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Institute of Reproductive Medicine

From the President's Desk

Application of Inflammasome Concept in Predicting Endometriosis during Adolescence: Future Thoughts

Dr. Baidyanath Chakravarty

Endometriosis is neither curable nor preventable. Given its unknown etiology, treatments usually aim at relief of pain and/or achieving pregnancy. However, unlike modern–day treatment of targeting lesions, research should plan at identification of potential victims with a view to provide relief to most distressing clinical manifestations like pain, volume of the disease and sub fertility. Currently, the possible hypothesized etiologies of endometriosis are, immunological, inflammatory, genetic and endocrinological, however, the precise mechanisms underlying origin and development of endometriosis remain mainly unknown.

At a cellular level, the main alterations in endometriosis are characterized by cell proliferation, inflammation, and angiogenesis, which are closely connected to each other and are caused by an alteration in sex hormonal signaling, which depend on the sustained activation of estradiol (E2)-dependent pathways and the disruption of those dependent on progesterone. A recent and detailed revision about the role of P4 and E2 in endometriosis describes the normal molecular hormonal regulation of the physiology of endometrium and its alterations in endometriosis that highlights inflammation as a known key contributor in the pathophysiology of endometriosis.[1] Presence of chronic pelvic pain and associated defects in endometrial receptivity and the decidualization process attests the above finding.

The molecular mechanisms involved in the pathogenesis of endometriosis are not completely understood; however, inflammation plays a significant role in the pathophysiology of the disease, mainly by altering the function of immune cells (macrophages, natural killer, and T cells) and increasing levels of pro-inflammatory mediators in the peritoneal cavity, endometrium, and blood. These immune alterations inhibit apoptotic pathways and promote adhesion and proliferation of endometriotic cells, as well as angiogenesis and neurogenesis in endometriotic lesions. In addition, IL-33 has emerged as a possible biomarker in a syngeneic mouse model of endometriosis.[2] Inflammasome has emerged as a key player in innate immunity as well as inflammation. Abnormal inflammasome activation, in absence of detectable infectious causes, might stand as one of the molecular mechanisms involved in establishing an abnormal endometrium, potentially leading to endometriosis.[3]

Overproduction of chemokines, prostaglandins, with recruitment of immune cells like activated macrophages, natural killer cells, and neutrophils to endometriotic sites have been rigorously reviewed in literature with regard to local inflammatory milieu; however, the role of neutrophils in the biology of endometriosis has not been clearly defined. The future research may throw optimum light on this issue in conjunction to ligand stimulated externalisation of DNA and neutrophil-extracellular-traps (NET) production to look into the inflammatory status.[4]

The initiation of inflammation and/or inflammasome activation in endometriosis, if any, will help to pre-empt the pitfalls of the host-defence mechanism to infection and dys-regulation of this process. Looking at the challenge of treating women with endometriosis, it is warranted to look at the genetic background. The women susceptible to the development of endometriosis perhaps carry a genetic signature that renders the endometrial cells to[1] manifest an 'activated' angiogenic phenotype

and increased potential to implant and penetrate,[3] resistance to progesterone,[4] increased potentiality to generate aromatase and heightened cellular sensitivity to estrogens, and[5] enhanced cellular survival with attenuated apoptotic decay. This directive/s may be evaluated with the modern day diagnostic tool/s like inflammasome and/or NET-osis to assess the inflammatory milieu by harvesting eutopic endometrial cells from menstrual blood of adolescent girls born in "high risk" (history of endometriosis in the family) families. Few therapeutic modalities currently available are aromatase inhibitors, LNG-IUS, SERMS etc. Progesterone receptor modulators, estrogen ligand modulator as targeted therapy for treating endometriosis are on trial.[4] Hence, a better understanding of endometriosis biology may allow identification of the potential victims at an early stage of life and offering them with possible preventive approaches. Perhaps also importantly, diagnosis and management of endometriosis may pave the development of essential conceptual tools towards future basic and clinical research.

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Editorial

Prof. (Dr) Gita Ganguly Mukherjee

Institute of Reproductive Medicine (IRM) is the brainchild of Prof. B N Chakravarty, a living legend of ART. This is a premier organization known very much not only for the patients friendly modern management but also for its academic activities. Under the leadership of Prof. B N Chakravarty Clinicians, Embryologists and Scientists are working together to make ART safer and effective.

Activities of IRM are:-

- Investigation and treatment of infertility including IUI and IVF. Uptil were more than 5000 IVF babies delivered. Every year there was grand baby show.
- From 1990 Training of Gynecologists from all over India in this specialty.
- From 1992 IRM became the FNB Training Centre. Trained FNB doctors are now working with repute in different parts of the country.
- Research activities with Kharagpur IIT & Hyderabad and Calcutta University.
- Phd training is going on in research collaboration with Indian Institute of Technology, Kharagpur, Bose Institute, Kolkata, IICB, Jadavpur and Calcutta University.
- Embryology course started at IRM since 2017. Participants of the course were from all over India and neighboring countries.
- Bimonthly seminars which were so educative that doctors used to look forward to attend.
- 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 stalwarts.

During covid situation most of the activities came to a grinding halt except Prof. Chakravarty who continued writing and completed the volume 4 of this book "Clinics in Reproductive Medicine and Assisted Reproductive Technology."

We are glad that gradually I.R.M. is back to normal again and patients are attending OPD. IUI and IVF have also been started.

Prof B N Chakravarty's 4th volume of the above book was released by His Excellency the Hon'ble Governor of West Bengal, Mr. Jagdeep Dhankhar on 1st October 2021.

Bimonthly seminar last held in the month of November 2021.

I am thankful to Prof. B N Chakravarty, Director of IRM for giving me the opportunity of editing the bulletin. With great enthusiasm I present the 1st issue after about 2 years of Covid situation. Here the contributors are Prof. B N Chakravarty who has written a very interesting chapter i.e. Research Module in Reproductive Medicine Life-time experience of the clinician from Stethoscope to Microscope, Dr. Ratna Chattopadhyay, a famous embryologist in India has contributed a chapter on Ovum Collection – Gateway to Invitro Fertilisation, Dr. Sunita Sharma, an expert in Infertility treatment has written on Pregnancy and Live birth rate are comparable in Young Infertile Women presenting with Endometriosis and Tubal Infertility,

Hope these deliberations will be beneficial to the readers and if necessary will provide with ample thought for modifying treatment and further research.

Wishing all Merry Christmas and Happy New Year and hope for COVID free 2022.

My Journey through IVF

Dr. Baidyanath Chakravarty

Introduction

My interest and journey in infertility started around 1965, 12 years after my medical graduation through association with Dr. Subhash Mukherjee, while both of us were posted in our respective disciplines at N R S Medical College, Kolkata. We liked each other because we had common interest in uncommon subjects of ill understood etiology and unfavourable outcome with treatment. For example, while Subhash had keen interest in endocrinological abnormalities, both in adolescent boys and girls like Turner's, testicular feminizing syndrome, intersex problems etc, I was more interested in surgical correction like congenital absence of vagina, reconstructive surgery of ambiguous external genitalia, and recanalization of blocked tubes, vas. etc.

The evolution of infertility and interest in the Management started around 1960, immediately after the second world war. The reasons for the new revolution were different in two different hemispheres of the world. On one side, technologies and interventions required in the ongoing warfare added new informations which helped in developing a society associated with rapidly available methods involving less manpower and physical activity. One example was "Information technology, transport and communication system". The Western society grabbed the advantage, and made life easy moving. "Easy life and richer society' possibly might have been a background for the genesis of three very important causes of 'female infertility',- Obesity, PCOs and 'Endometriosis'.

The picture was however, different in the Eastern Hemisphere. Here, the explosive increase in population demanded a situation of "task force" population control administration. Poverty, lack of sanitation, lack of development of sophistication, lack of nutrition, led to transmission of infection and of contagious diseases like malaria, kalazar, S.T.D. etc. Implementation of population control had to be achieved through undesirable procedures like vasectomy and tubectomy even in unmarried boys and girls, especially in many areas of poverty-stricken world. India was a glaring example of this unfortunate crisis which was believed to be one of the causes of fall of Mrs. Indira Gandhi's Government.

To balance the difference between the two hemispheres of the world, technologies and methods were introduced through different approaches for the management of infertility during this period. Population control in the developing countries like eastern Hemisphere and population stabilization in the West were attempted in two different ways.

In the western hemisphere women were treated with Human Menopausal gonadotropin which was isolated from menopausal urine. These achievements were credited to two individuals, Gemzell in USA and Lunenfeld in Israel. Clomiphene Citrate, initially believed to be an infertility agent, later proved to be a fertility enhancing drug. Extensive basic and clinical research on follicullogenesis, preparation of media, in-vitro growth of human oocyte, and clinical use of pelviscope for diagnosis of Pelvic pathology were introduced during this period.

As mentioned earlier, in the developing world, unexpected Global Population explosion, led to unplanned mass vasectomy, tubectomy, MTP etc. These procedures necessitated introduction of newer technologies in the treatment (reversal of blocked tubes and vas).

Pioneers in the above field were Victor Gomel in Canada, Robert Winston in Hammersmith, Green Armytage in London, Shirodkar in Bombay and many others. These are few names to be mentioned in introduction and popularization of different techniques of the procedure. For population control hormonal contraceptive tablets (OC pill) was a remarkable contribution which was introduced during this period, though the procedure had certain limitations.

Moreover, in developed areas of the world, changing life style and diet habits led to multiplying incidence

of PCO and endometriosis requiring special attention for fertility stabilization.

Management

Basically the procedures for management of problems in the two different hemispheres were:

(1) Ovulation inductions – based on the concept "Two gonadotropin – Two cell-theory" (West)- PCOS, endometriosis – more towards medical treatment

(2) Micro surgery for correction of tubal / vassal block (East) – pelvic inflammatory diseases – required more surgical attention

This brought in the concept of understanding the physiology of folliculogenesis, ovulation induction and also prevention of conception (both medical and surgical procedures)

Knowledge about physiology of folliculogenesis and ovulation

The concept of "two gonadotropin – two cell theory" was known in 1941, but practical application was implemented in 1960. The gonadotropins were:- FSH (Follicle stimulating hormone) for follicular growth and development (oestrogen) & LH for maturation of follicles. The two cells were:- granulose cells – for oestrogen production, and thecal cells – for progesterone and androgen production. These are basic hormones, essential for folliculogenesis and ovulation.

Impact of few other adjunctive hormones on reproductive axis (hypothalamic Pituitary Ovarian axis) were explained. These were initially and still essentially used for induction of ovulation in unsuccessful cases. The indications are:

a) Hypothyroidism – in hypothyroidism, SHBG is low and therefore androgen is elevated. Addition of drugs like Eltroxin, with primary ovulation inducing drugs (clomiphene, letrozole, gonadotropin) may benefit in successful induction.

b) Prolactin - Elevated Prolactin – borderline increase of prolactin will decrease basal level of gonadotropin and elevation of adrenal androgen, leading to non-ovulation. Supplementation with bromocriptine might lead to success in ovulation.

c) Hyperactive adrenal (as in few examples of PCOS) Dexamethasone in low dose in early follicular phase, with ovulation inducing drugs, may bring successful ovulation.

d) Insulin – A co-gonadotropin androgen elevator through hepatic suppression of SHBG or stimulation of IGF-1I in theca cells (not very much known at that time).

Hence, for ovulation induction in these specific situations, in addition to Clomiphene citrate (CC) and gonadotropin, - eltroxin, Bromocriptine, dexamethasone and occasionally metformin were used in appropriate doges, in these abnormal situations. Though controversial, estrogen 0.01 mg was added to CC (from d7 to d11) to counteract its anti-estrogenic effect on endometrium and to make cervical mucus more permeable for sperm penetration.

Wedge Resection in non-ovulation –

In intractable cases of anovulation, wedge resection was very popular. If properly performed the results were very encouraging with minimum side effects. Monitoring of ovulation and timing of ovulation trigger, esp for IUI – 3 procedures were followed. Standard procedure for IUI started becoming popular from 1975.

Parameters were:

- Insler's cervical mucus scoring
- Basal body temperature chart (BBT)
- Urinary LH assay (By color change on paper strip

Insler's cervical mucus scoring:

Two methods were popular

Cervical mucus is good biological mirror of follicular development and ovulation.

Daily observation of cervical mucus from the first day after menstruation ceases, is a good sign of the follicular measurement and also estradiol production. A nearly mature follicle (17-18 mm in diameter) will produce about 100-150 pg. Estradiol.

Observation of stretchability of cervical mucus (starting from D8 or D9 of menstrual cycle)

Everyday the cervical mucus is collected in between two fingers or tips of artery forceps, and maximum

stretchability without break should be around 10 cm (see figure-2) which indicates ovulatory cycle and oestradiol (100 to 150 pg in stimulated cycle) on the day before ovulation. That is the day when hCG should be given for ovulation trigger in the stimulated cycle. Ovulation is likely to occur 36 to 42 hrs. after the injection. We have seen a drop to appear at the central point of the stretched out cervical mucus. In addition, under the microscope, the mucus will appear as branches of a fern tree, (first and second order branches) (See figure-2,3,4 & 5). The exact time of injection will be indicated by –

- Appearance of stretched out cervical mucus preferably with a drop of mucus at the center in between two finger-tips or tips of artery forceps (see figure-2).
- Transparent mucus (see figure-1).
- Gaping cervical external OS (see figure-1).
- Second order branches indicate peak oestradiol level and exact timing of injection (triggering) (see figure-3,4 & 5).
- Figure-6 demonstrate microscopic appearance of cervical mucus after ovulation
- Dark background, branches broken, LH surge completed, effect of progesterone

Appearance under microscope:-

Hazy to dark background (see figure-6), migration of inflammatory cells, and addition of branching indicates appearance of progesterone and completion of ovulatory phases (extrusion of 1st polar body). Therefore, cervical mucus in pre-ovulatory period is said to be a "Biological mirror" of the endocrine status around the Period of Ovulation.

Another parameter of ovulation monitoring was BBT:

What is Basal Body Temperature (BBT)?

