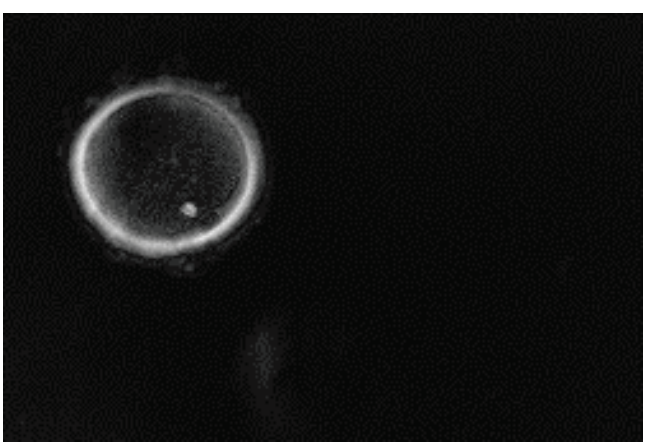


Fig 8: Meiotic spindle with good retardance

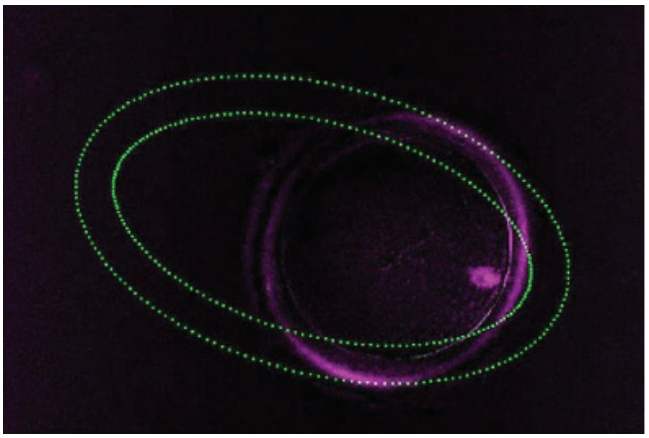


Fig 9: Inner layer of zona pellucida

The inner layer of ZP exhibits the highest birefringence in good-quality oocytes.[6] Zona birefringence intensity is higher in the conception cycle.[7]

A. b. 2. Time Lapse Imaging through Embryoscope

TL monitoring allows continuous non-invasive embryo observation without removing them from the optimum culture environment. The entire information on the cleavage pattern, morphological changes, and embryo developmental dynamics helps to identify the embryo with the highest implantation potential.[8]

Time lapse system (TLS) was first introduced[9] for intermittent observation of oocyte fertilization. Later on, morpho-kinetic evaluation allows us to observe both the morphological appearance of embryos at different stages of development as well as the timing of different cellular events, playing an important role in embryo evaluation.

Position correlation between embryo kinetics and euploidy has been reported.[10] A significant difference in the difference of first mitotic phase has been observed between euploid and aneuploid embryos.[11]

By TLI 9.7% increase in CPR was reported compared to traditional culture and morphology assessment alone.

Cochrane review reported[12] insufficient evidence of a difference in birth, miscarriage, stillbirth, or CPR between TLI and conventional incubation.

TLI excludes embryos with direct or abnormal cleavage and suboptimal development. Moreover, TLI reduces embryo stress and inter-observer variability.

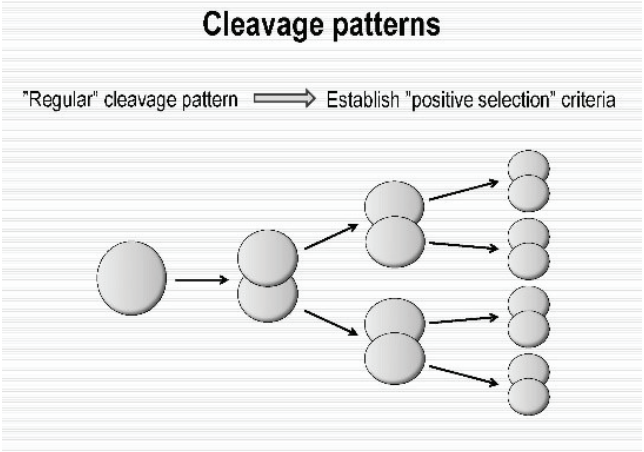


Fig 10: Regular cleavage pattern

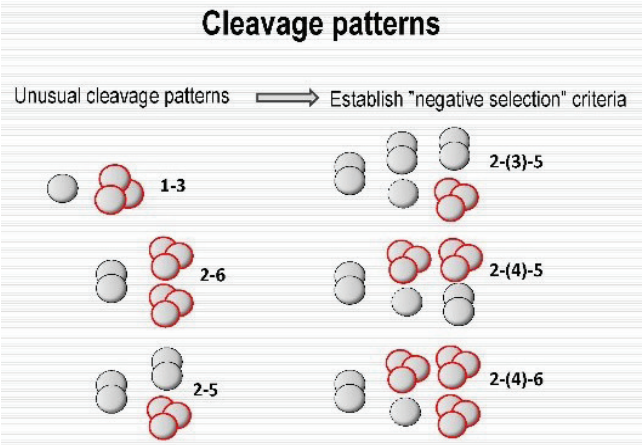


Fig 11: Unusual cleavage pattern

EEVA (early embryo viability assessment) test

Morphological evaluation of day 3 embryo based on automated analysis of early cell division timing. Good morphology day 3 embryos based on EEVA test is supposed to develop more usable blastocysts and significantly increase CPR and IR compared to conventional morphology assessment.[13]

A. b. 3. Morphometry

It is a computer assisted embryo scoring system, another non-invasive assessment technique to select embryos by measuring area of blastomere as well as embryos and, volume and roundness factor of the embryo, through analysing images of known implanted embryos. According to morphometric analysis, good D2 embryo having roundness factor ≥ 0.9, average zona pellucida thickness of 13.1 µm and surface area of the embryo 17,695.1 sqµ. For D3 embryo diameter of largest and smallest blastomere and volume of the whole embryo was evaluated[14] in 2011-13. A better prediction of implantation rate based on number and size of blastomere on D3 and correlation between total embryo volume and clinical pregnancy rate was observed by him. Primary aim is to evaluate morphometric variables in a larger data set and to determine possible link between total volume of transferred embryo and pregnancy rate. Though this method avoid intra-observer variability, but it is time consuming and due to the absence of required database this method is not very popular nowadays.

B. Embryo assessment by using OMICS technology

OMICS technology is used to develop multiple markers of embryos phenotype by measuring components either secreted in or consumed from the spent culture media by the embryos. Identification of embryos with the highest developmental potential is a challenge to ART specialists. A small proportion of embryos generated by ART possess the genetic and metabolic requirements needed to produce healthy offspring. The association between metabolites in culture media and constituents of an embryo with its developmental and implantation potential has already been observed. Assessment of best embryos by a non-invasive, rapid, and reliable method towards single embryo transfer, promoted metabolites studies requirement in IVF. It includes metabolomics, proteomics, and transcriptomics, and an invasive genetic approach to embryo assessment is Pre-implantation genetic screening.

B.1. Non-invasive

Metabolomics: Analysis of metabolic content of spent culture media has been proposed as a non-invasive diagnostic tool to assess embryo viability in IVF. Certain key ingredients in culture media are essential for the normal development of preimplantation embryos. Any change in the level of these ingredients and their metabolites is suggested as an indicator of the metabolic activity of the developing embryos during IVF. In ART, by metabolic study it has been observed that, embryos having early cleavage, increased glucose uptake and reduced glycolysis has higher pregnancy rate.[15] Another study reported, increased utilization of amino acids by d2 embryo indicated higher clinical pregnancy and live birth rate. Any deviation in the biological and environmental atmosphere of oocyte and embryo which may affect their viability can

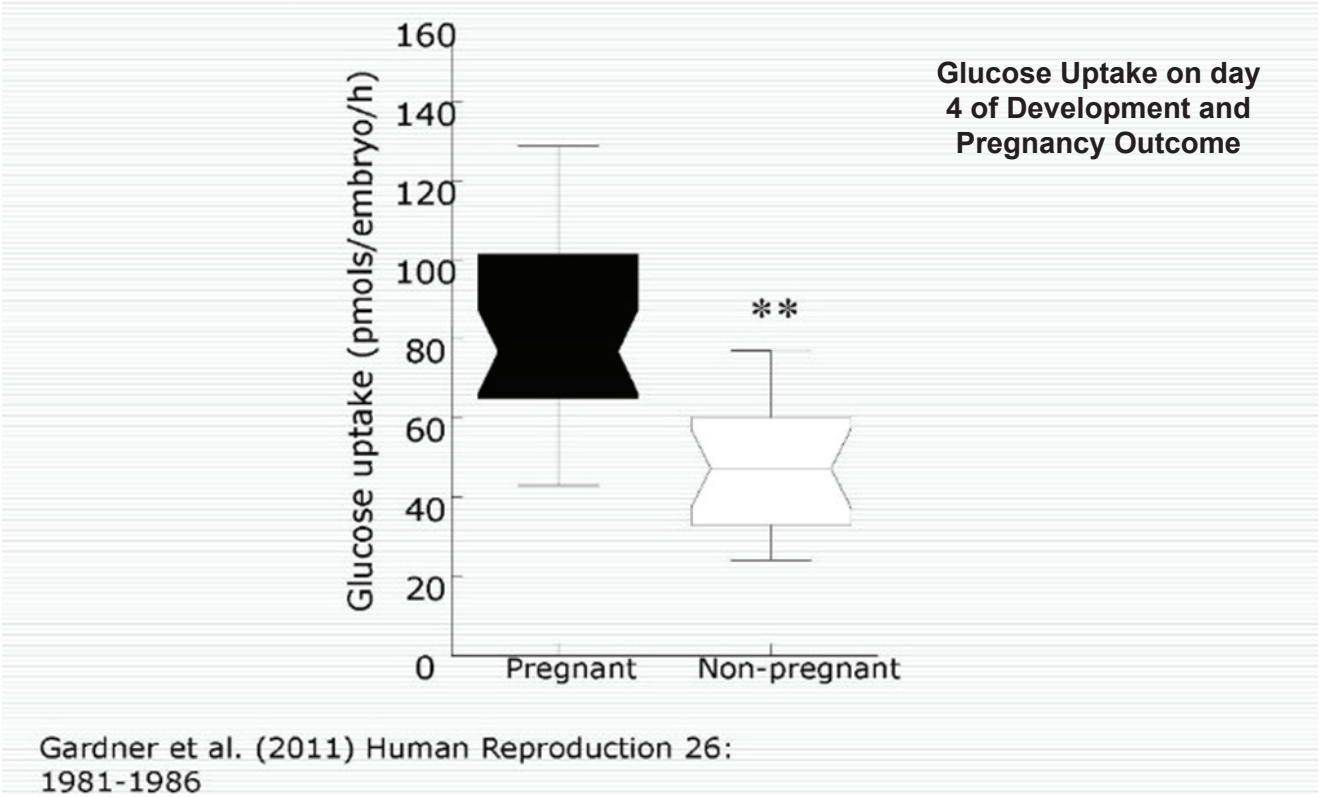


Fig 12: Embryos having early cleavage, increased glucose uptake, and reduced glycolysis are associated with a higher pregnancy rate

