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A RETROSPECTIVE STUDY TO FIND EMBRYONIC OUTCOME WITH DIFFERENT VARIABLES DURING IVF TREATMENT

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ABSTRACT

Infertility is defined as absence of conception after one year of regular intercourse. To check the efficacy of embryonic outcome, the different variables such as AGE, BMI, FSH, LH, P4, E2, AMH are measured. The purpose of the study is to know how different hormones, age, BMI influence on oocyte production and embryonic outcome.

A retrospective study to find embryonic outcome with different variables during IVF treatment. In this Retrospective study performed at sims hospital, the data are collected between Jan 2020-Dec 2021. Embryonic outcome of 100 blastocyst were analysed. The frequency of the different variable such as AGE, BMI, LH, AMH, FSH, P4, E2 is compared with D3 and D5 blastocytes and their significant is measured.

The total number of study population was 100. The majority of the population was found to be 31 to 40 age. The predominant BMI among study population of overweight (46%). The mean FSH was 7.88.62. The mean of LH was 5.725.57. The mean of AMH was 3.803.45. The mean of p4 was 4.105.66. The association between Age and BMI with hormone profile was found to be not significant. The clinical outcomes of fertilization rate D3 and D5 key performance indicator was found to be not significant and hormone profile variable. Using different variable, the embryonic outcome on D3 and D5 was found to be no significant.

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ABBREVIATIONS

1. IVF- INVITRO FERTILIZATION
2. ART-ASSESTED REPRODUCTIVE TECHNOLOGY
3. ICSI-INTRA CYTOPLASMIC SPREM INJECTION
4. BMI- BODY MASS INDEX
5. FSH- FOLLICULAR STIMULATING HORMONE
6. LH- LUTEINIZING HORMONE
7. P4- PROGESTERONE
8. E2- ESTRODIOL
9. AMH- ANTI MULLERIAN HORMONE
10. HPO-HYPOTHALAMIC PITUTARY OVARIAN AXIS
11. ET- EMBRYOS TRANSFER
12. HRT- H ORMONE REPLACEMENT THERAPY
13. HCG- HUMAN CHORIONIC GONADOTROPIN
14. PCOS-POLYCYSTIC OVARY SYNDROME
15. FET CYCLE- FROZEN EMBRYO TRANSFER CYCLE
16. LBR- LIVE BIRTH RATE
17. AFC- ANTRAL FOLLICLE COUNT

18. DOR- DECLINED OVARIAN RESERVE

19. CCCT- CLOMIPHENE CITRATE CHANGE TEST

20. WAA- WORLD AUTHORITY AND AGREEMENT

21. AFC- ANFA FERTILITY CENTER

22. ICI- INTRA CERVICAL INSEMINATION

23. IVFD- INVITRO FERTILIZATION WITH DONAR SPERMATOZOA

24. IUI- INTRA UTERINE INSEMINATION

INTRODUCTION

Infertility is defined as absence of conception after one year of regular intercourse. IVF involves using hormones to modify ovarian function in order to increase follicular growth and thus develop more than one oocyte. Over the past two decades, ovarian reserve has been identified as a key factor in the success of assisted reproductive technologies (ARTs). As a result, markers of ovarian reserve have evolved to become part of routine diagnostic testing performed prior to in vitro fertilization (IVF). The goal of such diagnostic procedures and assays is to identify women at high risk of having a poor response to controlled ovarian stimulation during IVF cycles, with subsequent risk of cancellation. Likewise, such testing can identify patients at risk for ovarian hyper-stimulation syndrome. IVF protocols are constantly under review in an attempt to improve follicular recruitment, whilist primarily to increase live birth rates. To check the efficacy in vitro fertilization, the following BMI, Age and hormones profiles [FSH, LH, P4, E2, AMH] are measured. in with BMI is the important key factor in assessing quality of oocyte produced and pregnancy rate.in previous studies, the obesity is the major cause for the infertility. it is widely regarded as a major global pandemic that has far-reaching implications well beyond the consequence of the patient's health (1).

