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International Journal of Human Reproduction, Research and Development (IJHRRD)

Volume 1 I Issue 1 I July 2021

CONTENTS

CASE REPORT

INTERNATIONAL JOURNAL OF HUMAN REPRODUCTION RESEARCH AND DEVELOPMENT

Global Scenario of In Vitro Fertilization (IVF)

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Introduction

In Vitro Fertilization (IVF) has made the entire globe a happier place to live by making the families complete. It brought immense joy to millions of couple and even larger number of people as the entire family is overjoyed.IVF is a dream come true revolution for infertile couples globally. Couples yearning to have a baby in their arms now will be able to fulfill their dreams.

IVF is a form of treatment for infertile patients available across the globe from nearly four decades. In simple explanation, it involves fertilization of gametes, both male (sperm) & female (oocyte) outside the body. The sperms are required to be procured by the male partner and oocytes are retrieved surgically after stimulating the ovaries, under transvaginal guidance and short anesthesia.

Assisted Reproduction Technology (ART) includes medical procedures and techniques for the management of infertility. Various advance techniques has been developed in the field of ART with the advancement of technology. Intra Cytoplasmic Sperm Injection (ICSI), Intra Morphological Sperm Injection (IMSI) are advanced IVF method. Third party reproduction is another remarkable therapy, which includes Sperm donation, oocyte donation, embryo donation and Surrogacy. There are also associated procedures like cryopreservation which include sperm, oocytes & embryos. These procedures are now modified from slow freezing to virtification, which is fast cryopreservation [1].

There are several advances in these procedures which include segmentation of IVF, where the IVF procedure is divided into segments to achieve better and safer results in select patients. Other advances include Oocyte activation, ICSI under spindle ICSI manipulator, where sperms are injected after viewing the spindle body, (PICSI - physiologically selected intracytoplasmic sperm injection).

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Advances in IVF globally

The first test tube baby of the world was born 44 years ago and since then this field has undergone many advances and development at a rocket speed in this short span of time. ICSI, Cryopreservation, Blastocyst Culture, Third Party reproduction, Endometrial Receptivity Array (ERA),Magnetic Activated Cell Sorting (MACS),Activated Oocyte Stimulation, advances in the line of treatment for the diseases causing infertility, Plasma Rich Protein (PRP) ,Time lapse Imagining, Pre-implantation Genetic Screening (PGS) and Diagnosis (PGD) and Fertility Preservation.

The development of ICSI in 1992 has changed the entire scenario of IVF management around the globe. ICSI has made it possible for millions of couples with male factor to conceive and have their own biological offsprings.

The incidence of male factor infertility is around 35% .The most common findings are oligo-, astheno-, teratozoospermia, a combination of the three, or even a complete absence of sperm cells in the ejaculate. All of the above can be treated with ICSI. The process involves injection of a single sperm cell directly into the oocyte. ICSI allows selecting the best morphological sperm to be injected. For male with low sperm count or with morphological abnormalities, ICSI is very helpful. Successful pregnancies from the use of these spermatozoa has pressed the boundaries of the application of ICSI to the most extreme aspect of male infertility, often encountered in cryptozoospermia, virtual azoospermia or of men with absolute azoospermia where surgical retrieval is required [2].

ICSI, its value has been affirmed in a variety of challenging situations, particularly for severe male factor couples wishing to have their own genetic child. Not least, over these last 25 years, ICSI has made possible the utilization of immature forms of the male gamete such as epididymal and testicular spermatozoa.

Blastocyst Culture

The embryos are cultured for 5 to 6 days in the embryology lab & then transferred. This requires the quality control of the lab to be of high standard & also the number of oocytes & in turn the number of embryos to be more in quantity to get the best results**.** The best embryo can be selected in this method as only the best quality embryos develop to this stage having many cell divisions. As the quality of embryos that form blastocyst are good, there is an option of transferring only single blastocyst. This avoids the complications associated with multiple pregnancies. This advanced method of blastocyst culture is simple & could be incorporated in most developing countries.

Endometrial Receptivity Array (ERA)

This assessment is utilized for increasing the implantation rates. It is based on study of endometrial tissue which is biopsied in the cycle prior to transfer cycle. The endometrial receptivity is studied on a molecular basis. This technique makes it possible to replace the embryos during the most receptive phase of the transfer cycle. The implantation is improved as the exact timing of receptivity is known.

Time Lapse Imaging (TLI)

TLI is a newer modality in the management of IVF, the embryos in this system are monitored

continuously right from early stages of the embryo every 15 to 20 minutes. As the embryo development is a dynamic process, TLI is very useful. As the embryos need not be removed from the incubator for evaluation, exposure of thermal effect, light, pH and gas is avoided. The embryos are evaluated regarding the morphology and growth with the help of imaging.

Need for IVF in all countries across the globe

The immense population surge in some developing & underdeveloped countries poses a question as to whether there is actually a need for such techniques, which will cause overburden to the countries & societies. There is also a need for having small family norms, which will force the couples to have involuntary infertility. These couples if confronted with postponement of conception or spacing between pregnancies, it will be vital to support these couples in need of pregnancy.

Every human being across the world has the fundamental human right to reproduction and having a child.IVF is a part of infertility management, every human, rich or poor, from developed country or developing country should have the option to avail these services.

Global Data Registration & Statistics

IVF is one of the most commonly practiced amongst the list of newer therapies. It is also one the highly sophisticated and has advanced many folds. There are numerous centers of IVF mushrooming all around the globe, more so in the Asian countries like China & India. Due to increased popularity of this treatment, it's imperative to maintain the global data.The need of the hour is establishing a systematic database of IVF across the globe. This will lead to a well formed draft of IVF cycles, implantation rates, pregnancy rates, miscarriage rates, live birth rate & take home baby rates. This will be a milestone to the preparation of a format to showcase the global ART and thus healthy comparison & a sense of healthy competition to do better is attained.

There are about 16 countries assessed to know regarding the wide disparity in the access to care in ART [3], in this study, many countries from Europe, Russia, Africa & Asia are included. There's a more recent observation (Deyer et al 2019) the actual number could be 275 cycles/million population [4].

IVF in Developing countries

There are numerous myths regarding infertility and the advanced treatments offered in the recent era amongst developing countries. It could be due to lack of education or due to the age old traditions and culture. There is a belief amongst policy makers that IVF is an expensive treatment with no good results and may affect women in an adverse way. The fertility management is not considered as a priority by the governments of these countries. More concern is given to management of vaccination, family planning, HIV, tuberculosis and malaria.

There is a necessity of cost effective IVF practice in these countries. There are methods developed towards reducing the cost of the procedures by doing minimal stimulation, using indegenious disposables and media along with doing batch IVF. Unfortunately there are no systematic randomized studies and evidence to promote and practice low cost IVF. The need of the hour is to develop promote cost effective IVF in these developing countries .There IVF services should be available to all infertile couples despite the difference in resources , the problem of infertility amongst population is worldwide and distributed across the

entire world. The Government and policy makers need to look at this issue seriously, try to provide the IVF treatment to the infertile couples and make important changes in the agendas to healthcare system.

Cost of IVF Globally

The cost of IVF is as important as the treatment itself. There is a wide discrepancy in the cost of IVF offered at different centers across the world. In the developed countries, the cost is much more higher while compared to the developing countries. Basically the cost of IVF comprises of the cost incurred to the center, the medicines including hormones and the disposable items which include the Retrieval needle, Embryo transfer catheter, sperm collection jar, falcon tubes, culture dishes. The centers expenses include the initial consultation, investigations, serial Sonography, ovum retrieval and embryo transfer. The average cost of IVF for 3 cycles ranges from $50,000/- in developed countries like USA to $5000/-, in developing country like India. These costs are including medicines & disposables. The latest data according to 2020 recording are on an average ranging from $15,000/- to $10,000/-. It's rarely lower than that and does not include the medicines cost which may be around $1,500 to $3,000 per cycle.

Global future of IVF

The future for IVF seems to be very exciting. Newer technologies are been conceptualized & planned to be executed. There may be a few cutting edge technologies possible in this era. The latest developing technologies are non invasive PGT-A & Artificial intelligence.

Non Invasive Pre implantation Genetic testing-Analysis is a method by which the mosaicism caused during testing which gives false negative reports is eliminated. This is a non invasive method to detect the aneuploidy in embryos. The culture media, in which these embryos grow, is tested for aneupoliody. There is no invasive method involved .This culture media is examined by next generation sequencing(NGS).This method of screening embryos is safer, non invasive, affordable, safer , accurate & easier to screen [5].

Artificial intelligence to improve results of IVF will be the future across the globe. The technique of cryopreservation of oocytes is picking up speed. By AI, these oocytes could be scored to choose the best out the frozen oocytes. Thus, by screening, there is the benefit of choosing the best oocytes which could be predicted for giving positive result in terms of pregnancy. Scoring half the participant's eggs, after looking at photos taken hours after the eggs were harvested and then predicting their odds of IVF success. Embryologist predicts potential of eggs from the remaining half of the eggs, analyzed through standard actuarial table calculations. This will allow them to then track these patients to compare fertilization, implantation and birth rates to learn if the AI is a better predictor. There is the requirement of tracing these patients later, which is after treatment, if there are live births as per the screening by AI.

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Facial masking and Covid-19: A lesson from Japan

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Introduction

I walk every morning at 05:30, when it is still dark with impending glorious sky of dawn. I found few working people are moving, someone by bicycle and someone walking. There is no crowding or traffic jam, but one thing is interesting to note that every one is wearing mask even in this early morning. With the first detection of novel corona virus in Japan on January 28, 2020, Japanese Government has declared to use basic counter-infection measures such as use of facial mask, regular hand washing, and keeping social distancing in daily life. In addition, our Government imposed strict measures to avoid "3Cs" for careful personal and social containment of this virus. Basically Japanese people are mask addicted and they have previous experience of infections such as influenza and SARS. Pursuing this new life style and avoidance of "3Cs" (closed spaces, crowded places, close-contact) in daily lives, work places, shopping malls, and restaurants are appreciable steps to counter infection [1].Japanese people are good at following rules and regulations and most of the people in Japan are maintaining these measures. I just wonder if these measures are acceptable, then why they are using mask in this early morning before dawn. Is it necessary or it is just a self-regulation of life for personal/social protection from novel corona virus (SARS-CoV-2). It is very simple to think but it makes sense and stimulating for all of us in this world who are suffering from the great disaster of this pandemic.

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Asia including Japan has largely managed to suppress the new corona virus through these mandatory measures in daily life and quarantines, such as international and domestic travel restrictions and aggressive contact tracing. East and South Asia comprise about a third of the global population and a small fraction of the world's Covid-19 deaths. In comparison, The US and Europe account for about 15% of the world's population and have half of the world's Covid-19 deaths. These differential scenarios in three different corners of the world may give us a clear window to see and realize how different governments are trying to squash outbreaks in their own ways and policies. It is easy to make rules/policy but it is difficult to abide and maintain them. We, the Japanese people, are trying to keep these simple measures and policies even in the early morning to protect ourselves, our society, and to contain corona virus.

In these days, facial masking became a close associate in our daily life on the streets, inside transportation, working places, shopping malls, restaurants and schools. There are some shopping malls and restaurants in Japan, we cannot enter without facial masking and if we forget, they serve mask to the customers. Every medical universities and/or institutions here in Japan are holding all meetings, lectures to students, clinical/research conference with facial masking and keeping social distancing. Even this pandemic changes our normal life to virtual life; a routine daily life with facial masking is appreciable and acceptable. Recently a group of researchers in Japan said they have used the 'Fugaku' supercomputer to demonstrate that wearing mask is effective in suppressing the spread of exhaled airborne droplets in places such as karaoke rooms, outdoors and in taxis [2]. This information may strengthen the simple idea that use of facial masking could be an effective countermeasure against SARS-CoV-2 transmission. When I see cute little kids of elementary schools are using mask and going to school with their mothers, I can easily presume how these innocent kids, without realizing the disaster of this pandemic, are keeping the basic measures to counter infection. Sometimes I remember one childhood proverb "if you want to go to heaven, please be a child". We, the adults, across the world need to learn the basics of corona prevention from these kids. Is this just a cultural difference among different countries to use the mask or it is an adulthood negligence or ignorance to clearly understand how to prevent the spread of this corona virus across the globe.

Researchers have assessed how effective various medical and consumer-grade facial masks are at protecting the wearer from exposure to particles similar in size to SARS-COV-2. Experts believe that facial masks effectively reduce viral transmission because they block a person from expelling the virus and help prevent a person from receiving the expelled virus. The protective effect of facial mask differs by type of material such as non-woven, urethan, and/or cloth. Among them,

non-woven masks that we usually wear in the hospital are more effective than other types, although it carries disadvantage of making people hard to breathe.According to Dr. Robert R. Redfield, the previous Director of the Centers for Disease Control and Prevention (CDC), "cloth face coverings are one of the most powerful weapons we have to slow and stop the spread of the virus-particularly when used universally within a community settings" [3]. This means we have a responsibility to protect ourselves, our families and our communities by regular using of facial masks.

Is facial masking truly necessary to contain the virus? It has been claimed that as SARS-CoV-2 continues its global spread, universal facial masking, one of the pillars of Covid-19 pandemic control, might help reduce the severity of disease and may confer a greater proportion of new infections to mild disease or asymptomatic disease. This hypothesis of facial masking could become a form of "variolation" that would generate immunity and slow the spread of the virus [4]. Since there is no difference in viral shedding between asymptomatic and symptomatic cases, facial masking seems to be a possible way to prevent transmission from asymptomatic infected people. Previous evidence related to other respiratory viruses indicates that facial masking can protect the wearer from becoming infected by blocking viral particles from entering the nose and mouth [4] . Epidemiologic investigations in Asian countries that became accustomed to population-wide masking during the 2003 SARS pandemic, have suggested that there is a close relationship between public masking and pandemic control [5]. The link between use of facial masking and reduction in disease severity is consistent with the theory of viral pathogenesis indicating that severity of disease is proportionate to the viral inoculum received. Depending on type, facial masking can filter out some virus containing droplets, reduce the inoculum that an exposed person inhales, and may contribute to increasing proportion of SARS-CoV-2 infections that are asymptomatic. The recent WHO Dashboard data indicates that there are 460,897 confirmed Covid-19 cases and 8,938 deaths in Japan (www.covid19.who.itl). In mid-July, CDC declared that use of universal facial masking may increase the rate of asymptomatic infection to >80%, a rate similar to that was also found here in Japan after implementing strict infection counter measures including facial masking. According to recent data (November 23, 2020) by the Ministry of Health in Japan, a total of 18,019 patients were hospitalized for Covid-19 nationwide with the number of severe Covid-19 patients requiring oxygen ventilation reached 331 (1.83%) and the remaining cases were either mild or moderately infected cases [6]. The most obvious way to spare society the devastating effect of Covid-19 is to promote measures to reduce both transmission and severity of illness. If this is true and prevention is still better then cure, then universal facial masking as has already been practicing in

Japan may be the best way to drive these frustrating people to become healthy asymptomatic cases and to avoid the pain of suffering from severity of illness.

With the prediction that increasing the proportion of cases in which disease is mild or asymptomatic would be a public health victory, universal facial masking like in Japan seems to reduce the rate of new infections and/or to keep people asymptomatic. Another important point to note that case-fatality rates in countries like Japan (0.72 per 100,000 people) have remained low with mandatory population wide masking, even with resurgence of cases after state of emergency was lifted [6]. This could be due to inhalation of low dose viral inoculum as a result of nationwide application of facial mask, subsequent induction of innate immunity resulting in mild infection supporting the process of variolation that was hypothesized before introduction of variola (smallpox) vaccine [4].

The long-term efficacy and acceptable safety of SARS-CoV-2 vaccine are still debatable. Even the efficacy of currently available vaccines against different corona variants is also unclear. While we await the results of further vaccine trials, any public health measure that could increase the proportion of asymptomatic SARS-CoV-2 infections may both make the infection less deadly and increase population-wide immunity without severe illness and deaths. Some important questions still remain, are we perfectly safe after corona vaccination and do we need to use facial masking after vaccination? We do not know the answer of the first question, because we still do not know the long-term efficacy the currently available vaccines. With this uncertain and critical dilemma in mind after vaccination, we ought to continue using facial mask.

At present, vulnerable people across the world are struggling to get corona vaccines. Most vaccination platforms uses a two-dose prime-boost approach to create an immune response against the virus S1 spike protein, the titers of which correlate with functional virus neutralization and increase with boosting [7,8]. To enable larger numbers of people to receive the first dose, delayed administration of second dose has been advocated and implemented by some [9]. A most recent study demonstrated that among previously uninfected seronegative individuals, anti-S-titers after single dose of vaccine were comparable to peak anti-S-titers in individuals with a previous natural infection who had not yet been vaccinated [10]. Among those with a previous SARS-CoV-2 infection, even asymptomatic, vaccination increased anti-S-titers more than 140-fold from the peak pre-vaccine levels [10]. This elegant study dictates the importance of first dose of any vaccine with potential acceleration of vaccine rollout. This study also indicated the significance in the generation of population with asymptomatic infection by mandatory use of facial mask in public as mentioned above.

Among different infection counter measures, practicing and nationwide use of facial masking like in Japan may be one of the fundamental public health protective strategies on this issue in order to protect our society and our future in this deadly pandemic period. Every country has its own policy to counter spread of any infection like SARS-CoV-2 and every person has his/her own human right to follow that policy. When we think about the personal, social and economical sufferings in response to current SARS-CoV-2 infection, we may need to learn some basic and simple policy from others in order to counter this deadly infection. The policies of Japan could be a good model to learn for all of us.

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OVARIAN RESERVE MARKERS

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ABSTRACT

The estimation of ovarian reserve is routinely performed through various tests, in an effort to predict the response and outcome in couples and counsel them. Most widely used tests are Antral Follicle Count (AFC), Anti-Mullerian Hormone (AMH) and basal Follicle Stimulating Hormone (FSH).