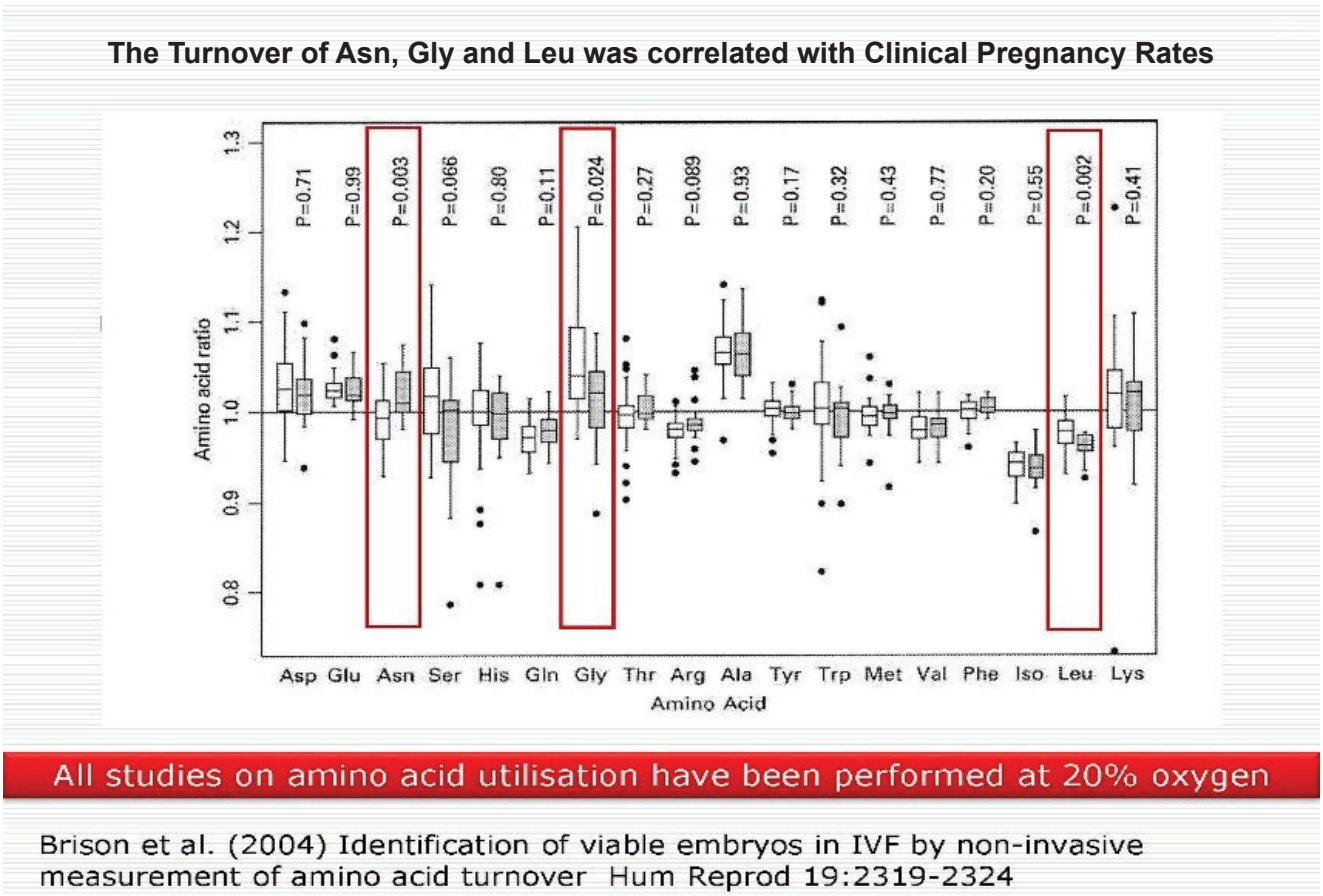


Fig 13: Increased utilization of amino acid by d2 embryos indicate higher clinical pregnancy and live birth rate

be detected by the metabolomics studies through different abnormal changes in the spent culture media.

Table:

Metabolic profiling of culture media can be analyzed by Raman and near-infrared spectroscopic technology. The association between culture media metabolomes and clinical outcomes following ART has already been reported.[16,17,18]

Metabolomics is the most widely practiced non-invasive OMICS technology, but its efficiency is still under debate and nowadays it is not a very popular technique for embryo assessment.

Proteomics: Study and analysis of complete set of expressed protein in a cell at a particular time. One of the promising non-invasive unconventional techniques of viability assessment of embryos. It helps us to detect viability and biological system of the embryo usually by immunoassay and mass spectrometry. This technique enable us to proceed one step ahead for single embryo transfer by identifying the viable embryo having the highest implantation potential. Measurement of components up taken from and secreted into spent culture media by a single embryo is the basis of proteomics to assess embryo health.

Secretomics: In ART it is the study of different factors in the spent culture media secreted by the developing embryos and helps us in assessing the viability of those embryos.

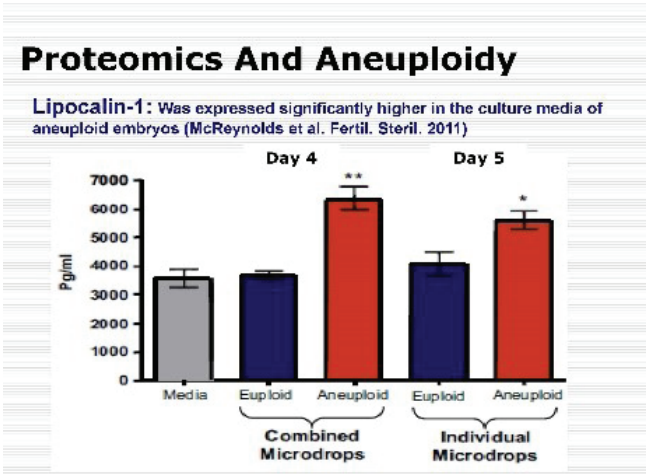


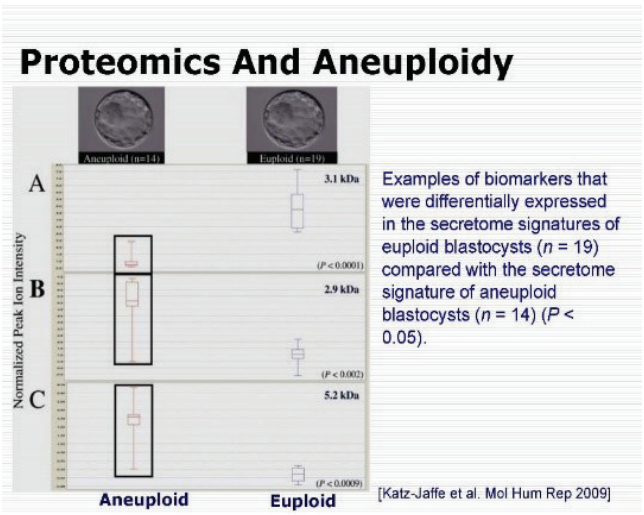
Fig 15: Expression of Lipocalin-1 in spent culture media

Soluble human leucocyte antigen-G (sHLA-G): Positive correlation between sHLA-G in the culture media (measured by ELISA) an increased embryo viability and improved pregnancy rate was suggested.[19,20]

Leptin: Secretion of leptin, a small pleitrophic peptide measured in spent embryo culture media had shown a positive correlation with blastocyst development.[21]

B.1. Invasive

Transcriptomics: Study of the RNA in embryo in any of its form in the cell. Transcriptom can vary with external environmental condition. Transcriptom of cumulus cells is used to study gene expression which is informative about the oocytes developmental potential.



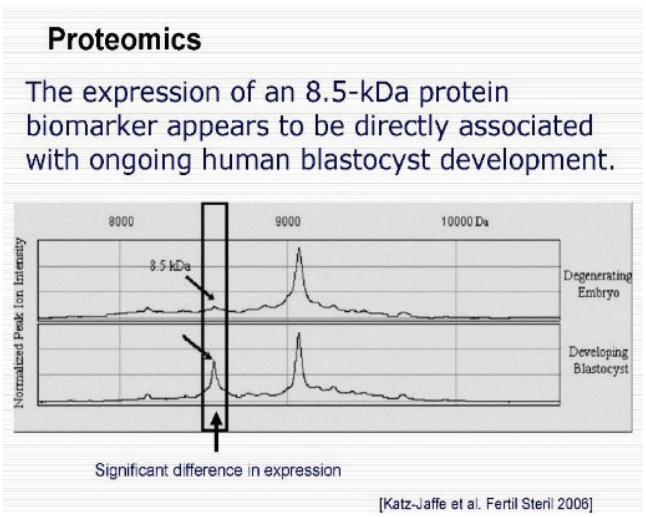
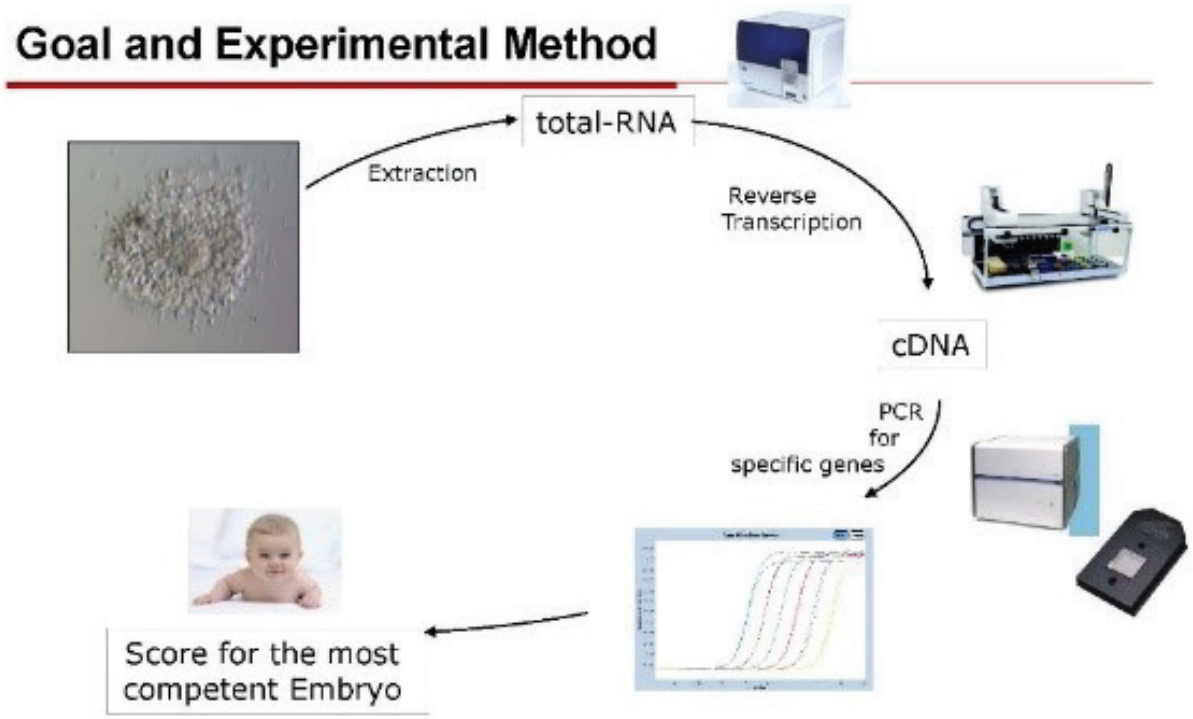


Fig 14: By expression of different protein biomarker we can differentiate euploid and aneuploidy embryos.



The strongest pregnancy predictive genes in 3 consecutive QPCR studies

23 genes were evaluated until now in 122 patients *

Study	Total n	% pregnant	Genes retained for pregnancy	PPV	NPV	Accuracy
1st	42	45%	SDC4 and VCAN	88	81	83
2nd	33	48%	EFNB2, CAMK1D and STC1	80	78	79
3rd	47	40%	EFNB2, GSTA3, GSTA4, PGR and GPX3	93	93	93

Example of a multiparametric pregnancy prediction model

Chance on pregnancy = -2.25846 + 0.79256 x EFNB2 + 0.09491 x GSTA4 - 0.09632 x PGR

→ Gene only models perform well

Fig16: By transcriptomics of cumulus cells, expression of above genes helps us to select the best quality oocytes, leading to best quality embryos.

Genomics: It is the study of complete set of genes or genomic materials of a cell. It involves DNA sequencing, mapping, function and expression of different genes. In ART, by genomics we study the DNA constitution/sequence of the embryo but whether an analysis of embryonic DNA sequence can generate information on embryo viability is still not known rather chromosome number and integrity can determine embryo viability by PGS. Some of these techniques are invasive and some are non-invasive.

Invasive Pre implantation Genetic screening:

1. Pre-implantation genetic testing for monogenetic disorder (PGT-M)
   - PCR: To diagnose specific genetic mutation

- FISH: To diagnose the chromosomal abnormality or to determine sex of the embryo for patients having x-linked chromosomal disease

2. Pre-implantation genetic testing for aneuploidy (PGT-A)
   - Cleavage stage embryos showed a high level aneuploidy
   - PGT-A select against these aneuploidy embryos to improve LBRs
   - PGT-A at Day 3 was not effective because,
     - Limited accuracy of FISH
     - Limited numbers of cells available for biopsy
     - Chromosomal abnormalities/mosaicism are at the peak of Day 3 cleavage stage embryos
3. Comparative Genomic Hybridization Analysis (CGH)
   - Conventional: Copy number of all chromosomes can be analysed but it takes 3-4 days to complete the procedure so cryopreservation of embryos is needed
   - Micro Array: Analysis is completed in 48hrs hence embryo cryopreservation is not required.

It has been reported that embryos selected by Array-CGH (A-CGH) are associated with higher incidence of clinical pregnancy compared to those selected without A-CGH.[22]

Non Invasive Pre implantation Genetic screening (NIPGS): Novel Approach to the genetic analysis to diagnose euploid embryo. Discovery of cell free DNA in blastocoel fluid and spent culture media has led to the invention of non-invasive pre-implantation genetic testing. It can reduce the embryo biopsy damage, cost of treatment and the use of micromanipulation. The limitations of this technique are lower quantity and lesser quality of cell free genetic material as the chances of contamination from granulosa cells are higher.

C. Microfluidic

It is a long lasting debate on "how to choose best environment for culture and the best embryo for transfer". Till date most of the laboratories evaluate embryo quality by its gross morphology before transfer but it may not reveal the truth. Combination of morphology and morphokinetics through Time Lapse Imaging is more authentic. Due to its higher installation and recurring cost this technique is not very popular in developing countries. Moreover, solely metabolism based scoring strategy for embryos have not made it yet to the clinics due to conflicting results on the validity of certain metabolic parameters. A lack of user friendly protocol and a high technicality of associated instrumentations.[23] Gradually, different parameters for embryo characterization and pre-implantation embryo culture, was tried to combine in one single and automated platform known as microfluidic. This technology, involves uses of devices containing nanolite micro-chambers or micro-channels for embryo culture mimicking in-vivo culture by establishing a dynamic environment.[24] Embryo assessment in microfluidic technology is done using OMICs technology by interfaced to spectroscopic techniques and by morphokinetics using Time Lapse Imaging. But this technology is not very popular due to lack of robust and convincing validation using human embryos, lack of user friendliness and automation of the microfluidic platforms and psychological constraint for embryologists to use microfluidic.