Obesity rates have increased dramatically in developed countries, and this trend is also now evident in developing countries, for example in India the prevalence of overweight increased from 8.4% to 15.5% among women between 1998 and 2015, and the prevalence of obesity increased from 2.2% to 5.1% over the same period (2). Most of the recent evidence categorically demonstrates that obese women are at an increased risk of sub-fecundity and infertility. This is mediated by an interplay between derangements in the hypothalamic pituitary ovarian axis (HPO), oocyte quality and endometrial receptivity (3). Indeed, poorer reproductive outcomes have been demonstrated to impact

obese women regardless of the mode of conception, encompassing natural conception, pregnancies achieved by ovulation induction, in vitro fertilisation (IVF) (4)

Analysis of follicular fluid assayed for various hormones and metabolites from patients undergoing IVF cycles demonstrates significant differences in obese patients compared with their normal-BMI counterparts. In a study by Metwally et al. (2007) (5), comparing IVF outcomes in obese and lower-BMI groups of patients under the age of 35 revealed that women who are obese had significantly lower oocyte utilisation rates and significantly more embryos discarded than the normal or overweight subgroups being analysed. Data correlating fertility outcomes and BMI are conflicting and complicated by relatively few sufficiently powered prospective trials. The lack of homogeneity and standardisation in definitions of obesity and study protocols has resulted in inconsistencies in study outcomes. Early research suggested that an increased BMI has a deleterious effect on fertility. In one study, Zaadstra et al. (1993) (6) analysed 500 women receiving treatment with donor sperm.

A 30% reduction in the rate of conception was demonstrated with each 0.1-point increase in waistehip ratio (WHR). Conversely, a recent retrospective Canadian study investigated the effects of BMI on gonadotropin requirements for ovarian stimulation and IVF outcomes. These included cycle cancellation, clinical pregnancy and live birth rates in various BMI groups, followed by a multivariant analysis adjusting to confounders such as age, presence of PCOS and duration of infertility. No significant difference in outcomes between normal, overweight and obese groups was demonstrated .

It is well known that a woman’s ability to conceive naturally decreases after the age Capsule. The rationale of focussing on women under the age of 35 is that one would expect the quality of oocytes to be consistently better than in an older age group . It is well known that fertility in women decreases with increasing age, as illustrated by the following statistics

- Infertility in married women aged 16–20 = 4.5%
- Infertility in married women aged 35–40 = 31.8%
- Infertility in married women over the age of 40 = 70%

A worldwide survey of IVF centres demonstrates most utilize antral follicle count as part of their practice but most do not consider it the best predictor of ongoing pregnancy rate (9). As women continue to postpone childbearing, thereby increasing the prevalence of agerelated fertility, ovarian reserve testing remains an important diagnostic tool in daily practice for IVF providers (10). Several methods have been established to predict IVF response, including basal follicle stimulating hormone (FSH) levels, anti-Mullerian hormone (AMH), and Age is, of course, a very sensitive marker of oocyte quality and a prognostic marker of assisted reproductive technology (ART) success. Age is, however, not the only important predictor of IVF outcomes. Functional ovarian reserve (FOR), a term reflecting the growing follicle pool, and, therefore, oocyte and embryo numbers, is also closely associated with IVF outcomes. This view is challenged by a recent study clearly showing that the quality of oocytes and number of oocytes retrieved is similar in older women undergoing IVF cycles. Infertile women up to 45 years with severely diminished ovarian reserve achieve better live birth rates than previously reported and should not be denied access to IVF based on elevated FSH levels alone (11,12).

AMH was discovered in the 1940s by Alfred Jost, who described the role of the hormone in the differentiation of gender in the embryo. It has also been proven that it has a strong influence on the function of ovaries, especially on the growth of follicles. This discovery opened a completely new spectrum of AMH use in gynaecology, from in vitro fertilization (IVF) to diagnostics of different ovarian diseases and cancers as well as its future use (13). Anti-Mullerian hormone (AMH) secreted by granulosa from small growing follicles in ovary, plays a very important role in maintaining the "follicle pool"[1]. Female mice with AMH deficiency exhibit a phenotype of premature depletion of primordial follicles[2]. The mechanism of the action of AMH on maintaining the "follicle pool" is