Keywords*:* Antral Follicle Count, Anti-Mullerian Hormone, Follicle Stimulating Hormone, Ovarian Reserve.

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INTRODUCTION

Delayed childbearing, voluntary or involuntary, iscommonlyseen in couples seeking fertility consultation. Ovarian Reserve Tests (ORTs) are routinely performed as part of the evaluation of women with infertility. Diminishing ovarian reserve is a phenomenon noted in women during mid to late thirties and at times earlier, reflecting decline in follicular count and subsequently, oocyte quality.[1] This age-related decline of follicles doubles when the numbers fall below a critical figure of 25,000 at approximately 37.5 years of age.[2]

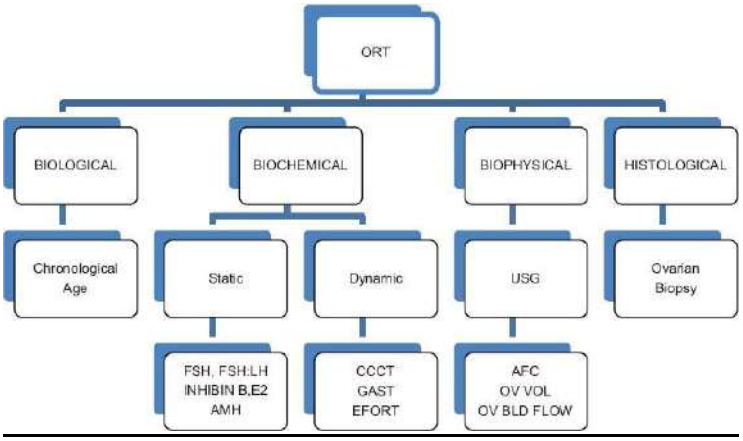
An ideal ORT should be economical, easy to perform, reproducible, and decisions based on them should help differentiate women with a normal and poor ovarian reserve. ORT's, thus help in identifying and counselling couples with negligible chance of conception against any expensive treatment option. However, the availability of multiple ovarian reserve markers suggests that none is ideal. Largely, these tests have been used in infertile women when they first visit a fertility clinic or prior to the first IVF attempt to predict ovarian response. More recently, ORT's are also being used in predicting hyper-response and thus using safe stimulation regimes to prevent OHSS.[3]

The initial evidence suggested that ORTs had a good predictive value for pregnancy.[4] However, in the recent years we have realised thatORT's can only be effective in predicting the ovarian response and not prediction of pregnancy or its outcome.[5] The interpretation of the results is further complicated by the lack of uniform definitions for poor or hyper-responders and uniform threshold values to identify abnormal results.

TYPES OF OVARIAN RESERVE TESTS

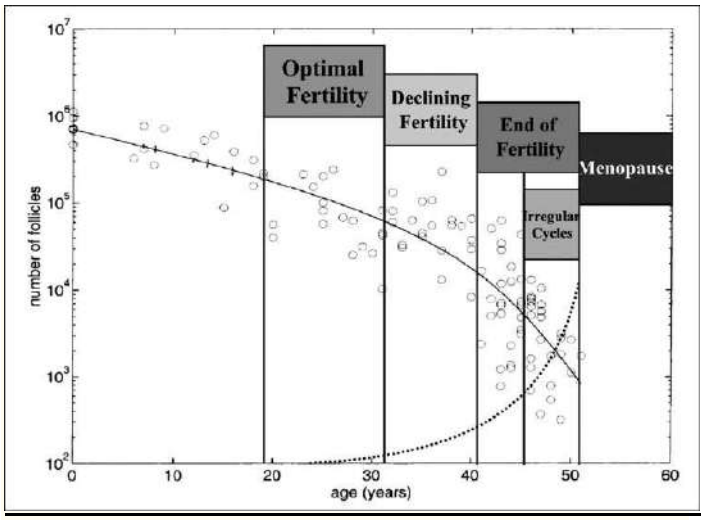
A literature search was made and a total of 308 articles were found.

Biological (age), biochemical, biophysical and histological tests have been used to identify ovarian reserve [Figure 1].



Age

Quantitative (solid line) and qualitative (dotted line) decline of the ovarian follicle pool, which is assumed to dictate the onset of the important reproductive events [Figure 2].



It is long established that ovarian reserve reduces progressively with age.[6] The capacity to reproduce in natural as well as stimulated ovarian cycles declines with maternal age, beginning in the late 20s and becoming more abrupt in the late 30s. Even though fertility does not decline uniformly in women, age is known to be the most important factor determining the pregnancy potential in regularly cycling women.[7] However, chronological age alone has a limited value in predicting individual ovarian responses.[8] This has led to the development and use of various biochemical and biophysical markers of ovarian reserve.

Anti-Mullerian Hormone

Anti-Mullerian Hormone (AMH) is a dimeric glycoprotein produced by granulosa cells of pre-antral (primary and secondary) and small antral follicles in the ovary. The production of AMH starts following follicular transition from the primordial to the primary stage, and it continues until the follicles reach the antral stages, with diameters of 2-6 mm.[9] With the decrease in the number of the antral follicles with age, AMH production appears to diminish and become undetectable after menopause.[10] AMH levels strongly correlate with AFC, measured by transvaginal ultrasonography (TVS).[3] It can be measured on any day of the menstrual cycle, unlike many other biochemical markers [11] and does not exhibit inter-cycle variability.[12] Various threshold values (less than 1.26 ng/ml), have been used to identify poor responders with 80–85% sensitivity and 65–95% specificity.[13] With better understanding of its clinical implications, AMH is now known to predict hyper-response as well, but unfortunately, its high sensitivity is yet to be proven.[3,14] The use of nomograms identifies the age-related physiological decline in the AMH levels, and abnormal deviations can be used for counselling couples wishing to delay childbirth.[15,16] Serum AMH, though cannot be used as a marker to predict pregnancy.[17]

A clear decline in AMH levels with age is noted and it is the earliest marker to show a decline longitudinally in women [18]. It is considered that at levels 0.5–1.26 ng/ml, AMH indicates perimenopausal transition within 3–5 years.[19] Levels within this range still suggest possibility of positive pregnancy results with ART. Of all the ORTs available, AMH has a unique place as it may be applicable as a screening test in a general population seeking fertility.[19]

Basal Follicle Stimulating Hormone

Basal FSH levels measured on day 2 or 3 of the menstrual cycle is used widely to assess the ovarian response to stimulation.[20] An increase in FSH levels occurs due to depletion of follicle. The measurement of FSH is easy, and is relatively inexpensive, though, it is known to have diurnal, intra- and inter-cycle variability.[21] There is no universally accepted cut-off

value to identify a poor response. A wide range in threshold values has been used to define abnormal levels of basal FSH. In regularly cycling women, FSH can predict a poor response adequately only at very high levels, and hence will be helpful only to a small number of women as a screening test. [22] It is understood that the ovarian aging begins several years before any elevation in FSH levels is noted and hence a normal test cannot rule out a poor ovarian response in some women. The usefulness of basal FSH in a general subfertile population or regularly cycling women as a screening test is unclear.[22]

Inhibin B

Inhibin B is a heterodimeric glycoprotein released by the granulosa cells of the follicle. Women with a low day 3 inhibin B concentration (<45 pg/ml) have a poor response to superovulation for IVF and are less likely to produce a clinical pregnancy.[23] Decreased levels ofInhibin B probably precedes the increase in the FSH concentration.[24] However, other investigators have failed to show any added predictive value for inhibin B as a measure of ovarian reserve.[25]At very low threshold levels, the accuracy in the prediction of a poor response is modest [20] and hence its routine use cannot be recommended.

Basal Estradiol

Basal Estradiol (E2) has been evaluated as a marker of ovarian reserve in women, prior to IVF. An elevated basal E2 level may mask abnormal FSH levels. Initial studies have shown an association between elevated basal E2 level and a poor ovarian response.[27] A large study showed that poor ovarian response was commonly seen in women with <20 or >80 pg/ml of E2.[27] A meta-analysis concluded that as basal E2 does not add to the predictive value and hence its routine use in clinical practice is not recommended.[19]

Clomiphene Citrate Challenge Test

Clomiphene Citrate Challenge Test (CCCT) is a dynamic test involving the administration of 100mg of Clomiphene Citrate from the fifth day of the cycle for 5 days. Basal FSH is estimated on day 3 of the cycle and again on day 10. Abnormal values on day 3 or day 10, or on addition of the two, is considered as a predictor of a poor ovarian response. However, a meta-analysis has shown that CCCT is no better than basal FSH in predicting ovarian response.[30] In addition, it has the drawback of being expensive, more time consuming and associated with the possible side effects of administered drugs.[31]

Exogenous FSHOvarian Reserve Test

This dynamic test involves the measurement of basal FSH and E2 followed by the administration of 300 IU FSH on day 3 of the cycle. E2 concentration is determined 24 hours later. It is found to be better than CCCT in predicting hyper-responders and inferior to the latter in predicting a poor response. This test is not recommended for routine clinical use.[3]

Gonadotrophin Releasing Hormone Agonist Stimulation Test

It involves the assessment of serum E2 on day 2 of the cycle followed by the subcutaneous administration of GnRHa (Triptorelin) 100 µg. SerumE2 level is again tested for 24 hours later (on day 3). A rise in E2 level is indicative of good ovarian reserve. It is found to have a good ability for the prediction of poor ovarian reserve but is not superior to inhibin B or AFC.[28]

ULTRASOUND PARAMETERS

Antral Follicle Count

In the early follicular phase, AFs are measured by transvaginal ultrasonography, by taking the mean of two perpendicular measurements. For the total AFC, the numbers of follicles in both ovaries are added. For long, AFC has been used as a marker of ovarian reserve.[18] A count of 8–10 is considered as a predictor of a normal response. Different diameters – those measuring 2–6 and 7–10 mm, are used to define AFs. There is no consensus regarding the size of AFs which truly represent ovarian reserve. With age, the number of AFs 2–6 mm in size declines and it was found to correlate with other markers such as FSH and CCCT. The number of AFsmeasuring 7–10 mm remains constant [30] and hence, the antral follicle count has been noted to be a more reliable marker of ovarian reserve. A limited inter-cycle variability, has been shown by repeated measurements.[30] When compared to the total ovarian volume and basal serum levels of FSH, E2, and Inhibin B on day 3 of the cycle, AFC is considered to have the best discriminating potential for a poor ovarian response. However, to predict the non-occurrence of pregnancy, antral follicle count, lacks the sensitivity and specificity.[29] A good predictor of hyper-response is considered to be a count of more than 14 AFs.[31] In the assessment of ovarian reserve, 3D ultrasound does not have any advantage over 2D.[32]

Ovarian Volume

The ovarian volume is measured by transvaginal ultrasonography applying the formula for an ellipsoid ($D1 \times D2 \times D3 \times \pi/6$). By measuring in three perpendicular directions, the volume of each ovary is calculated.Basal ovarian volume (BOV), is calculated by addingthe volumes of both ovaries. Till the perimenopausal period,the ovarian volume remains unchanged and does not add to the predictive value of AFC.[29,31] In women >40 years, a decline in the ovarian volume is noted.

Ovarian Vascularity

During ovarian stimulation,the ovarian Doppler flow in IVF cycles has been observed and studied. During stimulation, the increase in the Doppler flow noted is considered not to provide additional information to AFC.[33]

Ovarian Biopsy

The follicular density obtained by Ovarian biopsy done at laparoscopy or laparotomy is correlated with the ovarian volume in women >35 years of ageand also appears to reduce with age. Also, compared to women with tubal factor infertility, those with unexplained infertility have fewer follicles. The true follicular density may not be represented by the biopsy as the distribution of follicles is not uniform within the ovary.[33] An invasive ovarian biopsy is not

routinely recommended to be used as an ORT because it does not add to the information available through non-invasive modalities.[34]

DISCUSSION

Women in their mid to late 30s and early 40s constitute an important part of the infertile population and many of them require expensive treatments including assisted reproductive technologies. The primordial follicle pool can be estimated byperforming an ORT. With age,it is well understood that the ovarian follicular pool and hence fertility declines. However, there is a large individual variation in its onset.[1] Minimally invasive, inexpensive, easily measurable and good predictive value for the outcome being assessed are few qualities of the ideal parameter to estimate ovarian reserve. In order to identify high numbers of poor responders, majority of the ORTs need very high threshold levels.[21] Even though ORTs primarily have been used to identify poor responders,hyper-response has been successfully predicted by some ORTs. Hence, maximal ovarian stimulation in such women can be identified and avoided thus, minimizing the risk of life-threatening OHSS without compromising the pregnancy rate.[14]

Majority of the ORTs, including the most widely used basal FSH levels, show abnormal values late in a woman's reproductive life to be of practical help.[21] compromised ovarian reserve is indicated by abnormal results, hence, inflicting a huge emotional and financial burden to such couples as all interventions would be ineffective. No better predictive value was found with Basal E2 and inhibin B as compared to FSH. Though hyper response is predicted with some success by measuring FSH induced increase in the inhibin B level, the basal markers to predict the ovarian response, both poor and hyper, are AMH and AFC. Both, AMH and AFC were found to have a high sensitivity and specificity and are comparable in this regard.[3] Minimal intra- and inter-cycle fluctuations are noted inserum AMH levels and thus can be performed at any stage of the menstrual cycle. As compared to than other markers including AFC, they show distinct age-related declines at a very young age.[18] In infertile women with diminishing ovarian reserve,nomograms have been developed for AMH .At any giventime, it helps to identify in the general subfertile population and thus providing an opportunity to counsel couples appropriately regarding reproductive performance. When the chances of achieving a pregnancy are reasonably good effective modalities of treatment can be accessed by these couples.[15,16] In young women treated for malignancies with chemotherapy or radiotherapy, AMH is the only ORT found to be useful in evaluating the residual ovarian reserve.[34] Dynamic tests cannot be recommended as a diagnostic tool as they do not add to the value of baseline tests.[35]

Using multiple markers instead of single basal marker,as an attempt to improve the predictive value of ORTs was not found to be beneficial. It is clear from the current evidence that ORTs cannot be used as diagnostic tests for a poor ovarian reserve but should be used as screening tests, and the first IVF attempt remains the diagnostic tool to identify a poor response.[21] AFC and AMH can be used as diagnostic tools to identify hyper-responders and treatment strategy modified accordingly to minimize OHSS.[3,14]

The best predictor of pregnancy, despite the multitude of ORTs available, is age. In this regard, ORTs may have a poor predictive value due to the fact that chance of pregnancy after IVF depends on many other factors than ovarian reserve alone. Also, after an ORT, the first IVF cycle alone is used to usually assess the pregnancy outcome which may not adequately represent a woman's true reproductive potential.[21]

CONCLUSION

ORTs do have a moderate ability to predict poor and hyper-response.The information though can be used to counsel couples regarding their reproductive statusand also to influence the treatment protocol to be chosen. No couple should be denied ART services at least once irrespective of their ORT results.The present evidence shows that AFC and AMH appear to be the most useful markers of ovarian reserve. In addition AMH has the ability to be applied to the general population for identification of diminishing ovarian reserve before it reaches a critical level below which successful reproductive outcomes are difficult to obtain.

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Neonatal Uterine Bleeding and early onset of Endometriosis

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Abstract

In this review article we have try to get evidences for the relationship between neonatal uterine bleeding and early onset endometriosis. 3 -5% of the neonates are found to have neonatal uterine bleeding while occult bleeding was detected in 25 -65% in neonates.During early gestation cervical length is larger than the uterine corpus. When the cervix getscanalized, it becomes patent. Later on, it gets occluded by the thick mucus plug under the influence of progesterone. There is retrograde reflux of the endometrial cells due to pseudo-occlusion of the cervical canal. The endometrial responds to maternal hormone like oestrogen and progesterone. Hence, the endometrium can be either proliferative or secretory in nature. Children with neonatal uterine bleeding develop progesterone resistance which is genetic and leads to the development of early onset endometriosis. The endometrial progenitor stem cells remain dormant in the peritoneal cavity and later under the influence of the oestrogen which is produces at the onset of thelarche and develops into endometriosis. These children when they grow up progesterone resistance persists which may lead to high risk of developing poor placentation which leads to increased incidence of preeclampsia, preterm labour and FGR. Neonatal uterine bleeding is more common in post term babies and children of mother who have maternal preeclampsia and Rh incompatible mothers. Preterm babies have the lowest risk of developing neonatal uterine bleeding. Low birth weight babies are prone for DIE as they have placental insufficiency or chronic hypoxia.Earlier neonatal uterine bleeding was not given any importance but now we know it is a marker for early onset endometriosis and adverse reproductive events.

Keywords: Neonatal uterine bleeding; Retrograde menstruation; Early onset endometriosis; Adverse effects on pregnancy

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Introduction

A new theory to explain early onset endometriosis which occurs in pre-menarchial girls can be explained based on the finding of neonatal endometrium that shows proliferative orsecretory activity under the maternal hormone influence. These babies present with neonatal uterine bleeding, with shedding of endometrium, once when maternal progesterone withdrawal occurs. This happens in 3-5% of neonates which could be due to functional plugging of endocervical canal by mucus plug which in turn, leads to reflux of menstrual debris. Neonatal uterine bleedingis more often seen in postmature babies rather than preterm babies. Autopsy of female new born with hydro-metro-colpos showed ectopic endometrial implantation. The same pathogenesis is observed in adolescents with outflow obstruction to the Mullerian tract and they develop early onset endometriosis. Children with neonatal uterine bleeding develop progesterone resistance which is genetic and at later days, during their pregnancy they are at high risk of developing poor placentation which leads to increased incidence of preeclampsia, preterm labour and FGR. Neonatal uterine bleeding should arouse the suspicion of early onset endometriosis and adverse reproductive outcomes when they grow up and hence neonates with neonatal uterine bleedingshould be monitored.