D. Recent innovation by using Artificial intelligence (AI)

Embryo evaluation and selection is the most critical step to an embryologist before embryo transfer and cryopreservation. Even after embryo selection based on morphology, time lapse imaging and embryo biopsy with PGT, implantation rate is difficult to predict in an IVF cycle in spite of satisfactory endometrium.

Recent invention in the field of ART is an improvement and automatization of the embryo selection procedure by using artificial intelligence and machine learning, based on images of embryos with known implantation data. Full automatization of embryo scoring implies fewer manual evaluation and eliminates biases due to inter and intra observer variations. However, further larger studies are required to demonstrate reproducibility of the reported findings prior to wider clinical application.

Conclusion

Embryo assessment is one of the most critical procedures that play a crucial role in the success of IVF. Traditional embryo assessment is challenged by different factors, i.e. subjectivity low efficiency and limited predictive value. New 'non-invasive' techniques may provide valuable additional information to optimize embryo assessment and maximize the chances of IVF success. A perfect combination of morphological, metabolic and chromosomal status of an embryo can only evaluate its health and competence but that is far from reality in recent scenario. Embryo selection by morphokinetics and machine learning using artificial intelligence can be widely spread out in clinical setting in near future for single embryo transfer to achieve singleton pregnancy which is the ultimate goal in present ART practice.

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Low-dose Letrozole — an Effective Option for Women with Symptomatic Adenomyosis Awaiting IVF: a Pilot Randomized Controlled Trial

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ABSTRACT

Research question: Can low-dose letrozole reduce dysmenorrhoea, menorrhagia and sonographic features in symptomatic women with adenomyosis awaiting IVF?

Design: This was a longitudinal randomized prospective pilot study to explore the effectiveness of low-dose letrozole and compare it with a gonadotropin releasing hormone (GnRH) agonist in reducing dysmenorrhoea, menorrhagia and sonographic features in symptomatic women with adenomyosis awaiting IVF. The women were treated for 3 months, either with the GnRH agonist goserelin 3.6 mg/month (n= 77) or the aromatase inhibitor letrozole 2.5 mg three times weekly (n= 79). Dysmenorrhoea and menorrhagia were evaluated at randomization and followed up monthly using a visual analogue score (VAS) and pictorial blood loss assessment chart (PBAC), respectively. A quantitative scoring method was used to assess the improvement of sonographic features after 3 months of treatment.

Results: Both groups reported a marked improvement in symptoms after 3 months of treatment. In both the letrozole and GnRH agonist groups, VAS and PBAC scores decreased significantly over the 3 months (letrozole: P= 0.0001 and P= 0.0001 for VAS and PBAC, respectively; GnRH agonist: P= 0.0001 and P= 0.0001 for VAS and PBAC, respectively). Participants on letrozole had regular menstruation cycles, while most of the women who received the GnRH agonist were amenorrhoeic, with only four women reporting mild bleeding. Haemoglobin concentrations also improved after both treatments (letrozole P= 0.0001, GnRH agonist P= 0.0001). A quantitative assessment of sonographic features showed significant improvements following both treatments (diffuse adenomyosis of the myometrium: letrozole P= 0.015, GnRH agonist P= 0.039; diffuse adenomyosis of the junctional zone: letrozole P= 0.025, GnRH agonist P= 0.001). Women with adenomyoma also responded well to both therapies (letrozole P= 0.049, GnRH agonist P= 0.024), whereas the letrozole group responded comparatively better in focal adenomyosis when the outer myometrium was involved (letrozole P < 0.001, GnRH agonist P= 0.26). No noticeable side effects were observed in women receiving letrozole therapy. Additionally, letrozole therapy was found to be more cost-effective than GnRH agonist treatment.

Conclusions: Low-dose letrozole treatment is a low-cost alternative to a GnRH agonist, with comparable effects in improving the symptoms and sonographic features of adenomyosis in women awaiting IVF.

Introduction:

Adenomyosis is a common benign gynaecological condition of women of reproductive age. It is an oestrogen-dependent disease characterized by the presence of ectopic endometrial glands, and stroma in the uterine myometrium (Kitawaki, 2006). Signs and symptoms of this condition include menorrhagia (50%), dysmenorrhoea (30%), increased uterine size (60%) and infertility, and it significantly reduces quality of life (Harada et al., 2016; Li et al., 2019). According to Bird and colleagues, 35% of all patients with adenomyosis display no obvious symptoms (Bird et al., 1972).

An accurate evaluation of the prevalence of adenomyosis has not been carried out for a variety of reasons, first and foremost the fact that until recently the diagnosis could only be confirmed following hysterectomy. Even in women undergoing hysterectomy, the estimated prevalence varies from 8.8% to 61.5% (Upson and Missmer, 2020). In recent years, with an increasing use of transvaginal ultrasound (TVS) and magnetic resonance imaging, adenomyosis has been identified in 20.9-34.0% women with or without symptoms or infertility (Naftalin et al., 2012; Pinzauti et al., 2015; Puente et al., 2016). However, a recent meta-analysis of 25,600 subfertile women shows that the overall prevalence of adenomyosis is 10% when it occurs in isolation, 18% in women with endometriosis, 10% in those with coexisting fibroids, and 17% when both endometriosis and fibroids are present (Mishra et al., 2023).

It is estimated that as many as 50% of patients with dysmenorrhoea, menorrhagia and infertility suffer from adenomyosis (Eisenberg et al., 2017; Harada et al., 2016). There is no consensus about the optimum management in symptomatic women with adenomyosis and infertility. Dysmenorrhoea associated with adenomyosis may sometimes be so debilitating that pain management often takes precedence over fertility treatment. Treatments including combined oral contraceptives (COC) and progestins such as norethisterone, dienogest and levonorgestrel released from an intrauterine system provide symptomatic improvement; however, their role before IVF is not clear (Alborzi et al., 2010; Gurates et al., 2008; Polyzos et al., 2011; Vannuccini and Petraglia, 2019). Recently, an oral GnRH antagonist was also found to be effective in patients with symptomatic adenomyosis (Donnez and Donnez, 2020; Kavoussi et al., 2020). However, its role in infertile women is yet to be investigated by randomized controlled trials. GnRH agonist therapy has gained popularity in women undergoing fertility treatment to improve their clinical symptoms and likelihood of pregnancy (Andreeva and Absatarova, 2020).

Multiple studies have reported the advantage of adding GnRH agonist pretreatment in terms of pregnancy and live birth among women with adenomyosis who underwent IVF (Li et al., 2021; Mijatovic et al., 2010; Niu et al., 2013; Tao et al., 2015). Increased aromatase activity in adenomyosis (Kitawaki et al., 1997) has led to the use of aromatase inhibitors for symptomatic improvement but there is a dearth of studies involving aromatase inhibitors as a pretreatment in women with adenomyosis and undergoing IVF. Only two studies have used aromatase inhibitors in women with adenomyosis and infertility (Badawy et al., 2012; Kimura et al., 2007). An efficacy of letrozole was also observed in symptomatic endometriosis refractory to other medications, but when used long- term at a dose of 2.5 mg per day, it was linked to side effects (Alborzi et al., 2010; Ferrero et al., 2009).

The primary hypothesis of this longitudinal study was that low-dose letrozole (2.5 mg three times weekly) would be effective in reducing dysmenorrhoea and menorrhagia in infertile women suffering from adenomyosis who were awaiting IVF. The main objective of this study was to assess the effectiveness of low-dose letrozole in reducing dysmenorrhoea and menorrhagia.

Material and Methods

Settings and patient selection: This prospective, randomized, non-blinded study was conducted at the Institute of Reproductive Medicine, Kolkata, India, a tertiary-care hospital, from May 2022 to September 2022. This study was approved by the Institutional Human Ethics Committee and registered with the Clinical Trials Registry-India (CTRI/2019/01/016919, 12 May 2022). Written informed consent was obtained from all participants.

Couples reporting to the clinic for IVF treatment were screened for adenomyosis and recruited after

TABLE 1 BASELINE CHARACTERISTICS AND SONOGRAPHIC CRITERIA

Parameter	Letrozole (*n* = 79)	GnRHa (*n* = 77)	*P*-value
Age (years)	34.14 ± 3.43	33.41 ± 2.93	0.155
Body mass index (kg/m^2)	27.25 ± 3.18	26.72 ± 3.26	0.306
Duration of infertility (years)	7.6 ± 3.2	7.3 ± 2.35	0.506
Haemoglobin (mg/dl)	9.95 ± 0.59	10.13 ± 0.73	0.092
Anti-Müllerian hormone ng/ml	2.26 ± 0.59	2.17 ± 0.58	0.338
Bone mineral density (g/cm^2)	0.879 ± 0.161	0.834 ± 0.192	0.125
DEXA *z*-score	−0.93 ± 0.24	−0.97 ± 0.28	0.339
Associated symptoms			
Dysmenorrhoea	79/79 (100)	77/77 (100)	1
Menorrhagia	21/79 (26.58)	20/77 (25.97)	0.538
Miscarriage	32/79 (40.51)	28/77 (36.36)	0.357
Presence of endometriosis	54/79 (68.35)	49/77 (63.64)	0.325
Sonographic criteria[a]			
Globular uterus	36/79 (45.57)	32/77 (41.56)	0.366
Asymmetrical thickening of the myometrium	57/79 (72.15)	54/77 (70.13)	0.459
0.5–1 cm difference in wall thickness	38/57 (66.67)	37/54 (68.52)	0.498
1–2 cm difference in wall thickness	11/57 (19.30)	9/54 (16.67)	0.456
2–3 cm difference in wall thickness	7/57 (12.28)	6/54 (11.11)	0.542
>3 cm difference in wall thickness	1/57 (1.75)	2/54 (3.70)	0.480
Myometrial cyst	28/79 (35.44)	24/77 (31.17)	0.346
Echogenic subendometrial lines and buds	6/79 (7.59)	5/77 (6.49)	0.500
Subendometrial cyst	9/79 (11.39)	6/77 (7.79)	0.313
Hyperechogenic islands	17/79 (21.52)	19/77 (24.68)	0.391
Fan-shaped shadowing	11/79 (13.92)	12/77 (15.58)	0.332
Translesional vascularity on colour Doppler scanning	79/79 (100)	77/77 (100)	1.000
Adenomyoma	11/79 (13.92)	9/77 (11.69)	0.430
Irregular or interrupted junctional zone	66/79 (83.54)	58/77 (75.32)	0.142

Data are presented as mean ± SD or *n* (%). A Fisher's exact test and two-sample t-test were used to calculate the statistical difference between the groups.

[a] See *Bosch et al. (2019)*.

DEXA, dual X-ray absorptiometry (for bone density).

applying stringent inclusion and exclusion criteria. The inclusion criteria ensured that only symptomatic women below 40 years of age with a normal ovarian reserve were recruited, after informed consent. Women with fibroids, hydrosalpinx, enlarged uterus of a size larger than 12 weeks' gestation, congenital uterine abnormalities or anti- Mu€llerian hormone concentrations less than 1.1 ng/ml were excluded. All cases of adenomyosis were confirmed by two-dimensional TVS (SonoAceR7, Samsung- Madison, South Korea) equipped with a 4-9 MHz vaginal probe. The TVS exams were performed by the same operator using a standardized protocol during the period of day 5-10 of the menstrual cycle within 3 months of starting the IVF treatment. Adenomyosis was confirmed if one or more of the following diagnostic features were present: (i) a globular uterus, (ii) asymmetrical thickening of the myometrium, (iii) a myometrial cyst, (iv) echogenic sub-endometrial lines and buds, (v) a sub-endometrial cyst, (vi) hyperechogenic islands, (vii) fan-shaped shadowing, (viii) translesional vascularity on colour Doppler scanning, (ix) adenomyoma, or (x) an irregular or interrupted junctional zone (Table 1) (Bosch et al., 2019,2015). From 167 symptomatic women with adenomyosis, 156 who were refractory to treatment with either COC or progestins were recruited for the study (Figure 1).

Following randomization, these women were treated with the GnRH agonist or letrozole for 3 months. Patients were randomly allocated by the lead clinician (S.S.) into one of the two treatment groups (Figure 1) using a computer-generated random table. The letrozole group (Letroz 2.5 mg; Sun Pharmaceutical Industries, India AstraZeneca, United Kingdom) received 2.5 mg three times weekly for 3 months and the GnRH agonist group received goserelin (Zoladex 3.6 mg; AstraZeneca) 3.6 mg/month subcutaneously for 3 months.

Women receiving either of the treatments were advised to avoid pregnancy during the treatment period. Out of 79 women treated with letrozole, three were lost during follow- up, and out of 77 women treated with a GnRH agonist, two dropped out. All the participants were followed up monthly (Figure 1).