associated with its suppression of genes (encoding stimulatory growth factors) required for the cyclic recruitment of primordial follicles and a decrease in the sensitivity of primordial follicles in response to the stimulation of follicle-stimulating hormone (FSH). Therefore, the level of serum AMH can reflect ovarian reserve (14). Currently, AMH has been widely used as a golden maker for evaluating ovarian reserve of females, particularly in the field of assisted reproduction. This is because of the high sensitivity of the AMH concentration in reflecting ovarian reserve, which exhibits stable expression that is independent of the menstrual cycle and can be accurately and easily determined in serum. Not restricting the ovarian reserve, AMH can also serve as a useful marker in predicting the ovarian response to controlled ovarian stimulation, cycle cancellation and time of menopause.

However, reports regarding the predictive value of AMH on clinical pregnancy and live birth in assist reproduction are controversial. This may be due to inefficient exclusion of confounding factors. For example, when comparing the difference in pregnancy or live birth rate, the quality, quantity and in vitro culture time of embryos transferred (ET) should be considered. Successful pregnancy or live birth in assisted reproduction is determined not only by the quantity but also by the quality of oocytes retrieved. It has been widely acknowledged that anti-Müllerian hormone (AMH) is a golden marker of ovarian reserve. Declined ovarian reserve (DOR), based on experience from reproductive-aged women, refers to both the quantitative and qualitative reduction in oocytes.

This view is challenged by a recent study clearly showing that the quality of oocytes is similar in young women undergoing IVF cycles irrespective of the level of AMH. However, it remains elusive whether AMH indicates oocyte quality in women with advanced age (15).

Follicle-stimulating hormone (FSH) is an important part of the reproductive system.

It’s responsible for the growth of ovarian follicles. FSH is the same hormone that is contained in the injectable gonadotropins which are used to produce multiple eggs for infertility treatment. An

increase in FSH may also indicate a reduction in the production of good quality eggs and embryos for fertilization (16). A common reason for this is your age. As you age, your fertility starts to decline and fewer eggs mature in your ovaries. The quality of the eggs that remain is lower than during earlier years. By measuring a woman's baseline FSH on day

3 of the cycle (we do it on day 2, 3, or 4), we get an indication as to whether she has normal "ovarian reserve". We are looking at how hard her body needs to "step on the gas" early in the menstrual cycle to get a follicle growing. Therefore, if the baseline FSH is elevated the ovarian reserve (how many eggs are left) is reduced (sometimes the egg quality is also reduced). Some practical problems with the day 3 FSH test follows an abnormal result (high baseline FSH) tends to be very predictive of low egg quantity, a normal result does not necessarily mean that the egg quantity is good (17). There are a significant number of women with normal FSH values that have a reduced egg supply. The lower egg supply is not being reflected in their FSH value. This is why doing antral follicle counts and AMH levels can be useful. By doing multiple ovarian reserve tests, we are more likely to find an ovarian reserve problem. The second most problem is the value of FSH varies based on different laboratories for example and FSH of 11 in one laboratory may reflect good ovarian reserve – whereas a level of 11 in another lab using a different assay may indicate diminished ovarian reserve (18)

The relationship between elevated basal FSH and embryo quality remains a topic of heated discussion among practitioners of ART (19). Ovarian Reserve Screening involves the use of multiple tests to better understand the likelihood of achieving a live birth.There is no single test that is the best predictor of live birth. All test results should also be interpreted in the setting of age and prior performance during treatments. Low FSH and higher AMH levels and Antral Follicle Counts are associated with a better prognosis. High FSH and low AMH and Antral Follicle counts are associated with a worse prognosis. But in some cases Some authors suggest a negative effect of raised FSH on

the quality of embryos and therefore on IVF treatment outcome. We postulate that women with elevated FSH who respond well to ovarian stimulation and have embryos to transfer, have the same chance of conceiving like women of a similar age with normal FSH (20).