Incidence

Incidence of Neonatal uterine bleeding was reported between 3.3 – 5.3 % while occult blood was detected in 25.4 – 65.3% of the neonates[1].Neonatal uterine bleedingoccurs in < 08% of preterm babies, 4.4% among term babiesand 9.1% post term babies[2].

There was relationship between maternal preeclampsia (32% – 47.5%) depending upon the severity. Neonatal uterine bleeding is seen in 14% of babies of Rh incompatible mothers[3].

Anatomy of neonatal uterus and cervix

During the early gestation, uterine length will be less than cervical length. Cervix will be 2-2.5 times more than the corpus. When it gets canalised, the cervical canal becomes patent. Later, the cervix gets occluded by the thick mucus plug under the influence of progesterone[4]. This pseudo-occlusion is the predisposing factor for the retrograde menstruation which sheds the endometrial cells into the peritoneum via the tubes which is of significance in the pathogenesis of endometriosis.This can be extra- polated to adolescent age girls with Mullerian anomalies and outflow obstruction who have early onset endometriosis. The probable hypothesis which can be postulated is that either functional (or) anatomical outflow obstruction can increase the risk of retrograde uterine bleeding in the peritoneal cavity of the neonate.

In a Vaginoscopic study, Terruhn*et al* (1979) found ectropion of uterine cervix which is physiological at birth and at puberty. A case of neonatal endometriosis has been published by Arcellana*et al* where the foetus had hydro-metro-colpos and McKusick-Kaufman syndrome characterised by vaginal agenesis or stenosis. The foetus was found to have large pelvis cyst which contained brownish fluid and the post mortem of the neonate showed fragments of endometrium embedded over the ovaries, uterus and the serosa of sigmoid colon. Histopathology showed ectopic endometrial tissue.

Pathogenesis

Endometriosis is a chronic inflammatory disorder where there is presence of ectopic endometrial tissue which is oestrogen dependent and progesterone resistant. There are various aetiologies for origin of endometriosis like Sampson's spill theory, Meyer's metaplastic theory, Halban's lympho vascular theory, immunological theory of Semino, genetic theory,exposure to pollution especially dioxins and persistence of embryonic endometriosis as described by Signorile*et al*.

As per Sampson's spill theory, there is menstrual regurgitation which leads to implantation of the debris into the peritoneal cavity causing endometriosis. According to this theory, endometriosis cannot occur prior to puberty, as retrograde menstruation occurs only after puberty. But in reality, endometriosis is found to occur prior to puberty, as early as thelarche starts. The pathogenesis in this situation differs from adolescent and adult endometriosis [4].

Though the hormonal environment is the same for all neonates, why < 5% of the neonates have bleeding is really fascinating. Probably these new-borns are at high risk of developing endometriosis because of genetic factor or immune abnormality. The reason why neonatal uterine bleeding is rare despite high circulating levels of progesterone can be attributed to progesterone resistance present in a majority of neonates. Recent work indicates that neonatal uterine bleedingrepresents a significant biomarker for events that can occur later-on during adolescence.

Study by Ober and Bernstein demonstrated that the endometrium of new born show varying response to progesterone, ranging from proliferative (65%), secretory (27%) and decidual changes (5%) [5]. They also demonstrated the presence of clotted blood in the endometrial cavity. Because of the lack of foetal ovarian activity, authors concluded endometrial changes could be secondary to maternal hormones. Inspite of high circulating levels of progesterone, decidual changes are found to be rare, which could be attributed to the progesterone resistance, which is the key factor in endometriosis.

To date, no direct evidence for the role of endometrial stem or progenitor cells in the pathogenesis of endometriosis has been reported, but clinical observations supporting a link between neonatal uterine bleedingand adolescent endometriosis have been documented by the occurrence of pelvic endometriosis in a new born[5]. Epidemiological evidence also supports this hypothesis, because of the low risk of endometriosis in females born preterm[6] and the higher risk in females with a low birth weight[7].

Neonatal uterine bleeding causes tubal spillage into the peritoneal cavity and the stem cells or the progenitor cells get into the peritoneal cavity. They remain dormant till the time of thelarche, and the cells get activated under the influence of oestrogens and survive leading to early onset endometriosis. Estrogen production around the larche stimulates angiogenesis and promotes the development of endometriotic lesions. Earlier the occurrence of menarche, adolescents can develop aggressive endometriotic disease due to seeding of more multipotent endometrial mesenchymal stem cells or endometrial progenitor cells which are long lived. Relationship between neonatal uterine bleeding and early menarche is poorly understood. Serbian study showed preterm babies have lower risk of endometriosis as they have decreased incidence of neonatal uterine bleeding whereas, low birth

weight babies are prone for two-fold increase in DIE. Low birth weight babies have placental insufficiency and chronic hypoxia and they are related to neonatal uterine bleeding, which could be an early marker for early onset endometriosis. This could be due to epigenetic origin of endometriosis.

The concentration of progesterone in the fetal circulation increases dramatically to levels much higher than those found in the maternal circulation[8]. Some form of progesterone resistance prevents neonatal uterine bleeding. When there is sudden reduction in progesterone levels in new born, it leads to neonatal uterine bleeding. We have proposed that this is due to a developmental form of progesterone resistance, a concept that is supported by classic studies of pathology[9, 10]. This progesterone resistance is defined as the decreased responsiveness of target tissues like the endometrium to ever increasing levels of bioavailable progesterone[11], hence explaining the absence of any neonatal uterine bleeding in a majority of neonates.

Earlier, neonatal uterine bleeding was not given any significance, but now it is found to have association with early onset endometriosis.French and German literature searchrevealed that 3-5% of neonates showed neonatal uterine bleeding and 25-60%[12] showed occult bleeding which lasted for 3-7 days after birth. Neonatal bleeding is as a result of maternal hormonal withdrawal bleeding. The endometrial stem cells become disseminated into the pelvis and they remain quiescent till the girl attains menarche.

For the past 30 – 50 years, there has been a sparse paper published regarding neonatal uterine bleeding. Study by Huber *et al*in 1971[13] evaluated the post mortem histopathology of the uteri of foetuses, infants and children. 82 samples were obtained. In Immature fetus< 20 weeks, there was no glandular development. After these, proliferative changes were seen followed by secretory changes beyond 34 weeks of gestational age. This may not be similar to adult female. Soon after birth, the endometrium sheds off due to sloughing and bleeding occurs which is followed by thinning of endometrium with theabsence of glycogen. These changes might take almost 3 months and endometrium remains dormant up to or thelarche starts. Other studies have reported disintegration of secretory glandular structures and areas of pre-decidualised stromal cells in endometrium five days after birth. Endometrium is found to be thin, atrophic with scanty stroma (Kaiser *et al* 1974). The nucleus of endometrial glands increases in size along with gestation and plateaus at term. From third week of neonatal life till the age of seven, the endometrium becomes inactive. Then between the age of eight and eleven, the stromal cell nucleus enlarges. Foetal and infantileendometrial tissue may not react in the same way as an adult and may not reach the actual maturity.

Marsh and Laufer*et al*[14] have reported, laparoscopic proven endometriosis in 5 pre-menarchial girls between the age of 8 – 13 years. There was severe lower abdominal pain in these girls for a period of more than 6 months though they did not have any obstructive Mullerian anomaly. Thelaparoscopicappearances in these girls were of subtle type of clear or red vessels with extensive micro vascularisation which was excised and these girls showed post-operative reduction in pain.

Biopsies showed fibroconnective tissue, lined by mesothelium, granulation tissue, vascular proliferation, hemosiderin deposits and

macrophages but absence of glandular structure. Stroma is found to be more than glandular tissue in early onset endometriosis. Ovarian endometriosis has been reported in pre-menarchial girls. Pre-menarchial endometrioma could be due to the activation of endometrial cells that are shed into the peritoneum at the time of neonatal uterine bleeding. They remain dormant and get activated by the angiogenesis before menarche, ideally during thelarche when there is optimal threshold level of estrogen.

VEGF expression by endometrial cells varies during the phases of menstrual cycle with higher levels during the proliferative phase.Angiogenesis helps in implantation of the endometrial debris which has remained dormant since neonatal period and developed into early onset endometriosis. Because of extensive angiogenesis the lesions are red peritoneal deposits, haemosiderin pigments and even endometrioma can develop.

Recent studies have proved that adolescent endometriosis is becoming more common and severe which is an extrapolation of early onset endometriosis.

The challenge today is to test the hypothesis that neonatal uterine bleeding is a biomarker for subsequent reproductive disorders. Progesterone resistance is physiological in new born babies, and could be linked to foetal distress. If progesterone resistance continues beyond menarche there are chances of developing obstetrical placental syndromes when pregnancy occurs in teenage[15].Persisting biological immaturity until adolescence may result in defective deep placentation in the event of a very early pregnancy, increasing the odds of major obstetrical disorders, such as preeclampsia, low birth weight, or spontaneous preterm labor[16]. This requires the conduct of prospective studies aimed at ascertaining whether the progesterone response of the neonatal endometrium is a key factor for reproductive health in the adolescence and beyond[17].

The observation of the “little bleeding from the vagina” described in the lay press as perfectly normal, may indeed be “normal,” but it does not seem to be “unimportant,” or without consequences. For these reasons, its presence should be recorded carefully, and the hypothesis that this is an endometrial marker of fetal distress should be explored. Neonatal uterine bleeding is related to post mature and dysmature babies. Decidualization could be due to chronic foetal hypoxia as evidenced in post and dysmature babies. Hence neonatal uterine bleeding can be a marker for foetal distress in utero.

Only systematic recording of the presence or absence of neonatal uterine bleeding will allow the prospective examination of this hypothesis. Hence, there should be creation of an international registry for neonatal uterine bleeding, coupled with links in adolescence and first pregnancy, to determine whether this simple sign can identify patients at risk for adverse reproductive outcomes in future.

Diagnostic Delay

It must be stressed that the diagnosis of endometriosis in adolescents remains a challenge and is almost inevitably delayed for several reasons and sometimes for a very long period of time. This diagnostic delay is consequential, because it allows endometriotic implants to progress toward the more destructive stages of the disease, with an often-irreversible impact on the reproductive potential and the ovarian

reserve of these young women. Surgical treatment at that late stage is also more likely to have an additional negative impact on reproductive success. Recent studies have estimated the length of diagnostic delay in surgically treated endometriosis patients may be up to a decade. It would not be surprising that, taking into account the postnatal incubation time, the interval between the primary endometrial shedding and the development of active lesions may involve an even longer delay.

A survey conducted by the World Endometriosis Society reported that the average time between the onset of pain and the final diagnosis of endometriosis is 9.3 years[18]. When the endometrium shows resistance to the action of progesterone at birth, not only will the female new born not show any sign of either visible or occult neonatal bleeding, but this resistance is likely to persist till the onset of menarche and even beyond.

Conclusion

The progesterone response in the neonatal endometrium exhibits a spectrum of biological maturity, varying from an absent to a full response. This will result in menstrual-like bleeding at birth in a small minority of cases, whereas in the majority of new born girls, progesterone-resistance may persist till the onset of menarche. The presence or absence of neonatal uterine bleeding can have consequences: on one hand, tubal reflux and pelvic seeding of endometrial stem or progenitor cells in the neonate can increase the risk of early-onset endometriosis.

In conclusion, the fundamental question is whether the presence of neonatal uterine bleeding may represent a warning sign of the future development of early onset endometriosis and increase the awareness of a devastating disease in the young woman. It is well appreciated that endometriosis may be present for a long time before a diagnosis is established. Neonatal uterine bleedingshould not be ignored, but actually may represent an important biomarker for reproductive events that can occur during adolescence.

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Sonographic Panorama of PCOS to explain its endocrinal orchestra

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Abstract

Polycystic ovarian syndrome (PCOS) is one of the most controversial diagnosis in the females of reproductive age group. It is often either under or over diagnosed. The controversies are because of metabolic derangements along with endocrinal derangements and that too every component may have variable severity. This leads to different clinical presentations and also a huge variation in laboratory parameters. Though Insulin, androgen and luteinizing hormone (LH) are the main players in the entire syndrome, these may at different times and in different patients express differently and depending on their expression the patients with PCOS may have variable ultrasound presentations. Vascular endothelial growth factor (VEGF) and LH lead to increased stromal vascularity, hyperinsulinemia and LH lead to stromal abundance and hyperandrogeneima leads to more antral follicles.

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Introduction

Polycystic ovarian syndrome (PCOS) is one of the most controversial diagnosis in the females of reproductive age group. It is often either under or over diagnosed. The controversies are because of involvement of metabolic derangements along with endocrinal derangements. The severity of these derangements may vary patient to patient. Hyperinsulinemia is a major component of metabolic derangement. It is associated with lipid metabolism derangement. Androgen and luteinizing hormone (LH) derangements are primary endocrinal components of the syndrome. It is therefore that all the concerned specialists have evaluated this syndrome from a different perspective. As reproductive medicine specialist, our major concern is the impact of the syndrome on the female reproductive system. Considering this in 2003 ESHRE/ASRM meet in Rotterdam, a consensus was formed for the diagnosis of PCOS. According to this consensus, that still prevails as the latest one, PCOS is diagnosed by presence of two of the three following criteria[1].

- Oligoovulation (cycle length of longer than 35 days) or anovulation.
- Biochemical or clinical (diagnosed by acne/ hirsutism) hyperandrogenemia.
- Polycystic ovaries on ultrasound (ovary that is > 10cc in volume and/or has more than 12 antral follicles).

Moreover very interestingly, because any two of these three criteria may diagnose PCOS, four phenotypes have been defined for the syndrome.

- Type A – hyperandrogenism + oligo-anovulation + polycystic ovaries
- Type B – hyperandrogenism + chronic anovulation
- Type C – hyperandrogenism and polycystic ovaries
- Type D – oligo-anovulation and polycystic ovaries

Considering these, ultrasound does play a major role in the diagnosis of the syndrome in at least three of the four phenotypes. But the role of ultrasound in PCOS is much more than what has been presumed from this introductory discussion.

In 2018, during the ESHRE meeting certain very important amendments were suggested in context with the Rotterdam criteria, regarding diagnosis as well as management strategies. This also included change in the number of antral follicles per ovary to 20 from 12.

How and when is this scan done to confirm the diagnosis of polycystic ovaries?

For PCOS diagnosis, USG scan has to be done, when oestrogen and progesterone levels are at baseline, ovaries are silent, and have no active follicle or corpus luteum. It is preferred to do Transvaginal scan as transabdominal approach for ovarian assessment may miss at least 42% of the ovarian anatomical details.[2]All the scans are done using B mode ultrasound with colour Doppler, pulse Doppler, 3D ultrasound and 3D power Doppler.

Techniques for Baseline scan of Ovaries:

B mode ultrasound assessment of the ovaries consists of assessment of ovarian diameters and volume and counting of antral follicles as quantitative assessment and stromal echogenicity as qualitative assessment.

The transverse diameter is measured as longest diameter on the transverse section. Ovarian volume can be calculated by the formula (x x y x z x 0.523), where x, y and z are long, transverse and AP diameters in cms. (Figure 1)

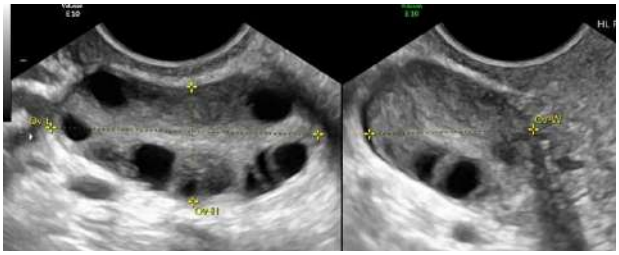

Figure 1: Orthogonal diameters of the ovary to calculate the ovarian volume.

Number of antral follicles are counted in the whole ovary by eyeballing while taking a 2D sweep across whole ovary.Follicular number per ovary (FNPO) with 2D or 3D have given identical results.[3]But when number of follicles is much more as in polycystic ovaries, the calculation using b mode scroll may be inaccurate.More exact value of AFC was acquired when counted by 3D US. Number of follicles > 12mm on day of oocyte retrieval correlated significantly with AFC counted by 3D US with inversion mode rather than 2D US[4, 5]. It has been shown that SonoAVC(figure2) took significantly less time to measure the size and record the number of antral follicles.(132 ± 56.23s vs. 324.47 ± 162.22s).[5]The over counting of follicles is prevented by this colour coding. Post processing can be used for the follicles that are missed out on automated counting. Further the size of the follicles can be a clue and can be correlated with the biochemical parameters of PCO.

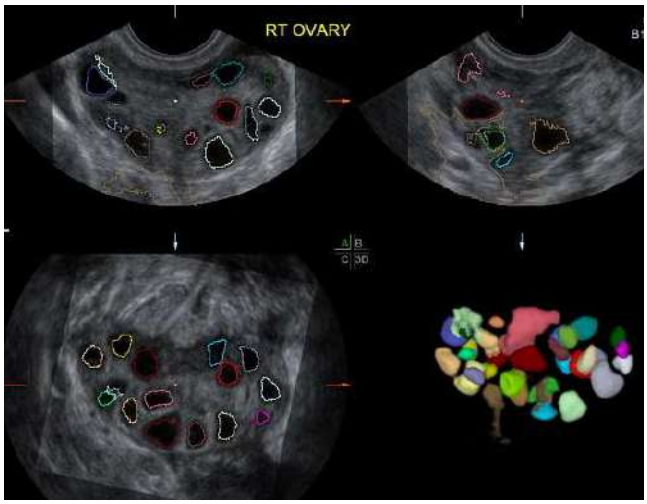

Figure 2: Sono AVC showing colour coded antral follicles.

Ovarian stromal echogenecity is assessed in comparison with the echogenecity of myometrium, especially if ovary and uterus are at almost same depth from probe. Normally ovarian stroma is hypoechoic or isoechoic to normal myometrium.