The primary outcome measures were the reduction of dysmenorrhoea in terms of visual analogue scale (VAS) scores, and of menorrhagia in terms of the pictorial blood loss assessment chart (PBAC). The secondary outcome was an improvement of

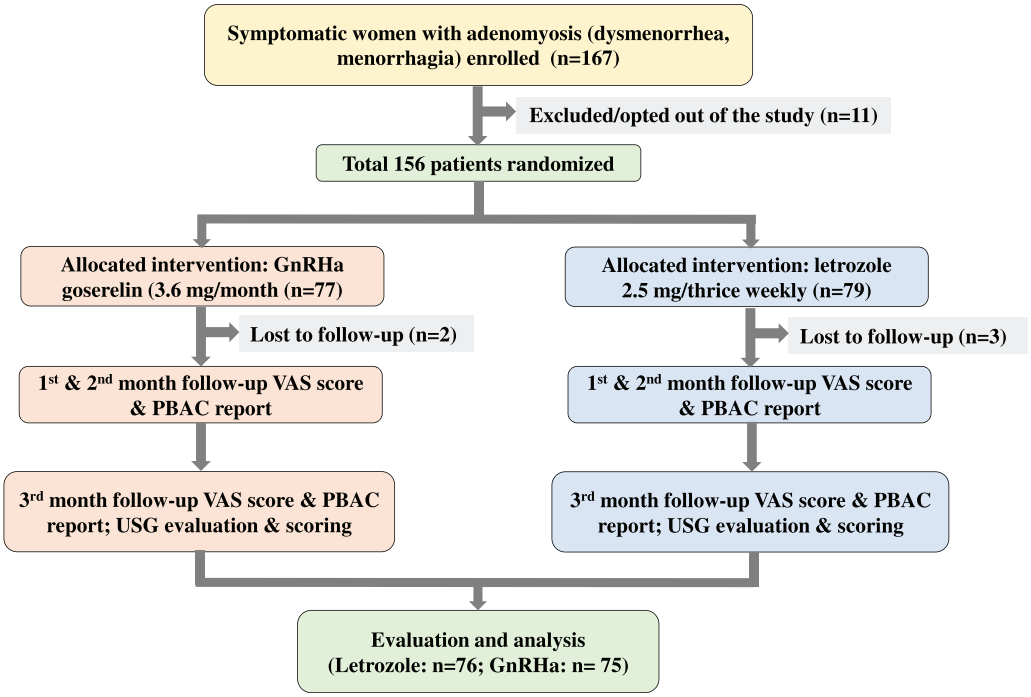


Figure 1: Flowchart for the participant recruitment and study design. Patients were recruited at the Institute of Reproductive Medicine, Kolkata, India between May and September 2022. GnRHa, gonadotrophin-releasing hormone agonist; PBAC, pictorial blood loss assessment chart; USG, ultrasonography; VAS: visual analogue scale.

sonographic features of adenomyosis. A GnRH agonist, which is conventionally used to treat symptoms associated with adenomyosis, was used to provide a parallel perspective.

Assessment of pain, menorrhagia, and ultrasonography features

Dysmenorrhoea and menorrhagia were evaluated for all the participants every month for 3 months. The severity of pain symptoms was assessed using a 10-point VAS scale, which is a subjective tool for the self-assessment of pain, with possible scores ranging from 0 (no pain) at the left extreme of the scale indicating the absence of pain, to 10 (severe pain) at the right indicating the worst pain. Severe dysmenorrhoea was defined as a VAS score of at least 7.6 (Cozzolino et al., 2019; Woodforde and Merskey, 1972). In women with menorrhagia, menstrual blood flow was assessed using the PBAC chart; menorrhagia was defined as a score of over 100 (Higham et al., 1990). Haemoglobin concentration was measured every month. Bone mineral density was measured before and after 3 months of treatment for all participants using bone density scan (dual-energy X-ray absorptiometry (DEXA)).

In this study, ultrasound-diagnosed adenomyosis was further assessed by the scoring method for disease classification and severity reported by Lazzeri and colleagues, with satisfactory sensitivity and specificity (Lazzeri et al., 2018). Adenomyotic lesions were given scores, ranging from 1 to 4, based on the number, size and extent of the lesions in the myometrium and/or junctional zone (Table 2). Two gynaecologists experienced in ultrasound (M.P.B. and K.C.O.), who were blinded to the patient's history and treatment, independently assessed the stored images and video clips in the database recorded by the lead investigator (S.S.). They reviewed the presence of adenomyosis, assigned types (diffuse, focal or adenomyoma), and gave appropriate scores based on the method described by Lazzeri and colleagues (Lazzeri et al., 2018). After a 3-month interval, the participants were re-evaluated independently by the same sonologists.

Sample size calculation and statistical evaluation

To determine the sample size, a preliminary study was carried out with 10 women who underwent letrozole therapy. The VAS scores before treatment and 1 month after treatment were entered into the sample size calculator (https://clincalc. com/Stats/SampleSize.aspx). A baseline VAS score of 8.4 ±

TABLE 2 SONOGRAPHIC ASSESSMENTS OF ADENOMYOSIS SEVERITY AND POST-TREATMENT FOLLOW-UP

Schematic mapping system of adenomyosis severity and scoring method for extension of corresponding type of adenomyosis[a]	L_{pre} Mean ± SD[b] (*n*/*N*)	L_{post} Mean ± SD (*n*/*N*)	G_{pre} Mean ± SD (*n*/*N*)	G_{post} Mean ± SD (*n*/*N*)	*P*-value	% Change from baseline (Let vs GnRHa)
N	**79**	**76**	**77**	**75**		
Diffuse adenomyosis: diffuse inside the myometrium and thickening of the uterine walls Score 1: 1 wall affected, ≤20 mm thick Score 2: 2 walls affected, ≤20 mm thick; or 1 wall affected >20 to ≤30 mm Score 3: 1 wall affected, >30 mm thick; or 2 walls affected, >20 to ≤30 mm thick Score 4: 2 walls affected, >30 mm thick; or the whole uterus affected and globally enlarged	3.5 ± 0.68 (71/79)	3.26 ± 0.51 (69/76)	3.41 ± 0.59 (67/77)	3.23 ± 0.47 (66/75)	L_{pre} vs L_{post}: 0.015 G_{pre} vs G_{post}: 0.039 L_{pre} vs G_{pre}: 0.379 L_{post} vs G_{post}: 0.7	Let: 3.70 ± 19.64 GnRHa: 3.64 ± 13.2 (*P* = 0.98)
Diffuse adenomyosis of the JZ: diffuse inside the JZ and thickening of the JZ Score 1: JZ_{max} >6 to ≤8 mm; or JZ_{dif} >4 to ≤6 mm; or diffuse JZ infiltration ≤20 mm in length Score 2: JZ_{max} >8 mm; or JZ_{dif} >6 mm; or diffuse JZ infiltration <20 mm in length; or <50% of the uterus Score 3: diffuse JZ infiltration >50% to ≤80% of uterus Score 4: total 80% JZ infiltration	3.32 ± 0.71 (64/79)	3.1 ± 0.48 (63/76)	3.52 ± 0.74 (58/77)	3.26 ± 0.57 (58/75)	L_{pre} vs L_{post}: 0.025 G_{pre} vs G_{post}: 0.01 L_{pre} vs G_{pre}: 0.10 L_{post} vs G_{post}: 0.06	Let: 3.02 ± 21.3 GnRHa: 4.54 ± 19.64 (*P* = 0.68)
Focal adenomyosis: focal lesions within the outer myometrium Score 1: 1 lesion ≤10 mm Score 2: ≥2 lesions ≤10 mm; or 1 lesion >10 to ≤20 mm Score 3: ≥2 lesions >10 to ≥20 mm; or 1 lesion >20 mm Score 4: ≥2 lesions >20 mm	2.67 ± 0.58 (3/79)	2.33 ± 0.58 (3/76)	2.5 ± 0.55 (6/77)	2.4 ± 0.55 (5/75)	L_{pre} vs L_{post}: <0.001 G_{pre} vs G_{post}: 0.26 L_{pre} vs G_{pre}: 0.062 L_{post} vs G_{post}: 0.44	Let: 11.11 ± 19.25 GnRHa: 0 ± 0 (*P* = 0.22)
Focal adenomyosis of the JZ: focal lesion in the JZ Score 1: 1 hyperechoic lesion; or cystic areas ≤10 mm Score 2: ≥2 lesions ≤10 mm; or 1 lesion >10 to ≤20 mm Score 3: ≥2 lesions >10 to ≤20 mm; or 1 lesion >20 mm Score 4: ≥2 lesions >20 mm	2.5 ± 0.71 (2/79)	2.5 ± 0.71 (2/76)	2.5 ± 0.71 (2/77)	2 ± 0 (2/75)	L_{pre} vs L_{post}: 1 G_{pre} vs G_{post}: <0.001 L_{pre} vs G_{pre}: 1 L_{post} vs G_{post}: <0.001	Let: 0 ± 0 GnRHa: 16.67 ± 23.57 (*P* = 0.4)
Adenomyoma Score 1: 1 lesion ≤20 mm Score 2: 2 lesions ≤20 mm; or 1 lesion >20 to ≤30 mm Score 3: 2 lesions >20 to ≤ 30 mm; or 1 lesion >30 to ≤40 mm Score 4: ≥1 lesions >40 mm	2.55 ± 0.52 (11/79)	2.36 ± 0.67 (11/76)	2.56 ± 0.53 (9/77)	2.33 ± 0.71 (9/75)	L_{pre} vs L_{post}: 0.049 G_{pre} vs G_{post}: 0.024 L_{pre} vs G_{pre}: 0.9 L_{post} vs G_{post}: 0.78	Let: 7.58 ± 17.26 GnRHa: 9.26 ± 18.84 (*P* = 0.79)

[a] The extension of each type of adenomyotic lesion in the external myometrium and in the JZ was divided into four grades (score 1–4) using a scoring system according to the transvaginal ultrasound features described by *Lazzeri et al. (2018)* (2).

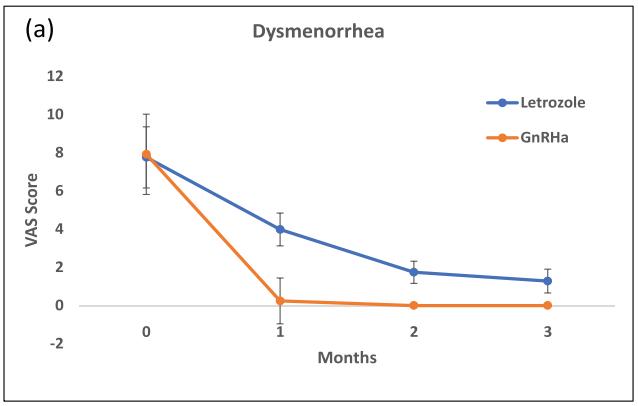
GnRHa, gonadotrophin-releasing hormone agonist; G_{pre}, GnRHa group pretreatment; G_{post}, GnRHa group post-treatment; JZ, junctional zone; JZ_{dif}, difference between the maximum and minimum JZ thicknesses; JZ_{max}, maximum JZ thickness; Let, letrozole; L_{pre}, letrozole group pretreatment; L_{post}, letrozole group post-treatment.

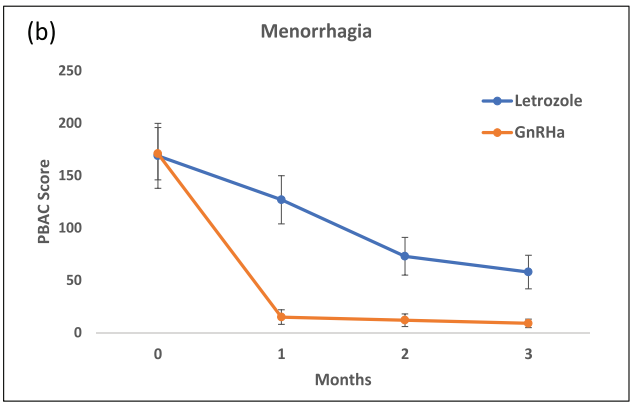
A Fisher's exact test and Paired and unpaired two-sample t-test were used to calculate the statistical difference between the groups.

[b] Data presented as mean +/- SD or n (%)..

1.17 and an expected 10% decrease after 1 month (assumed drop-out rate of 5%) were used to calculate the sample size. The target sample size was estimated to be 30 in each treatment group with an estimated power of 80% and acceptable alpha error of <0.05 and a beta error of 0.2. The sample size was 79 for the letrozole group and 77 for the GnRH agonist group.

Bland—Altman plots were used in this study to compare the letrozole and GnRH agonist treatments based on the differences in their effects on various sonographic criteria in terms of the severity score as a percentage of its decline from baseline. A Bland-Altman plot is often used to compare and visualize the agreement or difference between a new treatment or technique for measuring some outcome compared with the existing treatment (Giavarina, 2015). The plot is constructed by plotting the difference between the two methods against the mean of two treatments (Altman and Bland, 1983). The plot allows for a visual assessment of the agreement and the presence of any systematic bias between the two methods. The limits of agreement, which are defined as the mean difference + 1.96 times the standard deviation of the difference, provide a range of expected differences between the two methods.