Estradiol is a form of the hormone estrogen. It's also called 17 beta-estradiol. The ovaries, breasts, and adrenal glands make estradiol. During pregnancy, the placenta also makes estradiol. Estradiol helps with the growth and development of female sex organs, including the, uterus, fallopian tubes ,vagina, breasts .the main function of E2 is, it control the way fat is distributed in the female body. if the level of Estradiol is higher than normal may suggest early puberty ,tumors in the ovaries or testes and lower lever indicates ovarian failure, or premature menopause, which occurs when the ovaries stop functioning before the age of 40 and polycystic ovarian syndrome (PCOS), a hormone disorder with a wide range of symptoms that's also believed to be a leading cause of infertility in women (17).

In previous studies ,The increase in Estradiol shows increases the chance of pregnancy . Estrogens, including estradiol and estriol, progesterone, and glucocorticoids increase over the course of pregnancy and affect transcriptional signalling of inflammatory immune responses at the maternal-fetal interface and systemically. In some studies the increased or decreased Estrogen level in blood does not cause any impact In embryonic outcome and pregnancy rate (21).

LH is an important sex hormone. The elevated LH in the blood for couples undergoing ivf finding it hard Estrogens, including estradiol and estriol, progesterone, and glucocorticoids increase over the course of pregnancy and affect transcriptional signalling of inflammatory immune responses at the maternal-fetal interface and systemically conceive or maintain a pregnancy. The LH test is must for IVF treatment (22). A too-low or too-high LH level does not, in itself, automatically preclude a successful conception. High exposure of the genital tract to LH and E2 in the early follicular phase is

associated with a reduced chance of pregnancy ref, 1Elevated day 3 FSH/LH ratio is associated with inferior outcome in IVF treatment cycles and it could be used as an additional predictor of decreased ovarian reserve (23). IVF and fertility-challenged patients should not be too discouraged if their LH readings are out of the normal range (which can, in any case, vary from lab to lab). Many of our IVF patients have had babies, despite sub-normal LH scores (24) the present study has shown that serum LH measurements, in the mid-follicular phase during ovarian stimulation with recombinant FSH under pituitary suppression with leuprolide in normogonadotropic women undergoing assisted reproduction, cannot predict ovarian response, IVF/ICSI outcome, implantation, and the outcome of pregnancy. Therefore, the results do not support the need for additional exogenous LH supplementation in down-regulated women receiving a recombinant FSH-only preparation (25). On this basis, the analyses whether or not all patients need luteinizing hormone for follicular growth stimulation low serum LH concentrations on the day of trigger hCG has better fertilization rate. LH levels between 25th and 75th percenage have an influence on the average number of > 18 mm size follicles. However, the LH level on Day 1, Day 5 and Day of hCG does not affect the cycle outcome in COS with antagonist protocol of IVF cycle. Hence, LH estimation is not mandatory in ART cycles with GnRH antagonist protocol (26,27,28,29,).

Progesterone plays a key role in implantation through several mechanisms such as endometrial differentiation (1), myometrial quiescence (2) or immune modulation (3). It has an essential function for the onset of pregnancy and is thus widely used in luteal support of assisted reproductive technique (ART) cycles. progesterone level increases during pregnancy. But in some studies, high progesterone groups did not show difference in clinical pregnancy or miscarriage rates (30). High progesterone group had lower clinical pregnancy rate and similar miscarriage rate, despite having higher number of fertilized oocytes and better quality of embryos. Some results suggests that elevated progesterone-initiation-day E2 levels may negatively affect pregnancy outcomes during artificial cleavage-stage

embryo transfers. However, it is not necessary to monitor E2 levels when transferring blastocysts in artificial FET cycles (31). Some studies showed a negative impact of different variables, in terms of embryo quality and cumulative live birth rate (LBR), regardless of the ovarian response. Others linked the negative impact on clinical outcomes to cleavage embryo transfer, blastocyst or frozen-thawed embryo transfer . in our studies the d3 and d5 blastocysts are measured as embryonic outcome and the outcome measured is compared with different variables.

The aim of our study is to evaluate the impact of variables on the day of triggering on the probability of embryonic outcome in IVF.