The vessel selected for interrogation is a vessel that shows brightest colour on colour Doppler, is not close to follicle and is not in continuity with the main ovarian artery. (Figure 3) For colour Doppler pulse repetition frequency (PRF) is set at 0.3, wall filters lowest with optimum gains and balance settings. For pulse Doppler PRF is set at 1.3 and wall filter at 30 Hz as stromal flows at base line scan are low velocity flows. Pulsed Doppler is used for quantitative assessment of the flows - intraovarian resistance index (RI) and peak systolic velocity (PSV).

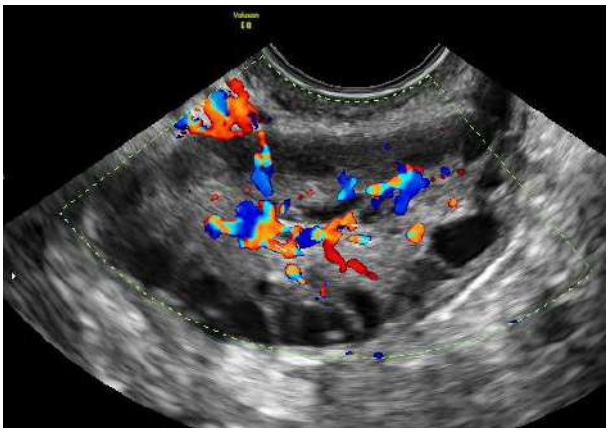

Figure 3: colour Doppler showing ovarian stromal flow.

3D ultrasound provides a new method for objective quantitative assessment of follicle count with Sono AVC, ovarian volume, stromal volume and blood flow in the ovary[6.] (Figure 4).The 3D power Doppler volume of ovary is used to calculate ovarian volume and stromal volume and to assess global vascular indices VI (vascularity index), FI (flow index) and VFI (vascularity flow index) (figure 5, 6).

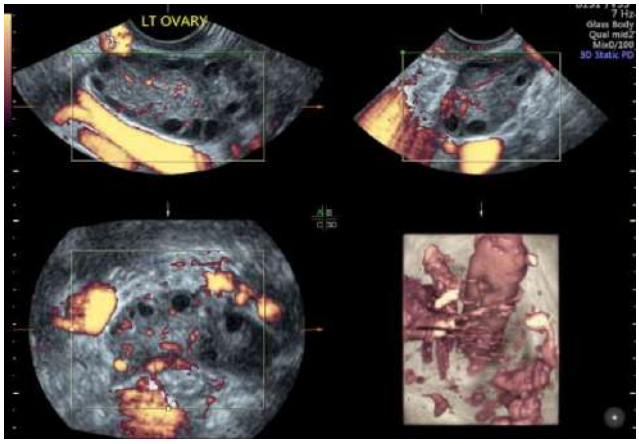

Figure 4: 3D power Doppler volume acquired by 3D ultrasound.

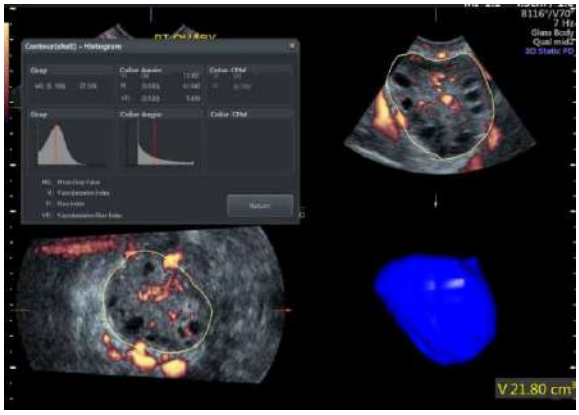

Figure 5: volume histogram of 3D PD volume of ovary showing VI, FI and VFI calculations.

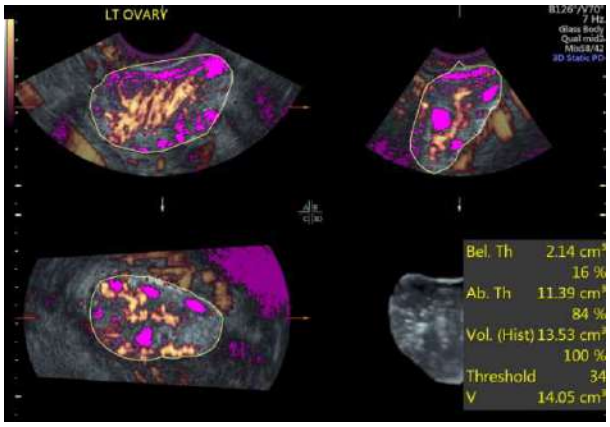

Figure 6: 3D power Doppler with volume of the ovary, VOCAL calculation of the same is done and then threshold volume is applied to calculate the stromal volume.

Controversies and interpretations related to ultrasound findings:

Ovarian volume:

"Enlarged spherical ovaries > 10 cc, has shown good correlation between ultrasound and diagnosis of polycystic morphology and histopathological criteria for polycystic ovaries[7]." But there are controversies regarding the ovarian enlargement in PCO. The currently accepted cut-off of >10 cm^3 was associated with 98.2% specificity, but only 45% sensitivity, in discriminating between normal and polycystic ovaries[7].

Since 2003, both a lower threshold of 7 cc[8]and a higher threshold of 13 cc[9] have been proposed as being more appropriate thresholds for polycystic ovarian morphology.

Concerning the ovarian volume setting the threshold at 7 cc offered the best compromise between specificity (91.2%) and sensitivity (67.5%) [7].Ovarian volume 6.6 cc has shown 91 % sensitivity and 91 % specificity for polycystic ovarian syndrome[10]. Moreover according to S.Kupesic, ovaries that are normal in volume can be polycystic as demonstrated by histological and biochemical studies (in 20%).

This discussion indicates that ovarian volume alone cannot be used as a parameter for diagnosis of polycystic ovaries. Polycystic ovarian morphology has therefore been found to be a better discriminator than ovarian volume between polycystic ovarian syndrome and control women[11].Morphological characteristics of polycystic ovaries consist of number and arrangement of antral follicles, stromal echogenecity and vascularity.

Antral follicle count (AFC):

According to Rotterdam criteria, antral follicle count of 12 / more (2 - 9mm) had been used as a characteristic for polycystic ovaries. Setting the threshold at 12 for 2 -9 mm FNPO (follicle number

per ovary) offered the best compromise between declines in the AFC because of atresia of these follicles. And therefore PCOS patients with advanced age may have an AFC < 12 also. Dewailly et al in 2011 showed that FNPO > 19 had a sensitivity of 81% and specificity of 92% for PCO respectively. But it also mentioned that the higher number is because of increased resolution of new scanners to identify follicles smaller than 2mm. Adding AMH > 35pmol/l as a cut off further adds to the accuracy with sensitivity of 92% and specificity of 97% for the diagnosis of PCO[13]. Another study published in 2013 suggested that an average value of 26 or more follicles per ovary is a reliable threshold for detecting polycystic ovaries in women with frank manifestation of PCOS. It's sensitivity and specificity for diagnosis of PCOS for FNPO (26) was 85% and 94% and for OV (10cc) was 81% and 84%[14]. But a very important and interesting statement in this study is that the lower follicle threshold may be required to detect milder variants of the syndrome. This variability in the antral follicle count can be explained by the variable androgen level in different patients of PCOS. This is because androgen is responsible for recruitment of preantral follicles to antral. At this stage a short understanding of pathophysiology and hormonal correlation of ultrasound findings may be helpful. using ROC (receiver operating characteristics) curves, the investigators reclassified the diagnostic threshold for PCO as a mean FNPO of 20 or more [with the area under curve (AUC) of 98.7%, specificity of 100%, sensitivity of 70%, positive predictive value (PPV) of 100%, and negative predictive value (NPV) of 91%] and an ovarian volume of atleast 13 cm3 (with AUC of 94.8%, specificity of 100%, sensitivity of 50%, PPV of 100%, and NPV of 85%) [9](Fig. 6.16).By using different threshold volume and AFC at different ages, best sensitivity and specificity for diagnosis of PCOS can be obtained.[15]

Pathophysiology:

Polycystic ovaries are a result of chronic anovulation. Mildly raised androgen levels in early follicular phase in PCOS patients can lead to recruitment of several follicles. Androgen leads to early follicular development but further progression is not normal due to hyperinsulinemia and/or other metabolic influence linked to obesity[12]. All these follicles do not become dominant, beyond 6-7 mm size; these follicles develop FSH receptors and therefore cannot grow with low FSH levels. The FSH levels are low because there is partial conversion of androgen to oestrogen and there is also cumulative effect of minimal oestradiol production by multiple follicles. This causes negative feedback mechanism for FSH and positive feedback for LH which leads to maturation arrest and premature luteinisation leading to atresia. Theca cells of these atretic and other follicles contribute to the stroma leading to stromal abundance.

Hormonal correlations of AFC and ovarian volume in PCO:

Antimullerian hormone (AMH) is a biomarker produced by granulosa cells of antral follicles. Antral follicle count and ovarian volume showed significant correlation with AMH, total testosterone and free androgen index[16.]

AMH, obesity, IR and high androgen levels relates to large size of antral follicle pool and ovarian volume on PCOS[16].

The mean follicular number per ovary (FNPO) of follicles 2 – 5 mm in size was significantly higher in polycystic ovaries than in controls, while it was similar within 6 – 9 mm range. Within 2 – 5 mm range, significant relationship was found between FNPO and androgens but FNPO in the range of 6 – 9mm was significantly and negatively related to body mass index and fasting serum insulin level.

Arrangement of follicles:

The antral and atretic follicles get arranged peripherally or are dispersed in the stroma and thus may categorize polycystic ovary as peripheral and general cystic pattern. In peripheral cystic

pattern there is typical garland like arrangement of follicles and in generalized cystic pattern, the follicles can be seen throughout the ovary[17].(Figure 7). Though one school of thoughts believe that peripheral cystic pattern polycystic ovaries and generalized cystic pattern polycystic ovaries have different histopathological and endocrine bases[18], another theory is different. According to this, the ovary is multifollicular in adolescence. This is because, during the pubertal transition, adolescents have relative androgenemia, insulin resistance, cystic ovaries and anovulatory cycles, which transition into an oestrogenic state later in puberty.[19]

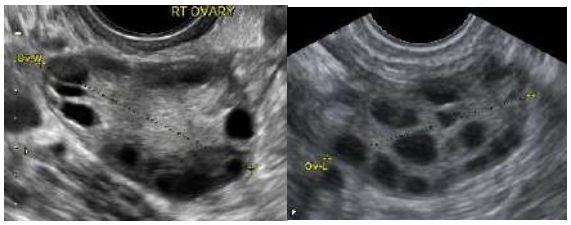

Figure 7: polycystic ovaries: peripheral cystic pattern, generalized cystic pattern.

Because of the pathophysiology explained earlier, follicles that are not mature are exposed to LH and undergo atresia and due to their exposure to LH the granulosa cells get converted into theca cells and adds to the stromal content. If it is left untreated, gradually the follicles in the central part of the ovary, in an effort and process of recruitment reach the periphery or are pushed out to periphery by expanding stroma and undergo atresia ultimately leading to peripheral cystic polycystic ovary[20], [21].Thus, multicystic ovary to generalized cystic PCO, to peripheral cystic PCO is a process of evolution of the disease. This indicates that the patients having more severe form of disease or long standing diseases have a peripheral cystic pattern and evidently will have worse hormonal milieu as compared to those who have generalised polycystic pattern.

Stromal abundance:

Stromal abundance has been described with polycystic ovaries since the first definition of the syndrome by Stein-Levanthal[22].

Stromal hypertrophy was recognized as a frequent and specific feature in ovarian androgenic dysfunction[23]. Another study also shows that patients having long standing PCOS and long standing anovulation have more dense stroma and this cardinal feature has been shown as presence of a bright, highly echogenic stroma on transvaginal ultrasound[24]. And even when stromal hyperechogenecity is seen in normo-ovulatory women it is not a variant of normal but a silent form of PCOS[25]. A study revealed that clinically and laboratory proven PCO showed the typical morphological changes in ultrasound examination in 91.1%; however, only 30 ovaries (16.6%) showed increased volume. In patients, 16 (8.8%) ovaries showed normal morphological appearance while the rest (91.1%) showed morphological picture of PCO in the form of detection of 10 or more cysts of 2–8 mm in diameter peripherally arranged around an echodense stroma[26].

Most severe form of stromal abundance, hyperthecosis, presents large ovaries with almost absence of cystic lesions: solid looking ovaries. These studies clearly indicate that stromal abundance is probably the most consistent feature of PCO.

Cause of stromal excess:

Hyperinsulinemia is a key factor to the pathogenesis of PCOS[27]. Insulin augments LH stimulated androgen production by stromal cells. Androgen in turn causes proliferation of stromal and theca cells. Theca cells of PCOS women hyper respond to gonadotrophins (LH) and produce excess androgens. This is due to an escape of their normal down regulation to gonadotrophins. This dysregulation is linked to excess of insulin and IGF-1 to increased stroma in the PCO.

Stromal volume was positively correlated with serum androstenedione concentrations in patients with polycystic ovarian syndrome[28]. Stromal abundance, one of the most consistent features seen on ultrasound in PCOS patients, can be correlated with hyperinsulinemia, high LH and hyperandrogenemia.

Assessing stromal abundance and hormonal relevance:

Increased stromal echogenecity for diagnosis of PCO has a sensitivity of 94% and specificity of 90% [29]. But assessment of this hyperechogenecity is subjective not only to the operator but also to equipment settings[24]; [30.]Though ovarian stroma can be stamped as hyperecoic when it is more echogenic than myometrium (figure 8a, b).

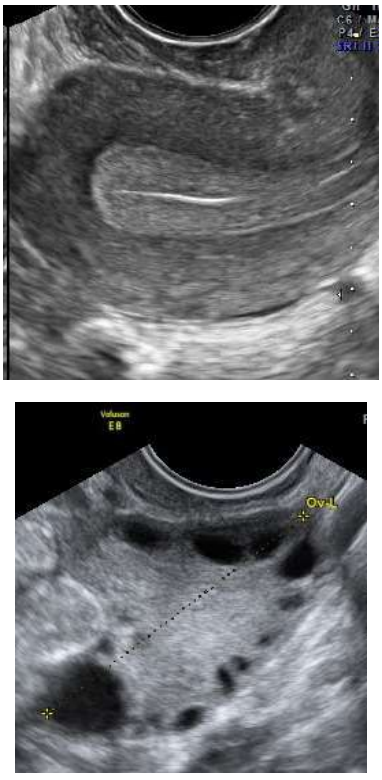

Figure 8a: B mode image of normal uterus and myometrium, b. B mode image of ovary, with hyperechoic stroma.

Moreover, recent studies have shown that mean stromal echogenecity or total ovarian echogenecity as measured by histogram are not different in controls and PCOS. But Stromal index (stromal echogenecity/total ovarian echogenecity was significantly higher in PCOS than controls[31].

Stromal abundance can be measured on ultrasound as stromal area in the most longitudinal section of ovary on 2D US. Stromal area of 4.6cms^2 has 91 % sensitivity and 86 % specificity for diagnosis of PCOS. Ovarian area of 5.3 cm^2 has 93%sensitivity and 91% specificity for diagnosis of PCOS[11]. Stromal area/ovarian area ratio of > 0.34 is diagnostic of PCOS and can be correlated with S. androstenedione[23].

Mean stromal area /mean ovarian area ratio of 0.34 and above also has a specificity of 100% in the same study[23].Moreover, S/A ratio also has a strongest correlation with S. androgens especially testosterone and androstenedione and insulin[32]. The proportion revealed between the stroma and the ovary surface in the median section (S/A ratio) had been indicated as a reliable marker for hyperandrogenism. Hyperandrogenic subjects showed higher values of stromal area and S/A ratio, with no difference in ovarian volume and ovarian area[33]. This parameter may be used in routine clinical practice for improving US diagnosis of PCOS[23].

Stromal abundance may be better assessed by stromal volume than with stromal area. 3D ultrasound provides a new method for objective quantitative assessment of follicle count, ovarian volume, stromal volume and blood flow in the ovary [34.] Stromal volume can be assessed by using threshold volume on vocal calculated ovarian volume.

A prospective study of 50 polycystic ovarian syndrome patients with 50 non PCOS patients showed p positive correlation between ovarian and stromal volumes and fasting and postprandial insulin levels. With Pearson correlation significance level of 0.01 (2 tailed) the correlation for

- ovarian volume to fasting insulin is 0.651

- ovarian volume to PP insulin is 0.409
- stromal volume to fasting insulin is 0.736
- stromal volume to PP insulin is 0.428

Stromal volume could be correlated with fasting insulin levels followed by ovarian volumes and AFC[35].The ovarian stroma/total ovarian volume ratio was the most accurate predictor of both hyperandrogenemia (area under the curve, 0.915; P < .0001) and hirsutism (area under the curve, 0.891; P < .0001).[36]A similar study has also been done earlier. Study by Pache et al has shown that the degree of insulin resistance can be correlated with ovarian volume and stromal echogenecity[37] .To add avery interesting study to this shows that obesity and insulin resistance may enhance follicular excess by dysregulation of AMH through pathway of hyperandrogenemia[16]. And another study to support this, in classic PCOS, follicular number was positively related to insulin resistance and biochemical hyperandrogenism[38].

Stromal vascularity:

Women with PCOS have an increased stromal volume and vascularity. In polycystic ovaries even on 3rd day of the cycle intraovarian stromal flow is seen with moderate to low resistance flow with RI of 0.50 – 0.58[39].3D ultrasound clearly showed higher AFC (median 16.3 v/s 5.5 per ovary), ovarian volume (12.56 v/s 5.6ml), stromal volume (10.79 v/s 4.69ml) and stromal vascularization (VI 3.85v/s 2.79%, VFI 1.27 v/s 0.85)[40]in PCOS patients.