We defined BBT as a marker of normal and abnormal endocrine function of a woman, undergoing treatment of infertility. Because at that time USG, RIA or EIA were not developed, BBT was considered as a mirror of many endocrine functions or dysfunction. For example, we know that progesterone and androgen are thermogenic hormones and oestrogen is a non-thermogenic hormone (in the follicular phase). In the earlier part of menstrual cycle, there is availability of higher level of oestrogen, whereas during the later

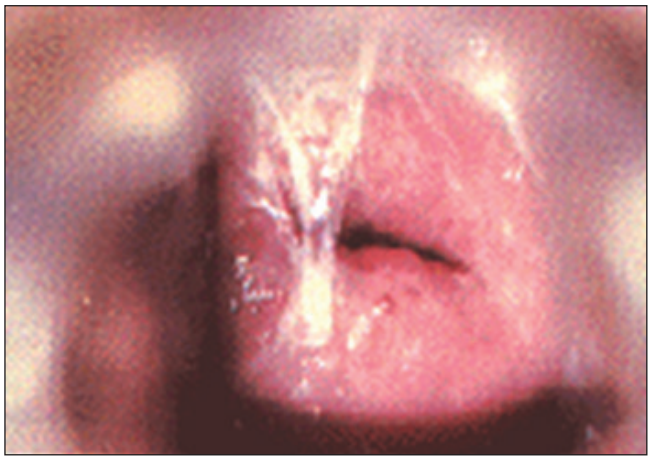


Fig-1: Gaping external OS; Thin transparent copious mucous - E2 peak

Fig-2: Stretchable thin mucous with drop formation at the centre - timing of trigger

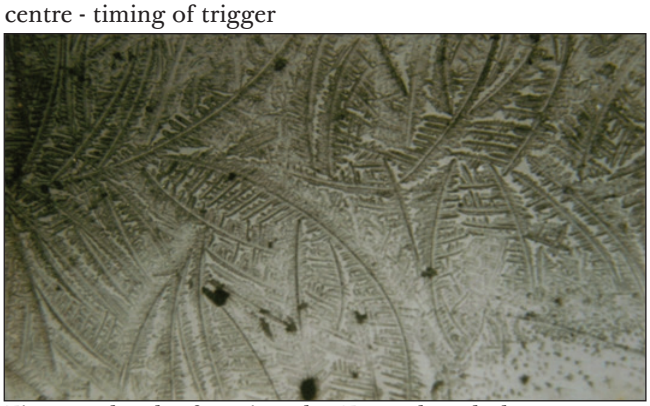


Fig-3: 2nd order fernning also E2 peak and plateau - timing of hCG

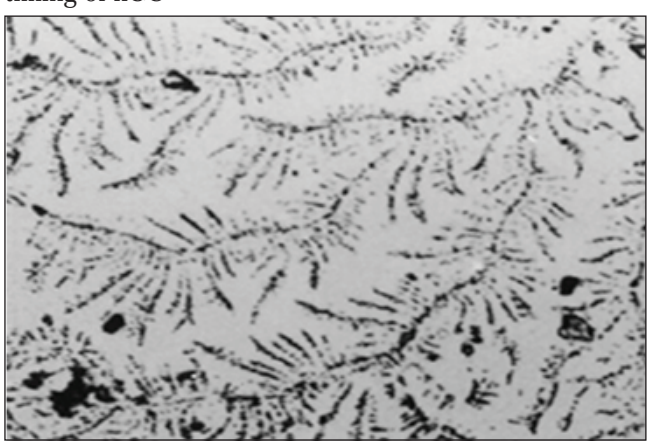


Fig-4: First order branching; clear transparent background – E2 high

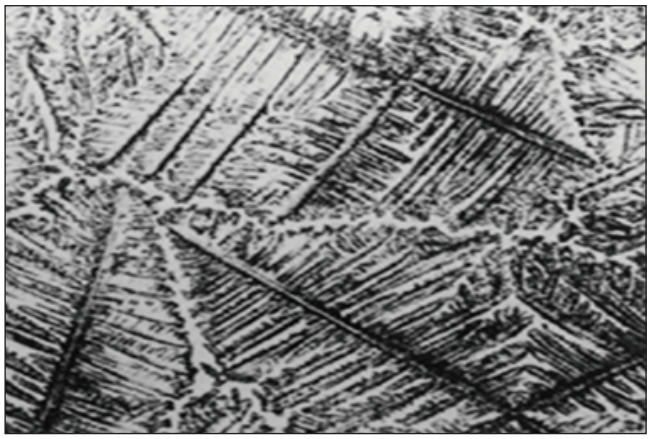


Fig-5: Second order branching; - E2 reached peak and plateau - LH surge starts

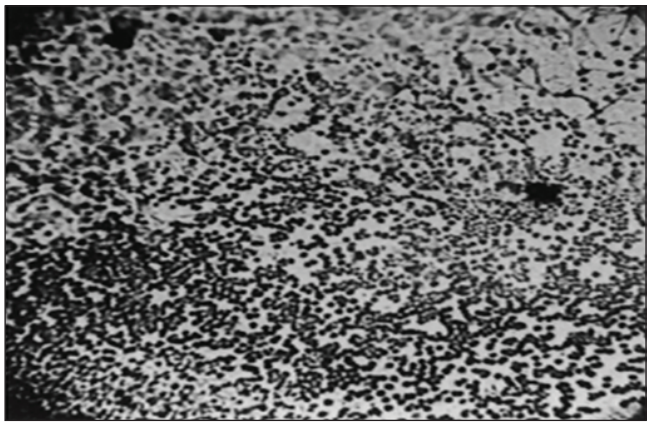


Fig-6: Dark background branches broken - LH surge completed; P effect

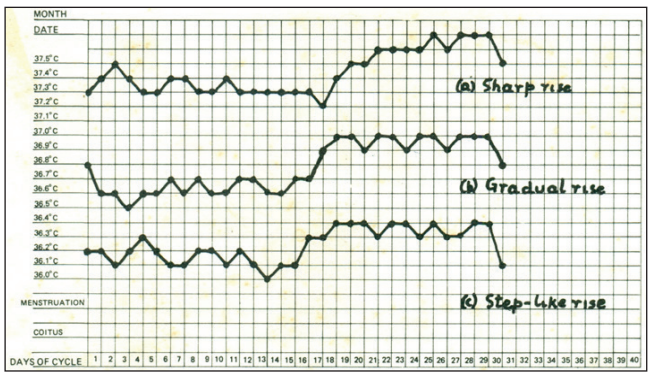


Fig-7: BBT chart indicating Ovulation with sharp-rise, gradual rise and step-ladder rise

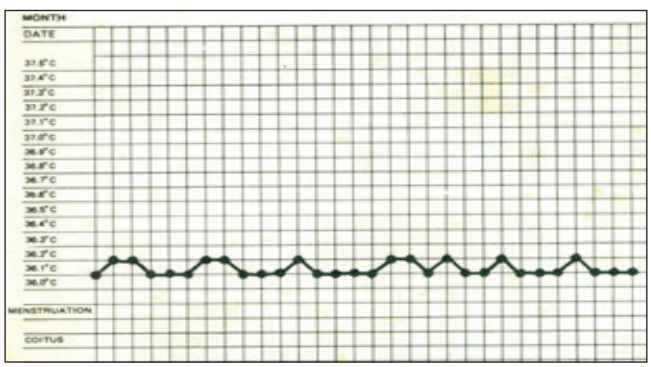


Fig-8: Anovulatory low, flat and monophasic BBT

part progesterone level is higher than oestrogen. Therefore the temperature chart will be designated as biphasic;- higher temperature in the later part (Luteal phase- progesterone), compared to the earlier part (follicular phase-oestrogen). Considering it is an instance of consistent phenomenon in an infertility work-up, we marked out different types of BBT in various types of disorders causing infertility (etiologic type).

BBT should be recorded through oral, rectal and axillary routes. Timing of recording is early morning, before any activity starts (thermometer at the bed side and measurement should be accurate as far as possible)

BBT was used as clinical marker of infertility in following cases. Typically ideal ovulatory BBT chart should be biphasic.

I) Typical ovulatory – biphasic

During the follicular phase, oestrogen is the prominent hormone while in the luteal phase progesterone is the controlling hormone. Therefore, in the earlier period of menstrual cycle the hormonal pattern will be non-thermogenic. During post ovulatory period, the temperature will be elevated above the basal level. The cut off has been considered as 97.80 F. This is known as biphasic pattern. This biphasic pattern has been considered as defining point of ovulatory type of BBT. The biphasic pastern will be of three sub types depending on the type and function of developing corpus luteum (a) sharp rise- (b) gradual rise (c) step-ladder like (Figure-7).

Abnormal varieties are as follows:-

(i) Typical anovulatory – flat- non biphasic

- Elevated flat – PCO
- Low flat- POF
- Discordant – Mixture of PCO and normal

(ii) Abnormal luteal – dysovulatory- Figure-12

- Short luteal – dysovulatory- Figure-11
- Discordant luteal

II) Anovulatory:-

Anovulatory BBT is of three types-anovulatory high monophasic, anovulatory low monophasic and discordant (See Figure 8, 9 & 10). Monophasic is of two types- elevated monophasic and low

monophasic. Elevated monophasic is due to persistent hyperandrogenism-PCO. Androgen is a thermogenic hormone. Low monophasic is due to hypoestrogenic (POF or hyperprolacinimia). Discordant- here the temperature is sometimes high and sometimes low due to an excess level of androgen, progesterone or oestrogen respectively.

Luteal phase BBT defect-short luteal and discordant luteal

The luteal phase defect is of two type- short luteal and discordant luteal (see figure-11 & 12).

Figure-11: Short luteal

Figure-12: Discordant luteal

Duration of normal luteal phase is of 7 days and level of mid-luteal progesterone is 15 ng/ml. A well-balanced follicular phase is prerequisite for an efficient luteal phase.

Short luteal and discordant luteal phases are inefficient for endometrial preparation and blastocyst implantation.

In summary, many endocrinological defects like PCO, POF could be detected on their pattern of BBT which helped as basic parameter for planning the treatment protocol.

Urinary LH assay (By color change on paper strip)

Paper strip LH estimation:- Urinary LH assessment from D10.

Presence of LH was indicated on change of color from white to orange depending on rising concentration of LH (indicating occurrence and gradual increase in density of orange color on the paper strip)

Focus on IVF

Beginning in 1950s, Around 1961, attention on management of infertility was gradually moving towards research on different aspects related to in-vitro fertilization and embryo transfer (IVF-ET). The move was initiated in 1959 when babies were delivered with injection of urinary gonadotropin In individuals in two countries of the world; - Gemzell In USA and Lunenfeld in Israil. Though successful, the procedure was laborious, expensive and not totally safe. Subsequently sporadic attempt of manufacturing

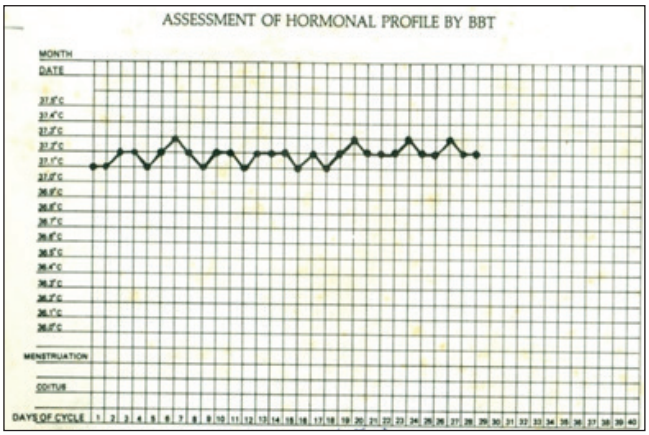


Fig-9: Anovulatory elevated, flat and monophasic BBT

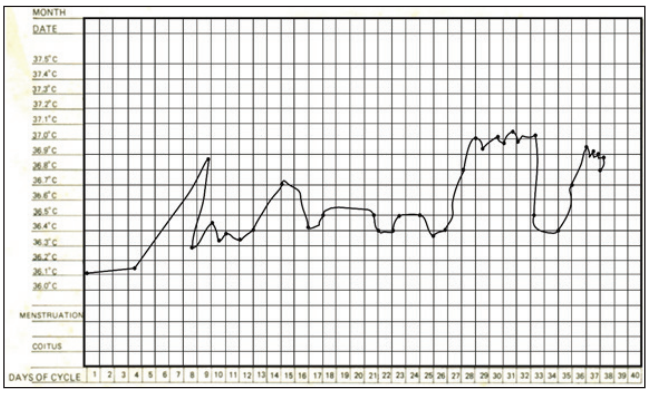


Fig-10: Discordant throughout non-ovulatory - non satisfactory for pregnancy

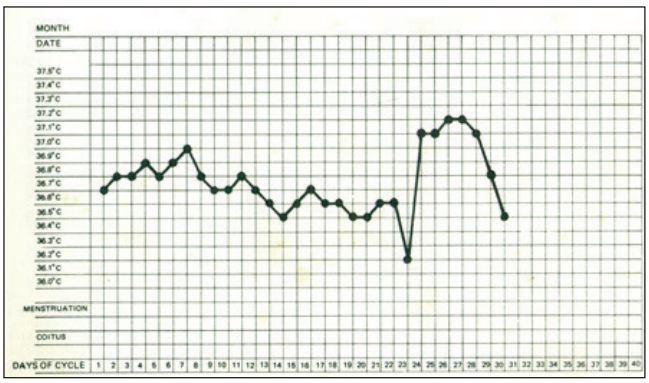


Fig-11: Short luteal

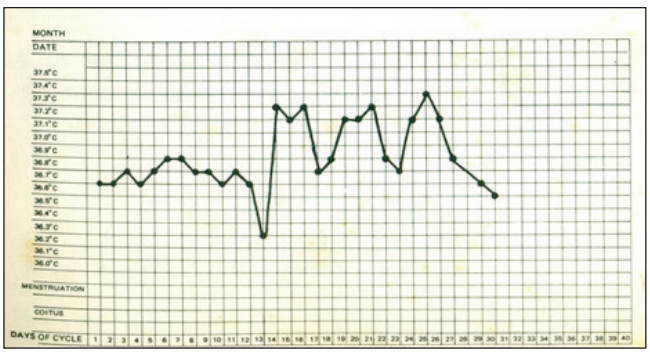


Fig-12: Discordant luteal

human pituitary gonadotropin (HPG) to replace human menopausal gonadotropin HMG came in vogue. This procedure also proved to be equally laborious and in practical too. Because 5 cadavers were required to prepare the requisite amount of human gonadotropin for induction of ovulation of one anovulatory cycle of hypogonadotripic hypogonoism to complete the treatment. In addition the method was not safe because of risk of infection and therefore the procedure was totally abandoned.

During the same period research on hormonal contraceptive was intensified because of population explosion and combined contraceptive tablets with combination of Oestrogen and progesterone was initiated. As mentioned earlier Clomephene Citrate (CC) was introduced presuming it to be and anti fertility drug. Infertility was certainly a hard topic and research started on both technology and hormones related to child birth. Simultaneously research went further and people started trying to fertilize the egg outside the body (extra corporeal fertilization or invitro fertilization). The test tube baby Louise Brown was born in Oldham in July 1978. Contemporary international pioneers were Dr Howard Jones of USA, Dr Carl Wood, Dr Trounson and Dr John Yobich of Australia and many others.

Research on IVF in Calcutta

Myself and Dr.Subhash Mukherjee started working on infertility since 1965. When both of us were posted at Nilratan Sarkar Medical College & Hospital. Subhash concentrated on basic aspect of endocrinology by oocyte maturation, oocyte growth invitro and extra corporal fertilization. I was working more on clinical aspect – like tuboplasty, wedge re-section, vaginoplasty, hysteroplasty etc. Finally we developed a common interest of starting IVF in India, a technology – which at that time was an ill understood subject globally. While the research by Calcutta duo (myself and Subhash) was developing, it was dealt with a blow when we were transferred; Mukherjee to Bankura and myself to Siliguri. Still we came to Calcutta in weekends to continue the work. In the midst of this confusion in continuing the work Subhash announced the birth of Durga alias Kanupriya, India's first and world's 2nd test tube baby on October 3, 1978. But few people believed that the research would have been possible in a power cut prone district without basic facility. Subhash however, could not accept the criticism and committed suicide in 1981. His death made me more adamant to carry the research forward. But I had also to face and share a lot of 'doubters'. As a surgeon by that time I had been successful in reconstructing cervix and vagina in a series of women born with cervico vagina atresia. Finally three of these women married, conceived, and successfully delivered viable babies (1990 to 2000).

Like Subhash I had also shared Ignonimity when I lectured about my success at Delhi, Vellore, Bombay people clapped politely but their lack of credence in research happening in Calcutta, was apparent. The same fate awaited my paper and publications abroad. The papers initially created excitement – which fizzled out learning that the researcher was from India. This made me more challenging and I continued my work with determination.

With a team of youngsters that involved Dr.Sudarshan Ghosh Dastidar, Dr.Siddhartha Chatterjee, Dr. Bani Kumar Mitra, Dr.Arup Majhi, Dr. Partha Goswami, Dr. Sanghamitra Ghosh, Dr.Ratna Chattopadhaya and young but senior associate late Dr.Subir Dutta. I started my IVF research in a small garage in my own CIT Road chamber at Moulali. Finally 'Imran' my first test tube baby was born on 3rd November 1986. Around the same period other Indian pioneers who developed IVF in India were Dr Indira Hinduja, Dr T C Anant Kumar, Dr Mehroo Hansotia, Dr Sadhna Desai, Dr Mohanlal Swarankar, Dr Kamala Selvaraj, Dr Sudarshan Ghosh Dastidar and many others.