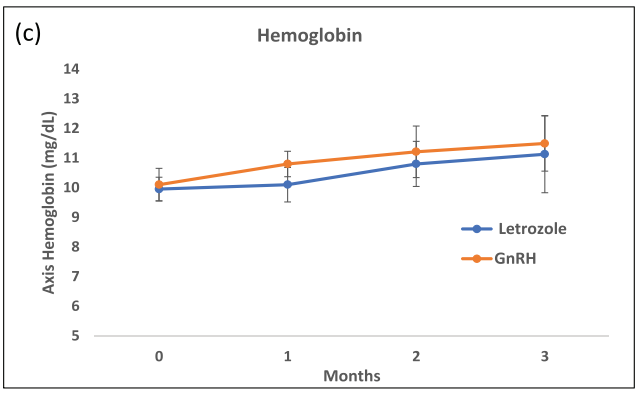


Figure 2: Improvements of symptoms during treatment with letrozole or GnRH agonist. (a) Dysmenorrhoea (after 3 months: letrozole P= 0.0001, GnRH agonist P = 0.0001), (b) menorrhagia (after 3 months: letrozole P= 0.0001, GnRH agonist P = 0.0001), and (c) haemoglobin (after 3 months: letrozole P = 0.0001, GnRH agonist P= 0.0001). Data are shown as means ± SD, and data were compared using an unpaired t-test. Letrozole: n = 79 at O months and n = 76 at post 3 months' treatment; GnRHa: n = 77 at 0 months and n = 75 at after 3 months' treatment. GnRHa, gonadotrophin-releasing hormone agonist; PBAC, pictorial blood loss assessment chart; VAS: visual analogue scale.

The horizontal line in the middle of the plot refers to the average difference in measurements between the two treatments. The further this value is from zero, the larger the average difference in measurements between the two methods (Riffenburgh and Gillen, 2020).

Statistical analyses were carried out using the statistical packages (MedCalc Software Ltd, Belgium) and GraphPad Prism 5.0 (Dotmatics, UK). The Fisher's exact test and Student's t-test were used for analysis, as applicable. A value of P < 0.05 was considered to be statistically significant.

Results

The baseline characteristics were comparable in the two treatment groups (TABLE 1). Dysmenorrhoea was present in all 156 women recruited for the study, but only 26.28% of women had complaints of menorrhagia (TABLE 1). A previous history of miscarriage was present in 38.46% of the study population, and 66.03% of women were diagnosed with coexisting severe endometriosis (stage III or IV). Most women had an irregular or interrupted junctional zone (79.49%), asymmetrical thickening of the myometrium (71.15%) and translesional vascularity on TVS. A globular appearance of the uterus (43.59%) and a myometrial cyst (33.33%) were also common. (Table 1). Following randomization, both groups had a comparable representation of women with different sonographic features and other parameters (Table 1).

After 3 months of treatment, both groups reported a marked improvement in symptoms. In the letrozole group the initial average VAS score of 7.76 § 1.6 was reduced to 1.74 § 0.58 following 2 months of treatment (Figure 2a) and was further lowered to 1.28 § 0.62 after 3 months, which was a significant improvement (P= 0.0001). In most of the women on the GnRH agonist, there was a sharp decrease from an average VAS score of 7.92 § 2.1 at randomization to a negligible score of 0.24 § 1.2 after 1 month of treatment. In the following 2 months of observation, no woman in this group reported any dysmenorrhoea. In the letrozole group dysmenorrhoea was also minimal after 3 months of treatment, but there was a statistical difference (P= 0.0001) between the two treatment groups based on the VAS scores (Figure 2a).

In women treated with letrozole, menorrhagia (PBAC score) also improved gradually over the course of treatment. During recruitment, the

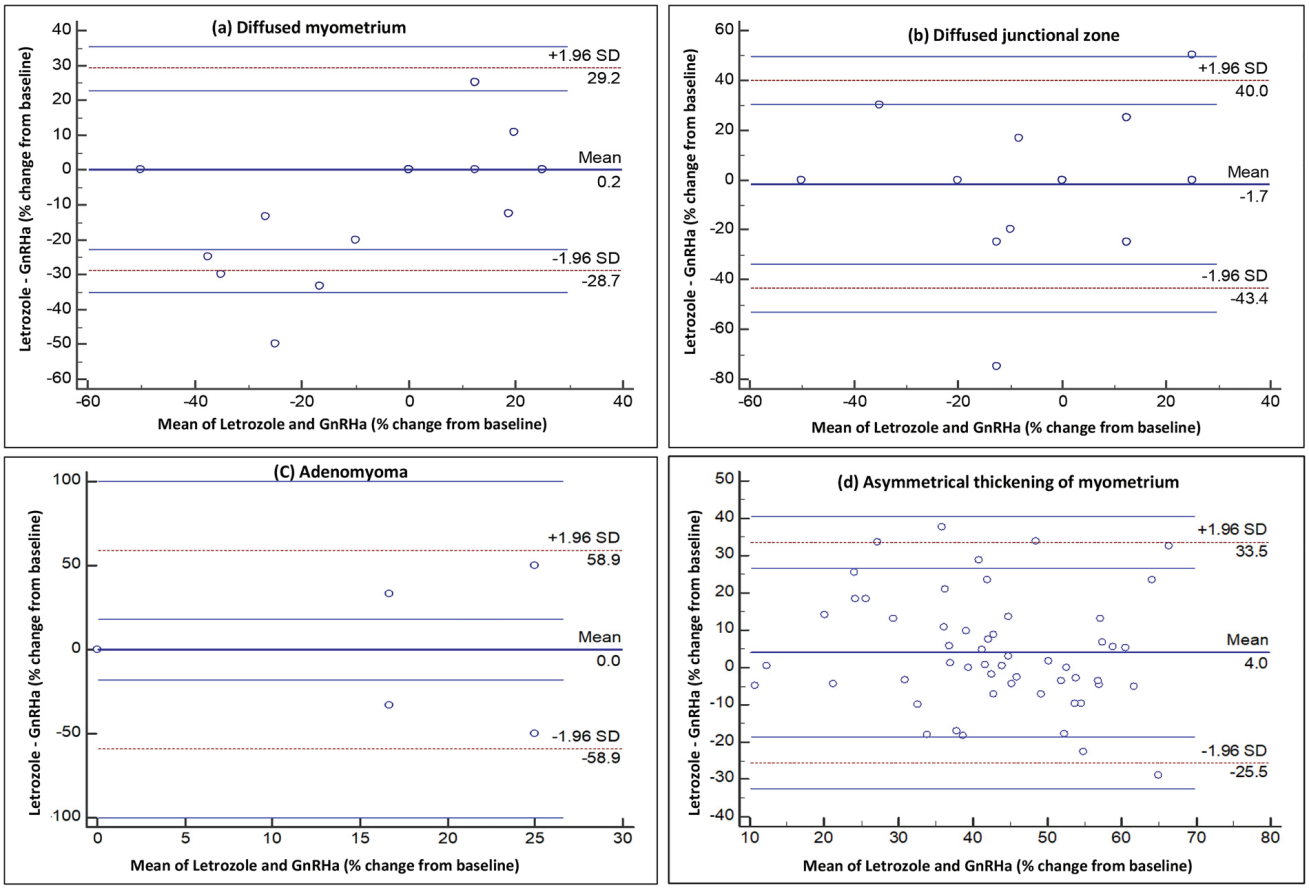


Figure 3: Bland-Altman plots of the difference between the letrozole and GnRH agonist treatments. (a) Diffuse myometrium, (b) diffuse junctional zone, and (c) adenomyoma, comparing the differences in sonographic severity score in terms of the percentage of decline from baseline between the letrozole and GnRH agonist groups. (d) Bland-Altman plot of asymmetry in myometrial thickness. Scatter diagrams of the differences (in percentage of baseline value) plotted against the averages of a mean percentage decline in value for the two treatments; horizontal lines are drawn at the mean difference (dark blue) and the limits of agreement (dashed). The maximum allowed differences (A and -A) for each treatment are marked in light blue. Treatments were compared using paired samples t-tests: (a) diffuse myometrium, P = 0.903; (b) diffuse junctional zone, P = 0.546; (c) adenomyoma, P = 1.0; and (d) asymmetrical thickening of the myometrium, P = 0.0567. GnRHa, gonadotrophin-releasing hormone agonist.

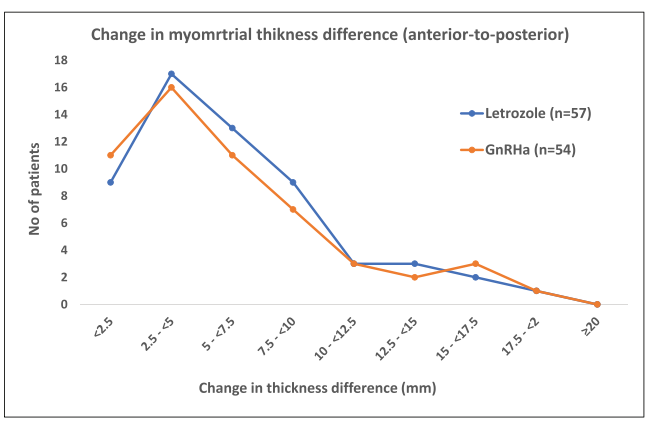


Figure 4: Regression in the difference between the myometrial walls (anterior to posterior) as seen on ultrasonography after 3 months' treatment. GnRHa, gonadotrophin-releasing hormone agonist

average PBAC score was 169 +31 in these women, and this was restored to a normal level of 58±16 after 3 months (P = 0.0001) (RGURE 2b). During the 3 months of treatment with the GnRH agonist, only four women had mild bleeding, whereas the others were amenorrhoeic, and the average PBAC score at recruitment (171+25) was significantly less after 3 months of treatment (9 ± 4; P = 0.0001) (RGURE 2b). The post-treatment PBAC score was calculated based on only those four women with minor bleeding in the GnRH agonist group; on comparing them with the scores from letrozole group, there was a statistical difference between the two treatment groups (P = 0.0001).

Blood haemoglobin concentrations were also observed to be significantly improved (P = 0.0001) in women treated with letrozole (9.95 ± 0.4 at baseline

and 11.13±1.3 after 3 months) or the GnRH agonist (10.10.55 at baseline and 11.49 0.93 after 3 months; P=0.0001) (Figure 2C). There was no significant difference between the two treatments based on haemoglobin concentrations after 3 months of treatment (P = 0.053).

Furthermore, the effectiveness of both the letrozole and the GnRH agonist was evaluated separately for women with only adenomyosis and for those who had both adenomyosis and endometriosis (stage III or IV). The response, as measured by the VAS, PBAC and haemoglobin concentrations did not show any significant difference between the two treatments in either the group of patients. with adenomyosis alone or those with adenomyosis and coexisting endometriosis (Supplementary Figure 1).

The assessment of sonographic features showed significant improvements after treatment in both the letrozole and GnRH agonist groups (TABLE 2, Supplementary Figure 2). Most of the participants in this study had diffuse adenomyosis, and significant improvements were observed following both treatments (diffuse adenomyosis of the myometrium: letrozole P=0.015, GnRH agonist P = 0.039; diffuse adenomyosis of the junctional zone: letrozole P=0.025, GnRH agonist P=0.01) (TABLE 2). Moreover, there was no difference in the effectiveness between the two treatments after 3 months (diffuse adenomyosis of the myometrium: P=0.7; diffuse adenomyosis of the junctional zone: P=0.06) (TABLE 2). Women with adenomyoma also responded well to both therapies, and the size of the adenomyomas was significantly reduced (letrozole P=0.049, GnRH agonist P=0.024) (TABLE 2); however, there was no observable difference between the two treatment groups (P=0.78). The letrozole group responded comparatively better in terms of focal adenomyosis when the outer myometrium was involved (letrozole P< 0.001, GnRH agonist P = 0.26), while improvements were noted in the GnRH agonist group when the junctional zone was involved (GnRH agonist P < 0.001) (TABLE 2). However, the number of cases with focal adenomyosis of the outer myometrium or junctional zone was much lower (n = 9 and n = 4, respectively). Bland-Altman plots also revealed that, for both types of diffuse adenomyosis and adenomyoma, letrozole and the GnRH agonist were considered to be comparable, having similar effects, and no significant differences were observed between them (Figure 32-c) (diffuse myometrium, P=0.903; diffuse junctional zone, P = 0.546; adenomyoma, P = 1.0).

All participants with an asymmetry in myometrial thickness (the difference between the anterior and posterior wall thicknesses) were observed to have a variable degree of reduction. The decrease in asymmetrical thickening of the myometrium fell into two groups: a change of between 0 and <7.5 mm, which was considered as a minor reduction, and a change of between 7.5 and >20 mm, which was considered as a major reduction. A cut-off value of 7.5 mm was determined empirically based on the clinical correlation between the improvements in symptoms and the asymmetrical thickening of the myometrial layer. A total of 30.63% of the women had a major reduction of between 27.5 mm and 20 mm (Figure 4). The remaining patients had minor reductions of less than 7.5 mm (Figure 4). The mean baseline asymmetry in thickness for these patients was 22.5 ± 0.5 mm in the letrozole group and 23.5±0.74 mm in the GnRH agonist group. The Bland-Altman plot revealed that the two treatments had similar effects on myometrial asymmetry with no significant differences after 3 months (Figure 3d). The mean percentage. reductions in the letrozole group and GnRH agonist group were 46.19 ± 15.7 and 40.9± 16.2, respectively, and were statistically insignificant (P = 0.8).