Obesity is a chronic disease with an increasing prevalence worldwide; it also negatively affects female fertility. Compared with normal weight women, overweight or obese women are at a higher risk of infertility, abortion, pre-eclampsia, gestational diabetes and other pregnancy-related or obstetric complications. Obese women are more likely to experience ovulatory dysfunction, disruptions in the hypothalamic–pituitary–ovarian axis, and oocyte quality defects. Increasing evidence has shown that obesity affects clinical outcomes after in vitro fertilization (IVF) procedures [. Obesity is usually determined based on the body mass index (BMI), calculated as weight in kilograms divided by height in metres squared [6]. According to the World Health Organization, normal weight is defended as 18.5≤BMI≤24.99 kg/m2, overweight as 25≤BMI≤29.9 kg/m2, and obesity as BMI≥30 kg/m2. However, according to the Chinese Ministry of Health, the Chinese have a lower BMI than comparable European populations; thus, BMI as an indicator of modern metabolic diseases functions different in the Chinese. The Working Group on Obesity in China has formulated its own BMI classification criteria, defining underweight as BMI

The advanced maternal age effect on assisted reproductive technology (ART) and pregnancy outcomes is clear because of age-related increases in the frequency of chromosomal aneuploidy in the embryo. However, the paternal age impact has been controversial. Increased incidences of de novo autosomal dominant mutations, autosomal aneuploidy, copy number variant, and Linked disease in the offspring of advanced paternal age cases were reported by Justin et al.Furthermore, an association between advanced paternal age (40 years) and trisomy 21 was reported by Halve et al. However, the impact of sperm findings, ART and pregnancy outcomes because of paternal age varies among reports. Regarding semen analysis findings, studies have and have not reported sperm findings' effects in advanced paternal age cases. The paternal age effect on pregnancy outcome in intrauterine insemination was investigated by Belloc et al. and the multivariate analysis demonstrated that paternal age influences pregnancy outcomes. Regarding ART and pregnancy outcomes, some studies have reported that in vitro fertilisation (IVF) affects embryogenesis and pregnancy outcomes, whereas others have not reported the abovementioned effect. Some studies have reported that intra-cytoplasmic sperm injection (ICSI) affects embryogenesis, but does not affect pregnancy outcomes, and others reported that neither culture results nor pregnancy outcomes are affected It was reported by Park YS et al. that paternal age only affected pregnancy outcomes in ICSI.A metanalysis of the studies published in August 2019 that showed an association between advanced paternal ages and an increased risk of spontaneous miscarriage was reported by Nadia; although, the paternal age effect was less pronounced than that observed with advanced maternal age . On the other hand, it was reported by Halve et al. that paternal age was not clearly associated with ART or pregnancy outcomes because of confounding factors such as maternal age. Therefore, the confounding factors were excluded, such as maternal age and embryonic condition, which could affect * ART and pregnancy outcomes, and paternal age effect on ART and pregnancy outcomes was investigated. Furthermore, considering the sperm findings' effects, abnormal semen examination cases were excluded with reference to the World

Health Organization WHO guidelines 2010, and only IVF was used. This study's primary outcomes were to determine whether paternal age affects high-quality blastocyst rate and miscarriage rate in IVF. Furthermore, normal fertilization (2 PN (pronuclei)), clinical pregnancy and live birth rate as secondary outcomes in IVF were investigated.

Hormones and inflammatory mechanisms are implicated in the major events of female reproductive function, including ovulation, menstruation, embryo implantation and pregnancy. Increasing evidence shows that hormonal aberrations and a hyperinflammatory state may lead to derangements of the immune-endocrine cross talk among endometrium, myometrium and cervix, and between the decidua and trophoblast, predisposing to pregnancy complications. Therefore, the aim of the current review was to assess whether inflammatory mechanisms and hormonal and metabolic dysfunctions occurring in uterine (endometrium, myometrium, cervix) and placental tissues in women with uterine fibroids, endometriosis, adenomyosis, PCOS and unexplained infertility may contribute to pregnancy disorders. Since other uterine conditions associated with obstetric complications, such as uterine malformations , synechiae and Asherman syndrome, work mainly through mechanisms other than inflammatory, endocrine and metabolic pathways, they are not part of the present review.