Fig-13: My 1st Test Tube baby

Financial and legal constraints

This was a time of strict foreign exchange monitoring. Only £5 lb was allowed for foreign travel. Importing machines and disposables was next to impossible.

Little things like single use embryo transfer catheter made of plastic would have an import duty of £300 slapped on each. This type of turbulence continued till 1992. So it was not until 1989 that the second test tube baby was born in our unit. But when Dr.Monmohan Singh, the then Finance Minister liberalized foreign exchange policy, I could deliver 4 test tube babies in a month. Finally people stopped doubting my work.

Initiating I.RM. for further expansion

After my retirement, I put my retirement benefit for acquiring a plot of land in Salt Lake for research facility. A Deputy Secretary who was my patient helped me acquiring 2880 Sq.Ft. of land in DD Block, Salt Lake, Kolkata and to get permission for a five-storied building. Building was constructed over 4 years and was inaugurated in 1989. The flow of patients suddenly increased and to accommodate research, clinic and academic activities required some extra space.

2nd building in HB Block (new IRM)

The then Government headed by Sri Jyoti Basu, CM helped me in providing 7200 Sq.Ft. of plot in HB Block to construct our second building.

Our activities started attracting students from across the country

Two post doctoral students come every year for FNB course through National Selection in Delhi.

Additional academic activities

We are recognized for PhD course in reproductive medicine by Calcutta University and West Bengal University of Health Sciences. In addition we are also involved in collaborative research with SMST, IIT Kharagpur, IICB Jadavpur and Bose Institute, Kolkata.

PhD students from these Institutions also work in our laboratory.

Dr.Manjushree Chakravarty initially an active senior obstetrician and gynecologist and infertility specialist now 'silent help' (due to her present indisposition) in all our clinical, academic and research activities.

Finally

My current approach in infertility management is to identify 'markers' which could 'predict success' and also to find out measures which may 'prevent' failures.

In summary

I had an 'inspiring ambitious but a challenging journey'.

Acknowledgement

I sincerely thank the effort of Dr Shaktirupa Chakraborty for helping me in compiling the chapter.

Fig-14: IRM Old Building


Fig-15: IRM new Building

Ovum Collection - A Gateway to In-vitro Fertilization

Dr Ratna Chattopadhyay, Dr Sanghamitra Ghosh & Dr B N Chakravarty

Learning objective: Ovum pick-up (OPU) is a surgical intervention by which female gametes or oocytes are retrieved from the ovaries under USG guidance and collected outside the body for in vitro fertilization in Assisted Reproductive Technique practice.

The main objective of this chapter is to make the team members aware about the importance of understanding and coordination between themselves, the time of ovulation trigger and OPU.

Moreover, the team members in OT should learn the operation and function of different equipments used in OPU.

The laboratory staffs should know when they should communicate and warn the clinician to avoid unwanted situations during OPU.

To recover almost 100% oocyte without any complication is the ultimate learning objective of the present chapter.

Introduction

Ovum pick up can be defined as the "Gateway to the world of assisted reproductive technology (ART) procedure" by which we collect optimum number of good quality oocytes for in vitro fertilization (IVF). For practicing ART, gametes from both the partners are to be collected outside the body and then fertilized in the laboratory to generate embryos.

Male gametes or sperms are easy to collect at any time when they are present in the ejaculate. Otherwise, surgical retrieval of sperms is to be done in case of azoospermia to assist fertilization outside the body. But female gametes or oocytes are highly precious, and collection outside the body cannot be done at any time or any place. After proper preparation, when oocytes are mature enough, oocyte collection can be done under appropriate aseptic protection and arrangements in the OT so that the collected oocytes can be carried to the laboratory immediately after, for identification, gradation, and fertilization. This procedure is known as "OVUM PICK UP" (OPU) or oocyte retrieval.

History

- Recovery of oocytes was first started in 1940s by Miriam Menkin through laparotomy to assess the fertilization process of oocyte in laboratory.
- Aspiration of follicle with needle and tubing and oocyte collection following wedge resection of ovary were tried during 1940s till 1970s.
- The first test tube or IVF baby, Louise Brown, was born in 1978 from oocytes retrieved through laparoscopy by Dr. Steptoe.
- First IVF boy, Alastain MacDonald, was born on 14th January, 1979, through laparoscopic ovum pick up.
- Laparoscopic technique was gradually developed and adopted during 1970–1980s to evaluate preovulatory oocytes in stimulated and nonstimulated cycles. It is less invasive and more cost effective than laparotomy.
- In mid 1980s, ultrasound was introduced for oocyte retrieval.
- First transvesical approach was tried using abdominal ultrasound guidance. The technique was invasive and cumbersome, but it was the stepping stone to introduce ultrasound guidance for ovum pick up.
- Transvaginal oocyte retrieval by abdominal sonography was done in 1981 by Lenz and his colleagues.

- Then transvaginal oocyte recovery using transvaginal sonography was first introduced by Pierre Dellenbach et al. in 1984.
- Transvaginal sonography guided oocyte retrieval (OCR), which is the standard procedure till date, was started in 1985 by Wikland.

For successful and uneventful OPU an experienced team and proper equipments are needed. The team should consist of an ultrasonologist (operator), a nurse, and an anesthetist. It is recommended that at least one person in the room is trained in advanced life support.[1]

Ultrasonography (USG) System And Transducer

USG system for OPU should be fitted with a high-frequency transvaginal ultrasound transducer. The ultrasound system should have the following abilities:

- To adjust the field of view and zoom
- To adjust the focal zone to the region of interest
- To control image gain adjustment
- To adjust acoustic power, color and power Doppler capabilities
- To adjust the mechanical and thermal indices on the screen
- To display the needle guide super imposed on the field of view
- To print or save images

The transducer or vaginal probe should have a frequency range of 5–8 MHz and abdominal probe with a frequency range of 2–6 MHz. Good quality sonographic gel should be incorporated on the tip of transducer and cover with a specific, sterile latex-free cover before starting OPU. The needle guide is then attached to the probe. It may be a permanent one, which can be sterilized, or it may be a disposable one.

Needle

Usually single lumen, 17–18 gauge needle is used for OPU, sharper and narrower diameter of the tip is associated with less pain.[2,3] (Fig. 1).

Tubing should be translucent to see the content and color of the aspirate. Needle guide should ideally be disposable. Prior to OPU, patency and aspiration power of the needle should be checked.

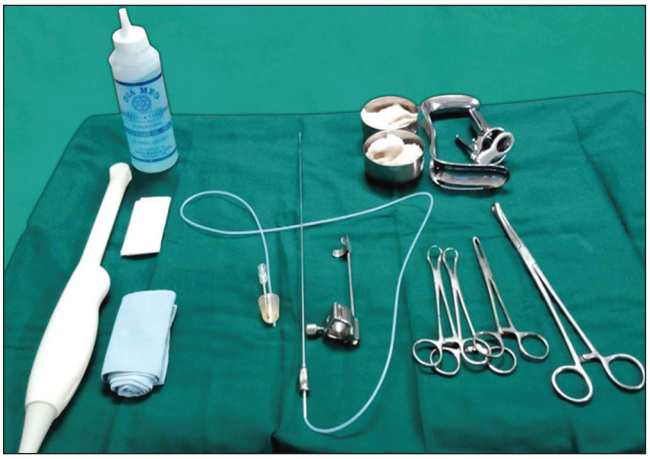


Fig. 1: Vaginal probe, needle, and gel used for OPU

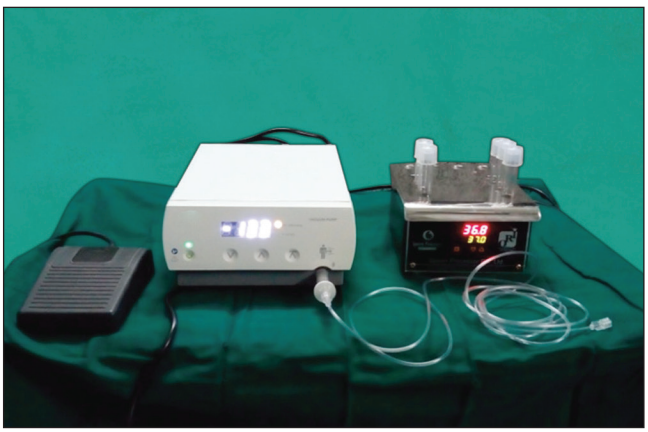


Fig. 2: Aspiration pump

Suction Pump

A stand-by suction pump should be kept in the OT to avoid unwanted situation in a busy ART clinic. Still no optimum suction pressure is yet decided, usually maintained between 100 and 130 mm Hg. It has been reported that higher pressure (140 mm Hg) was not associated with damage to the oocytes.[4]

Manual suction was used in the beginning; then came suction pump operated by foot pump (Rocket CraftTM Medicals, UK). Nowadays sophisticated suction pumps with adjustable suction pressure are used in the ART centers for OPU (Cook Aspiration Pump) (Fig. 2).

OPU Procedure

Steps before Scheduling the OPU Procedure

- Thorough pat ient 's hi s tor y about diabetes, hypertension, bleeding disorder, and cardiorespiratory disorder is needed.
- Clinical check up is mandatory if there is any suggestive history.

- Previous records of OPU whether there is any history of ovarian hyperstimulation syndrome (OHSS), failed oocyte retrieval, difficult accessibility to ovaries, and complications should be checked.
- Pelvic scan 4–6 months prior to OPU is to be done to see accessibility of ovaries and any associated pathology.
- Serological investigation for HIV, HBSAg, HCV, and VDRL of both partners must be checked.
- Signed consent form is mandatory.

Timing of OPU

After controlled ovarian stimulation, when at least one follicle is ≥17 mm diameter and two or more follicles are ≥14 mm diameter, ovulation trigger is to be done by 10,000 units of human chorionic gonadotropin (hCG) or 0.2 mg gonadotropin-releasing hormone (GnRH) agonist. Dual stimulation may be applied. OPU should be done within 34–38 hours of ovulation trigger.[5] Most authors recommend a 36-hour gap between ovulation trigger and ovum pick up. Timing of OPU plays a key role in the retrieval of competent oocytes.

Before starting the procedure, patient should be asked about the timing of hCG or GnRH agonist injection. If there is any confusion, serum level of βhCG should be assessed, less than 23 mIU/mL hCG suggests inadequate hCG administration,[6] but routine hCG testing is not recommended.

If ovulation trigger is done with GnRH agonist, serum LH level should be measured on that day. LH estimation should be repeated on the day of OPU; if it is below 5 mIU/ mL, trigger should be repeated with recombinant hCG.[7] If there is any apprehension of follicle rupture, TVS should be performed before starting of follicle puncture.

Few prerequisitions:

- Patient position: lithotomy or semi-lithotomy
- Emptying the bladder for better resolution and to avoid injury
- IV line should be opened and Ringer's lactate or normal saline should be started
- Speculum examination is must to exclude any discharge or pathological condition
- Cleaning or sterilizing the vagina is necessary before OPU, to prevent bacterial or fungal contamination, by warm normal saline[8,9]
- Povidone or culture media are used by some clinicians
- These agents may affect cell membrane and may not be safe for oocyte health[10,11]

Sedation

Precautions should be taken regarding the time of recovery and safety of the agents regarding oocyte health, etc. Sometimes conscious sedation is preferable for the patients as the time of recovery is shorter[12] in comparison with GA. Thiopental Sodium or Propofol are used for general anesthesia. Conscious sedation for OPU is a suitable option for most of the patients,[13] especially for obese patients or in patients where deep sedation induces hiccups, though it is rare.

Deep sedation is priority for patients suffering from:

- Extreme anxiety
- Severe endometriosis and pelvic adhesion
- In case of ovaries with many follicles and difficult accessibility

Risk benefit ratio should be considered before selecting the type of sedation, but we should consider patient's choice. Overall evidence does not support one particular method or technique over another.[13]

Before starting OPU we should check:

- Patency of the needle
- Suction pressure
- Proper connection of the entire system
- Proper connection of needle guide and USG probe

During Procedure

OPU should be done in a well-equipped OT under the supervision of an experienced team consisting of an anesthetist, well-trained nurse, operator, and another doctor to manage any emergencies. OT should be semidark and should have 22–23°C room temperature. Representation of the transducer should be from the lower part of the monitor, which helps to control the transducer's manipulation in a better way. Better visualization of the anatomy is of crucial importance to avoid injury of the vessels and intestine. Before starting the OPU, the pelvis should be scanned to assess the anatomy. A panoramic view

of the ovary is needed first and then a closer look is required. The ovary should occupy 75% of the field to ensure visualization of the intrapelvic part of the needle during OPU. Doppler study is beneficial, when there is any doubt about the vascular structures, to avoid injury.[14] Perifollicular blood flow can also be assessed by Doppler study, which is an important indicator of oocyte competence.[15]

Aspiration Technique

The probe is gently introduced per vagina after draping with sterile plastic cover and attaching with a needle guide. It is placed in such a way that there should be no space between the transducer and ovarian cortex. It will help to avoid the injury to intestinal loop. The needle should be pushed gently through the guide to puncture the vaginal wall just below the ovary, and then puncture of the nearest follicle is done in one movement (Fig. 3).

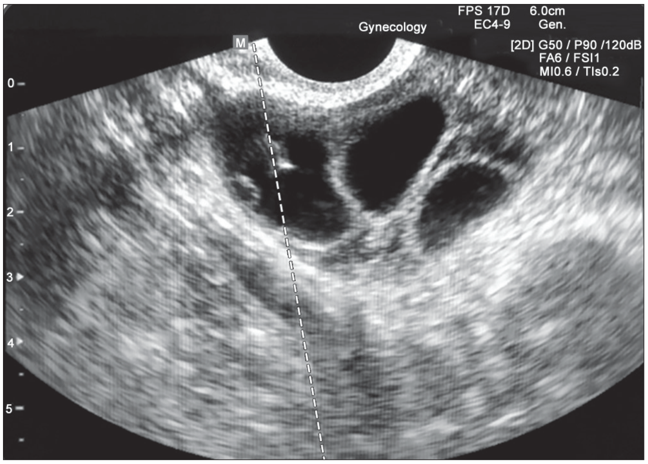


Fig. 3: Needle tip is visualized within the follicle

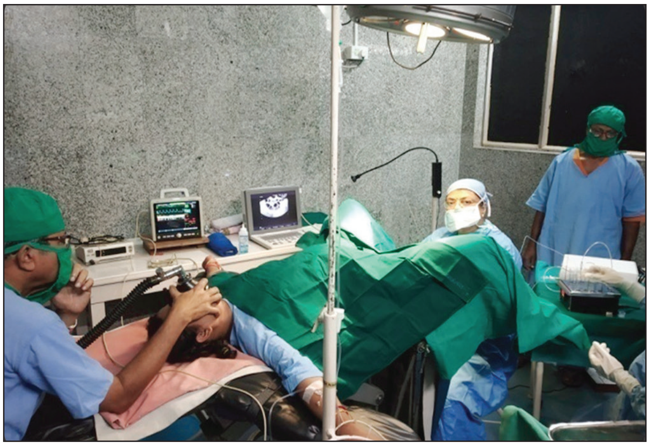
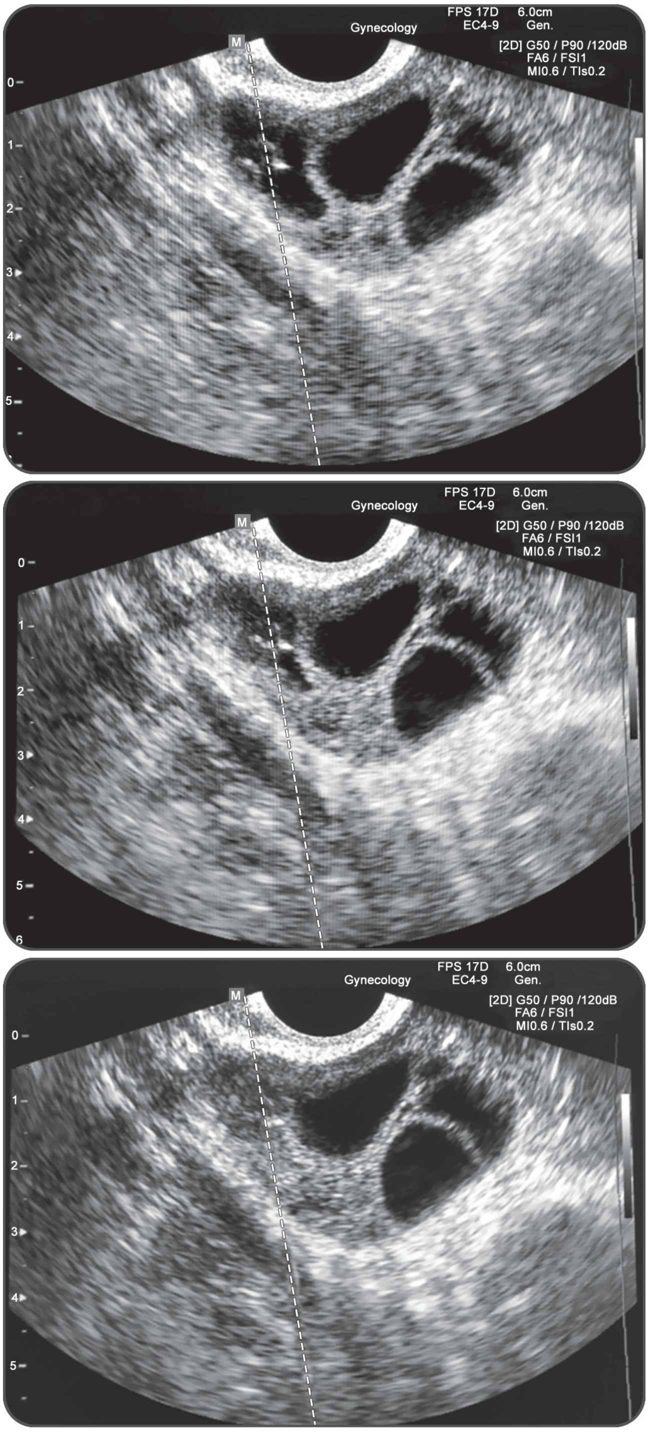