A DEXA scan of hips did not show any difference in bone mineral density pre- and post-treatment in the two groups (letrozole P=0.125, GnRH agonist P=0.545). However, eight women in the GnRH agonist group (10.66%) complained about hot flushes, mild myalgia and joint pain, while none in the letrozole group had such complaints. Three women treated with letrozole (3.95%) developed a functional cyst (1.6-2.4 cm).

A major difference in the cost of therapies was noted in this study. In India, the cost of letrozole (2.5 mg three times weekly for 3 months) was about $21 per patient during the study period, whereas the cost of GnRH agonist (goserelin acetate 3.6 mg/month for 3 months) was about $400, which is several times costlier than letrozole.

Discussion

In this longitudinal prospective study, both low-dose letrozole and the GnRH agonist were found to be successful in managing the symptoms of dysmenorrhoea and menorrhagia in women suffering from adenomyosis and awaiting IVF treatment. VAS and PBAC scores were significantly lower after treatment compared with the scores at randomization (Figure 2a, b). Haemoglobin concentrations in the participants in both treatment groups also improved significantly (Figure 2c). A significant decrease in the severity scores on ultrasound for diffuse adenomyosis, adenomyoma and asymmetry of the myometrium was observed after treatment in the low-dose letrozole treatment group and GnRH agonist group (Table 2, Figure 4).

Aromatase inhibitors have been suggested to be effective in adenomyosis that is resistant to other therapies (Tosti et al., 2016). They target the increased aromatase activity in both eutopic and ectopic endometrium, thereby regulating oestrogen production (Kitawaki, 2006). The first use of an aromatase inhibitor in combination with a GnRH agonist was successful in controlling abnormal uterine bleeding and uterine volume in a young adenomyotic patient (Kimura et al., 2007). Badawy and colleagues compared letrozole and a GnRH agonist in adenomyosis for 3 months and observed that the letrozole was as effective as the GnRH agonist in reducing the uterine volume and symptoms of adenomyosis (Badawy et al., 2012). In agreement with Badawy and colleagues, the current study also did not observe any significant difference in efficacy between letrozole and GnRH agonist treatment. However, Badawy and colleagues evaluated the treatment effect based on sonographic changes in uterine/adenomyoma volume, and the improvements in symptoms were qualitatively expressed (Badawy et al., 2012). Conversely, in this study, the improvements in symptoms were quantitatively evaluated using VAS and PABC scores.

In the present study, improvements in disease severity were also determined quantitatively by applying a previously established scoring system (Lazzeri et al., 2018), based on ultrasound features of the uterus. The current observations are in agreement with earlier studies, where significant changes in sonographic features following 3-6 months of letrozole therapy were reported (Badawy et al., 2012; Ferrero et al., 2014). Several studies have reported an association between the severity of symptoms and sonographic features (Naftalin et al., 2016,2014; Pinzauti et al., 2015). In contrast, Exacoustos and co-workers found no significant correlation between the severity or extent of the symptoms and the number of sonographic criteria, although they did observe a correlation between the severity of diffuse adenomyosis and the symptoms (Exacoustos et al., 2020). In the present study, most of the women had diffuse adenomyosis with 3-4 sonographic criteria representing the severity of the disease, which is further correlated with the symptoms (Supplementary Figure 3).

Previous studies have observed the formation of ovarian cysts in 24-50% of women on letrozole monotherapy (2.5 mg/ day) for 2-3 months (Alborzi et al., 2010; Gurates et al., 2008). To avoid such complications, aromatase inhibitor therapy in combination with a COC, and progestins have been widely used ("American College of Obstetricians Gynecologists (ACOG) Committee Opinion No. 663: Aromatase Inhibitors in Gynecologic Practice," 2016; Amsterdam et al., 2005; Bosch et al., 2019). However, such combined therapies have been reported to be associated with a higher incidence of hypo-oestrogenic side effects (Bosch et al., 2019; Zhao et al., 2021). Moreover, COC/progestins may affect the pregnancy rate and live births in women awaiting IVF treatment (Farquhar et al., 2017).

Like several studies involving endometriosis, Badawy and colleagues also used 2.5 mg of letrozole daily for 3 months (Badawy et al., 2012). In contrast, the current study used letrozole in a dose of 2.5 mg three times weekly to establish the lowest effective dose with minimal side effects while achieving an overall improved quality of life in terms of symptoms for these women. This reduced dose of letrozole monotherapy was observed to have a comparable effect to that of conventional GnRH agonist therapy. It was observed that only three women (3.95%) had developed functional cysts (<2.5 cm). Similar to the current study others have also reported minimum hypo-oestrogenic effects such as hot flushes with aromatase inhibitors (Polyzos et al., 2011) relative to a GnRH agonist. Although, GnRH agonist pretreatment may improve the pregnancy rate in

IVF (Sallam et al., 2006), there are concerns that it could lead to a significant suppression of ovarian activity, leading a greater need for gonadotrophin therapy and a reduction in both the number and quality of oocytes (Mijatovic et al., 2010; Salim et al., 2012). It may also trigger a flare-up effect, potentially worsening the symptoms (Cantor et al., 2019). In contrast, letrozole does not have such drawbacks and the current findings showed that it provides symptomatic relief, enabling immediate IVF treatment.

A possible limitation of this study is the lack of proper blinding of the lead clinician and participants due to the different routes of administration of the drugs tested, but both sonologists were blinded to the patient history and the treatment received. In the absence of a universally accepted diagnostic modality and classification system for adenomyosis, this study used ultrasound-based severity scoring as proposed by Lazzeri and collaborators (Lazzeri et al., 2018). Furthermore, the assessment and scoring of the sonographic features were carried out based on the database-stored images/video clips instead of real-time data. Another limitation of this pilot study is the unavailability of data from these two therapies on their impact on the IVF outcome. However, future follow-up studies could be conducted on the subsequent IVF outcomes of such patients to determine whether the treatments have a comparable impact on success rates. The strengths of this study are its prospective nature, the randomization of the participants at recruitment, and the longitudinal follow-up. Moreover, this study for the first time attempts a quantitative assessment of symptoms and the severity scoring of sonographic features to assess the effectiveness of low- dose letrozole in the treatment of adenomyosis.

Conclusion

This study provides evidence that low- dose letrozole is a useful, cost-effective option with minimal side effects in symptomatic women with adenomyosis awaiting IVF. Additionally, the letrozole therapy compares favourably with the conventional GnRH agonist treatment. Furthermore, women treated with letrozole have an uninterrupted menstrual cycle and quick relief of symptoms, allowing them to proceed to IVF without any further delay. Due to its significant therapeutic efficacy and minimal side effects, this low-dose letrozole therapy may also be explored for the management of symptomatic adenomyosis in general. Therefore, these findings warrant further interest from the clinical community, and require multicentric randomized controlled trials with a large number of participants.

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Overview of Genetics in Infertility

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GENETICS OF FEMALE INFERTILITY

Genetic, endocrine, physiological, anatomical, and immunological abnormalities of the reproductive system can affect a woman's likelihood of becoming pregnant and delivering a living child. Successful reproduction requires the precise regulation of complex processes essential for development of functional gonads and other reproductive organs, sex determination, gametogenesis, neuroendocrine competency, and ability to carry a pregnancy. Oogenesis is a process by which the mammalian egg becomes competent for fertilization and involves complex interaction between the oocyte and somatic cells that surround it, including the interplay of multiple transcriptional regulators. The disruption of these transcriptional regulators leads to ovarian dysgenesis or disorders of sex development. Multiple genes have been implicated in the production and maturation of germ cells, defects in which can cause accelerated cell apoptosis and follicle atresia, resulting in primary ovarian insufficiency (POI). Functional disruption at the level of the hypothalamus or pituitary glands leads to hypogonadotropic hypogonadism, small ovaries, amenorrhea, and infertility. Other pathologies, such as disruption of reproductive tract development, endometriosis, uterine fibroids, polycystic ovary syndrome, or autoimmune factors, may have a negative impact on implantation and pregnancy, leading to infertility or recurrent pregnancy loss. Genetic abnormalities leading to infertility in females comprise of large chromosome abnormalities, deletion and duplications, and DNA sequence variations in the genes that control numerous biological processes implicated in oogenesis, maintenance of ovarian reserve, hormonal signalling, and anatomical and functional development of female reproductive organs. The euploid maternal genome and the cytoplasmic components are essential for normal embryonic development—otherwise recurrent fertilization failure, recurrent implantation failure may happen.

Role of genetics in female infertility can be divided into into different subgroups—

1. Female sexual development-

Normal karyotype (46XX) has been found in 92% of women with Mullerian anomalies and abnormal in 7.7% of these women.[1] Mutation in HOXA10 and HOXA11 gene have been identified in patients with uterine malformations. Heterozygous pathogenic variants in HNF1B, LHX1, WNT4, WNT7A, and WNT9B have been reported in individuals with uterine malformations, including Mayer-Rokitansky-Kuster-Hauser syndrome.[2]

X chromosome and female infertility-

Different Chromosomal abnormalities such as monosomy X (Turner syndrome), cytogenetically visible deletions and duplications, and balanced and unbalanced X-autosome rearrangements (mosaicism) are associated with an accelerated loss of primordial oocytes during female fetal development, resulting in streak gonads at birth[3,4] presenting later as primary or secondary amenorrhoea. They account for close to 10% of cases of premature ovarian insufficiency (POI).[5] Approximately an entire X chromosome are missing in 50% of affected womenand have a karyotype of 45,X. About 25% have a partial deletion of one X chromosome, while about 20% have varying degrees of mosaicism, most commonly a 45,X/46,XX karyotype.[6] A small group (5%) of affected women carries an XY cell line. Seventy-six percent of the patients with mosaic Turner syndrome had two detectable ovaries and larger ovarian volumes, compared to girls with 45,X karyotypes, of whom only 26% had ovaries detected by ultrasonography. Girls with mosaicism more frequently had spontaneous thelarche (50%) and menarche (38.5%) than those with 45,X karyotypes. There are, however, reports of individuals with 45,X karyotypes spontaneously conceiving and delivering healthy infants, indicating that although women with mosaicism are more likely to conceive spontaneously, women with monosomy may also be fertile. Over 90% of women with Turner

syndrome who have spontaneous pregnancies have a mosaic karyotype.

XY Gonadal Dysgenesis—

XY individuals with femalephenotype with external genitalia and Mullerian structures, but dysgenic and non-functional gonads, lack of spontaneous pubertal development, absent breast development, primary amenorrhea, and infertility (commonly known as Swayer syndrome). Approximately 10–15% of individuals with XY gonadal dysgenesis have deletions or pathogenic sequence variants affecting the SRY gene on the Y chromosome.

Androgen insensitivity syndrome (AIS) – 46XY karyotype with inactivating mutation in the gene encoding the intracellular androgen receptor (located on the long arm of the X chromosome). It presents with primary amenorrhoea with female phenotype and short blind vagina.

XX Gonadal dysgenesis-

Phenotypic expression and severity of impaired development of gonads and oogenesis may depend on the type of alteration (loss of function vs. gain of function) and the effects on specific protein domains.

Defects in oogenesis and segregation defect can cause female infertility. Defect in DNAdamage repair gene cause early apoptosis leading to POI. The gradual loss of cohesions or cohesion-related proteins such as SGO2, has been implicated in meiotic non-disjunction and age-related aneuploidy and infertility. DNA repair genes such as MCM8, MCM9, XRCC4, and MSH5 who present with a non-syndromic primary ovarian insufficiency.[7–9] Deficiency in MCM8/MCM9 leads to oocyte loss due to unrepaired double-strand DNA breaks.

Multiple oocyte-specific transcription factors, including FIGLA, NOBOX, LHX8, SOHLH1, and SOHLH2, also control follicular development and play a role in cytoplasmic maturation, regulating the expression of maternal-effect factors essential for future embryonic activation.[10]

Patients with pathogenic variants in the FSHB (FSH beta subunit) and the FSHR (FSH receptor) genes develop follicles up to preantral stages, but further maturation is blocked. Defects in FSHB lead to a low production of FSH, resulting in hypogonadotropic hypogonadism, whereas changes in FSHR cause hypergonadotropic hypogonadism.

Hypogonadotropic hypogonadism is a rare disorder characterized by the deficiency of gonadotropin-releasing hormone (GnRH) due to its impaired production, secretion, or function. Kallmann syndrome is characterized by a defective sense of smell in approximately 50% of patients with hypogonadotropic hypogonadism.

Also, Nucleotide sequence variations in the human zona pellucida genes may cause defects in the formation of zona pellucida and subsequent infertility.

2. Reproductive ageing:

POI cases vary from complete failure of germ-cell development, resulting in primary amenorrhoea, to an accelerated decline in germ cell numbers, leading to the cessation of ovarian function before age 40 (Crisponi et al., 2001). Several genetic factors have been reported in such cases, including FOXL2, STAG3, FOXO3a, and X-linked genes, such as FMR1 and BMP15 located at Xp11.2. Among them Fragile X mental retardation gene (FMR1), located at Xq27.3 needs to be mentioned. It involves trinucleotide (CGG) repeat sequence mutation. Normally FMR1 gene contains approximately 30 repeats. Repeats between 55 to 200 is responsible for the disorder. The prevalence of premutations is approximately 15% among women with familial POI and lower, but still significant (1-7%), in those having no family history of POI.[17] Screening for the FMR1 premutation should be offered in all women with POI.