Luteinizing hormone (LH) plays a key role in gonadal function. LH in synergy with follicle stimulating hormone (FSH) stimulates follicular growth and ovulation. Thus, normal follicular growth is the result of complementary action of FSH and LH.

FSH is frequently used in assisted reproductive technology (ART). The most commonly used protocol in ART consists of controlled ovarian hyper-stimulation (COH) with daily injections of recombinant human FSH (r-hFSH) to induce multiple follicle growth in the ovaries. To prevent premature LH surge and premature ovulation, gonadotropin-releasing hormone (GnRH) agonist or antagonist is injected daily. The pituitary down-regulation (endogenous pituitary suppression) that is achieved with GnRH

analogues creates an environment where LH is deficient or very low and which may be detrimental to the development of normal healthy follicles. It has been shown that growing follicles become increasingly sensitive to and ultimately dependent on, the presence of LH for their development. Documented results associate poorer outcomes with patients whose LH concentration was low, after pituitary suppression was achieved with GnRH analogue treatment.

The availability of recombinant human LH (r-hLH) has paved a way for supplementation of LH in down-regulated IVF cycles. Several recent studies have evaluated the role of r-LH in women undergoing GnRH analogue/r-hFSH therapy and IVF and observed variable results. One such study observed that supplementation with r-hLH showed lower levels of cumulus cell apoptosis than treatment with FSH alone, possibly indicating improved oocyte quality in LH-supplemented cycles. Reduction in apoptosis of cumulus cells in the r-hLH group might be the result of lower levels of follicular fluid vascular endothelial growth factor (FF VEGF-marker of maturity and quality of occytes) that is produced by granulosa and theca cells in response to FSH, LH, human chorionic gonadotropin (hCG) and proliferative and apoptotic factors. All these studies point that LH may be crucial in COH. The poor outcome of COH includes increased age (above 35 years), poor ovarian reserve, poor response to previous ART cycles, genetic variations and hormonal status majorly LH, FSH, estradiol and anti-Mullerian hormone (AMH). Overall, these studies suggest that LH supplementation could be beneficial for a particular sub-population, including older patients and poor responders. This might be due to the better ooctye quality resulting from a restored follicle at the end of stimulation in these ART patients. These findings reinforce that the use of the r-hLH in ART should be guided by a rationale that is based on the need of the patient.

Although recent researches have facilitated better understanding of supplementation of LH with FSH hormone and effect on fertilization and implantation, there is still a paucity of information on its usage in ART patients. In this review, we looked into the multiple roles that LH plays complementary to FSH to better understand the LH requirement in patients undergoing ART.

ADJUVANTS

The ovary comprises of two cellular components, which are stimulated independently by LH and FSH, leading to the production of ovarian steroids. Androgen production from cholesterol and release during follicular genesis is dependent on the stimulation of the theca cells by LH and FSH

Ovarian steroidogenesis in the preovulatory follicle takes place through LH receptors on theca and FSH (possibly plus LH) receptors on granulosa cells. The steroidogenic acute regulatory protein (StAR protein) is the primary regulator of production of androstenedione, which subsequently diffuses into granulosa cells to serve as an estrogen precursor. In the preovulatory follicle, cholesterol in theca cells arises from circulating lipoproteins and *de novo* biosynthesis. FSH is responsible for follicular growth and estrogen formation. FSH may be crucial at an earlier stage of follicular development, perhaps earlier in the follicular phase, to induce the aromatase enzyme that converts androgen to estradiol. During the later stages of follicular growth, activins and estradiol, the predominant estrogen in humans, enhance the actions of FSH.

Concept of follicle stimulating hormone threshold and role of luteinizing hormone

The concept of the FSH “threshold” proposed by Brown postulated that in gonadotropin therapy, the ovary has a minimum requirement level (threshold requirement) for FSH below which

follicular development does not occur. More recent studies also confirm that follicular growth does not occur below the threshold levels.