Increased ovarian stromal blood flow in PCOS may be because of over expression of vascular endothelial growth factor(VEGF), which modulates the permeability of theca cells and increase insulin like growth factor 1 (IGF 1)[41]. Elevated LH levels may be responsible for increased stromal vascularization due to neoangiogenesis, catecholaminergic stimulation and leukocyte and cytokine activation. In 22% of GCP PCO intraovarian vessels are not recognized[42]. And this can be explained by the milder form of derangement in the GCP PCO patients. Stromal vascularity is significantly higher in women with PCOS who are hyperandrogenic and lean rather than normoandrogenic and obese[43].Ovarian stromal FI is higher (33.94 v/s 29.30) in hirsutes than in normoandrogenic PCOS women.

In PCOS women with obesity the vascularity was lower than in normal weight women (VI 3.25 v/s 4.51%, VFI 1.22 v/s 1.56)[40, 44.] 3D vascularization quantification has been found to be more sensitive than 2D vascularization quantification[45.]It is evidently this differential ovarian stromal vascularity that that explains variable sensitivity of PCOS patients to ovulation induction drugs as stromal blood flow velocity after pituitary suppression is an independent predictor of ovarian response[46].Measurement of ovarian stromal flow in early follicular phase is related to subsequent ovarian response in IVF treatment[47].Ovarian stromal PSV after pituitary suppression is predictive of ovarian responsiveness and outcome of IVF treatment[48]. Kupesic has shown correlation in the ovarian stromal flow index and number of mature oocytes retrieved in an IVF cycles and pregnancy rates[48].

Tonic secretion of LH in early follicular phase in PCOS is also associated with theca and stromal cell hyperplasia and consequent androgen production. Hyper secretion of androgen leads to increased follicular recruitment and vasoconstrictive effect on the uterine arteries. It is thought that this effect is due to activation of specific receptors in arterial walls and collagen and elastin deposition in smooth muscle cells. Uterine artery PI is > 3 and sometimes the diastolic flow is absolutely absent, even in later phases of the cycle this effect continues. This leads to inadequate perfusion of the endometrium and is thought to be responsible for blastocyst implantation failure and high abortion rate in PCOS. Uterine artery resistance is higher in obese than in lean patients and is also associated with hyperinsulinemia, high

triglycerides, low high density lipids and higher heamtocrit values.

To summarize

Hyperinsulinemia leads to enhanced gonadotrophin stimulated steroid production in granulosa cells and theca cells, leading to increased androgen level, more antral follicles and in turn leading to increase AMH[49] .More antral follicles in turn produce more oestrogen through granulosa cells of multiple antral follicles or by aromatization of excess androgen. This leads to negative feedback to FSH, preventing growth of follicles and positive feedback to LH, leading to atresia of follicles, stromal abundance and further increase in androgens through the theca cells.
Though Insulin, androgen and LH are the main players in the entire syndrome, they may at different times and in different patients express differently and therefore depending on their expression the patients with PCOS may have variable ultrasound presentations. LH, along with VEGF leads to increased stromal vascularity, LH along with hyperinsulinemia leads to stromal abundance and hyperandrogeneima leads to more antral follicles.

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INTERNATIONAL JOURNAL OF HUMAN REPRODUCTION RESEARCH AND DEVELOPMENT

Comparison of Oocyte and Embryo Quality and Reproductive Outcome in PCO and Normoresponder Patients Undergoing IVF-A Retrospective Case-Control Study

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ABSTRACT

This is a retrospective study done on patients enrolled for IVF (in vitro fertilization) from March 2016 to Jan 2021 in our IVF unit. Aim of this study is to evaluate quality of oocytes in Polycystic ovarian syndrome (PCOS) patients and embryo quality in PCOS patients having normal semen parameters and their comparison with age matched normo - responders. Study population includes all PCOS patients diagnosed according to Rotterdam's criteria who underwent IVF at our centre and the controls included normo-responders who were age matched with the PCOS group .Total 30 cases were included in both the groups. The data were retrospectively collected and appropriate statistical tests (Mann Whitney and chi square) applied using Epi-info software. Results- There is no difference in the rate of mature oocytes obtained (67.20% vs 70.45%, p=1.00) and top quality embryos formed (70.1vs 92.9%, p=0.106) between the PCOS and normo-responder group. The clinical pregnancy rates were also similar between the two groups .

Conclusion: PCOS patients yield higher global oocytes as compared to normo responders. Oocyte and embryo quality are comparable between the two groups. Reproductive analysis shows similar pregnancy rates, while freeze all strategy in the PCOS group helped in preventing severe OHSS (ovarian hyperstimulation syndrome)

Keywords: PCOS, normo- responders, oocyte quality, embryo quality, mature oocytes, top quality embryos, clinical pregnancy, OHSS (Ovarian hyperstimulation syndrome).

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INTRODUCTION

PCOS (polycystic ovarian syndrome) is a disorder that globally affects 6-26% of reproductive-aged women and almost 70% of these women are not diagnosed. [1–3] It affects the metabolic, reproductive and psychological health of females significantly. With the availability of highly sensitive ultrasound machines, the diagnosis of PCO morphology has become more frequent and is recognized as a common factor in various phenotypes of the PCO spectrum. There are multiple follicles growing at a time in PCO morphology which can be recruited. Controlled ovarian hyperstimulation (COH) in PCO females leads to development of a higher number of follicles but the quality of the oocytes is poor. This leads to lower pregnancy rates and higher abortion rates. [4–6]

Studies have shown that PCOS affects oocyte competence owing to faulty dialogue between the cumulus cells and the oocyte and an impairment of the follicular micro-environment.[7, 8]

There have been various studies that have looked into the oocyte quality in PCO patients by evaluating the fecundation, implantation rate and pregnancy rate. But the results have been conflicting. [9–13] which resulted in some studies showing better oocyte quality and reproductive outcome, while vice versa in others.

This study was conducted with the aim to evaluate the quality of oocytes, quality of embryo and reproductive outcome in pcos patients in comparison with normo responders.

MATERIALS AND METHODS

Present study is a retrospective study done at sterling genesis and Oasis Fertility centre. All patients who underwent IVF at the centre from March 2016 to February 2021 were enrolled in the study. Patients were divided into two groups-PCO group and Normo responder group. Revised 2003 Rotterdam criteria were used to classify a patient having polycystic ovaries syndrome. [14]Total 34 patients met the Rotterdam.4 out of them were excluded because of associated male factor. So, total 30 PCO patients having no male factor infertility were taken for statistical calculation. Normo responders having age matched with the PCO group in the next indexed case were included for comparison. 30 patients were included in the study. All patients underwent antagonist protocol. The choice of gonadotrophin for stimulation was left to the consultant. Chi square test and student t-test was applied wherever necessary for comparison.

Exclusion Criteria-

- Couples with male factor infertility
- Couples undergoing PGT cycles.
- Female with poor ovarian reserve.
- H/o endometriosis.
- Donor cycles.

RESULTS

There were total 30 patients included for analysis in each group i.e PCOS group and normo responder group.

The median age of the females in the PCOS and normo responder group was 32 years (IQR 29 to 34 years in the PCO group and 31 to 33 years in the normoresponder group). (Table 1.1)

Table-1.1 Demographic profile

VARIABLE	PCOS GROUP	NORMORESPONDER GROUP	Man-whitney U (p-value)
	N=30	N=30	
AVG AGE Median (IQR)	32(29-34)	32(31-33)	424(P=0.24)
AVG BMI Median (IQR)	24.80(22-28)	25(22-28.90)	504(P=0.93)
AVG AMH Median (IQR)	5.7(3.78-7.92)	2.00(1.8-2.5)	49.50(P<0.0001)

Median (IQR) BMI was 24.8 (22-28) and 25 (22-28.9) in the PCOS and normo responder group respectively. There was no significant difference in the age and BMI of the two groups. (Table-1.1)

The median AMH in the PCOS group was significantly higher (5.7) as compared to the normoresponder group (5.7 vs 2) (p<0.0001). (Table-1.1)

There was no significant difference in the number of females with primary or secondary infertility in both the groups (p=1.00). (Table-1.2)

Table-1.2 Prevalence of primary and secondary infertility in the two groups

VARIABLE	PCOS GROUP (N=30)	NORMORESPONDER GROUP	P-VALUE
PRIMARY INFERTILITY	19	18	1.00
SECONDARY INFERTILITY	11	12	

There was no significant difference in the percentage of mature oocytes per patient or the percentage grade I quality of embryo on day 3 in both the PCOS and the normo responder groups (p=1.00 and p=0.11 respectively). (Table-2)

Table-2 Oocyte and embryo quality

VARIABLE	PCOS GROUP N=30	NORMORESPONDER GROUP N=30	P-VALUE	OR
	N%	N%		
%MATURE OOCYTES PER PATIENT	67.20 (20)	70.45 (21)	P=1.00	1.10
%IMMATURE OOCYTES PER PATIENT	32.8 (10)	29.55 (9)		
% GRADE I QUALITY EMBRYO ON DAY 3	70.1 (21)	92.9 (27)	P=0.106	3.85
% HIGHER THAN GRADE I EMBRYO ON DAY 3	29.9 (9)	7.1 (3)		

In the normo responder group, there was 7 times more chance of the first embryo transfer being fresh as compared to the PCOS group (OR=7; p=0.003). (Table-3) There are more chances of"" freeze all" in the PCOS group.

Table-3 Fresh vs Frozen transfers in both groups and Reproductive outcome

VARIABLE	PCOS GROUP N=24	NORMORESPONDER GROUP N=30	P-VALUE	OR
	N%	N%		
1ST EMBRYO TRANSFER-FRESH	41.66 (10)	83.33 (25)	P=0.003	7
1ST EMBRYO TRANSFER-FROZEN	58.33 (14)	16.66 (5)		
% CONCEIVED IN FIRST TRANSFER	33.33 (8)	20 (6)	p-0.424	2
% DID NOT CONCEIVE IN FIRST TRANSFER	66.67 (16)	80 (24)		

DISCUSSION

In our retrospective study we did not find any statistically significant difference in the quality of oocytes retrieved after controlled ovarian stimulation between PCOS group and normo-responder group. Various studies have shown that oocytes in PCOS syndrome show defective folliculogenesis which may have a negative effect on the competence of the oocytes. [4–6, 15, 16]

Studies have shown that PCOS is associated with alteration in gene expression involved in chromosome alignment and segregation during meiosis and mitosis and cell cycle check points.[7,8]A meta-analysis showed that patients with PCOS had the same pregnancy rate as other patients who underwent IVF, hence possibly polycystic ovaries produce enough competent oocytes after COH .[17]

In our study we found that the total numbers of mature (MII) oocytes retrieved are higher in PCOS group as compared to the normoresponder group. However there is no difference in the rate of mature and immature oocytes between the two groups. Our results are in accordance with the prospective study done by sigala et al.[18], and two others.[9,10]The rate of top quality embryos were also similar between the PCOS group and normoresponder groups, although the number was higher in the PCOS group.

Meta-analysis done by Roque et al showed better reproductive outcomes and lower rates of OHSS(ovarian hyperstimulation syndrome) with elective FET as compared to fresh transfer[19].In our study also the percentage of frozen transfers in the first cycle were significantly higher in the PCOS group as compared to the normoresponder group. The clinical pregnancy rate is similar between the PCOS and normo responder group in our study which corresponds with the prospective study done by Sigala et al. [18]

None of the patients in the PCOS group had OHSS in our study.

Strength of our study is that it has normo responder as controls; age matched with the PCOS patients. The demographic profile is similar between the two groups. We have not included patients with BMI more than 34 kg/m2 and hence the probable effect of obesity on oocyte quality in PCOS patient is removed. PCOS patients having male factor infertility were excluded from the study.

Limitations- It is a retrospective study with a smaller sample size, hence it cannot be extrapolated to a larger population and results need to be interpreted with caution. We have not calculated the miscarriage rate which may give an indirect measure of the quality of gametes.

CONCLUSION

PCOS patients yield higher global oocytes as compared to normo responders .Oocyte and embryo quality is comparable between the two groups. Pregnancy rates are similar between PCOS and normo responder groups. Freeze all strategy reduces the risk of severe and moderate OHSS.

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INTERNATIONAL JOURNAL OF HUMAN REPRODUCTION RESEARCH AND DEVELOPMENT

COVID Testing In Pregnancy: Lessons Learnt from One Year Analysis At A Tertiary Care Centre

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INTRODUCTION

Coronavirus disease (COVID-19) is an infectious disease caused by a newly discovered coronavirus SARS-CoV-2 found in both avian and mammalian species. They resemble each other in morphology and chemical structure: for example, the corona viruses of humans and cattle are antigenically related. There is no evidence that human corona viruses can be transmitted by animals.[1, 2]

Corona viruses were originally grouped into the family Coronaviridae based on the crown or halo-like appearance of glycoprotein studded envelope on electron microscopy.

Most people infected with the COVID-19 virus will experience mild to moderate respiratory illness and recover without requiring special treatment. Older people and those with underlying medical problems like cardiovascular disease, diabetes, chronic respiratory disease and cancer are more likely to develop serious illness.[3, 4]

ONE YEAR DOWN THE LINE

- After the declaration of the pandemic worldwide, a lockdown was declared in major cities of Maharashtra like Mumbai, Pune from 21th March 2020. At the beginning of the pandemic, our hospital had neither the facilities nor the necessary resources to test patients for COVID-19.
- As a result, we managed patients symptomatically and transferred high risk patients to designated COVID facilities for further management.
- Initially, testing was available only in the Fever OPD and not in the obstetric department.
- The ICMR guidelines of April 2020 recommended testing of all pregnant women above 34 weeks of gestation and likely to be delivered in the next 5 days, or those hailing from hotspots/ containment areas with likely

contact to positive case, even if asymptomatic.

- Our institute is continuing testing according to ICMR guidelines(updated with time) till today[5]
- Obstetric COVID positive patients were managed according to the standard protocols
- Health care workers followed the standard guidelines during managing the patients like use of PPE kits, face shields, N 95 masks, etc.[4]
- During this year, positive cases showed rising trend with decrease toward end of 2020
- Unfortunately, 2021 didn't make Corona go away and a second wave started.

AIMS and OBJECTIVES:

- To assess the impact of screening on detection of corona virus infection in Symptomatic (Group A) & Asymptomatic (Group B) obstetric patients.
- To analyse trends in detection and to discuss challenges in implementation of testing.
- To find the incidence of COVID positive patients in symptomatic and asymptomatic pregnant women.

METHODOLOGY

Type of study: Retrospective Observational Study.
Centre of Study: Hindu Hrudaysamrat Balasaheb Thackarey Medical College (HBTMC) and Dr R.N. Cooper Hospital, Mumbai.
Duration of Study: 1st April 2020 to 31st March 2021 (one year)
Sample Size: All the patients admitted in Labour Ward, ANC and Gynaecology ward under Obstetrics and Gynaecology department.
Swab collection location and technique:
As per the directives of the ICMR Guidelines for COVID-19 testing in pregnant women, our hospital, a Non COVID designated centre, started testing all pregnant patients at entry point in Labour ward casualty/triage area by collecting naso-pharyngeal swab for COVID-19.[5] The swabs were collected with a swab stick and sent in transport medium for COVID-19 testing by Real time PCR. Samples were processed according to the standards prescribed by GOI. Based on the results, patients with positive results were triaged and managed as per the standard protocols for further care.

Inclusion Criteria:

- Pregnant patients registered in our hospital and admitted for antenatal care OR
- Pregnant patients registered with us and presenting in labour OR
- Unregistered patients presenting in labour (direct/ referred from other health care facility)

  AND
- Patients willing to get COVID swab testing.

Those with any symptoms were put as Group A, others Group B

Exclusion Criteria:

1. Patients refusing consent for swab test.
2. Patients who were already tested positive for COVID.

Management Protocol[6, 7]

- Patients were admitted and managed as per their symptoms according to standard protocols. Labour management was as per standard guidelines and Caesarean section was done only for obstetric indications.[6]
- Symptomatic and suspected patients were delivered in a separate isolation room; operative procedures in suspected cases were performed in a separate designated

operation theatre as per standard guidelines. [7]

- Precautions as per standard protocols were implemented in Labour Ward and Operation Theatre: wearing Personal Protective Equipment (PPE), N 95 masks and face shield.
- Patients with early pregnancy failure were managed by emergency check curettage in Operation Theatre. In order to reduce the risk of transmission to the healthcare workers, anaesthetists preferred Saddle block over intravenous sedation as the method of anaesthesia.Symptomatic patients post-delivery or surgeries were then shifted to designated isolation wards. Asymptomatic patients were shifted to respective post-natal Ward or Caesarean Section Ward.
- Neonatologists and anaesthetists were informed about symptomatic patients in advance of delivery or caesarean section to ensure proper care of patients, neonates and health care workers as per standard protocols
- Asymptomatic patients were discharged as per standard protocols.