Fig. 4: Aspirate collection via Teflon tubing in round bottom tube



Figs. 5A to C: Gradual collapse is observed

The objective of OPU is to retrieve maximum numbers of oocyte with minimum puncture of the vaginal wall.

Though more studies are needed for the safety and utility of follicle curetting, but it may increase the number of retrieved oocytes as well as the number of mature oocytes. It also eliminates the chance of blockage of the needle lumen by granulosa cells. Follicle curetting is the clockwise and then anti-

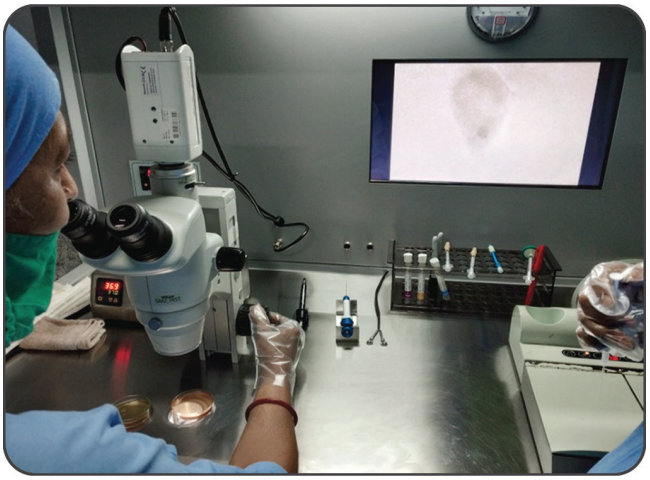


Fig. 6: OCCC identified in the follicular aspirate

clockwise rapid rotation of the needle within the follicle after complete aspiration of the follicular fluid.[16]

Vacuum suction should be used before puncturing of the follicles. Suction pressure usually should be calibrated between 120 and 140 mm Hg and kept constant during the procedure. Operator controls the suction pressure through the foot pump.

Aspirate should be collected through Teflon tubing in sterile round bottom tube kept in test tube rack at 37°C (Fig. 4). Gradual collapse of the follicle is observed (Figs. 5A to C). When the follicle is fully drained and collapsed, the needle is oriented to the next follicle, or the needle should be withdrawn slightly without negative suction pressure, to the cortex to puncture other follicles.

The aspirate is immediately sent to the IVF lab at 37°C via pass-through window and scanned under stereodissecting microscope to identify oocyte-corona cumulus complex (OCCC) (Fig. 6).

It is preferable to remain within the ovary, rather than repeated ovarian penetration to avoid ovarian surface bleeding. It is safe to start OPU with the ovary nearest to the probe rather than the ovary with largest follicle. Follicle less than 10 mm can only be punctured when there is a risk of OHSS, otherwise there is usually no benefit of oocytes retrieved from follicle less than 14 mm. The whole procedure should be as gentle, fast and steady as possible.

Usually aspiration is done without rinsing the follicle with media, to avoid infection and unnecessary consumption of media. But in poor responders, when follicle number is less than 5, open flushing is recommended. A good communication is needed between OT and Lab staff, about the presence of oocyte or cells in the aspirate. When there is any suspicion about premature rupture and fluid is seen in the Pouch of Doglus (POD), oocytes can be retrieved from fluid aspirated from POD.

At the end, proper checking should be done whether all the follicles are punctured and if there is any internal bleeding. Speculum examination followed by vaginal compression or packing with roller gauge is needed in case of vaginal bleeding. Sometimes hemostatic suture is required. Analgesic is to be administered if the number of follicles is more than or equal to 10 and in case of patient having endometriosis.

OPU in In Vitro Maturation Cycles

It is known as immature oocyte collection (IOC). When the follicles are within 10–14 mm in size, oocyte collection is done. The suction pressure should be high and puncture needle should be rigid, short and beveled. Bleeding is usually more due to high-suction pressure and more puncture, needed to maximize the number of retrieved oocytes and the procedure is associated with more pain and chance of infection.

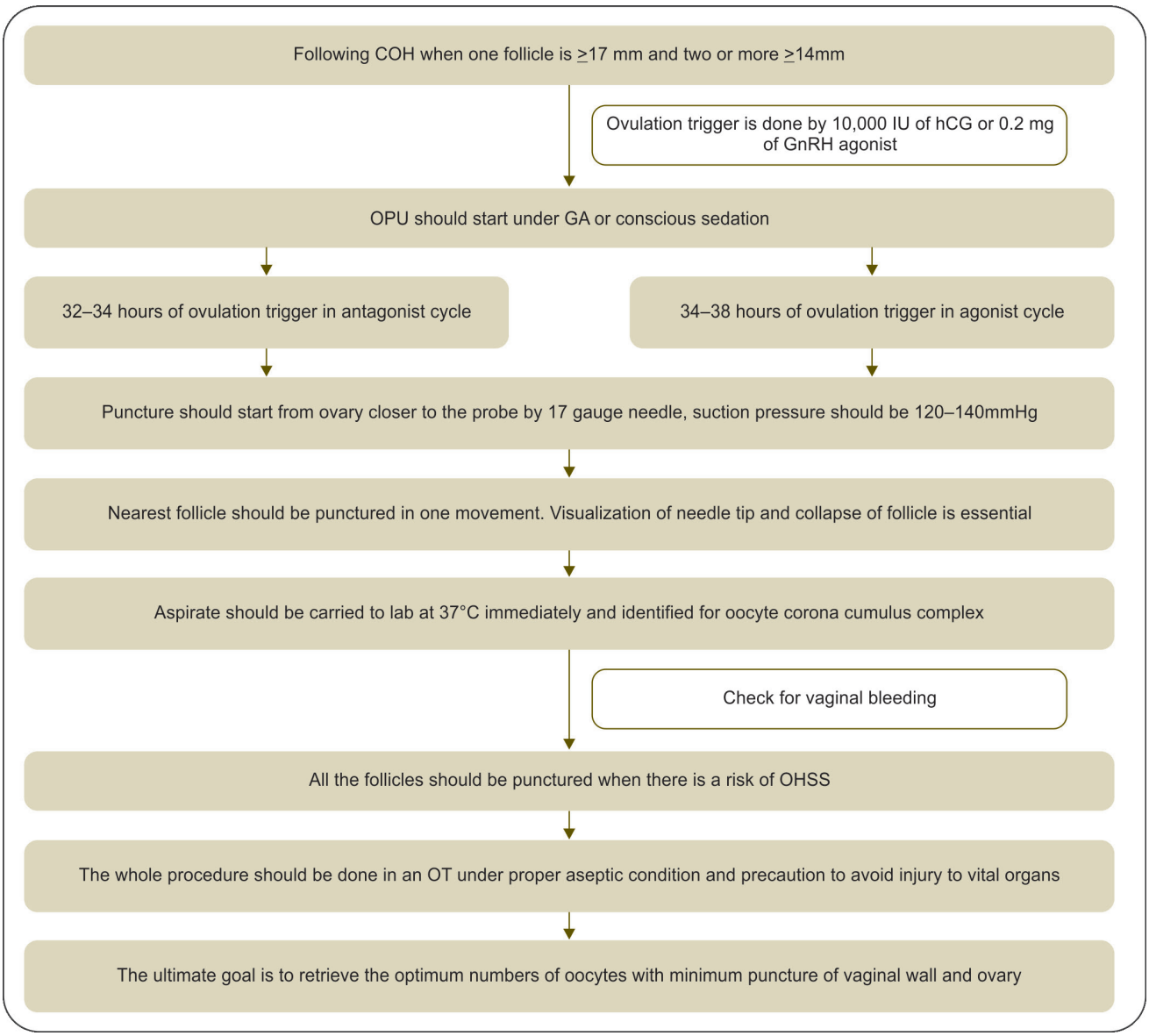
Post Procedure Steps (Flowchart 1)

- Patient should be in the bed for at least 2 hours under the supervision of nurse who will check abdominal distension, blood pressure, pulse rate, etc.
- Bleeding per vagina or urine retention may be a common feature and should be managed accordingly.
- Antiemetic or Analgesic should be administered in case of vomiting and pain.
- Proper advice should be given about estrogen/progesterone therapy or day of embryo transfer or cryopreservation.
- Advice for further USG in case of risk of OHSS.

Some Precautions

- When there is hydrosalpinx, it should be removed or clipped before OPU[17] and if identified on the day of OPU, embryo cryopreservation is the best option.

Steps of OPU



COH, controlled ovarian hyperstimulation; GnRH, gonadotropin-releasing hormone; hCG, human chorionic gonadotrophin; OHSS, ovarian hyperstimulation syndrome; OPU, ovum pick up

- In case of HIV or hepatitis infection, operator, and embryologist should wear double gloves and culture the oocytes and embryos in separate incubator.
- In endometriosis, puncture of the cysts should be avoided; if aspirated it may compromise oocyte quality or may increase the chance of contamination.
- Dermoid cysts should not be aspirated for the same reasons.
- It is not clear whether ovarian stimulation followed by OPU increases the chance of recurrence in borderline ovarian tumor or not.
- In case of purulent aspirate, though not very common, the aspiration should be stopped, needle should be changed and opposite ovary can be aspirated followed by embryo cryopreservation. The patient should be under antibiotic coverage.
- Increased bleeding may happen in lean women and women with polycystic ovary syndrome (PCOS).[18,19]

Trouble Shooting

- When the needle tip is not visualized Withdraw the needle and ensure the guide is in place allowing the needle to move in the same sector as the ultrasound beam

- When the suction fails
  - —— Follicle curetting should be done to remove any blockage by the granulosa cells
  - —— If still the suction fails, the needle should be withdrawn outside, followed by removal of any blood clot by "retrograde flush"
  - —— Patency of needle is checked by aspirating culture media
  - —— Double lumen needle can be flushed without taking it outside
- When first few aspirates are devoid of oocytes and any cells

  Further aspiration should be stopped

  - —— We have to be sure whether ovulation trigger was proper or not
    - Timing of trigger was wrong
    - Administration of improper or inadequate medicine for trigger
  - —— Serum hCG should be tested immediately or blood LH can be checked in case of agonist trigger
  - —— If the tests reveal that the patient did not receive the trigger, ovulation trigger should be done in a proper way and OPU should start after 36 hours
  - —— When premature rupture of the follicles happen with presence of fluid in POD, oocytes may be rescued from the POD fluid

Complication

Any invasive procedure may have complications though these are not common in OPU. The most common minor complication is bleeding per vagina, may be from the puncture point but it can be easily checked by compression or vaginal packing.

Next common complication is infection especially following OPU in a patient with endometrioma, any puncture of endometriotic cysts may cause contamination. OPU in a patient with a history of PID may contribute infection. Puncture of hydrosalpinx or accidental puncture of bowel loop may lead to septicemia.

OHSS, though a known common complication following OPU, is the effect of controlled ovarian hyperstimulation.

Psychiatric episode including vague palpitation, tachycardia, and aphasia can be experienced following OPU.

Injury to urinary tract and internal iliac vessel, pseudoaneurysm, and vertebral osteomyelitis are very rare complications.

Conclusion

OPU is a simple but sophisticated technique for collection of female gametes in in-vitro fertilization.

Timed ovum collection in sterile way by a skilled ART specialist with the help of a high resolution USG machine recover a good number of mature oocyte which is the base of successful ART cycle.

Individualisation of stimulation protocol timed and proper ovulation trigger, required time gap between trigger and OPU and ultimately a well-coordinated, experienced team are essential for optimum recovery without complications.

Key notes

Ovum pickup is done usually 34½ hrs to 36 hrs after the ovulation trigger when atleast 2-3 follicles attain the optimum size following controlled ovarian stimulation.

Anesthetist should be careful enough about the medical history of the patient scheduled for OPU and about the selection of anesthetic drugs for OPU.

Gynaecologist or sonologist performing the OPU should be skilled enough about the procedure to make it smooth and successful.

Proper timing of ovulation trigger and ovum pickup are the key factors to recover good quality or mature oocytes.

The clinician doing the OPU should be careful about empty follicle syndrome and endometriotic cyst to avoid unwanted situation.

Smart and aspirate of all the accessible follicles with precaution may help to achieve almost 100% recovery without complication.

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Pregnancy and Live Birth Rates are Comparable in Young Infertile Women Presenting with Severe Endometriosis and Tubal Infertility

Dr Sunita Sharma, Dr Sourav RoyChoudhury, Dr M. Padmaja Bhattacharya, Dr Ratna Chattopadhyay, Dr Baidyanath Chakravarty

Abstract: This prospective observational study included 294 women with severe endometriosis and 358 women with tubal factor as control who underwent IVF. Follicular fluid samples were collected during oocyte retrieval and cytokines and angiogenic factors estimated. The groups were sub-stratified based on age. Number of metaphase II oocytes, grade I/II embryos, pregnancy rate, miscarriage rate per pregnancy and live birth rate were compared. Significantly elevated levels of cytokines and angiogenic molecules were observed in younger endometriosis patients when compared to tubal group (p<0.001). Number of MII oocytes (p<0.003) and grade I/II embryos (p<0.001) were observed to be significantly lower in these women when compared with matched controls. Despite of higher levels of inflammatory cytokines, angiogenic molecules, fewer MII oocytes, and grade I/II embryos, the younger endometriosis patients had similar pregnancy [OR: 0.81 (95% CI: 0.54-1.22) p=0.31] and live birth rate [OR: 0.78 (95% CI: 0.5-1.2) p=0.26] when compared with matched controls. In contrast, endometriosis patients of age ≥35 years had significantly less likelihood of live birth [OR: 0.47 (95% CI: 0.25-0.9) p=0.02] and pregnancy rate [OR: 0.46 (95% CI: 0.22-0.95) p=0.03] respectively when compared with the matched controls. It appears that Women with severe endometriosis have even chance of successful pregnancy if diagnosed at early age and sought for assisted reproductive technology to reduce its adverse effect on reproductive outcome.