Following optimum FSH stimulation, there is follicular recruitment, growth, selection and dominance. Subsequent development of this cohort during the follicular phase becomes dependent on continued stimulation by gonadotropins. Increasing FSH concentrations should surpass the threshold level to initiate the final gonadotropin-dependent phase of follicular growth.

There is a secretion of increasing amounts of estradiol during this phase. The peripheral estradiol levels are increased with feedback inhibition of FSH secretion. The maturing follicle inhibits FSH secretion leading to a fall in its levels below threshold, thus stopping less mature follicles from maturing.

Further, it has been shown that FSH threshold is not fixed for any given follicle, but depends on the developmental stage and varies over time. The follicles exhibit different degrees of FSH sensitivity at the time of recruitment; highest need for FSH is at the early antral stage and declines in the late antral stage. The follicle with the highest sensitivity will benefit most from increasing FSH levels and will subsequently gain dominance.

The suggested reasons for the response of ovarian follicles to certain FSH level than to a specific dose are fluctuating levels of the endogenous production of gonadotropin, and up-regulation of its receptors due to FSH administration.

Although FSH can induce follicular growth even without LH, there is evidence that the follicles may have developmental deficiencies like abnormally reduced estradiol production and lack of ability

to luteinize and rupture, following hCG stimulus. Hence, a certain amount of LH exposure is necessary for optimal follicular development.

Another possibility is that FSH stimulates the production of progesterone by driving cholesterol conversion into the steroid pathway. Early increased exposure to progesterone can advance the endometrium, leading to asynchrony of embryo development to endometrial development and the reduction of implantation. LH stimulates the conversion of progesterone into androgens, which can be further aromatized to estrogens. The addition of LH may benefit the endometrium by decreasing the risk of a premature progesterone increase and therefore improve the likelihood of implantation and clinical pregnancy.

Concept of luteinizing hormone therapeutic window

The concept of the LH therapeutic window has been explained in brief. Though studies support the use of r-hLH in addition to r-hFSH in GnRH antagonist protocols in ovarian follicular development, these studies are fewer in number. There is also no clear-cut guideline regarding the optimum levels of serum LH and timing of its supplementation are fewer in number. This is an area that warrants further research. Studies have shown that serum LH levels should be between 1.2 IU/L and 5.0 IU/L, for optimal development follicle in cycles where endogenous LH is suppressed.

Some of the recent studies suggest that the indicators for adding LH to an ART cycle are mid follicular (day 6) hypo-response to long GnRH agonist, no follicles > 10 mm, E2 < 200 pg/ml, endometrial thickness < 6 mm and baseline serum LH < 1.2 IU/ml on day 6.

A recent meta-analysis of seven randomized controlled trials (RCTs) done by Hill *et al.* on the use of LH in ART in advanced patient age group concluded that five RCTs were in favor of adding

LH in ART therapy in patients of advanced age group. However, it is critical that add-back LH is administered in appropriate patients as an excess of LH can cause suppression of granulosa cells and follicular atresia.

Follicle stimulating hormone polymorphism

The FSH receptor (FSHR) gene is thought to play a significant role in the success of ovarian stimulation and can be used as a marker to predict differences in FSHR function and ovarian response to FSH. Patients with unfavorable genotypes are reported to require higher doses of r-hFSH to overcome relative ovarian insensitivity. The FSHR gene contains two important single nucleotide polymorphisms (SNPs) in exon 10, which are in linkage disequilibrium and change two amino acids at positions 307 and 680. Women with the 307 Ala and 680 Ser SNPs are associated with reduced COH outcomes, the 680 SNP Series specifically associated with lower clinical pregnancy. These patients when undergoing ART are characterized by higher basal FSH serum concentrations, higher administered amounts of FSH required and higher risks of hypo- or hyper-responses. Up to 35% of patients requiring ART are detected with alternatively spliced FSHR products. Genotyping the FSHR Asn680Ser SNP, together with some additional novel markers (e.g. transcript levels), may therefore provide a means of identifying a group of poor responders before infertility treatment is initiated.