Table 1: COVID positive patients detected in Dept of OBGY

	Month/Year	Symptomatic Cases : GROUP A	Asymptomatic Cases: GROUP B	No Of Positive Cases: TOTAL
1	April 2020	6	16	22
2	May 2020	10	137	147
3	June 2020	9	112	121
4	July 2020	5	83	88
5	August 2020	3	45	48
6	September 2020	2	30	32
7	October 2020	3	18	21
8	November 2020	0	4	4
9	December 2020	2	33	35
10	January 2021	1	5	6
11	February 2021	0	4	4
12	March 2021	2	24	26
	TOTAL	**43**	**511**	**554**

Chart 1A: SYMPTOMATIC COVID POSITIVE PATIENTS:

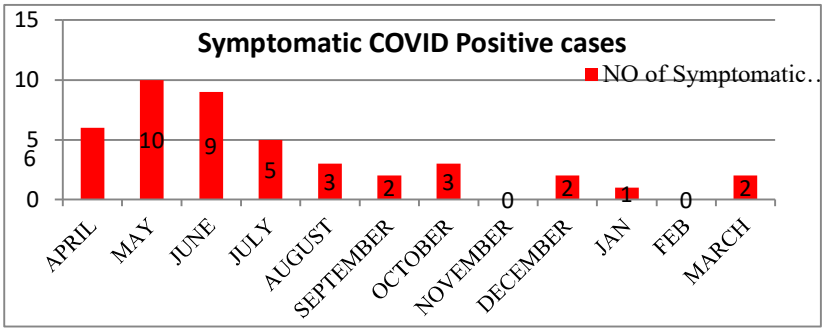


CHART 1B: ASYMPTOMATIC COVID POSITIVE PATIENTS

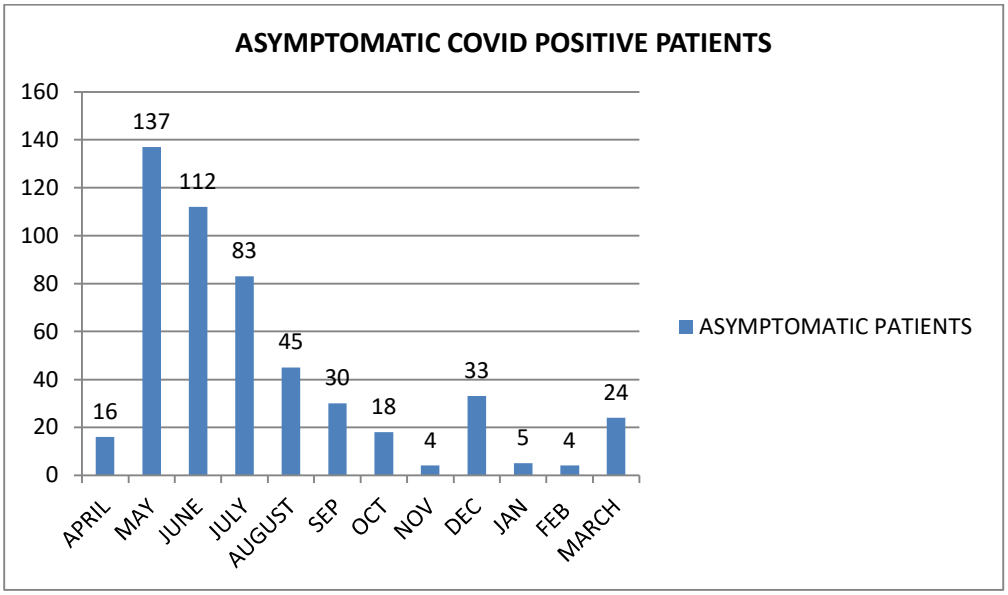


TABLE 2: Analysis of Monthly Trends Based On COVID Testing In Patients:

	Month/Year	No Of Positive Cases	Total Patients Screened	Total Patients Admitted
1	April 2020	22	161	516
2	May 2020	147	543	543
3	June 2020	121	361	361
4	July 2020	88	330	330
5	August 2020	48	461	461
6	September 2020	32	494	494
7	October 2020	21	486	486
8	November 2020	4	435	435
9	December 2020	35	398	398
10	January 2021	6	303	303
11	February 2021	4	260	260
12	March 2021	26	331	331
	TOTAL	**554**	**4563**	**4918**

Figure 2: Monthly Trend in Pregnant Patients

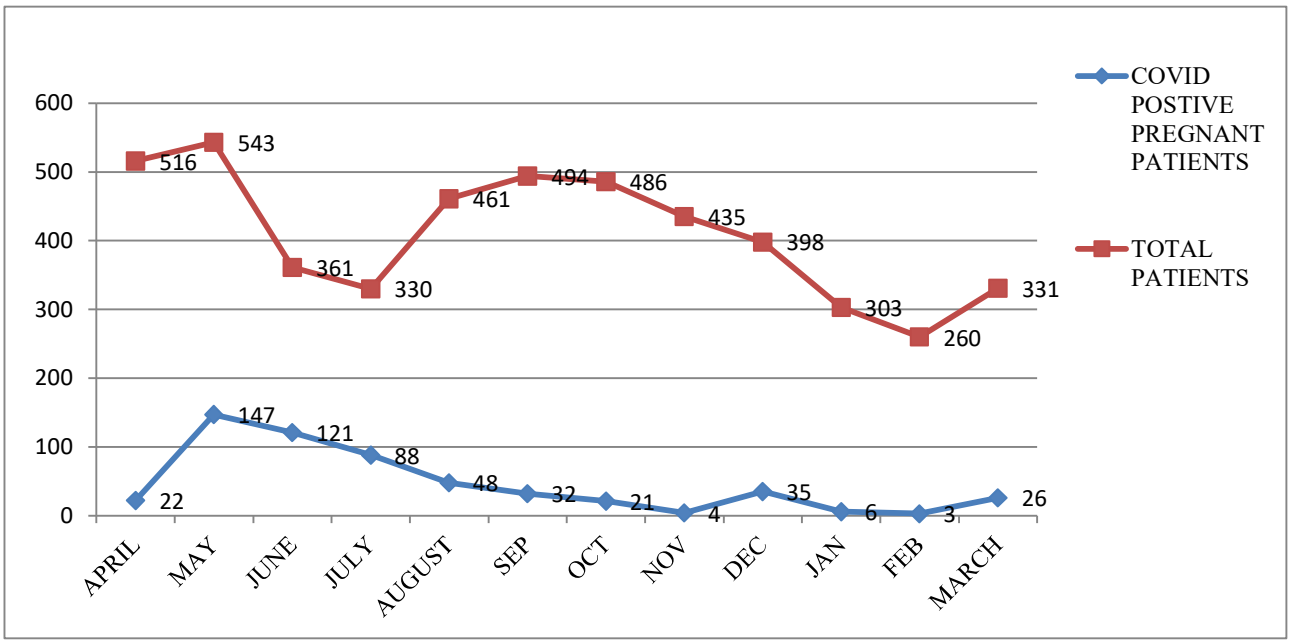
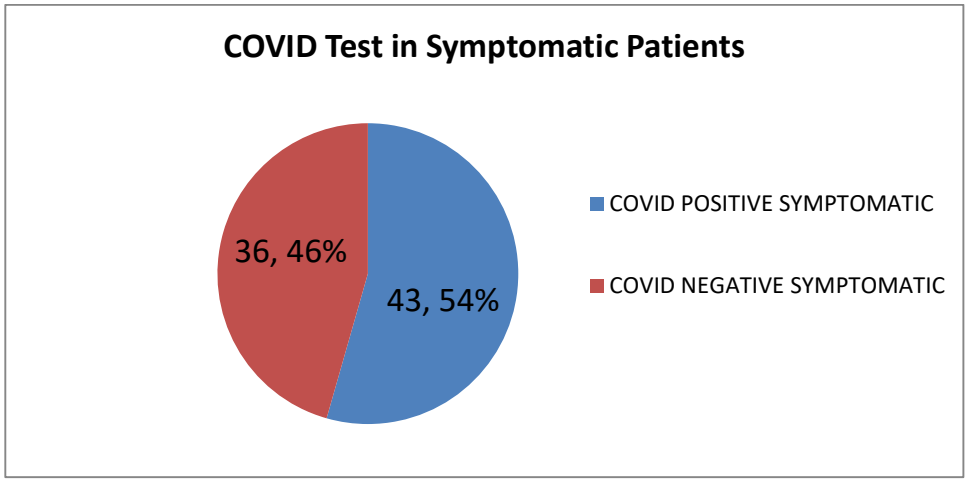
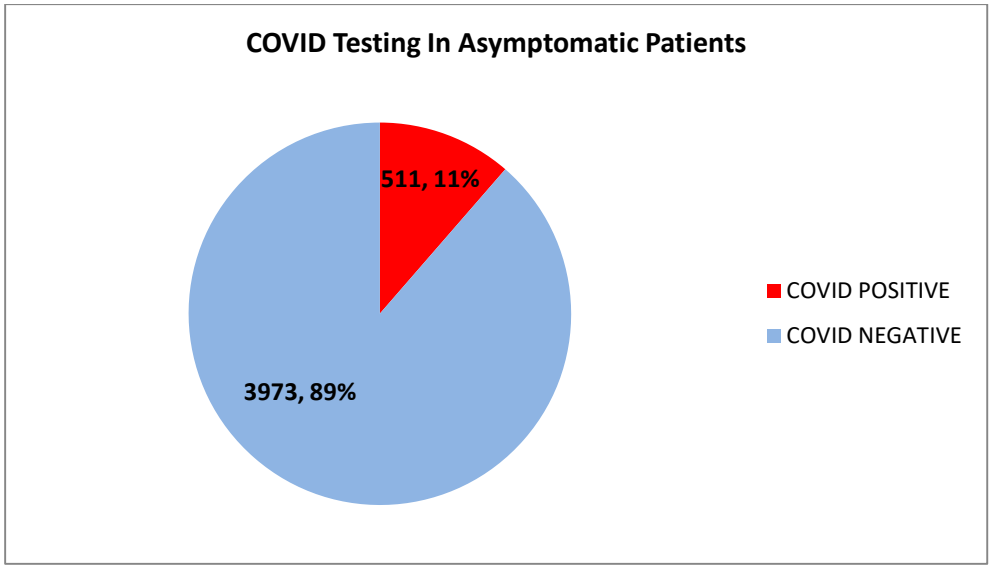


Table 3: COVID testing results for patients in dept of OBGY

	SYMPTOMATIC	ASYMPTOMATIC	TOTAL
COVID POSITIVE	43	511	554
COVID NEGATIVE	36	3973	4009
TOTAL	79	4484	4563





RESULTS

- we had total 4918 patients admitted in OBGY department from April 2020 to March 2021
- Testing of 4563 patients was done for COVID throughout the year, 92.78% being testing rate.
- In month of April 2020 systematic testing was not started so all patients in April were not tested.
- Testing of all pregnant women admitted to the obstetric department commenced from 22nd April 2020.
- In this one year we detected and managed 554 COVID positive patients.
- In 554 COVID positive patients,43(7.76 %) were symptomatic as shown in figure 1A and rest 511(92.23%) were asymptomatic as shown in figure 1B.
- There were 431cases of deliveries (both vaginal and caesarean section) and rest 124 included check curettages for first trimester miscarriages, antenatal cases and gynecology patients.
- Table 2 shows the number of COVID positive patients managed at our non-designated COVID hospital. It shows the trend over one year with rising trend till May following which there was plateau which settled around 2020 year end. Unfortunately 2021 year shows again rising trend of positive cases mimicking trend seen in 2020, possible second wave.
- 79 patients were having symptoms of which 43 were COVID positive (54.43%)
  - 0.94 % was incidence rate of symptomatic COVID positive patients (43) amongst the total patients tested.
- 511 asymptomatic COVID positive patients were detected out of 4484 asymptomatic patients screened (11.39%).

Discussion

When the WHO declared COVID-19 a pandemic and lockdown was implemented in India, the COVID-19 swab testing facility was not available at our tertiary care institute. As the number of cases started rising in Mumbai, swab testing for COVID-19 was made available in our hospital. Initially, the testing was limited to Fever OPD, so testing of asymptomatic pregnant patients could not be done. Only pregnant

women with symptoms of COVID 19 infection were tested in the Fever OPD and managed as per the standard protocol.

From 22nd April 2020, testing of all admitted pregnant patients for COVID-19 was started in our OBGY Department as per ICMR guidelines[5] .The swabs were collected in the Labour ward Casualty area on admission of pregnant women.

The various issues about sample collection that we encountered were:

- Incomplete form filling
- Illiterate patients unable to give accurate information
- Patients and relatives not knowing the exact address
- Language barrier
- Patients could not or choose not to give correct contact numbers.
- Sample spillage

Initially, the samples were tested in one designated MCGM COVID-19 laboratory. However, due to heavy workload, ICMR then permitted private laboratories to test the COVID-19 samples. Since then, samples are now sent to other private laboratories providing free testing for MCGM patients.

The incidence of symptomatic COVID-19 positive patients in our study was 7.76%. Symptoms include fever, sore throat, cough, breathlessness, running nose, headache, weakness, fatigue, diarrhea, loss of smell etc. However majority were asymptomatic (511) as seen in Fig 1B. Thus, despite the large number of COVID-19 positive patients, the prevalence of symptomatic patients was less. Further, most of the symptomatic patients had symptoms that were mild in nature; one patient had persistent fever had mastitis. One patient, a case of eclampsia with caesarean section had severe symptoms of fever and breathlessness with X Ray suggestive of early ARDS and cardiomegaly. The patient was on anticoagulants, higher antibiotics, steroids and diuretics and but couldn't survive.

The COVID positive patients who were asymptomatic were managed conservatively with medicines like vitamin C, Zinc, etc and were discharged as per routine protocols established by MCGM. Prevalence of asymptomatic patients (92.23%) was more as compared to symptomatic patients hence we believe that asymptomatic pregnant patients must be tested for COVID infection. This is important as they may be carriers, develop complications later and may spread infection to their own child also.

In our hospital, all pregnant women were not tested for COVID-19 till 20th April, when the first case, a case of chronic ectopic was diagnosed to be positive on high index of suspicion. It was only with the revised ICMR guidelines that universal testing of all admitted pregnant women was started from 22nd April with administrative sanction.In the same month, 22 pregnant patients were diagnosed positive till 30th April, and 147 in May 2020. Thus, implementing testing for all pregnant patients has helped us identify COVID positive patients and plan management.

Our data shows in one year we have managed 554 COVID positive pregnant women despite our hospital not being a dedicated COVID facility. The high incidence of positivity in symptomatic group is not unexpected. However, the fact that 92.33% of COVID positive patients were asymptomatic, highlights the need for mandatory testing for pregnant women.

Meticulous filling of forms and careful sample collection and dispatch is important for accurate reports and reduction of turn-around time for testing is also important.

A systematic review and metaanalysis of 13 studies of COVID in pregnant women suggested a high rate of maternal and neonatal complication in infected individual and the need for multicenter cohort studies. The estimated second epidemic wave should be faced keeping into account the available scientific information to reduce the burden of disease in vulnerable population groups, including pregnant women and neonates. [8]

Although our hospital is not a designated COVID-19 centre, our study revealed that there was a high incidence of asymptomatic positive pregnant women. Hence all patients should be treated with proper precautions due to high incidence of asymptomatic positive, often diagnosed post-delivery.

Measures adopted include:

- Clinical assessment of patients in triage area and admitting only if strongly indicated,
- Collecting COVID-19 swabs of all admitted patients and isolating symptomatic patients,
- Delivering symptomatic labor cases in separate area (pending swab reports),
- Making sure that list of all collected swabs with requisite patient details is maintained and reports are traced.
- Making it mandatory for all residents/ staff to wear appropriate PPE kit, N95 mask, face shield and gloves in the triage area, labour ward and during delivery by vaginal delivery or by caesarean section.

CONCLUSION

Based on our experience, we find that there is a need for testing all admitted pregnant women as most of COVID-19 positive pregnant women are asymptomatic. By testing all pregnant women requiring admission, proper management of patients, neonates and other pregnant women can be ensured. This eventually benefits everyone: patients, health care workers and society.

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Conflicts of Interest: None

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Syphilis in Pregnancy; Estimating the disease burden

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Abstract

Background: Maternal syphilis has a severe impact on pregnancy outcome, primarily as spontaneous abortion, still birth and congenital syphilis. It also shows the effectiveness of health system functionality. **Objective:** The present study was conducted to know the trend of syphilis seroprevalence in antenatal women. **Methods:** The study was hospital based retrospective Observational studywhere 54,713 samples were tested for syphilis by VDRL during the study period of 6 years from 2015 to 2020. **Results:** Out of total 54,713 sample tested for syphilis during the study period, 260 were found positive so the cumulative prevalence of syphilis was 0.47%. Seroprevalance of syphilis in years 2015 to 2020 was 0.1%, 0.6%, 0.56%, 0.31%, 0.87% and 0.27% respectively. 259 cases were delivered during the study period among which 36 (13.9%) were pre-term, 216 (834%) were term and 7 (2.7%) were post-term delivered.**Conclusion:** Still the prevalence of syphilis in pregnant women is varied during the study period so routine screening of all pregnant women during ANC visit is recommended to reduce the incidence of congenital syphilis & perinatal complications.

Keywords: Syphilis, VDRL, Seroprevalance, ANC Visit.

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Introduction

Syphilis, caused by Treponema pallidum is a classical example of a sexually transmitted disease (STD) that can be successfully controlled by effective public health measures due to the availability of a sound diagnostic test and effective and economical treatment options. [1] Maternal syphilis has a severe impact on pregnancy outcome, primarily as spontaneous abortion, still birth and congenital syphilis. [2]In 2012 WHO suggested replacement of the term congenital syphilis with "mother to child transmission of syphilis" to stress its importance. [3] To prevent perinatal complications, Screening of asymptomatic antenatal women is recommended.[4] The screening of syphilis and treatment in antenatal care has been called as one of the most cost-effective way to reduce fetal and infant mortality and morbidity in the developing world.[5]

Advancement in the diagnostic modalities, availability of the treatment and increase in public awareness has changed the prevalence of syphilis in India. But in some regional areas, syphilis still remains as major public health problem.[6]Two recent reports from New Delhi, India have found a constant trend in syphilis from 2001-2009 and 2005-2009, respectively [7, 8]. India is committed to the WHO SDG-4 by 2030 which entails elimination of mother to child transmission of syphilis among others. In order to plan, strategize and execute accordingly, it is imperative to know the current prevalence of the disease and how has the trend been under the existing government protocols for identification and treatment. Keeping this fact in mind the present study was planned to estimate the trend of 6 year's seroprevalence of syphilis among pregnant females attending Antenatal Clinic in a tertiary care hospital in Central India.