Diminished ovarian reserve: BMP15 and GDF9, members of the transforming growth factor-beta (TGFβ) superfamily, are involved in a cascade of regulatory processes directing granulosa cell proliferation, steroidogenesis, cumulus expansion, and apoptosis. GDF9 has been implicated in the transition from the primary to the secondary follicle stage. Human GDF9 and BMP15 gene variants, mostly heterozygous, have been detected in up to 10% of women with hypergonadotropic ovarian failure.[11]

3. Multigenic factors:

Polycystic ovary syndrome (PCOS)- The genome wide association studies identified susceptibility loci

on chromosome 2p16.3, 2p21 and 9q33.3. Additional studies identify 16 independent signals in 15 genomic regions associated with PCOS including signals near important reproductive hormone genes FSHR, LHCGR and FSHB.

Endometriosis— The most recent meta-analysis identified 14 genomic regions associated with disease risk. Endometriosis is an oestrogen-dependent disease and Oestrogen receptor 1 (ESR1) is the predominant receptor for oestrogen action in the endometrium. Estrogen and progesterone receptor polymorphism are important causative factor.

4. In RIF:

Different genetic association also found in RIF. Oocytes from women with loss-of-function pathogenic variants in WEE2 gene fail to complete MII exit and form pronuclei, resulting in fertilization failure. The TUBB8 gene encodes a beta-tubulin subunit that is essential for oocyte meiotic spindle assembly. Oocytes of patients with pathogenic variants in TUBB8 show no spindle formation or disorganized spindle and commonly fail to extrude a polar body. Loss-of-function variants of PATL2 were reported in infertile females affected by oocyte germinal vesicle arrest and primary infertility. These are some causes of post-fertilization pre-implantation embryonic failure.

Outline of treatment

Peripheral blood Karyotype is the basic investigation in clinically suspected female. Different gene sequencing method such as Next generation sequencing (NGS) or whole exome sequencing (WES) is important to identify clinically relevant genetic variants. FMR1 gene mutation should be performed in POI cases. PGD (now called preimplantation for monogenic disorder PGT-M) is helpful in identifying euploid embryo in known genetically affected individuals. But according to ESTEEM trial 2017, evaluation of oocyte euploidy in general population with age group between 36 to 41 years by arrayCGH does not improve clinical outcome.

In mullerian anomalies with absence of uterus, surrogacy is the only option. Whereas, in POI patients, donor egg IVF is the option. In hypogonadotropic hypogonadism, ovarian stimulation with HMG followed by IUI or IVF is the treatment option.

In Turner syndrome, fertility option is IVF with donor oocytes. Oocyte donation programs cite pregnancy success rates of 24-47%, which are consistent with pregnancy rates in women undergoing IVF who do not have Turner syndrome.[12] Miscarriages are most often due to the presence of ahypoplastic or bicornuate uterus and a thinner endometrial lining than that typically seen in pregnant women without Turner syndrome. Due to the comparatively small uterine size of women with Turner syndrome, it is recommended to transfer single embryo to prevent undue complications. More recently, there have been reports of women with Turner syndrome who have functional ovaries undergoing IVF using their own oocytes – autologous IVF. Predictors for successful autologous IVF include a mosaic peripheral blood karyotype, normal serum FSH and AMH levels, and spontaneous puberty. Negative predictors for successful autologous IVF are monosomy or a structurally anomalous X chromosome, elevated FSH levels, low AMH levels, and lack of spontaneous puberty.

Fertility preservation is a big trend now for impending POI. But timing for oocyte retrieval (for Fertility preservation) is controversial. There have been several case reports of adolescents (before ovarian failure) and adults with Turner syndrome undergoing ovarian stimulation followed by both oocyte and ovarian tissue cryopreservation for later IVF. However, there is currently no evidence regarding the safety of ovarian stimulation regimens in pre-pubertal females, and it is unknown what effect these treatments may have on overallpubertal development or adult height. An alternative is laparoscopic ovarian wedge resection with subsequent cryopreservation.

Karyotyping should be offered in all the couples with RIF.[13] Those found to carry structural rearrangement like balanced translocation, should be offered PGT-SR. On the other hand, women aged more than 40 years are often producing embryos with aneuploidy despite the fact that both the partner may carry normal karyotypes. Therefore, selection of the best quality of embryo in this case can be done by PGT-A. However, the evidences are showing controversial results regarding the benefits of PGT-A. It's important to note that, even if any person opts for PGT-A, the

ideal biopsy can be obtained from trophoectoderm of a blastocyst, rather than from blastomere of day3 embryo.[14]

GENETICS OF MALE INFERTILITY

In male, the fertility potential requires proper functioning of the hypothalamo-pituitary axis, testes and the outflow tract. All of them are controlled by several genes. The genetic landscape of male infertility is highly complex as semen and testis-histological phenotypes are extremely heterogenousand atleast 2000 genes are involved in spermatogenesis.

1. Hypothalamo-pituitary dysfunction:

Kallmann syndrome is charecterized by hypogonadotropic hypogonadism, due to impaired gonadotropin-releasing hormone secretion and anosmia. Given, the X-linked recessive (KAL1 gene-most frequent) and sometimes autosomal dominant and recessive mode of transmission, men with should receive genetic screening, as recommended by European Urology Association (EUA).[15] This can estimate the risk of transmission to the offspring. In men with Kallmann syndrome, the spermatogenesis can be induced by hormonal treatment like pulsatile GnRH therapy or gonadotrophins (hCG with or without FSH). However, initiation of spermatogenesis can take long time (6-24 months) in some cases. Given ease of administration and cost, gonadotrophin is preferred to pulsatile GnRH therapy. In most cases natural conception is possible after hormone therapy, even when the sperm parameters are subnormal.

2. Obstructive azoospermia:

Obstruction in pathway has been observed in 10 to 20 % of azoospermic men. Congenital bilateral absence of vas deferens (CBAVD) is associated with obstructive azoospermia (OA) and is associated with cystic fibrosis (CF) in most of the cases. The incidence widely varies depending on the ethnicity. In the Caucasian, the CF is the most common genetic defect, often caused by the mutation of the gene, CF transmembrane conductance regulator (CFTR), which controls the ion channels. This gene, located on the chromosome number 7, influences the development of the Wolffian ducts, particularly, the ejaculatory ducts, seminal vesicles, vas deferens and part of the epididymis and therefore, it is not surprising why CFTR mutation leads to OA.

There is a phenotypic diversity in CAVD and at least five phenotypes are known to be associated with male infertility: (1) congenital bilateral absence of the vas deferens (CBAVD) with normal kidneys, (2) CBAVD with unilateral renal anomalies (CBAVD URA), (3) congenital unilateral absence of the vas deferens (CUAVD), (4) CUAVD URA, and (5) CBAVD/CUAVD with ejaculatory duct obstruction. Of all these phenotypes, CBAVD is reported in 1%–2% of infertile men and 6-10% of men with obstructive azoospermia.[16] Earlier, we observed renal anomalies in 9% of Indian men with CAVD. Renal anomalies were comparatively higher (50%) in CUAVD compared to CBAVD (10%). No major CFTR gene mutations were detected in Indian men with CBAVD URA.[17]

CFTR gene mutations are detected in 60%–70% of isolated CBAVD, and 30%–40% of CBAVD cases may have genetic etiology other than CFTR.[18] F508del is the most commonly reported CFTR gene mutation in Caucasian men with CBAVD, whereas IVS 9 c. 1210 12[5] is the most commonly reported CFTR variant in non Caucasian men with CBAVD.

Routine testing can detect many, but not all of the mutations. Because of the autosomal recessive pattern of transmission, if the genetic defect is detected in the male partner, the female partner should be tested before proceeding for ICSI.[24] If the female is the carrier, the risk of the baby being affected with CF is 50% whereas if the female partner is normal, the risk is 0.4% because of transmission of undetected mutations.

Because CBAVD may be the reason for OA, in men with azoopsermia with semen volume less than 1.5 ml and pH less than 7.0 (because of associated seminal vesicle aplasia, careful examination of vasa is needed, because clinically often the diagnosis of CBAVD can be missed. In high risk situations, prenatal diagnosis or preimplantation genetic diagnosis (PGD) are indicated.

3. Testicular failure:

30 to 40 % of azoospermic men have testicular failure. The common genetic causes are structural

and numerical abnormalities of sex chromosomes and deletion of genes located in Y chromosome.

Sex chromosome abnormalities- The most common congenital cause of hypogonadism in male is **Klinefelter's syndrome (KS)** with incidence 1:1000 male birth.[24] Klinefelter syndrome with 47,XXY karyotype or its variants is the most common aneuploidy and is seen in about 14% of azoospermic men. It is also the most common sex chromosomal abnormality associated with azoospermia. The usual karyotype is 47,XXY but sometimes mosaic (46,XY/47,XXY) or additional X chromoso me (48,XXXY) have also been reported. The underlying factor is the nondisjunction of the sex chromosomes during meiosis. The cause of nonobstructive azoospermia (NOA) is because of atrophy of seminiferous tubules, germ cell damage and Leydig cell dysfunction. It leads to hypergonadotropic hypogonadism.[19]

In men with KS, particularly those with mosaic form, low levels of spermatogenesis can be present inside the testicles. Such sperms can be recovered by testicular sperm extraction (TESE) or preferably with microscopic TESE (micro-TESE) and can be used for intracytoplasmic sperm injection (ICSI). With such techniques, spermatozoa can be retrieved from 50% men with KS, leading to pregnancy and live birth rate of 50%.[20] However, the babies born out of these procedures are at risk of having 47,XXY abnormalities because of presence of sperms carrying abnormalities in sex chromosomes (24,XY sperms) and autosomes (disomyfor chromosomes 13, 18 and 21). Therefore, sperms obtained from KS men should be checked for aneuploidy by sperm fluorescent in-situ hybridization (FISH) and if appropriate, preimplantation genetic testing (PGT) should be offered.

Apart from chromosomal numerical aberrations, structural aberrations such as deletions, duplications, inversions, and translocations are commonly seen chromosomal abnormalities in about 5% of infertile men.[21] Robertsonian translocations, where long arms of two acrocentric chromosomes fuse to form a long chromosome with a single centromere, are seen in 0.8% of infertile men. The incidence is predominantly seen in oligozoospermic men compared to azoospermic men. The most commonly observed Robertsonian translocations are der (13;14) and der (14;21), of which der (13;14) is predominant.[22]

Microdeletions on the long arm of the Y chromosome (Yq11)—

Y chromosome microdeletions are deletion in the q11 region of the long arm of the Y chromosome (Yq11). Y chromosome gene mutations are the most common "molecular genetic cause" of severe male factor-infertility. Approximately 7.4% of infertile men show Y chromosome microdeletions worldwide. The prevalence of such defect is 6% in men with severe oligospermia and 9.7% in men with azoospermia.[23] Among the infertile men, 55% of men with maturation arrest and Sertoli cell only syndrome have Y chromosome microdeletions. Several studies have shown that microdeletions in AZFc region are most common (maximum of 80%), followed by AZFb (maximum to 5%) and AZFa (maximum to 4%).[24] In the long arm of the Y-chromosome (Yq), the region AZF contains several genes controlling the spermatogenesis. Deletions involving this region remove one or more of the genes partially or completely and are known as Y-chromosome microdeletion (YMD).[24] YMD is not found in men with normozoospermia and is extremely rare with sperm concentration more than 5 million/ml. The most common variety is AZFc deletion followed by deletions of AZFb, AZFb+c and AZFa+b+c. AZFa complete microdeletion causes Sertoli cell only syndrome whereas that of AZFb causes spermatogenetic arrest. On the other handAZFc deletion can be associated with oligospermia or azoospermia. Genetic testing for YMDshould be offeredMen with azoopsermia and oligospermia (concentration <5 million/ml) and if such deletions are detected, genetic counseling should be done, because of the risk of transmission from father to son. Apart from YMD, a new type of Y chromosome deletion known as the 'gr/gr deletion' has been described, which can predispose to oligospermia and testicular tumours.[19]

Other genetic problems- In infertile male, karyotype may sometimes reveal 46,XX pattern because of DNA translocation from part of Y chromosome to X chromosome. In contrast, 46,XY male can have disorders of sexual development (DSD) because of enzyme deficiency (17,20 desmolase, 17-α-hydroxysteroid dehydrogenase) and gonadal

dysgenesis (due to various genetic mutations). Leydig cell hypoplasia because of Inactivating LH receptor mutations can also cause spermatogenetic failure.

Qualitative defects of sperm is also expressed from genetic changes in sperm. An interesting syndromic phenotype that gained attention in recent times is multiple morphological abnormalities of the sperm flagella (MMAF), which was first proposed in 2014.[25] MMAF is a type of asthenoteratozoospermia with a mosaic of flagellar morphological defects such as absent, short, bent, coiled, and irregular flagella without systemic ciliary defects such as primary ciliary dyskinesia.