Adapted from the article published in Reproductive Sciences entitled: "Pregnancy and Live Birth Rates Are Comparable in Young Infertile Women Presenting with Severe Endometriosis and Tubal Infertility" (Reprod Sci. 2020 Jun;27(6):1340-1349).

Introduction:

Endometriosis, a debilitating disease of reproductive age, is characterized by the presence of uterine epithelial and stromal tissue outside the uterine cavity. Its exact prevalence is unknown, but up to 10% of the general female population and as many as 50% of sub-fertile women could be affected by endometriosis.[1,2] Prediction of infertility in women with endometriosis is challenging,[3] although there is an indisputable relationship between the two.[4] The Cause–effect relationship between endometriosis and infertility is complex and yet to be fully understood. Endometriosis can alter tubo-ovarian anatomical relationship due to adhesions.[5] As endometriosis is an inflammatory disease, local and systemic inflammation by activation of macrophages,[6] T cells,[7] and natural killer cells[8] with increased cytokines such as interleukins 1, 2, 6, and 8 (IL-1, 2, 6 and 8), vascular endothelial growth factor (VEGF) and tumor necrosis factor α (TNFα) in pelvic cavity[9,10] as well as oxidative damage[11,12] can adversely affect oocyte quality.[13] As granulosa cells constitute the primary component of ovarian follicles, secretion of several interleukins and other mediators of inflammation by them in endometriosis may contribute to poor quality of follicles, oocytes, and embryos thus finally adversely affecting the fertilization and implantation in women with endometriosis.[14–16]

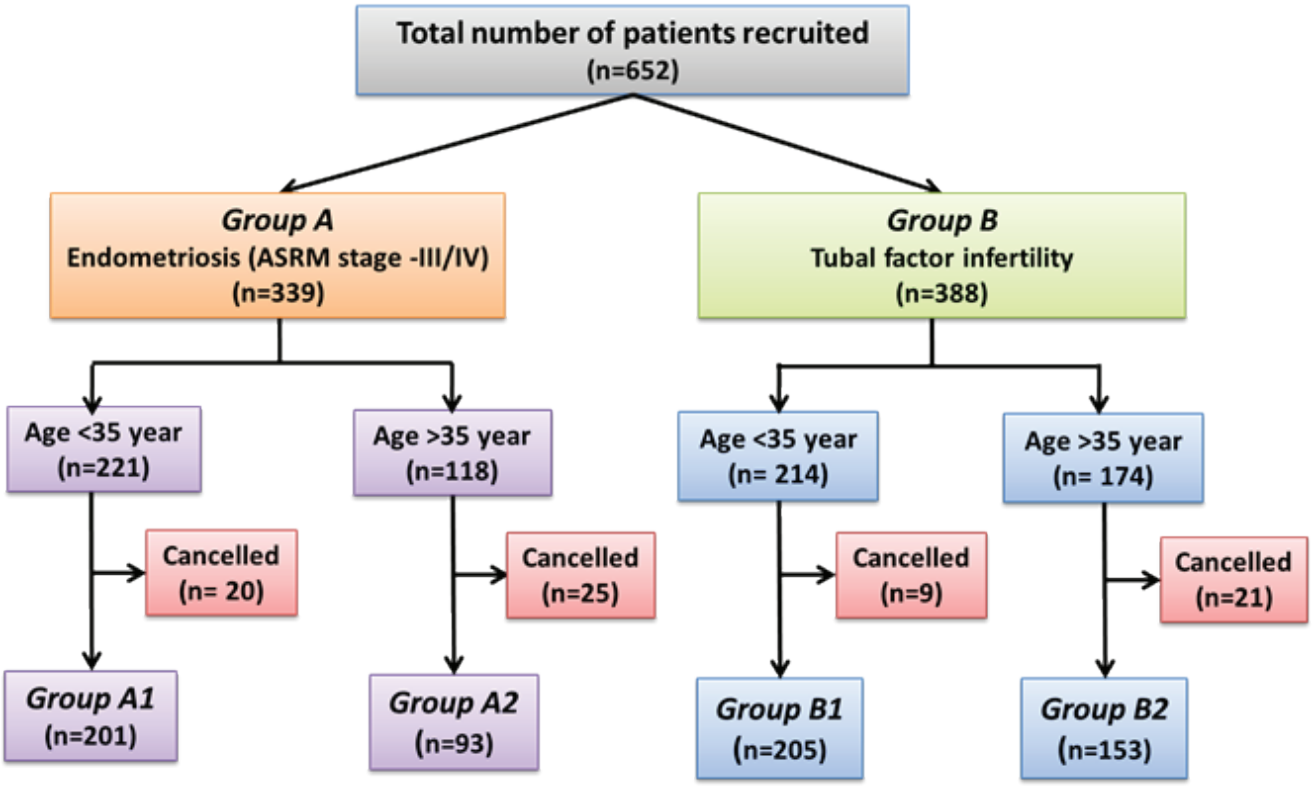

Figure 1: A flowchart depicting patient recruitment scheme in different groups

The primordial follicular reserve has been found to be significantly lower in ovaries with endometrioma,[17] which may further lead to reduced oocyte yield observed in endometriosis patients during *in vitro* fertilization (IVF). Assisted reproductive technology (ART) outcome is reported to be poor in infertile women with endometriosis[18–20] due to both diminished number and poor quality of oocytes retrieved. One of the recent studies suggests that women with endometriosis had lesser chance of a live birth when compared with unexplained subfertility, and increasing severity of the disease further influence the live birth in endometriosis women.[20] In contrast, some reports suggest that ART outcomes are not that unsatisfactory in endometriosis.[21,22] It was observed that, when sufficient numbers of good quality embryos are available, pregnancy rates seem to be comparable to the controls.[23] However, there is no clear evidence that endometriosis, the disease itself, causes poor IVF outcome. Moreover, several co-factors such as age, ovarian reserve, oocyte and embryo quality, etc. influenced by the disease, may be responsible for the poor pregnancy outcome in these women. It will be of interest to evaluate if younger women with endometriosis have better chance of IVF success if treated in time. This, in turn, may help to counsel the patients to seek the earliest opportunity for IVF treatment and or early pregnancy at a younger age to reduce the adverse effect of endometriosis on reproductive outcome. This current prospective observational study aims to evaluate whether severe endometriosis at a younger age with a lower ovarian reserve and abnormal cytokines affect IVF outcome.

Materials and methods:

This prospective observational cohort study included 652 infertile women who underwent IVF at a tertiary infertility center, Institute of reproductive medicine, Kolkata. Necessary approval was obtained from the Institute Ethics Committee (Approval no: IRM/IEC/BNC-IHP-49). Written informed consent was obtained from all the participants prior to their recruitment in this study. Infertile women undergoing the first cycle of ART were recruited for this study during the period of January 2012 to August 2018. Initially, a total of 727 patients were recruited. They were divided into two groups, women with severe endometriosis, Group A (n=294), and women with tubal factor as the sole cause of infertility as controls, Group B (n=358), following exclusion of canceled cycles in each group (Fig.

1). The power analysis yield was satisfactory for the study with the sample sizes in each group. Endometriosis was diagnosed laparoscopically and staged using the American Society for Reproductive Medicine guidelines.[24] We have included women who underwent surgery for endometriosis (82%), including cystectomy/ drainage/ electrocoagulation /sclerotherapy for deep-infiltrating endometriosis (DIE), whereas rest of the endometriosis patients had only diagnostic laparoscopy (18%). The patients who had only diagnostic laparoscopy done were already diagnosed elsewhere and further surgical treatment was not performed as they were asymptomatic. In patients with ovarian endometriosis, the stripping technique was used to excise endometriomas. The endometrioma was drained with aspiration, and the cyst wall was dissected by gentle traction and counter-traction using two 5-mm grasping forceps and then the diagnosis was confirmed using histology. The control group consisting of 358 women, had normal ovulation and tubal factor infertility but without any sign of endometriosis. Women with tubal factor infertility, included in this study, had either salpingectomy for ectopic tubal pregnancy or tubal blockage in the absence of genital tuberculosis. Tubal patency was evaluated by laparoscopy or hysterosalpingogram. Women with age < 18 years or >40 years, AMH <1.1 ng/ml, adenomyosis, fibroids, gross hydrosalpinx or with male factor infertility were excluded from the study. For further analysis, patients in the two groups were subdivided based on their age, endometriosis as Group A1 (<35 years), Group A2 (≥35 years), and controls as Group B1 (<35 years), Group B2 (≥35 years).

All patients underwent long protocol down-regulation with a gonadotropin-releasing hormone (GnRH) agonist leuprolide acetate (Lupride 4, Sun Pharmaceuticals, Mumbai, India) started in the mid-luteal phase. Following confirmation of adequate down-regulation, controlled ovarian stimulation was initiated with recombinant follicle-stimulating hormone (rFSH) (150- 300IU) (Gonal F, Serono,Geneva, Switzerland). Serial folliculometry was started from day 6 of gonadotropin stimulation and dose titrated accordingly. Human chorionic gonadotropin (hCG) (Pregnyl, Organon, Netherlands) was administered for final follicular maturation when the average diameter of at least one leading follicle reached 18 mm. The oocytes retrieval was done under the guidance of transvaginal ultrasound, 36-hour post hCG administration. Oocyte corona cumulus complex was retrieved, cultured, and inseminated with semen prepared by double swim-up technique. Oocyte count and maturity, fertilization rate, and embryo quality were assessed. In each patient from both the groups, 3 grade I embryos were transferred on day 2 or 3. Luteal support started on the day of embryo transfer with intravaginal progesterone gel (90 mg; Crinone; Serono) daily. The serum β-hCG level was done 14 days of embryo transfer and following positive result, luteal support was continued up to 12 weeks of pregnancy.

Collection and preparation of follicular fluid and serum

Follicular fluid samples were collected from both the groups during oocyte retrieval and analysis. Levels of cytokines and angiogenic factors were assessed in the follicular fluid of patients undergoing embryo transfers. The follicular fluid samples were centrifuged at 800×g for 10 min and the supernatant fluid was then divided into small aliquots and frozen at –80 °C until further use. Before analysis, precipitate was removed from follicular fluid by centrifugation and filtering followed by measurement of protein content. Concentrations of pro-inflammatory cytokines (IL-2, IL-8, IL-12, IL-1β, TNF-α, IFN-γ), anti-inflammatory cytokines (IL-4, IL-6, IL-10) were estimated using commercially available kits (BD Biosciences, USA). The follicular fluid was also analyzed for angiogenic molecules such as VEGF and angiogenin (Quantikine, R&D Systems Inc., USA). Adrenomedullin (ADM) was measured (DRG International Inc., USA). All analyzes were performed according to the manufacturer's protocol. Appropriate dilutions were made to obtain readings in the linear range of the assays. All estimations and calibrations were performed in triplicate. The sensitivity for IL-8, IL-12, IL-1β, TNF-α, IL-2, IFN-γ, IL-4, IL-6, IL-10, VEGF, angiogenin and ADM detection by the assay used was 0.8, 2, 0.8, 2, 1, 1, 2, 2.2, 2, 5, 6 and 6 pg/ml, respectively.

The live birth rate was taken as the primary outcome measure. Clinical pregnancy rate and miscarriage rate per pregnancy were considered as secondary outcome measures. Cycle characteristics like dose of gonadotropin, peak estradiol at hCG

trigger, number of metaphase II (MII) oocytes, fertilization rate, number of grade I/II embryos were also compared. Clinical pregnancy was defined as presence of cardiac activity in a fetus on an ultrasound scan performed at 6-7 weeks of gestation. Live birth was defined as a cycle with a live fetus delivered after 26 completed weeks of gestation since deliveries before 26 completed weeks are mostly non-viable in our scenario. Following conception, women attended the institute for routine follow up for antenatal checkups. We also noted obstetrical complications like antepartum hemorrhage (APH), postpartum hemorrhage (PPH), pre-eclampsia (PET), intrauterine growth restriction (IUGR), severe preterm delivery, intrauterine demise (IUD) and and diabetes mellitus.

Statistical analysis

SPSS 17.0 (IBM software, USA) was used for statistical analysis. Normality of data distribution was assessed using the Shapiro–Wilk test. Comparison between groups was carried out using Student's t-test and Kruskal–Wallis test, as applicable. Unadjusted odds ratios were calculated using MedCalc 14 (MedCalc Software, Belgium). Similarly, adjusted odds ratios were calculated separately by performing multivariable logistic regression analyses by adjusting for age, ovarian reserve (AMH and AFC), the number of mature oocyte and embryos. Statistical significance was defined as $p<0.05$.

Results:

Baseline and cycle characteristics are shown in Table 1. Characteristics of endometriosis in all patients and the surgery done for endometriosis were also mentioned in Table 2. Severe endometriosis patients of Group A were further segregated into Group A1: age <35, Group A2: age ≥35, similarly for patients with tubal blockage (controls), Group B1: age <35, Group B2: age ≥35. AMH levels were observed to be significantly lower in both the Group A1 and A2 endometriosis patients as compared control

Table 1: Baseline characteristics

	Endometriosis		Controls		p value
	Group A1 (<35 years) (n=201)	Group A2 (>35 years) (n=92)	Group B1 (<35 years) (n=205)	Group B2 (>35 years) (n=153)	
Age	30.65 ±2.98	37.25 ±2.03	30.64 ±2.5	36.96 ±1.8	NS
Duration of marriage	7.56 ±3.13	7.9 ±3.09	7.6 ±2.35	8.01 ±2.1	NS
BMI	23.6 ±3.05	23.56 ±2.72	24.39 ±3.69	24.09 ±3.42	NS
AFC	8.61 ±2.37	6.96 ±2.17	15.6 ±6.54	9.2 ±2.27	A1 vs. B1 <0.01 A2 vs. B2 <0.05
AMH	2.49 ±0.79	1.88 ±0.49	2.77 ±0.64	2.04 ±0.71	A1 vs. B1 <0.01 A2 vs. B2 <0.05
Dose of gonadotropin required	2390.78 ±1148.29	3076.40 ±728.14	2056.71 ±1100.99	2359 ±1100.48	A1 vs. B1 <0.01 A2 vs. B2 <0.01
E2 on day of hCG	1620.68 ±1102.49	1103.87 ±533.38	1836.05 ±882.28	1288.06 ±590.4	A1 vs. B1 <0.05 A2 vs. B2 <0.05

Table 2: Characteristics of endometriosis

Characteristics of endometriosis	Endometriosis (Group A1; < 35 years) (n=201)	Endometriosis (Group A2; > 35 years) (n=93)
Unilateral endometrioma	62 (30.84%)	26 (27.96%)
Bilateral endometrioma	40 (19.90%)	29 (31.18%)
DIE	49 (24.38%)	21 (22.58%)
Others	50	17
Size of endometrioma*		
<2 cm	(25%)	(21.25%)
2-3 cm	(45.13%)	(52.5%)
3-4 cm	(29.86%)	(26.25%)

*Others- Pouch of Douglas obliteration, adhesions

groups, Group B1 and B2, respectively. Required doses for gonadotropin were also observed to be significantly higher in both the endometriosis groups of as compared to respective control groups. The reduced pregnancy rate was also observed in women with endometriosis when compared with controls (p<0.06).

Pregnancy complications like severe preterm delivery, IUGR, APH, PPH, PET, IUD and gestational diabetes mellitus were observed to be comparable between severe endometriosis women and patients with tubal infertility (Table 3).

Severe endometriosis patients show a lower trend of live birth rate while compared with tubal infertility though not statistically significant [OR: 0.71 (95% CI, 0.49–1.02) p=0.06]. Lower pregnancy (statistically not significant) and comparable miscarriage rates were also observed between the two groups [OR: 0.72 (95% CI, 0.52–1.01) p=0.06; OR: 1.2 (95% CI, 0.58–2.47) p=0.61, respectively] (Table 4, 5 and 6). The reproductive outcome is also stratified according to the phenotype of endometriosis and is presented in Table 5. Significantly elevated levels of cytokines (IL-1β, TNF-α, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12, IFN-γ) and angiogenic molecules (VEGF, ADM, angiogenin) were observed in these endometriosis patients when compared with tubal group (p< 0.001) (Table 7).