Material and Methods

It was a hospital based retrospective observational studyconducted over a period of six year from January 2015 to December 2020 in tertiary care teaching hospital in Central India. All pregnant women who attended the antenatal OPD in last 6 years at our institute and screened for syphilis were included in this study. Screening for syphilis was performed by standard Venereal Disease Research Laboratory (VDRL) test. Samples positive for VDRL were subjected to a quantitative VDRL test using serum dilutions from 1 in 2 upto 1 in 64. [9] Thus, apositive VDRL was considered to indicate syphilis.Record files of VDRL positive pregnant women were studied, the data retrieved was collected, tabulated and analyzed year wise.
Institutional Ethical Committee has approved the study.

Statistical analysis
Data were analysed and statistically evaluated using SPSS software, version 25 (Chicago II, USA). Data which was quantitative was expressed in mean, standard deviation while qualitative data were expressed in percentage.

Observations & Results

Out of total 54,713 samples were tested for syphilis during the study period, 260 were found positive so the cumulative prevalence of syphilis was 0.47%. Year wise distribution of prevalence is shown in table 1. Seroprevalence of syphilis from 2015 to 2020 was 0.1%, 0.6%, 0.56%, 0.31%, 0.87% and 0.27% respectively. Most of the VDRL cases were seen in the age group of 21-30 years (n=181; 69.6%) followed by >30 years (n=57; 21.9%) while 22 cases were <20 years old. Rural (n=128; 49.2%) and urban (n=132; 50.8%) distribution of VDRL positive cases was almost equal. Out of 260 mothers found positive by VDRL, 91 (35.0%) were primi, 140 (53.8%) were multigravida and 29 (11.1%) were grand multipara. 259 cases were delivered during the study period among which 36 (13.9%) were pre-term, 216 (834%) were term and 7 (2.7%) were post-term delivered (Table 2).

Discussion

Present study was planned to know the trend of seroprevalence of syphilis in ANC cases attending a tertiary care hospital in Central India. Sero-reactivity of syphilis among pregnant females is highly variable from being as low as 0.02% to as high as 12.1% among the whole world population. [10] But in India as compared to other countries syphilis is having low seroprevalence of 1.9% which was reported in ANC patients by Kumar G et al. [11]

Overall prevalence of VDRL positive cases was 0.47% in our study which is lower than study done by Chen XS et al [12] (1.6%), Khan S et al [1] (0.7%) from Southern India while higher then Manimegalai M et al [13] (0.1%), Fatima N et al [14] (0.15%) and Nair N et al [15] (0.36%). Mehta KD et al [16] reported prevalence of syphilis as 0.48% from their study which is similar to our study.

Study by Chopra S et al [6] from North India reported significantly declining trends in VDRL reactivity in among ANC clinic cases from 0.69% in year 2002 to 0.24% in 2012 year. Study by Khan S et al [1] also reported declining trend in seroprevalence of syphilis in ANC cases. But finding of our study reported that in recent years seroprevalence of syphilis in ANC cases was not reduced significantly and it varied from 0.1% to 0.27% during year 2015 to year 2020.

Although prevalence of syphilis among pregnant women is declining in India due to greater awareness and better education of women about the features and complications of syphilis—by both doctors and nursing staff during antenatal visits but out study showed varied prevalence of syphilis in our hospital. This may be due to the fact that our hospital being a tertiary care hospital is catering to varied population from urban and rural area with wide geographical distribution of high risk population.

However, as syphilis can cause adverse outcomes of pregnancy in 80% of the cases, including stillbirths, abortions, perinatal death and neonatal infections in a significant number of cases, the importance of screening antenatal women for syphilis should always be highlighted.[17]

Proportion of pregnant women attending at least one ANC visit has been increased in India from 75% in 2007-2008 to 96% in 2010; however, still WHO recommended four visits are attended by significantly fewer women [18] which might result in fewer women getting treated for syphilis. Global WHO estimates show that 66% of adverse pregnancy outcomes due to syphilis occurred in women with at least one ANC visit but whom either not were screened or did not receive complete treatment. [19] Therefore, it is important to make efforts towards ensuring complete follow-up as well as adequate treatment of the VDRL reactive pregnant women in addition to universal access to early ANC.

As compared to HIV, syphilis has about 3-20 times greater infectivity per sex act. Its duration of transmissibility is significantly reduced after treatment. Henceforth, treating syphilis plays an important role in aborting the transmission chain. [20]

Conclusion & Recommendation

Present study concluded that as the prevalence of syphilis was not continuously declining and as screening and treatment of syphilis are inexpensive and cost effective even in the low prevalence areas. Therefore, despite the low prevalence of syphilis seropositivity, efforts should be taken to continue to make the screening facilities and treatment readily accessible, for antenatal women and other high risk population.

Table 1: Seroprevalence of syphilis infection from the study period January 2015 till December 2020

Year	No. tested	No. positive by VDRL	%
2015	6725	7	0.1
2016	10113	64	0.6
2017	10644	60	0.56
2018	10698	33	0.31
2019	8474	74	0.87
2020	8059	22	0.27
Total	54713	260	0.47

Table 2: Distribution of VDRL positive cases according to type of delivery

Year	Total positive subjects delivered in that year	Pre-term	Term	Post-term
2015	6	1(16.7%)	5 (83.3%)	0
2016	57	11 (19.3%)	43 (75.4%)	3 (5.3%)
2017	54	8 (14.8%)	46 (85.2%)	0
2018	42	6 (14.3%)	35 (83.3%)	1 (0.4%)
2019	66	7 (10.6%)	56 (84.8%)	3 (4.5%)
2020	34	3 (8.8%)	31 (91.2%)	0
Total	259	36 (13.9%)	216 (83.4%)	7 (2.7%)

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Conflicts of Interest: None

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INTERNATIONAL JOURNAL OF HUMAN REPRODUCTION RESEARCH AND DEVELOPMENT

Multiple Primary Cancers of the Female Genital System: A Rare Case Report

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Abstract

Multiple primary cancers of female genital system are very rare occurrence. The PubMed-indexed English literature report only 13 such cases. In this article we report a single case of multiple primary cancers of the right ovary, cervix and endometrium with three distinct histological patterns.

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Introduction

Multiple Primary Cancers (MPCs) also referred to as Synchronous/ Metachronous primary neoplasms refers to the condition in which more than one primary tumor occurs at the same time or in succession in one or multiple organs within the same patient. These neoplasms should be histologically discrete. MPCs are usually seen in head and neck with overall frequency of 2-17% and are rare to be seen in genital system with incidence if 0.6% to 5.4%[1]. Dual gynecological neoplasms are observed occasionally with the most commonly reported combination of endometrial-ovarian neoplasms [1, 2]. The incidence of triple or more synchronous primary neoplasms of the female genital system is highly uncommon, with only 13 such cases being reported in the PubMed-indexed English literature [1]. We report a single case of multiple primary cancers of the right ovary, cervix and endometrium with three distinct histological patterns.

Case Report

A 55-year-old multiparous, postmenopausal woman reported to our emergency department with complains of gradually increasing huge lump in abdomen. She noticed it 3 months back associated with weight loss, loss of appetite and increasing generalized weakness . She also had complained of postmenopausal bleeding per valium since 6 days. Her past medical, surgical and family history was not significant. On per abdomen examination, a cystic, non tender pelvic mass with restricted mobility was palpated measuring approximately 25 cm x15 cm. On per speculum examination bleeding through os was seen and cervical polyp of about 1cm x 2cm was seen protruding through the os, it bleeds when touched, On per vaginum examination same large1x2 cm was felt, cystic mass of about 25x 25x15 cm was felt which was not mobile with movement of uterus. Laboratory findings showed a elevated CA-125 level of 43.5 U/mL (normal - range: 0–35 U/mL).

USG showed a large multiloculated cystic lesion in the abdominal and pelvic cavity displaced bowel loops posteriorly, bilateral ovaries not visualized separately. CT scan showed a large well defined thick walled multiloculated hypoattenuating cystic mass in abdominopelvic cavity showing small solid component infero- laterally on right side and multiple thick enhancing septations within showing morphological and locoregional extent- size approx. 28 cm x 28 cm x16.7 cm displacing bowel loops posterolaterally. On IOTA scoring, risk of malignancy was found to be 80.5%.

The surgical staging operation consisted of total abdominal hysterectomy, bilateral salpingo-oophorectomy. During the laparotomy, the pelvic mass was found to be originating from the right ovary. The uterus was bulky and the left ovary was grossly normal. There was no ascites or other gross intra-abdominal lesions. All resected specimens were examined for histopathological analysis. Histopathological examination showed Complex hyperplasia with atypia of the uterus (Figure 1). Both ovaries were examined, and the microscopic appearance of the right ovary revealed granulosa cell tumor (Figure2). Bilataeral fallopian and right ovary appeared normal. Microscopic examination of the cervix

showed carcinoma in situ (Figure3).

Figure 1
Histopathological examination showing complex hyperplasia with atypia of the endometrium (H&E stain)

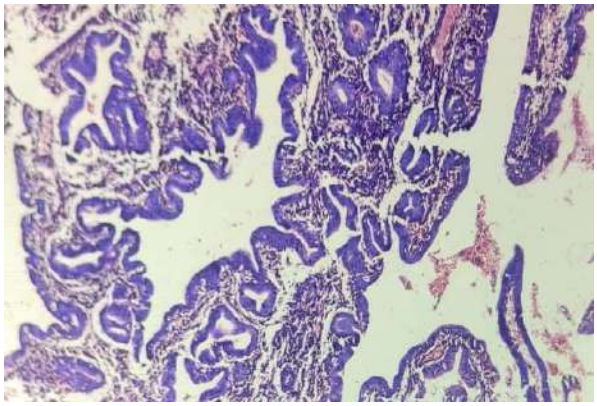
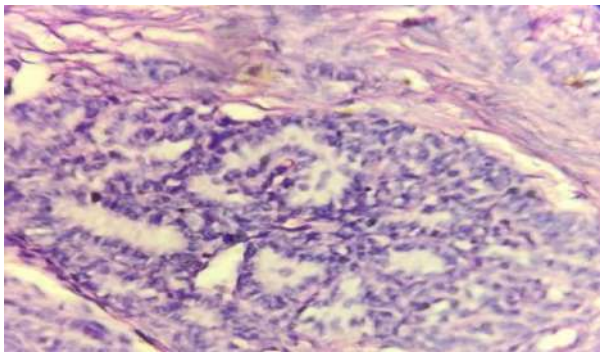


Figure 2
Histopathological examination showing granulosa cell tumor of right ovary (H&E stain).

Coffee Bean cells **Call Exner Bodies**



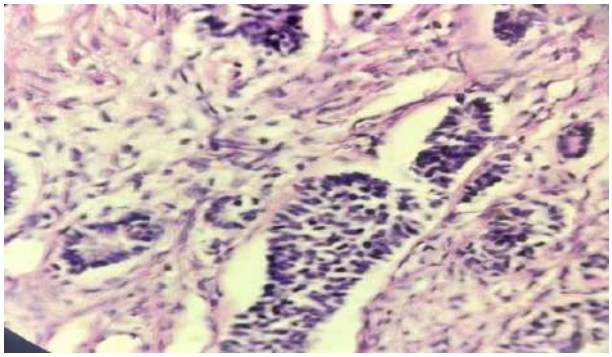
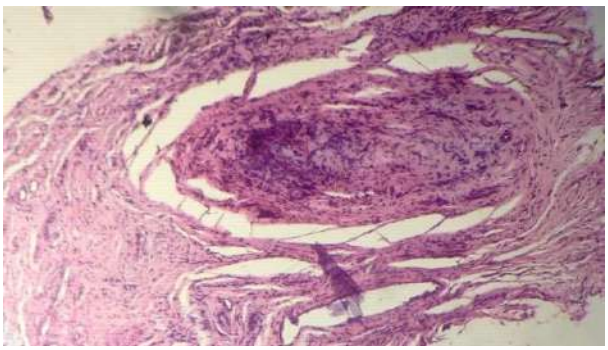


Figure 3 Histopathological examination showing carcinoma in situ of the cervix (H&E stain)



Therefore, the final histopathological diagnosis was triple synchronous primary stage 1Agranulosa cell tumour of right ovary (T1NxMx), stage 0 carcinoma cervix and complex endometrial hyperplasia with atypia of uterus.

Postoperatively patient was monitored and recovered well. She was discharged and advised monthly follow up for early diagnosis of any recurrence.

Discussion

Synchronous Primary Neoplasms are rare to be seen in the genital system with incidence of double synchronous primary gynecological neoplasms ranging from 0.6% to 5.4% [1]. Triple or more synchronous primary neoplasm of female genital system is exceptionally rare to occur. The PubMed-indexed English literature reports only 13 such cases [3-13] till date including 10 cases of triple synchronous neoplasms, 2 cases of quadruple [7, 10] and 1 case of quintuple [9] synchronous neoplasm. This study reports a rare combination of granulosa cell tumor of right ovary with complex endometrial hyperplasia with atypia with carcinoma cervix in situ

The etiology of synchronous primary neoplasms of the female genital system remains poorly defined. Mullerian tissues which have similar embryological origin may respond as a single structural entity to carcinogenic, hormonal, therapeutic, or other triggering factors in genetically predisposed individuals [1]. In this study, there were three different histological subtypes identified in the surgical specimens which may explain potential role of epigenetic factors.

For the purpose of assisting clinicians and pathologists in distinguishing synchronous primary neoplasms from related metastatic foci, one major and 4 minor criteria are suggested: These criteria include either one major criterion or all the four minor criteria. The one major criterion is the existence of distinct histological types of the neoplasms. The four minor criteria include (a) neoplasms which are limited to primary locations, (b) absence of direct extension between neoplasms, (c) absence of lymphovascular neoplastic invasion, and (d) absence of distant metastasis [14, 15]. In the case discussed in present study all the criteria, both major and minor were met confirming the diagnosis of multiple primary cancers.

Since management and prognosis of synchronous primary gynecological neoplasms and related metastatic diseases vary substantially, it become utmost important to differentiate them .Synchronous primary gynecological neoplasms have better survival rates than metastatic or advanced primary ones [17, 18, 19]. This may be attributed to the younger age of presentation, earlier disease stage, and lower disease grade at the time of clinical diagnosis [17]. The neoplasm with the poorest prognosis determines the prognosis of a triple neoplasm. [5].

Management should be largely individualized taking into consideration several parameters, such as age of patient, disease type, disease stage, disease grade, and extent of the neoplastic invasion [10]. Surgical debulking, adjuvant radiotherapy and adjuvant chemotherapy remain the main modalities of management.

Majority of the cases of granulosa cell tumor of ovary are stage I at diagnosis having good prognosis with a 5 year survival rate ranging from 90-100% and 10 year survival rate ranging from 84-95% [16]. Surgery is the first step of treating a granulosa cell tumor of the ovary and aims to remove as much of the tumor as possible. Additional treatments such as radiation therapy, chemotherapy, or hormone therapy- may follow surgery depending on the severity and extent of the original tumor, or if the tumor regrows after surgery.

Hysterectomy is the treatment of choice for hyperplasia with atypia in patient who have completed family as was in our case. Fertility sparing

treatment of endometrial hyperplasia and cancer is desirable in younger patients with PCOS, chronic anovulation and patients with infertility. Surrogate staging technique with MRI is employed. Continuous progestin therapy with progestins like megestrol acetate for atleast 3-6 months is the most reliable treatment for reversing complex or atypical hyperplasia.

Management of carcinoma cervix in situ includes modalities like cold knife conization, loop electrosurgical excision procedure (LEEP), or laser surgery. Hysterectomy for women who have completed their family or do not desire fertility as was seen in case discussed in this study.

Conclusion

Multiple primary cancers of genital system though extremely rare to occur, should always be kept in mind when evaluating neoplasms of the female genital system. Detailed and careful examination of the surgical specimen plays a key role in diagnosis the condition. Synchronous primary neoplasms should be differentiated from metastatic and recurrent tumors as it changes the treatment modality and prognosis of the disease. Hence counseling and educating patients regarding importance of regular follow up for early diagnosis and timely intervention.

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INTERNATIONAL JOURNAL OF HUMAN REPRODUCTION RESEARCH AND DEVELOPMENT

ABDOMEN A PANDORA'S BOX: AN UNUSUAL PRESENTATION OF OVARIAN CYST AT LSCS.

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ABSTRACT

It's common for women of childbearing age to get ovarian cysts, and it's not unusual for pregnant women to find out that they have an ovarian cyst during their Antenatal period.

Most adnexal masses are asymptomatic and spontaneously resolve before the 16th week of amenorrhoea. On the other hand, some cases are persistent forms which can cause complications for the mother and fetus. The most common types of ovarian cysts are called functional namely, follicular cyst and Corpus luteum cyst.

Our case is areferred primigravida, full term with deranged Liver function test. Despite four ultrasonography being done in her antenatal period in private, there was no mention of any adnexal mass. She was induced with PGE2 pessary but at 3 cms was found to have Meconium Stained Liquor. She landed up with a LSCS i/v/o fetal distress, her abdomen was nothing less than a PANDORAS BOX

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INTRODUCTION

It's common for women of childbearing age to get ovarian cysts and it's unusual for pregnant women to find out that they have an ovarian cyst while they are pregnant. Fortunately most of the ovarian cysts are simple cyst.

An ovarian cyst is a fluid-filled sac in the ovary.The two most common types of ovarian cysts are called functional and includesfollicular cyst form when an ovarian follicle (the tiny sac where the egg grows) doesn't open to release the egg and continues to grow into a cyst.[1]Corpus luteum cysts-A "corpus luteum" develops after an ovulation helps to produce hormones for the growth of the baby or to maintain menstruation. Sometimes, this fluid of empty follicle does not shrink and results into cyst.