Genetic association in Globozoospermia is also observed. Atleast 3 genes have been associated with this form of teratozoospermia in humans: SPATA16, PICK1 and DPY19L2.

4. Other role of genetics in male infertility:

Men with sperm defects are at risk of developing cardiovascular morbidities, diabetes, metabolic diseases, repeated hospitalization and early mortality. There are studies suggesting increased risk of cancer in infertile men and these include lymphoma, extragonadal germ cell tumours, peritoneal cancers and testicular cancers. The exact pathogenesis of testicular cancer in men with infertility is unclear.[23] Genetic mutations and chromosomal aneuploidies may be linked with such malignancies.[27] These include instability of Y-chromosome, abnormal Hiwi protein and DNA mismatch repair. Another possible explanation is the "testicular dysgenesis syndrome" (TDS) which is characterized by abnormal semen parameters and urogenital abnormalities like cryptorchidism, testicular germ cell tumour (TGCT) and hypospadias. The pathogenesis of TDS starts probably from the embryonic life because of endocrine disruption mediated by the environmental factors and all these can increase the risk of both infertility and TGCT in TDS.[19]

5. Recommendations for genetic testing in infertile male:

The aim of genetic testing in infertile male is to inform the patients about possible risks, to improve the success rate of the ART treatmentand to avoid the birth of children affected with a severe genetic disease. Men with azoospermia are at the highest risk of being carrier of genetic anomalies (25%), wheras this risk progressively decreases with increasing sperm count.

Personal history from the patient should be taken. Detailed pedigree chart should be drawn. It is also important to know about infertility in siblings or other family members. This information may suggest a possible chromosomal aberration such as translocation or a monogenic disease like Kallmann syndrome. In all men with sperm concentration less than 5 million/ml, karyotype should be offered to diagnose sex chromosomal and sometimes autosomal defects. Y chromosome microdeletion test should be offered in men with severe oligospermia and nonobstructive azoospermia. If AZFa or AZFb microdeletions are present, surgical sperm retrieval is discouraged. CFTR gene mutation testing should be done in case of nonobstructive azoospermia because of CBAVD but all the mutations may not be detected by the commonly performed tests. In addition men with unilateral absent vas and normal kidney should be offered this test.[19]

More research in the field of geneticswill provide us with a better understanding, as well as a better defining how great the risks are of transmitting infertility orpossibly genetic anomalies to the next generation.

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ART & Surrogacy Bill in a Capsule

Dr Meenakshi Karan, Dr Alina Bhattacharjee

Came into effect on	• 20th December 2021
Purpose	• Regulation of ART clinics & Banks • Prevention of misuse • Safe and ethical practices • Regulation of Research & Development
AUTHORITIES	• NATIONAL ART AND SURROGACY BOARD • NATIONAL REGISTRY • STATE ART AND SURROGACY BOARD • APPROPRIATE AUTHORITY
APPROPRIATE AUTHORITY	• Grant, suspend or cancel registration • Enforce standards of care • Investigate complaints of breach • Take legal action against misuse of ART
Levels of ART clinics	• Level 1 • Level 2 • ART BANKS
LEVEL 1 CLINIC	• Only IUI can be carried out as a procedure
Level 2 clinic	• Surgical retrieval of gametes • Handling the oocyte outside the human body; • Use sperms for fertilization of oocytes; • Transfer of the embryo into the reproductive system of a woman; • Carryout storage of gametes or embryos or • Perform any kind of procedure or technique involving gametes or embryos.
Eligibililty to avail ART services	• Female-21-50 years • Male-21 to 55 years
Eligibility for donor	• Oocyte donor: 23-35 years • Semen donor: 21-55 years • Only to one commisioning couple • Only once in lifetime
Qualifications of clinicians in LEVEL 1 CLINIC	• Medical post-graduate with degree in Obstetrics and gynaecology
Qualifications of clinicians in Level 2 clinic	• Medical post-graduate in OBGY • Record of performing 50 OPU under supervision • Experience of 3 years in ART Clinic • MCH/DM/Fellowship with 3 years experience in ART clinic
Qualifications of embryologist	• Post graduate in clinical embryology with 3 years human lab experience in gametes • PhD in clinical embryology with 1 year human lab ART experience • MBBS with post graduate in Clinical embryology with 2 years human ART lab experience • Post graduate in life science or biotechnology with 1 year full on site clinical embryology training with 4 years human ART lab experience
Staffing of ART level 2 clinic	• Gynaecologist • Embryologist • Andrologist • Counsellor • Anaesthetist • Director
ART Bank staffing	• Registered Medical Practioner
ART level 1 equipments	• Microscope • Centrifuge • Refrigerator

ART level 2 clinic	• Microscope, Incubator (2), Laminar air flow, Sperm counting chambers, centrifuge, Refrigerator, Equipment for cryopreservation, Ovum aspiration pump, USG machine with transvaginal probe and Needle guard, Test tube warmer, Anaesthesia resuscitation trolley
ART Bank	• Centrifuge machine • Incubator • Laminar Air flow • Microscope
Offences	• Abandon, disown or exploit child born through ART • Sell or purchase or import human embryos or gametes • Exploit commisioning couple, woman or gamete donor • Transfer human gamete or embryo into male person or animal
Penalties	• 1st contravention-5-10 lakh rupees • Subsequent contravention-3 -8 years imprisonment and 10-25 lakh fine
Surrogacy act	• Came in to effect on 25TH DECEMBER 2021
Requirements for surrogacy	• Medical indication necessitating surrogacy • Indian origin couple • Altruistic surrogacy • Certificate of essentiality • Eligibility certificate for mother • Insurance coverage for 36 months
Eligibility for surrogate	• Ever married woman with child of her own • Age 25-35 years • Once in her lifetime • Medical and pyschological fitness • No donation of own gametes
Eligibility of intending couple	• Male age: 26-55 years, Female age: 23-50 years • No surviving child either biological or through adoption • Child-Mentally /physically challenged/life threatening illness
Offences and penalties	• Commercial surrogacy, advertisement • Exploitation of child or surrogate mother, abandonement • Sale, import of gametes, Sex selection • Imprisonment of 10 years and fine upto 10 lakhs • Subsequent offence-suspension of registration upto 5 years • Commercial surrogacy-imprisonment 5 years and fine 5 lakhs

Work Statement from April 2023 to June 2023

1. GYANECOLOGICAL AND OBSTETRICS CASE

Total no. of Patient's attended: ***422***

- Infertility 370
  - — Primary 290
  - — Secondary 80
- History of Recurrent Spont Miscarriage (≥ 3) 12
- History of Unexplained Spont Miscarriage (≥ 1) 08
- Primary Amenorrhoea 03
- Secondary Amenorrhoea (Without infertility) 09
- Other Gynaecological case 20

Infertility

Female Factor: 175 (47.29 %)
Male Factor: 140 (37.83 %)
Unexplained: 35 (9.45 %)
Combined Factor: 20 (5.40%)

Female Factor **175 (47.29%)**

- PCO 35 (20.00 %)
- Endometriosis 28 (16.00 %)
  - Early: 12
  - Advanced: 10
  - Advanced with Adenomyosis 06
- Tubal Block 58 (33.14 %)
- POF 25 (14.28 %)
- Uterine Anomalies 06 (3.42%)
- Fibroid 11 (6.28 %)

Male Factor **140 (37.83%)**

- Azoospermia 54 (38.57%)
- Asthenospermia 25 (17.85%)
- Oligospermia 22 (15.71%)
- Oligoasthenospermia 21 (15.00%)
- Oligoteratozooasthenospermia 07 (5.00%)
- Aspermia 03 (2.14%)
- Pyospermia 08 (5.71%)
- Sexual Dysfunction 04 (2.85%)
- Retrograde Ejaculation 02 (1.42%)

Age Factor of Female Partner: (n=370)

- Less than 25 Yrs: 45 (12.16%)
- 25-35 Yrs: 254 (68.64%)
- 36-40 Yrs: 48 (12.97%)
- More than 40 Yrs: 23 (6.21%)

Duration of Infertility: (n=370)

- Less than 5Yrs: 210 (56.75%)
- 5-10 Yrs: 102 (27.56%)
- More than 10 Yrs: 58 (15.67%)

Residential Status: (n=370)

- Urban: 258 (69.72%)
- Rural: 112 (30.27%)

2 OBSTETRIC CASES (Pregnancy following investigation & treatment excluding IVF)

A. Confirmation of Pregnancy 76

- Following treatment of Infertility: 59
- Following previous foetal wastage (Spont): 17

Treatment modalities for achieving these pregnancies

- Following medical treatment (Induction Ovulation): 13
- Following Surgical Treatment: 02
- Following Hydrotubation: 07
- Following HSG & Laparoscopy: 08
- During investigation: 15
- Intrauterine Insemination: 31

B. Complication in Ongoing Pregnancy

- Threatened Abortion: 04
- Hypertension: 16
- Diabetes Mellitus: 13
- Sub Clinical Hypothyroidism: 11
- RH Negative: 03
- Pulmonary Hypertension 01
- Psoriasis 01

3 IVF UNIT

OCR: 91

Embryo Transfer 114

- Fresh 09
- FET 105

Pregnancy 37

- Fresh 03 (33.33%)
- FET 34 (32.38%)

Cancellation due to Failed Stimulation: (Poor Responder) 03

Discontinued other than poor responder 05

Fresh ET cancelled due to 82

- Due to OHSS 4
- Endometrium abnormality: 27
  - Thin endometrium (<7 mm) 03
  - Thick endometrium (>14 mm) 19
  - Endometrium Polyp 05
- Endometriosis/ Adenomyosis 51

Protocol

- Long 52
- Antagonist 39

IVF Cycles

- Conventional IVF 85
- PESA/ICSI Cycles: 03
- Ejaculated ICSI Cycles: 03

4 USG UNIT

- Folliculometry: 443
- Antenatal Monitoring: 215
- Pelvic Scan: 310

5 IUI UNIT

Total No. of Patients Treated: 271

	N	Preg
• AIH:	217	21 (9.67%)
• AID:	54	10 (18.51%)

6 SURGICAL UNIT

- Hysteroscope: 08
- Laparoscopy + Hysteroscopy: 08
- Ectopic pregnancy 01
- D+E+C: 04
- Mc Donald/Shirodkar: Stich 02
- LUCS 22
- Laparoscopy + Vaginoplasty 01
- Laparoscopic Cystectomy 02

Achievements:

Publication in A Comprehensive Handbook of IVF –

i) Non Conventional Methods of Embryos and Oocyte Assessment- A Chapter by Dr. Ratna Chattopadhyay in ' A Comprehensive Handbook of IVF - Jaypee Brothers'

Papers accepted in ESHRE 2022:

1. P Chakraborty, S Sharma, S Kalapahar, I Mitra, S Ghosh, R Chattopadhyay, K Chaudhury, Presence of stage III/IV endometriosis associated with systemic inflammation poses as individual risk factor for coronary heart disease in Indian women Hum. Reprod. Volume, (2023)

2. R Chattopadhyay, I Mitra, S Sharma, G Bose, S Ghosh, S Kalapahar, K Chaudhury, P Chakraborty, Predicting live birth rate using combined Day3-spent embryo culture medium metabolomics and cumulus cell gene expression based algorithm to optimize embryo selection in unexplained infertility, Hum. Reprod. (2023)

3. S Kalapahar, S Sharma, R Chattopadhyay, I Mitra, S Ghosh, M Dam, S Kalapahar, K Chaudhury, P Chakraborty, Administration of low molecular weight herapin alleviate immune physiology in recurrent implantation failure modulating CCL2 - CCR2 axis during frozen embryo transfer: effort from clinic to bench, Hum. Reprod. (2023)

4. Sunita Sharma, Sourav Roy Choudhury, M. Padmaja. Bhattacharya, Shubhendu Hazra, Pratip Chakraborty, Ratna Chattopadhyay, Arup Kumar Majhi, Impact of localization of diffuse adenomyosis on reproductive outcomes and pregnancy complications: A prospective cohort study of 585 patients after frozen embryo replacement cycles Hum. Reprod (2023)

Publications in peered reviewed Journal

1. Sunita Sharma, Sourav RoyChoudhury, M Padmaja Bhattacharya, Shubhendu Hazra, Arup Kumar Majhi, Kamal C Oswal, Ratna Chattopadhyay: Low-dose letrozole – an effective option for women with symptomatic adenomyosis awaiting IVF: a pilot randomized controlled trial, RBMO, Vol-47 (1), July,2023

On-going Projects

1. A new approach towards pain and infertility management in women suffering from endometriosis and adenomyosis (PI Dr. Sunita Sharma, Co-PI: Dr. Sourav Roy Choudhury)

2. TSLP modulates polarization of macrophage to M2 phenotype: A potential beneficial outcome during recurrent implantation failure (PC Dr. Ritobrata Goswami, Asst. Prof. Indian Institute of Technology, Kharagpur, PI Dr Pratip Chakraborty, Co-PI: Dr. Koel Chowdhury Prof., Indian Institute of Technology-Kharagpur. Dr. Sunita Sharma, Consultant, Institute of Reproductive Medicine, Kolkata).