Live birth [OR: 0.78 (95% CI: 0.5-1.2) p=0.26], pregnancy rate [OR: 0.81 (95% CI: 0.54-1.22) p=0.31] and miscarriage [OR: 1.12 (95% CI: 0.5-2.92) p=0.67] were comparable between Group A1 and B1 (Table 4 and 6). In contrast, live birth [OR: 0.47 (95% CI:

Table 3: Obstetric complications

	Endometriosis (Group A) (n=64)	Tubal (Group B) (n=101)
PPH	8/64	9/101
APH	9/64	11/101
PET	9/64	8/101
IUGR	10/64	12/101
Severe preterm	2/64	3/101
IUD	5/64	7/101
Diabetes	4/64	6/101

Postpartum haemorrhage (PPH), Antepartum haemorrhage (APH), Pre-eclampsia (PET), Intrauterine growth restriction (IUGR), Intrauterine demise (IUD). All these data are per ongoing pregnancy.

Table 4: Reproductive outcome parameters

	Groups	<35 years	>35 years	p value
M II Oocytes	Endometriosis	6.63 ±3.21	4.64 ±1.80	<0.001
	Tubal block	7.67 ±2.50	5.69 ±2.57	<0.01
	p value	<0.001	<0.001	
Grade I/II embryo	Endometriosis	3.38 ±1.38	2.61 ±1.12	<0.001
	Tubal block	4.2 ±1.45	3.55 ±1.31	<0.01
	p value	<0.001	<0.001	
Fertilization rate	Endometriosis	73%	63%	<0.001
	Tubal block	76%	72%	<0.01
	p value	<0.01	<0.001	
Implantation rate	Endometriosis	19.59%	10.11%	<0.01
	Tubal block	22.93%	18.24%	NS
	p value	NS	<0.01	
Pregnancy rate	Endometriosis	64/201 (31.84%)	16/92 (17.20%)	<0.01
	Tubal block	75/205 (36.58%)	47/153 (30.7%)	NS
	p value	NS	<0.02	
Miscarriage rate per pregnancy	Endometriosis	12/64 (18.75%)	4/16 (25%)	NS
	Tubal block	12/75 (16%)	9/47 (19.15%)	NS
	p value	NS	NS	
Live birth rate	Endometriosis	48/201 (23.88%)	11/92 (11.95%)	<0.05
	Tubal block	59/205 (28.78%)	35/153 (22.87%)	NS
	p value	NS	<0.05	

Table 5: Comparison of various cytokine levels and angiogenic factors in follicular fluid

Parameters	Endometriosis (<35 years) Group A1	Endometriosis (>35 years) Group A2	Tubal (<35 years) Group B1	Tubal (>35 years) Group B2
IL-β 1 (pg/ml)	94.48 ±3.74*#	113.2 ±3.67$	75.48 ±9.64	77.77 ±9.6
TNF-α (pg/ml)	44.44 ±1.91*#	52.07 ±1.47$	30.82 ±4.06	31.71 ±5.27
IL-2 (pg/ml)	5.48 ±0.35*#	9.40 ±0.30$	6.36 ±0.18	6.40 ±0.30
IL-4 (pg/ml)	3.41 ±0.26*#	6.42 ±0.28$	2.27 ±1.23	2.47 ±1.26
IL-6 (pg/ml)	27.39 ±1.30*#	37.69 ±1.46$	25.43 ±4.6	26.45 ±4.02
IL-8 (pg/ml)	18.85 ±1.02*#	26.26 ±2.32$	8.35 ±2.48	9.09 ±2.77
IL-10 (pg/ml)	2.55 ±0.27*#	6.52 ±0.34$	1.68 ±0.70	1.52 ±0.68
IL-12 (pg/ml)	54.38 ±2.33*#	72.23 ±2.01$	34.86 ±6.01	34.96 ±4.15
INF-γ (pg/ml)	11.09 ±0.86*#	17.28 ±0.79$	8.33 ±2.33	8.90 ±2.80
VEGF (pg/ml)	501.00 ±3.48*#	617.41 ±5.24$	412.93 ±15.72	411.64 ±17.85
ADM (pg/ml)	419.00 ±5.18*#	523.47 ±6.18$	357.09 ±13.2	411.32 ±14.98
Angiogenin (pg/ml)	262.56 ±6.44*#	405.27 ±3.82$	203.45 ±9.82	250.60 ±21.42

*Endo <35 vs. Tubal <35; # Endo <35 vs. Endo > 35; $ Endo >35 vs. Tubal > 35 *, #, $=p<0.0001

Table 6: Odds ratio comparison for pregnancy outcomes parameters

Comparison	Pregnancy rate	Miscarriage rate/ pregnancy	Live birth rate
Endometriosis (<35years) vs. Endometriosis (>35years)	OR: 2.21 (95% CI: 1.19-4.11) p=0.01*	OR: 0.69 (95% CI: 0.19-2.52) p=0.57	OR: 2.31 (95% CI: 1.14-4.7) p=0.02*
Endometriosis (<35years) vs. Tubal block (<35years)	OR: 0.81 (95% CI: 0.54-1.22) p=0.31	OR: 1.12 (95% CI: 0.5-2.92) p=0.67	OR: 0.78 (95% CI: 0.5-1.2) p=0.26
Endometriosis (>35years) vs. Tubal block (>35years)	OR: 0.47 (95% CI: 0.25-0.9) p=0.02*	OR: 0.71 (95% CI: 0.18-2.72) p=0.61	OR: 0.46 (95% CI: 0.22-0.95) p= 0.03*
Tubal block (>35years) vs. Tubal block (>35years)	OR: 1.13 (95% CI: 0.83–2.03) p=0.24	OR: 1.24 (95% CI: 0.47–3.22) p=0.65	OR: 1.36 (95% CI : 0.84–2.2) p=0.2

[a]OR: Odds ratio, CI: 95% confidence interval, * p<0.05 is considered as significant

0.25-0.9) p=0.02], pregnancy rate [OR: 0.46 (95% CI: 0.22-0.95) p=0.03], were significantly low in Group A2 when compared with the matched controls (Group B2), although, miscarriage rate was found to be comparable [OR: 0.71 (95% CI: 0.18-2.72) p=0.61] (Table 4 and 6). Moreover, using a stepwise multivariable logistic regression analysis adjusted for age, baseline AMH, AFC, number of mature oocyte and embryos, these differences were further extended (Table 6).

When the inflammatory molecules were compared in these sub-groups, a significant elevation of cytokines (IL-1β, TNF-α, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12, IFN-γ) and angiogenic factors (VEGF, ADM, angiogenin) were also found to be significantly higher in younger women with severe endometriosis (Group A1) when compared to their matched controls (Group B1) (Table 7). IL-8, IL-12 and ADM were observed to be the most important molecules in follicular fluid affecting oocyte and embryo quality (p<0.001). The number of M II oocytes and grade I/II embryos were significantly lower in Group A1 when compared with B1 (p<0.003 and p<0.001, respectively). Similar results were observed in Group A2 versus B2 for M II oocytes (p<0.001) and grade I/II embryos (p<0.001) (Table 4).

Discussion

Published reports indicate a great deal of controversy of ART outcomes in women with severe grades of endometriosis. The majority of these reports, while compiling their results, did not consider two important confounding factors, including age of the patient and phenotype of the disease. Women with severe endometriosis may be subcategorized in few phenotypes, which have been outlined in the Table 2. In this study, we have not considered the outcome of ART treatment with regard to phenotypes in detail. We conducted a cohort- based study to evaluate the impact of age on ART outcomes in women

with severe endometriosis and compare the results with age-matched tubal factor infertility. In elderly women (Group A2; ≥ 35 years) both live birth and pregnancy rates were significantly much less (11% & 13.5% respectively) in endometriosis compared to their age-matched tubal control group, Group B2 (Table 4 & 6). However, in younger age group (<35 years) the results were comparable in both the categories (Group A1 vs. B1) (Table 4 & 6). Moreover, using stepwise multivariable logistic regression analysis adjusted for age as well as baseline AMH, AFC, number of mature oocytes and embryos, the differences in live birth rates between the two groups were further extended (Table 6). Cytokines and angiogenic molecules were found significantly higher in severe endometriosis when compared to tubal controls. Surprisingly, even high levels of these molecules did not affect pregnancy or live birth rate in younger endometriosis patients, Group A1 (<35 years) (Table 4 and 6). Additionally, even when oocyte and embryo quality were observed to be poor in young endometriosis patients, the live birth rate was similar when compared with the control (Group B1). While stratifying endometriosis women according to their different phenotypes, women with only endometrioma had marginally better pregnancy and live birth rates compared to DIE or other types of endometriosis (Table 5). This observation has also been corroborated by other studies.[25–27]

Endometriosis is considered to have an adverse effect on fertility.[20,28–30] This is evident from the earlier studies, which showed that the disease primarily affects ovaries at the molecular, histological and morphological level.[31,32] Multiple pathologies attributing to poor IVF outcome can be due to altered peritoneal and follicular milieu, leading to abnormal ovulation, poor oocyte quality, finally resulting in low-grade embryos, decreased fertilization and implantation rates. In a recent study by Muteshi et.al, women with endometriosis reportedly had fewer oocytes retrieved, lower number of blastocysts transferred and a significantly reduced incidence of implantation rates were recorded when compared to women with unexplained subfertility.[20] Assessment of peritoneal fluid in pelvic endometriosis revealed presence of elevated levels of pro-inflammatory cytokines and activated macrophages.[25] In addition, follicular environment and oocyte quality in endometriosis is affected due to metabolic and hormonal dysregulation, which further leads to migration of abnormal levels of pro- and anti-inflammatory cytokines.[33] Our previous study is in agreement with these findings.[34] We observed significantly higher levels of pro-inflammatory (IL-1β, TNF-α, IL-2 IL-8, IL-12, IFN-γ) and anti-inflammatory (IL-4, IL-6, IL-10) cytokines in follicular fluid of women in severe endometriosis (Table 7) compared to controls who underwent IVF.[34–36] We also found that the intra-follicular angiogenic molecules, including VEGF, ADM, and angiogenin were significantly elevated in endometriosis of both the age groups (Group A1 and A2). Recent studies have negatively correlated the role of VEGF and ADM for poor ovarian response and IVF outcome.[34,37,38] We have observed increased levels of inflammatory and anti-inflammatory cytokines in DIE and other phenotypes. Although contradictory observations exist, our observations are in agreement with several previously published studies which suggest existence of high levels of cytokines in the follicular fluid in advanced endometriosis.[35,39–42] It is suggested that chemokines secreted by the ectopic lesions stimulate the infiltration of macrophages that further contribute to the development of the disease, and this may promote inflammatory agents.[43] In one of our earlier studies, we reported that elevated follicular fluid cytokine levels were also observed in patients with endometriosis irrespective of the stage of the disease.[34] A study suggests endometrioma seldom induce inflammation in nearby follicles and no evidence has been observed of presence of increased cytokine concentrations in the ipsilateral follicle.[25] Interestingly, in our current study pregnancy and live birth was observed to be marginally better in women with the only endometrioma as compared to other phenotypes (Table 5).

In the present study, the ovarian reserve was also noted to be significantly reduced in endometriosis patients when compared to controls. Regardless of the increased levels of inflammatory molecules and fewer oocytes and scarcity of good quality embryos, the live birth rate was not affected in younger endometriosis patients (Group A1, <35 years) when compared with younger controls (Group B1). However, the detrimental effect of endometriosis was observed to be more prominent in higher age group patients (Group A2, ≥35 years) when compared with tubal infertility patients of the

same age, Group B2 (Table 4, 6 and 7). Poor ovarian reserve, lower oocyte yield, fewer numbers of good quality embryos and reduced live birth seem to be more associated with the age and/or duration of the disease in severe endometriosis (Table 4). However, we could not specifically correlate the above findings with duration of the disease more often because of late diagnosis, although it may be considered that older women must have been suffering for a longer period of time. Therefore, it seems more likely that 'duration' of exposure to the severity and not the 'severity' alone is suggestive of being responsible for adverse reproductive potential in elderly women.

A meta-analysis by Barnhart et al. observed a fewer number of oocytes with lower fertilization, implantation and pregnancy rates in women with endometriosis undergoing IVF in comparison to tubal factor infertility (12.7 vs. 18.1%).[44] But significantly improved pregnancy outcome is observed nowadays, thereby questioning the appropriateness of these findings in today's ART scenario. The Society of Assisted Reproductive Technology (SART) database (2000- 2011) reported that endometriosis was associated with a statistically decreased but clinically insignificant difference in 'per embryo transfer' pregnancy and live birth rate in comparison to tubal factor infertility.[21,45,46] Surprisingly SART database during recent years has concluded that endometriosis alone had similar or slightly higher live birth rate compared to other causes of infertility undergoing IVF. However, when confounding infertility factors for example, age and phenotype of the disease, were taken into account with endometriosis, live birth rates are lowered significantly.[45] This observation did not address the severity of the disease, past therapy or presence of endometrioma. Therefore, the impact of severe endometriosis on IVF outcome cannot be ascertained from this retrospective analysis. In a more recent study, Muteshi and colleagues have reported that women with endometriosis are 24% less likely to have live birth when compared to those with unexplained subfertility. Moreover, they suggested that the live birth further drops with increasing severity of the disease.[20] Polat et.al observed similar pregnancy and live birth rate in endometriosis of different stages and extent when compared with tubal infertility.[47] Roux et.al in their study conducted on women with rAFS Stage III and Stage IV endometriosis, concluded that live birth rate was only significantly affected with rAFS stage IV endometriosis in presence of active smoking and poor ovarian reserve.[48]

All studies quoted above have tried to compare ART outcome in women with advanced endometriosis and matched non-endometriotic women serving as controls. The conclusion of the studies quoted above, when taken together, are, to a large extent, confusing. These studies did not subcategorize advanced endometriosis with regard to their age or phenotype of the disease. Our study also included rAFS stage-III and stage-IV endometriosis women treated with IVF, and we found reduced live birth rate mostly affecting women aged 35 years or more (GroupA2). Conversely, in younger women with severe endometriosis (Group A1) comparable ART outcome was observed when compared with controls. Therefore, it appears that age is one of the primary determining factors in prognosticating fertility in women with severe endometriosis.

A study by Opoien et al. showed similar pregnancy and live birth rates in women with of all stages of endometriosis in the absence of endometrioma who underwent IVF/ICSI compared to women with tubal factor infertility.[21] Patients with endometriomas, were reported to have significantly lower pregnancy and live birth rates.[21,48] However, the exact age subgroup has not been specifically mentioned in these reports. In our study, 53% of women have endometrioma (50.75% in < 35 and 59.14% in ≥35 age group). Despite more than half of the study population having endometrioma, comparable pregnancy and live birth rates were observed in the younger subgroup compared to controls. This again confirms the impact of age rather than severity or phenotype of the disease playing a significant role in reproductive potential in women with severe endometriosis.