Endometrioma or chocolate cysts, in women who have endometriosis are filled with old blood, so they can be darker in colour. Ovarian cysts develop on the surface of the ovary and are solid, like a ball of muscle. Commonly, functional cyst or luteomas are observed during pregnancy. Benign cystic teratomas, serous cystadenoma, paraovarian cyst, mucinous cystadenoma and endometrioma are other different types of cyst which are observed.[2] Malignancy in pregnancy is generally a germ cell tumour or borderline epithelial ovarian tumour.

The torsion of ovarian cyst is the commonest complication during pregnancy. The ovarian torsion is defined as partial or complete twisting of ovary on its ligamentous supports, often resulting in impedance of its lymphatic and venous outflow and arterial inflow leading to stasis, venous congestion, haemorrhage, necrosis and sometimes cyst rupture. [3]The exact etiology of torsion of ovary is not known and diagnosis is often difficult due to non-specific presenting features. The various predisposing factors often seen associated with torsion are moderate to large size of ovarian mass, long pedicle and free mobility. The patient presents with severe, colicky, unilateral and acute pain abdomen that is usually non remitting but can wax and wane in cases of incomplete or intermittent ovarian torsion. On pelvic examination, one might find tender cystic mass separated from uterus. The incidence of ovarian torsion is 5 per 10000 pregnancies and its risk increases 5 times during pregnancy.[4] The ovarian torsion is more commonly seen in reproductive age group. The majority of the cases presented in pregnant (22.7%) than in non-pregnant women (6.1%).

CASE

A 25 year old, primigravida, married since 3 yrs with 36.2 weeks of gestation was presented in casualty with chief complaints of pain in abdomen. There was no history of nausea, vomiting, fever, syncopal attack, bladder, bowel complaints, no discharge or bleeding per vaginum. Patient was a known case of PIH on Tab. Labetelol 100 BD since 20 days. There was no significant past, personal or surgical history.Her previous menstrual cycles were normal. Her obstetric history was uneventful. On examination she was conscious and oriented. Her BP was 140/94 mm of Hg, pulse rate was 92/minute, afebrile. Urine Albumin was1+ and no pre monitoring symptoms. Bilateral knee jerks were normal. Abdominal examination revealed uterine height corresponding to 34weeks of gestation with cephalic presentation. Foetal heart sounds were 144bpm regular and Abdomen was irritable.

On per Vaginum Examinations was admitting tip of finger, cervix was uneffaced and show was present.

Ultrasonography was suggestive of SLIUG of 32 weeks 1 day in cephalic presentation with estimated fetal weight 2.8 kg. Placenta Anterior and liquor wasadequate.

All preliminary investigation were within normal limits.Decision of induction of labour with PGE2 pessary was taken in view of Pregnancy Induced Hypertension with IUGR.Induction of labour with PGE2 gel was done. After 6 hours, patient went into active labor, was 3 cm dilated however liquor was meconium stained and fetal heart sounds were 100-120bpm, irregular suggestive offoetal distress.Decision of Emergency lower segment caesarean section was taken in view of Meconium Stained Liquor with foetal distress.

Intraoperative findings-

After the delivery of the baby and closure of uterine incision, bilateral fallopian tubes and ovaries were examined. Right tube and ovary were found to be normal however left fallopian tube was stretched upon a mass extending into the pouch of douglas. Left ovary could not be visualised separately and this left ovarian mass was exteriorised which was15*20*20 cm. It was cystic in nature, thin walled with no solid component grossly visible.Patients husband was explained about the intraoperative findings and an intraoperative consent for left ovarian cystectomy taken.Hydro-dissection was done, cyst wall was separated from ovarian tissue by blunt and sharp dissection. Left ovarian Cystectomy was done and abdomen was closed. Patient's postoperative period was uneventful. She was given injectable antibiotics for 5 days. Patient was discharged on 12th postoperative day. Histopathology report was found to be mucinous cystadenoma.

DISCUSSION

Frequency of ovarian tumours being coexistent with pregnancy is 1:10000 and among these frequencies of being malignant is approximately 1:15000 to 1:32000 pregnancies.[5] Most common ovarian masses encountered during pregnancy are functional cysts of ovary.

The other ovarian masses in order are benign cystic teratomas, serous cyst adenomas, paraovarian cysts, mucinous cystadenomas and endometriomas. The mucinous cystadenomas are one of the benign epithelial ovarian tumours which tend to be unilateral and multilocular with smooth surface and contain mucinous fluid. They comprise 12%-15% of all ovarian tumors. Around 75% of all mucinous tumors are benign, while 10% are borderline and 15% are invasive carcinomas.[6] .The benign mucinous tumors are most common in the third to fifth decades of life and may be 20-30 cm in size.

Giant cysts are found in less than 1% of the cases of ovarian cysts with pregnancy. The most common and serious complication of benign ovarian cysts during pregnancy is torsion. Other complications which might occur are rupture of cyst, infection, malignancy, impaction of cyst in pelvis, obstructed labour and malpresentations of fetus.

On review of literature the studies are lacking to guide proper management of such cases. Some studies favour surgical intervention for fear of above stated complications while others recommend conservative management because most of the cysts found during pregnancy are corpus luteal cysts and they regress spontaneously by 16 weeks of gestation. Occurrence of ovarian torsions is most common during first trimester and fewer during second and third trimester.

MANAGEMENT

Management of ovarian cysts depends on the size of cysts. Most of the cysts having diameter of less than 5 cm and which have benign looking picture on ultrasonography (USG) can be managed conservatively and careful follow up can be done as most of them resolve spontaneously over time. Cysts measuring more than 10cm are resected to prevent the complications like torsion, rupture and increase chances of malignancy. Management of cysts with diameter between 5-10 cm is controversial.[7] If they have USG picture of solid components, papillary excrescences, if the cysts contain septae and nodules, then it's better to resects them because of increase in risk of malignancy. On sonography, if the cyst appears like simple cyst, it should be monitored by sonography. However it is to be kept in mind that even these cysts may necessitate emergency laparotomy and exploration if complications like torsion, rupture or necrosis arise as seen in as many as 50% cases.

These days due to advent of modern techniques such as MRI, trans vaginal colour Doppler, high resolution ultrasound, conservative management has become quite easy. Based on the gestation period, management of ovarian cysts can also be done. Most common ovarian cysts encountered during pregnancy are corpus luteal cysts. They usually resolve up to 12-16 weeks so follow up can be done till then. There is an additional advantage of waiting till 16 weeks as by this time implantation of pregnancy is more secure and there are less chances of abortion. Persisting ovarian cysts beyond this gestation are managed by simple cystectomy or ovariotomy as indicated till 28 weeks. Beyond this gestation, risk of preterm labour is there if surgical option is considered. In case of emergency, such as torsion, rupture, hemorrhage, necrosis or features of malignancy, laparotomy has to be taken up regardless the period of gestation.

CONCLUSION

Abdomen being a Pandora's Box, Maternal Health can be affected by unusual and unexpected presentations, like incidental ovarian cyst. This probably led to non progress of labour and fetal distress. Despite four USG's are done in her Antenatal period in private, there was no mention of any adnexal mass. We should be prepared for such surprising things and also should take necessary actions.

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Conflicts of Interest: None

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Wandering Dermoid Cyst

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Abstract

Wandering dermoids are extremely rare, their incidence being 0.4 % of all ovarian teratomas. They tend to occur in young women of reproductive age group, although cases have been reported in pre-pubertal and elderly patients. The proposed theories for their occurrence includes, dermoids arising from supernumerary ovaries, from displaced germ cells and auto amputation followed by re-implantation. Mostly they are asymptomatic but some may present with discomfort, non-specific pelvic pain, abdominal mass and pressure symptoms. It may be complicated by torsion, rupture, chemical peritonitis, malignant changes and rarely infection. Here we are reporting a case of wandering dermoid, an incidental finding during laparoscopy done for secondary infertility, associated with endometriosis and the same was managed by excision of both endometriosis and dermoid.

Keywords: Dermoid; wandering; uterosacral dermoid; associated endometriosis

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Introduction

Dermoid cyst is one of the most frequently occurring ovarian cysts and it is the most common germcell neoplasm of the ovary. It constitutes 10-15% of the ovarian tumours and tends to occur in women of reproductive age group, although cases have been reported in extremes of age. Dermoids are seen mostly in one ovary, loculated with smooth surfaces containing sebaceous material, hair, bone, calcification, thyroid tissue, and bronchial mucosa[1]. Rokitansky nodule is seen inside the cyst and tuft of hair arises from the same[2]. Wandering dermoid cysts are extremely rare; their incidence reported being 0.4% of all ovarian teratomas[2]. These extragonadal teratomas occur most commonly in the omentum[2]and less commonly in the fallopian tube, uterus, diaphragm, liver, mediastinum, and thymus. So far, there has been report of only 4 cases of teratomas arising from the utero sacral ligament. [1, 3, 4]

Dermoid cysts may be complicated by torsion, rupture, chemical peritonitis and malignant change, but it is rarely complicated by infection. It occurs in 1% of mature cystic teratoma and the infecting organisms are most likely coliforms, actinomycosis, brucella and salmonella.

Dermoid cysts contain tissues from all three embryonic layers-endoderm, mesoderm and ectoderm. They are slow growing tumours and most of the dermoid cysts are asymptomatic and detected incidentally. Symptoms related to dermoids are abdominal discomfort, vague pelvic pain, mass abdomen, irregular bleeding and pressure symptoms.

Here, we are reporting a case of wandering dermoid which was an incidental finding at laparoscopy done for secondary infertility.

Case report

Mrs. X, married for 7 years was referred to us as a case of secondary infertility for diagnostic hystero-laparoscopy.

Her menstrual history was regular with normal menstrual flow. She had H/O 2 abortions in the past; one was an induced abortion in 2011 at 2 months amenorrhoea due to radiation exposure. Another one was a missed abortion in 2017 for which evacuation was done.

She had H/O TB cystitis with proximal urethral stenosis for which she had been undergoing serial urethral dilatation for quite some time. Now, she is on Tablet. Tamicept 0.4 mg (tamsulosin-an alpha1 receptor antagonist) per day.

As far as her infertility was concerned, she had under gone ovulation induction with IUI many times.

Her USG done on 23.02.2021 showed bilateral PCOS with a small cyst measuring 2 x 2 cms in the left adnexal region suggestive of para ovarian cyst. Her general examination was normal with normal BMI. Patient did not show icterus, lymphadenopathy and oedema. Vitals were stable. Breast, thyroid and spine examination were normal. On speculum examination: Cervix and vagina appeared healthy. Vaginal examination showed normal sized uterus and all fornices were free.

In view of prolonged secondary infertility, she was suggested diagnostic hystero-laparoscopy. All her basic blood investigations were within normal limits. Laparoscopy showed normal size uterus, tubes and ovaries. Para-ovarian cyst reported in USG was not seen. Uterosacrals on both sides had superficial endometriotic deposits. There was a cystic lesion of size measuring 2 x 1.5 cms in the pouch of Douglas attached to the uterosacral ligament on the right side, which could be the probable para ovarian cyst shown in USG. Initially it was thought to be an endometriotic cyst arising from the right uterosacral ligament. The same lesion was excised along with utero sacral endometriosis. Specimen was removed through lateral pelvic port. During its removal, the cyst wall ruptured and the contents were found to be thick sebaceous fatty material along with hair, confirming the nature of the cyst to be a dermoid. The cyst with its entire content was removed in to and sent for HPE. Other abdominal organs were normal. Chromo-tubation was done which showed bilateral tubal spill. Hysteroscopic findings were also normal.

Fig: 1 - Showing the Presence of Utero sacral wandering dermoid cyst with endometriosis

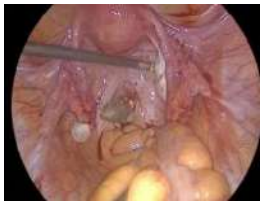


Fig 2: Excision of wandering dermoid cyst

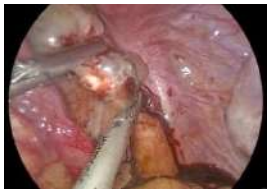
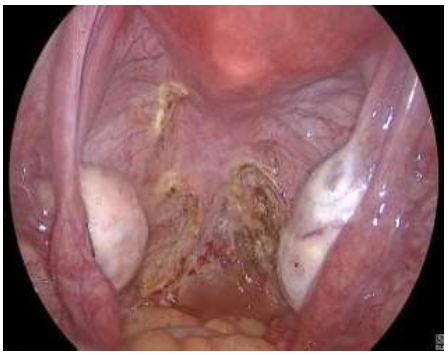


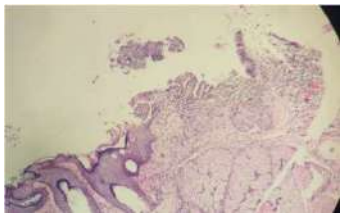
Fig 3: Post-excision



Post-operative period was uneventful and she was discharged next day.

HPE showed mature cystic teratoma and endometriosis of uterosacral ligament

Fig 4& 5: Benign cystic teratoma



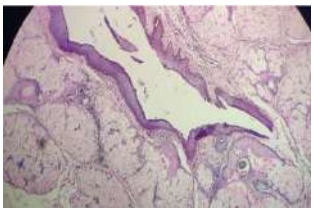
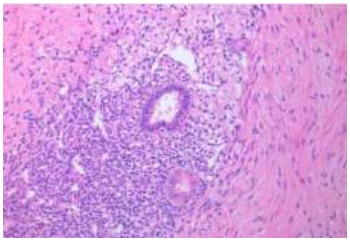


Fig 6:Endometriosis of utero sacral ligament



Discussion

Teratomas arise from toti-potential gonadal cells, and rarely extra gonadal teratomas have been reported in the midline[1]. Transmigration of germ cells in utero, can lead to teratoma formation which supports its congenital origin. It is very rare to have teratomas over the uterosacral ligament. Heller et al first described one such case in 1989, followed by 3 other cases that have been reported to date.[5]

Several theories exist to explain their occurrence. The first theory proposes dermoids developing from supernumerary ovary; the second theory proposes primary dermoids originating from displaced germ cells and the third theory as proposed by J. K. Thornton is autoamputation and re-implantation of an ovarian dermoid cyst as a result of torsion. If torsion of the tumour is sub-acute, an inflammatory response may occur which causes the tumour to become adherent to the surrounding structures with new collateral circulation[6]. The tumour may, and then become completely detached from its original blood supply, thus resulting in a wandering dermoid cyst. The auto-amputation mechanism is especially attractive when there is absence of ipsilateral ovary[7]. Among all the proposed etiologies torsion of pre-existing dermoids which cause auto-amputation, later re-implants in a different place leading to wandering dermoids. They are generally slow growing tumours and grow up to the diameter of 5–10 cm, which may rarely reach giant size.

Dermoids are usually asymptomatic and its diagnosis is incidental as it is in our case. Dermoid becomes symptomatic only when it undergoes torsion. Rarely nonspecific pelvic pain, AUB and abdominal masses are the presenting features[8].

Mature cystic teratomas are more prone for torsion, rupture leading to chemical peritonitis and infection than other ovarian tumours. 3.2–16% of cases present with torsion[8, 9] which could be due to the long pedicle and leads to severe abdominal pain. The mobility of dermoid could be attributed to the soft consistency, smooth surface and lack of adhesions to the neighbouring structures. When a dermoid undergoes torsion, it becomes gangrenous and show haemorrhagic infarction. Intermittent twisting can lead to partial torsion and symptoms may subside once de-torsion occurs. Pain recurs when subsequent torsion occurs.

Less than 1% of the dermoids are malignant. Malignant transformation is rare and can be approximately seen in 2% cases[8]. Dermoids are also associated with other types of ovarian tumours[9].

Imaging helps in easy diagnosis due to the presence of fat and calcification[9, 10]. By ultrasound, cystic lesion with echogenic Rokitansky nodule projecting into the cyst can be picked up[11]. Other USG findings are echogenic mass with sound attenuation or echogenic bands due to hair. CT and MR imaging will be confirmatory for the diagnosis of dermoids due to the presence of fat[11, 12]. On CT, due to fat attenuation, calcification may be seen in the wall of the dermoid which is pathognomonic. 56% of cases will show the presence of teeth or bone.[13]Laparoscopy is the ideal way of removal of ovarian dermoid cyst which is beneficial over laparotomy.[6]Single port laparoscopy has been found to be more cosmetically acceptable than conventional

laparoscopy[14]. Dermoid cysts can be enucleated without reducing the ovarian reserve.

Most common sites for wandering dermoids are the omentum and 27 such cases have been reported. A case of wandering dermoid of the ovary attached to the urinary bladder presenting as obstructed labour have been reported and, in such situations, dermoid cyst has to be removed during caesarean section[1]. The differential diagnosis of dermoid cyst has to be thought about in any pelvic masses. In our study the origin of dermoid could be extragonadal as there was no evidence of ovarian tissue in the HPE. As per literature evidence, dermoids of uterosacral ligaments are more common in elderly rather than young woman and it is found to be more common in the right side as it is found in our study[15].

Conclusion

Though wandering dermoid cysts are rare, we must always consider the differential diagnosis of dermoid when we come across the pelvic masses. USG, CT and MRI will help in the diagnosis of dermoid cyst. The presentation may be varied depending upon the size of the mass, torsion and rupture. Most often it is asymptomatic and an incidental finding and rarely larger lesions can be symptomatic. Smaller asymptomatic lesion can be followed up. Larger lesion should be removed as they are prone for complications, ideally through laparoscopy. Dermoid enucleation will not reduce the ovarian reserve. Less than 2% of cases undergo malignant transformation. Wandering dermoids are almost incidental finding during laparoscopy as it had occurred in our patient.

Extra gonadal teratomas have been reported in the midline[1]. Transmigration of germ cells in utero, can lead to teratoma formation which supports its congenital origin. It is very rare to have teratomas over the uterosacral ligament. Heller et al first described one such case in 1989, followed by 3 other cases that have been reported to date.[5]

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Legends for figures:

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Fig 2: Excision of wandering dermoid cyst
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