Surprisingly, it has been reported that even though the number of mature oocytes retrieved in women with endometriosis is low, fertilization and implantation rates were observed to be comparable.[49] While in the current study, irrespective of the number of mature oocytes retrieved, fertilization rates in both age groups of endometriosis women were found lower when compared with their respective tubal control groups. But implantation rates were observed to be poor in endometriosis Group A2 (≥35 years) when

compared with either respective tubal group (Group B2) or younger endometriosis group (Group A1) (Table 4 and 6). Muteshi et.al also demonstrated reduced follicular recruitment, decreased likelihood of blastocyst stage transfer and impaired implantation in women with advanced endometriosis.[20] Hamdan and others reported higher cancellation rates (about three times) and fewer numbers of mature oocytes in endometriosis patients.[50] There are various proposed mechanisms behind fewer oocytes retrieval and lower ovarian reserve in severe endometriosis or unoperated endometriomas.[51,52] It is hypothesized that this poor ovarian reserve or response may be due to disturbances in ovarian vascularization resulting in reduced availability of gonadotropins.[51] It may be possible that extensive endometriotic lesion involving ovarian cortical tissue may affect vascularity by causing ovarian cortical destruction or distortion. Surgical treatment of endometriosis though reduces the bulk of endometriotic lesion and improves symptoms, but it may further reduce ovarian reserve by reducing vascularity through cauterization and use of surgical sutures which may affect ART outcome.[53,54] The present study also noted higher gonadotropin requirement and increased cycle cancellation in severe endometriosis groups. 13.27% started-cycle was canceled in endometriosis group, while the cancellation rate was 7.73% for the tubal group. Most of the cancelled patients in endometriosis group were from the higher age group further highlighting the impact of age on ovarian reserve.

However, summarizing the literature reports, the poor outcome in endometriosis, being an inflammatory disease, can be attributed to poor oocyte quality, diminished ovarian reserve and impaired endometrial receptivity. In our study, it was observed that in younger group of women (Group A1), even with advanced endometriosis, pregnancy and live birth rates was not affected in spite of dysregulation of inflammatory cytokines, and angiogenic factors and availability of mature good quality oocytes and embryos. Diminished ovarian reserve and low number of oocytes retrieved did not affect the pregnancy or live birth rate observed in this subgroup of endometriosis. However, in the older age group patients with advanced endometriosis (Group A2), significantly lower live births were observed. This implies that age may be one of the primary deciding factors in determining the fertility outcome in endometriosis.

As age advances, decline in quality and quantity of eggs seems to be responsible for declining fertility in elderly patients. Endometriosis is a relatively common cause of female infertility, which causes progressive loss of ovarian reserve especially in longstanding cases which can further worsen the efficiency and can impair reproductive outcome at older age. Exact duration of the disease is often difficult to ascertain because of its delayed diagnosis. Therefore, earliest opportunity should be sought to reduce its adverse effect on reproductive outcome by counseling them for early pregnancy. Early conception may not be feasible for many women. However, fertility preservation through ovarian cortical biopsy may be an alternative option in future. Research is ongoing to identify possible 'markers' for prediction (e.g. genomics, proteomics, etc.) of endometriosis in young girls in endometrial cells harvested from menstrual blood in adolescent age born in 'high risk' families. If identified, it may also be possible to protect these potential young victims against 'severity' of endometriosis through non-invasive targeted medical management, which are currently in trial.

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Authors' roles:

S Sharma conceived the study and contributed toward the study design, patient selection. S Sharma and S Bathwala performed the study, manuscript drafting and critical discussion. S RoyChoudhury performed data analysis, manuscript drafting and critical discussion. R Bhattacharyaa, Shovandeb Kalapahar and I Saha helped with the data analysis and manuscript drafting. R Chattopadhyaya and BN Chakravartya did critical discussion and proofreading the article.

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Institute of Reproductive Medicine has recently received a project funded by Department of Science and Technology, Govt. of India (SEED/WS/2019/540) to investigate "A new approach towards pain and infertility management in women suffering from endometriosis and adenomyosis"

Dr. Sunita Sharma, Senior Consultant, IRM (PI)
Dr. Sourav Roy Choudhury, Senior Scientist, IRM (Co-PI)

Background:

Adenomyosis is a common benign gynecologic condition characterized by the presence of endometrial glands and stroma within the uterine smooth muscle myometrium and histologically, it is defined as occurrence of islands of ectopic nonneoplastic endometrial tissue (endometrial glands and/or stroma) within the uterine myometrium at different depths and accompanied by hyperplastic and hypertrophic smooth muscle. Adenomyosis and endometriosis patients suffer from chronic dysmenorrhea, are associated with severe pain and poor embryo implantation rate due to increased aromatase expression and thereby resulting in poor endometrial receptivity. This leads to poor quality of life and further affects their reproductive outcome.

There is an impending necessity for identifying a proper clinical management strategy to treat these symptomatic women. Moreover, understanding the molecular interplay during implantation is a prerequisite to improve the present poor reproductive outcome where surrogacy/adoption remains the only hope of motherhood for these patients. Letrozole, an aromatase inhibitor, brings a new hope for the women suffering from adenomyosis and endometriosis. Letrozole can inhibit aromatase, which otherwise produce estrogen, responsible for proliferation of the disease and associated pain there by relive severe pain as well as help in fertility management, which in turn can improve their reproductive outcome. The dysmenorrhea associated with adenomyosis as well as endometriosis may sometimes be so debilitating that the pain management takes precedence over fertility treatment. Medical management of pain associated with these disease processes takes longer time and thus losing the precious years of reproductive age.

We are investigating the impact of a lower dose of letrozole (2.5 mg/thrice weekly) on pain and heavy menstrual bleeding in infertile women with adenomyosis awaiting IVF. The proposed treatment seems to relive the women from severe pain as well as help in fertility management, which in turn can improve their reproductive outcome.

We compared the efficacy of this low-dose letrozole protocol with the standard treatment of GnRHa that has already been demonstrated to be useful in these.

About the project:

Date of start: 07/09/2021 (date of release of part of 1st instalment)

Duration of the Project: 3 years

Total cost of the project: Rs. 29,37,700

Assets developed and equipment acquired: Multi wavelength microplate reader, (Thermo Fisher)

Manpower recruited: One project assistant (working since 1st Jan 2022)

Social outreach component: Patient awareness through seminar/workshop

Human resources development & skill enhancement trainings: CME workshop, seminars etc.

Objectives of the proposal:

- To assess the quality of life in terms of chronic

pain level, menstrual irregularities and reproductive status in Indian women with endometriosis and adenomyosis.

- To examine the molecular markers of implantation in endometrium of patients with endometriosis and adenomyosis, and compare them with the controls.
- To evaluate the effects of the proposed treatment approaches in endometriosis and adenomyosis on the improvement of pain, implantation markers at molecular level and compare them with the marker levels before treatment.
- To further assess and compare the post treatment implantation and reproductive outcome for the proposed treatment approaches in endometriosis and adenomyosis undergoing in vitro fertilization.

Methodology & systems approach

- 20 women with only adenomyosis, 20 women with adenomyosis and endometriosis have directly received treatment under the project and their improvements in terms of dysmenorrhea (VAS score) and menorrhagia (PBAC score) also assessed.
- Reduction in terms of sonographic features in these symptomatic patients are also being compared for post treatment improvements.
- Endometrial biopsy samples have been collected form 55 patients to assess their implantation markers in future.
- Immunohistochemistry and ELISA based molecular marker assessment experiments are being at the standardization stage.

Initial observation

- A total of 425 infertile women were screened and from them 55 women were enlisted for the study comprising of 20 women with only adenomyosis, 20 women with adenomyosis and endometriosis and 15 women with tubal factor (controls) who are receiving letrozole therapy for 3 months. 15 women from each group i.e., total 30 women has completed the treatment and 5 more from each treatment group (total 10) are undergoing the treatment
- Pain and bleeding have been significantly reduced in these women and their menstrual pain and abnormal bleeding are quantitatively assessed. Endometrial tissue samples are also collected from all the 55 women before starting of the treatment and again 30 women from the same group who have completed the treatment.
- Both the treatment groups, adenomyosis and adenomyosis with endometriosis reported marked improvement in symptoms. VAS scoring showed a significant stepwise decrease in pain from baseline mean value of 8.76±2.1 in the adenomyosis only group during each follow-up, and the dysmenorrhea was negligible after 2 months of treatment (2.2±1.38; Fig:1) and comparable with controls after 3 months (1.44±1.22). Women suffering from adenomyosis and endometriosis also experienced similar pattern, where VAS score of 9.24±2.1 were restored to normal after three months of letrozole treatment (Figs: 1&4).
- The PBAC scoring for menorrhagia significantly improved post 3 months of treatment with letrozole (Figs: 2&4) with marked improvement observed at the second follow-up visit in both the groups. Both adenomyosis only and adenomyosis with endometriosis group experienced heavy menorrhagia (PBAC: 174±31 and 191±45, respectively) before the treatment and their menstrual blood loss levels were restored to the levels (51±16 and 62±22, respectively) that are comparable to normal (Figs: 2&4). Blood haemoglobin levels were also observed to be improved in women treated with letrozole in both the groups (Fig: 3&4).
- The majority of women had 3-4 sonographic criteria on TVS (median of 3), such as irregular or interrupted junctional zone (79.49%), asymmetrical thickening of the myometrium (71.15%), globular uterus (43.59%), and myometrial cyst (33.33%). Marked reductions in the sonographic severity scores for diffuse adenomyosis, adenomyoma, and asymmetry in myometrial thickness were observed following both the treatment. The effects of both the treatments were comparable in terms of severity of sonographic features (Fig:5).
- Standardization process of immunohistochemistry is underway and

representative photomicrographs are shown in figure 5 where increased expression of estrogen receptor α in eutopic endometrial tissue specimen from women with adenomyosis is shown (Fig:6).

- A patient awareness program was conducted on 12th January 2022 at the Institute of Reproductive Medicine, as part of the patient outreach program under the project (Fig:7).
- A continuing medical education (CME) workshop on the "Assessment and Future Direction Towards Pain and Infertility Management in Women Suffering from Endometriosis and Adenomyosis" was also held on 13th February 2022. This CME workshop aimed to train the obstetrician & gynecologists, and infertility clinicians on diagnosis and new approaches for adenomyosis treatment (Fig:8).

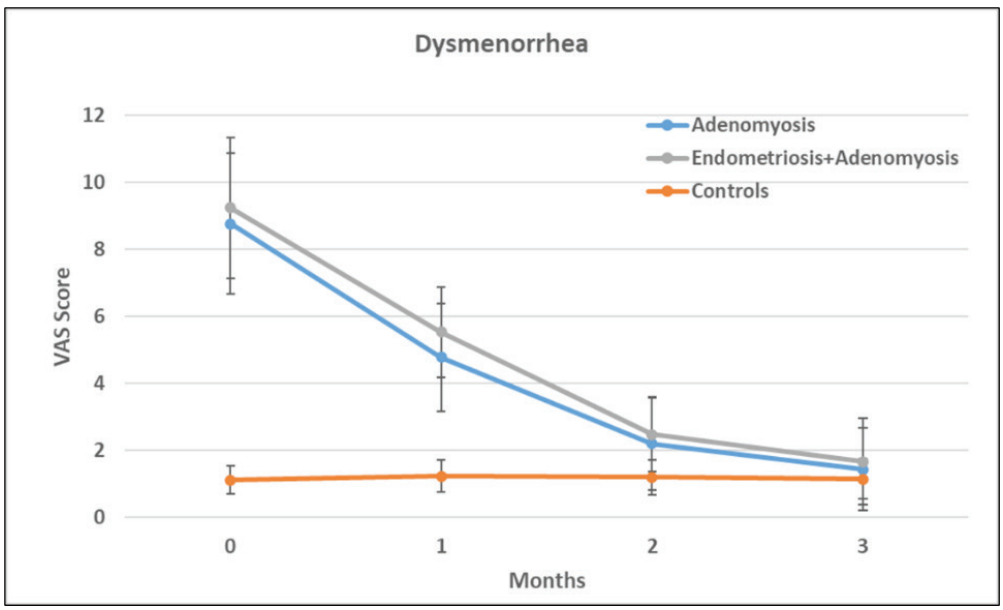

Figure1: Significant decline in dysmenorrhea (VAS score) over the 3 months of treatment period in the women with only adenomyosis, adenomyosis with endometriosis and comparing them with control patients. Post 3 months of treatment, VAS score in the symptomatic groups were restored to normal and were comparable with that of the controls

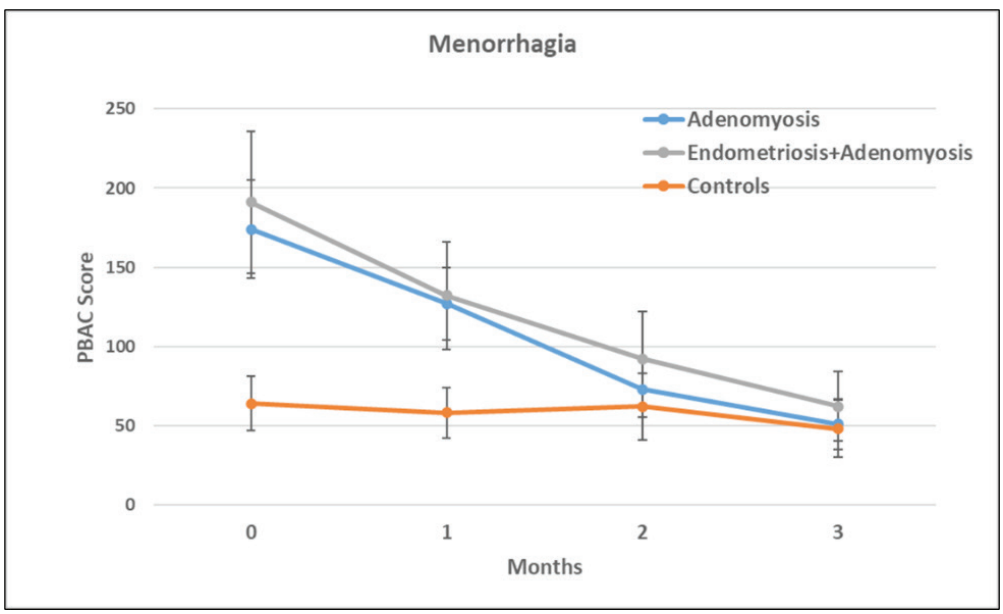

Figure 2: Significant decline in menorrhagia (PBAC score) over the 3 months of treatment period in the women with only adenomyosis, adenomyosis with endometriosis and comparing them with control patients. Post 3 months of treatment, PBAC score in the symptomatic groups were restored to normal and comparable with that of the controls

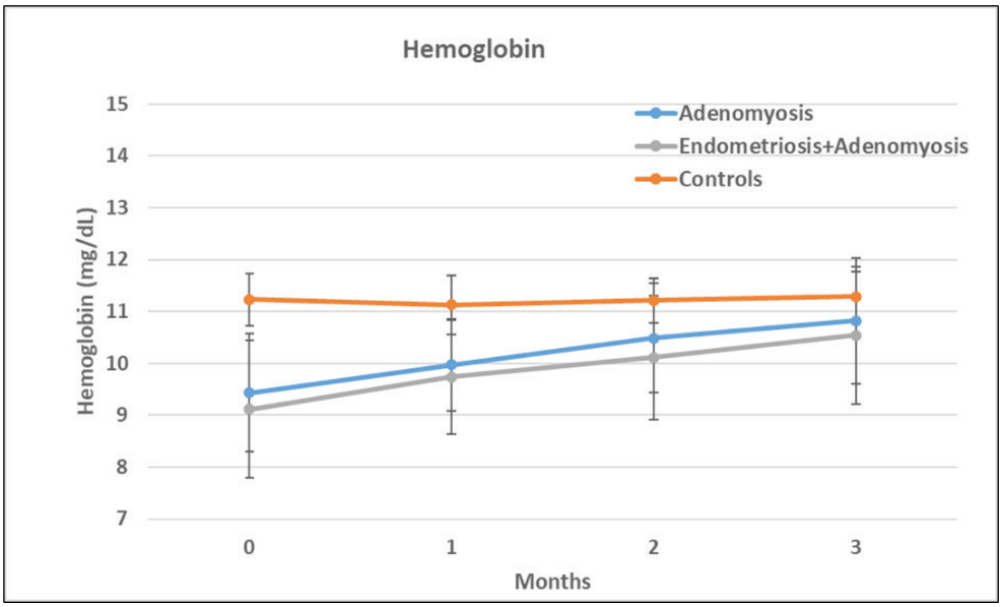

Figure3: Improvement in blood hemoglobin levels (Hb%)) over the 3 months of treatment period in the women with only adenomyosis, adenomyosis with endometriosis and comparing them with control patients. Post 3 months of treatment, Hb% in the symptomatic groups were comparable with that of the controls

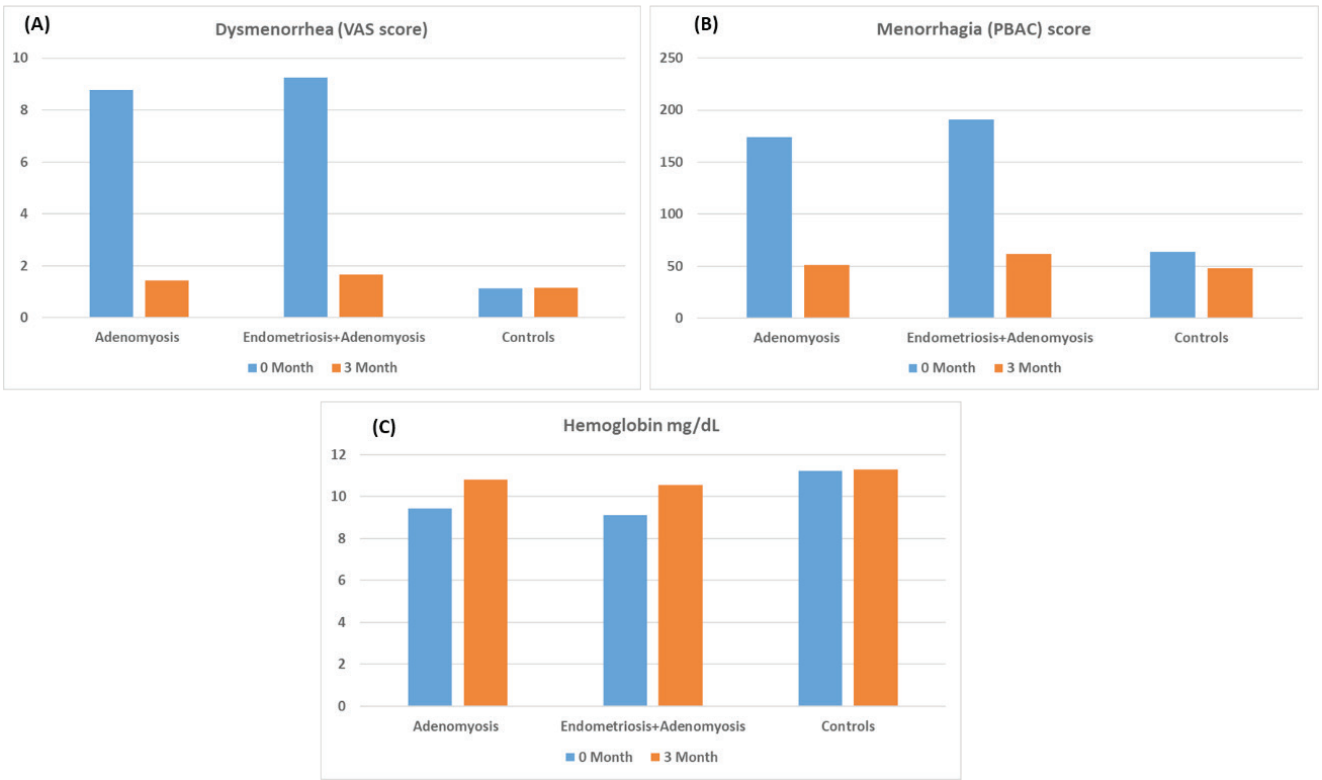

Figure 4: Direct comparison of post 3 months of treatment low dose treatment with baseline (A) VAS, (B) PBAC scores and (C) Hb% in both the adenomyosis and adenomyosis with endometriosis; values were comparable with that of controls

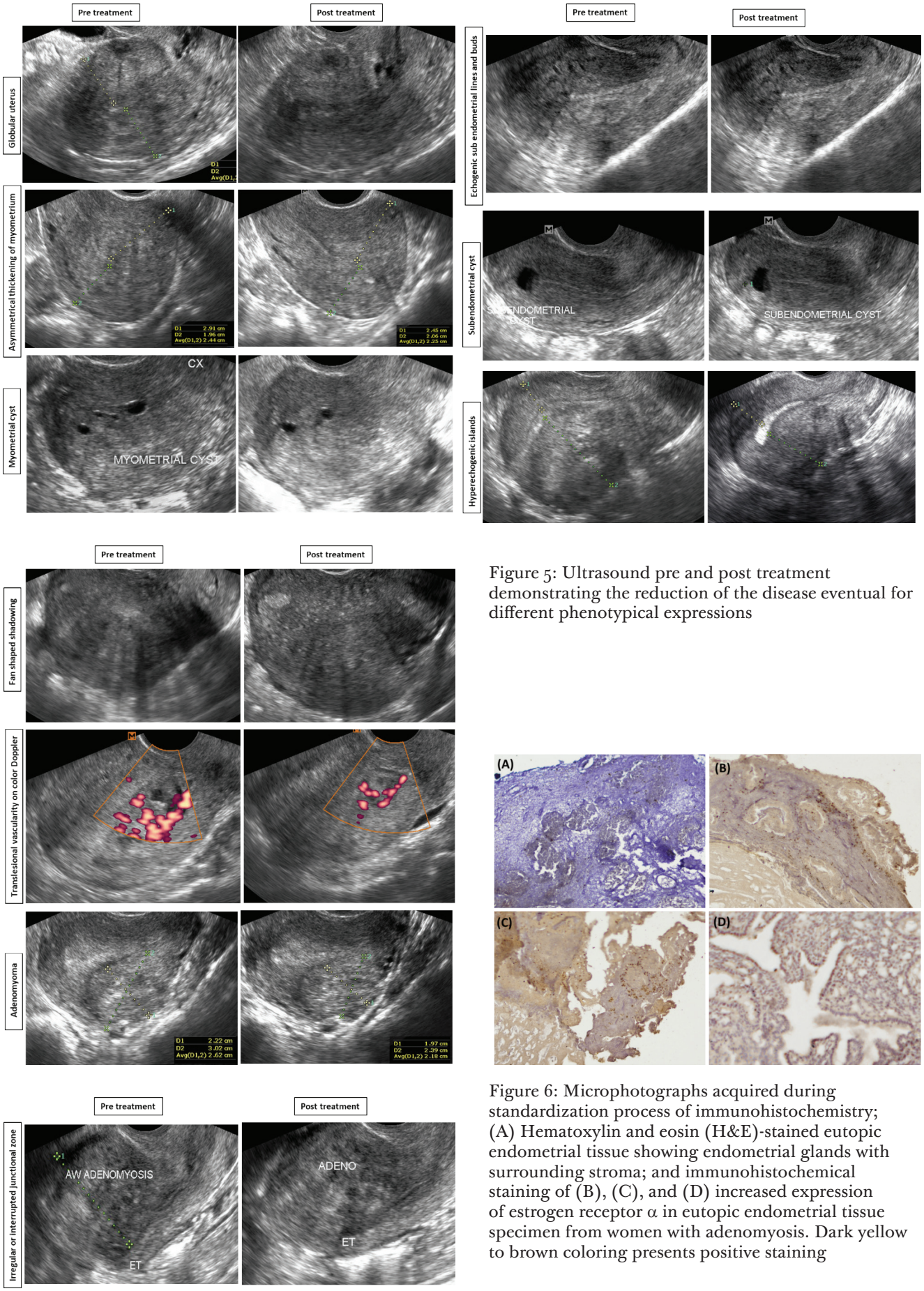

Figure 5: Ultrasound pre and post treatment demonstrating the reduction of the disease eventual for different phenotypical expressions

Figure 6: Microphotographs acquired during standardization process of immunohistochemistry; (A) Hematoxylin and eosin (H&E)-stained eutopic endometrial tissue showing endometrial glands with surrounding stroma; and immunohistochemical staining of (B), (C), and (D) increased expression of estrogen receptor α in eutopic endometrial tissue specimen from women with adenomyosis. Dark yellow to brown coloring presents positive staining

Figure 7: Patient awareness program conducted on 12th January 2022 at the Institute of Reproductive Medicine, as part of the patient outreach program under the project

Figure 8: CME workshop on diagnosis and new treatments of adenomyosis held on 13th February 2022 at the Institute of Reproductive Medicine

Work Statement of Patients for the month of July to September 2021

1. GYANECOLOGICAL & OBSTETRICS CASE

Total no. of Patient's attended: 397

- Infertility 315
  - Primary 224
  - Secondary 91
- History of Recurrent Spont Miscarriage (≥ 3) 24
- History of Unexplained Spont Miscarriage (≥ 1) 15
- Primary Amenorrhoea 05
- Secondary Amenorrhoea (Without infertility) 14
- Other Gynaecological case 24

Infertility

Female Factor:	134 (42.54%)
Male Factor:	114 (36.19%)
Unexplained:	46 (14.60%)
Combined Factor:	21 (6.67%)

Female Factor 134 (42.54%)

- PCO 33 (24.62%)
- Endometriosis 23 (17.16%)
  - Early: 12
  - Advanced: 04
  - Advanced with Adenomyosis 06
- Tubal Block 53 (39.55%)
- POF 16 (11.94%)
- Uterine Anomalies 06 (4.48%)
- Fibroid 03 (2.24%)

Male Factor 114 (36.19%)

- Azoospermia 46 (40.35%)
- Asthenospermia 21 (18.42%)
- Oligospermia 17 (14.91%)
- Oligoasthenospermia 16 (14.03%)
- Oligoteratozooasthenospermia 07 (6.14%)
- Aspermia 01(0.88%)
- Pyospermia 03 (2.63%)
- Sexual Dysfunction 01 (0.88%)
- Retrograde Ejaculation 02 (1.75%)

Age Factor of Female Partner: (n=315)

- Less than 25 Yrs: 39 (12.38%)
- 25-35 Yrs: 215 (68.25%)
- 36-40 Yrs: 46 (14.60%)
- More than 40 Yrs: 15 (4.76%)

Duration of Infertility: (n=315)

- Less than 5Yrs: 133 (42.22%)
- 5-10 Yrs: 136 (43.17%)
- More than 10 Yrs: 46 (14.60%)

Residential Status: (n=315)

- Urban: 259 (82.22%)
- Rural: 56 (17.77%)

2. OBSTETRIC CASES

(Pregnancy following investigation & treatment including ART)

A. Confirmation of Pregnancy 141
- Following treatment of Infertility: 124
- Following previous foetal wastage (Spont): 17

Treatment modalities for achieving these pregnancies
- Following medical treatment (Induction Ovulation): 16
- Following Surgical Treatment: 04
- Following Hydrotubation: 08
- Following HSG & Laparoscopy: 06
- During investigation: 15
- Intrauterine Insemination: 27
- IVF-ET including FET Cycle: 48

B. Complication in Ongoing Pregnancy
- Threatened Abortion: 04
- Hypertension: 06
- Diabetes Mellitus: 10
- Sub Clinical Hypothyroidism: 07
- Toxoplasma Infection: 02
- RH Negative: 02

C. Viable Delivery and the obstetric career of the Mother
- Following infertility: 10
- Following previous foetal wastage: 08

Perinatal outcome of viable pregnancies:
- Alive: 18
- Neonatal Death:
- Still Born:

3 IVF UNIT

Total no of Cycle:	164
Total no of index cycles:	23
Total no of FET cycles:	76
OCR:	88
Cancellation due to Failed Stimulation: (Poor Responder)	04
Discontinued other than poor responder	0
Embryo Transfer including FET	99
ET cancelled due to OHSS & poor endometrial thickness (< 7 mm)	15

IVF Cycles	n	Preg
• Conventional IVF	6	1 (16.67%)
• PESA/ICSI Cycles:	5	1 (20%)
• Ejaculated ICSI Cycles:	8	2 (25%)
• Blastocyst Cycles:	17	7 (41.17%)
• Donor Semen:	4	1 (25%)
• Surrogacy (including FET cycle)	4	2 (50%)
• FET Cycles :	76	36 (47.37%)

4 USG UNIT	
• Folliculometry:	383
• Antenatal Monitoring:	523
• Others:	156
(Includes Endometriosis, Fibroid etc.)	

5 IUI UNIT		
Total No. of Patients Treated:	219	
	N	Preg
• AIH:	201	21 (10.44%)
• AID:	18	07 (38.89%)

6 SURGICAL UNIT	
• Laparoscope:	01
• Hysteroscope:	12
• Laparo + Hystero:	12
• D+E+C:	11
• Mc Donald/Shirodkar:	02
• LUCS	18

Achievements

- The fourth volume of Dr B N Chakravarty book "Clinics in Reproductive Medicine and ART" has been released.
- Dr. Mrs Sunita Sharma has been glorified with Robert G. Edwards Prize Paper Award for the best paper published in RBMO in 2019.
- Dr B N Chakravarty have been felicitated with the ISAR Ratna Award as a mark of Life Time Achievement by Indian Society for Assisted Reproduction

Publications in peered reviewed Journal

1. Comparative Evaluation of Metformin and Letrozole in a Rat Model of Polycystic Ovary Syndrome; Chakraborty Pratip, Chatterjee Sujan, Ipsita Chaterjee, Patra Debajyoti, Chakravarty Baidyanath, Kabir Syed N; Indian Journal of Diabetes and Endocrinology; Volume 1 Number 1, January - June 2019; pg. 19-27
2. Scientific Reports: published (2019) 9:6276 – High frequencies of Non Allelic Homologous Recombination (NAHR) events at the AZF loci and male infertility risk in Indian men – Deepa Selvi Rani, Singh Rajender, Kadupu Pavani, Gyaneshwer Chaubey, Avinash A, Rasalkar, Nalini J.Gupta, Mamta Deendayal, Baidyanath Chakravarty and Kumarasamy Thangaraj.
3. Pregnancy and Live Birth Rates are Comparable in Young Infertile Women Presenting with Severe Endometriosis and Tubal Infertility; Sharma S, RoyChoudhury S, Bathwal S, Bhattacharya R, Kalapahar S, Chattopadhyay R, Saha I, Chakravarty B; Reproductive Sciences (2019)
4. Novel NR5A1 pathogenic variants cause phenotypic heterogeneity in 46, XY disorders of sex development. Sudhakar VSD, Shveta J, Phanidharnath R, Umesh K, Raghavendra S, Isha K, Sree N, Jaishree D, Gupta NJ, Chakravarty BN, Khadikar V, Imam S,Rajesh K, Mamata D, Datar CA, Dada R, Yogendra S, Anuranjan A, Tharangaraj K; Sex Dev (2019)
5. Intrauterine Infusion of Human Autologous Peripheral Blood Mononuclear Cells Improves In Vitro Fertilization Success in Infertile Women" Swarup K Chakrabarti, Sanghamitra Ghosh, Shovandeb Kalapahar, Gunja Bose, Saeeda Wasim, Tushar K Das, Ratna Chattopadhyay, Baidyanath Chakravarty. Journal of Stem Cells (2020).
6. Prevalence of Y chromosome microdeletion in azoospermia factor subregions among infertile men: Saurav Dutta, Pranab Paladhi, Samudra Pal, Gunja Bose,Papiya Ghosh, Ratna Chattopadhyay, Baidyanath Chakravarty, Sujay Ghosh. Mol Genet Genomic Med. 2021;00:e1769.
7. Novel variations in spermatogenic transcription regulators RFX2 and TAF7 increase risk of azoospermia. Samudra Pal1 • Pranab Paladhi1 • Saurav Dutta, Gunja Bose, Papiya Ghosh, Ratna Chattopadhyay,Baidyanath Chakravarty, Indranil Saha, Sujay Ghosh. Journal of Assisted Reproduction and Genetics, ttps://doi.org/10.1007/s10815-021-02352-5

Important grants received from Ministry of Science & Technology, Govt, of India

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).