Luteinizing hormone polymorphism

The LH receptor gene is known to carry as many as 282 SNPs. In 1991, Pettersson and Söderholm identified a common genetic LHβ variant or v-βLH owing to the alterations in two polymorphic base changes in the β subunit gene leading to changes in the amino acid sequence, Trp8Arg and Ile15Thr. They had initially suggested this discovery as an immunological anomalous LH form.

The short half-life of v-βLH may be linked to the presence of extra glycosylation signal into the β subunit that could lead to an addition of the second oligosaccharide to Asn13 of the β protein. It has been found that there is more potency of the overall LH activity of v-βLH at the receptor site; however, its duration is shorter *in vivo.* Previous clinical trials conducted to determine the impact of this variant on reproductive health reported its association with ovulatory disorders, premature ovarian failure, hyperprolactinemia, luteal insufficiency, menstrual disorders, endometriosis and infertility. An observational study noted low response in some women following ovarian stimulation, resulting in a greater need for r-hFSH (>2500 IU). In another preliminary study, the total r-hFSH consumption was elevated during ovarian stimulation due to the presence of v-βLH. Based on the findings, the researchers indicated the potential of v-βLH as a marker of ovarian responsiveness to r-hFSH. This role of v-βLH, if validated by further research, could thus facilitate clinicians in identifying patients requiring exogenous LH addition during ovarian stimulation.

Optimizing follicle stimulating hormone dosing

Various studies suggest four parameters of FSH administration management involved in the risk of multifollicular development: (a) the choice of the FSH starting dose, (b) the duration of the starting, dose before stepping up or stepping down, (c) the rate of increase in FSH dose at each increment and (d) the reduction of the FSH dose once a follicle has been selected.

In an attempt to prevent the risks of overstimulation and multiple pregnancies, it is crucial to use a low starting dose of FSH, and to use small increments in the daily dosage.

Exogenous luteinizing hormone supplementation

LH is important in regulating steroidogenesis throughout follicular development; adequate LH is particularly important for oocyte maturation.Most of the Asian assisted reproduction practitioners make use of both long agonist and antagonist protocols for ovarian stimulation; majority using the former approach. Published literature on the beneficial effects of exogenous LH in patients with previous suboptimal response or low baseline serum LH concentrations is more extensive in long agonist protocols. Documented results associate poorer outcomes with patients whose LH concentration was low after GnRH agonist treatment.

The Asia Pacific Fertility Advisory Group in 2011 strongly recommended r-hLH co-treatment with r-hFSH in patients with a history of poor response as in:

1. Suboptimal response on day 6 in long agonist cycles
    - absence of >10 mm follicles
    - endometrial thickness of <6 mm
    - estradiol levels <200 pg/mL
2. r-hLH may also be beneficial in women aged >35 years undergoing ovarian stimulation with long agonist or antagonist protocols.

Poor responders and low ovarian reserve

Many factors are linked to a decreased ovarian response and hence, it is difficult to identify poor responders. Although several tests have been suggested, none can indicate it accurately.

Some putative biomarkers to identify poor responders include (i) LH concentrations either at baseline or day 6 mid follicular (ii) AMH levels and (iii) antral follicle count (AFC). Wong *et al.* recommended that further research is needed in patients with suboptimal response based on the following biomarkers: (i) AFC < 6 in both ovaries; (ii) AMH concentration <1.5 ng/mL; and (iii) LH polymorphisms.

Poor ovarian reserve is estimated to occur in about 9-26% of the ART procedures. Evidence indicates that r-hLH and r-hFSH co-administration in these patients may help in improving ongoing pregnancy rates in poor responders and women of advanced age.

However, further studies are needed in this regard as some studies report that the available evidence is not enough to validate the effectiveness of r-hLH in subjects with poor response undergoing ART.

Advanced reproductive aged patients

A recent systemic review and meta-analysis concluded that the inclusion of r-hLH to FSH stimulation enhanced the clinical pregnancy and implantation rates in ART cycles in patients aged ≥35 years. Similar results were reported in many other randomized trials. Similarly, a Cochrane review reiterated the usefulness of r-hLH in poor responders and advanced aged women at risk of spontaneous miscarriage.

An open-label randomized controlled study found that r-hLH is beneficial in improving the implantation rate in women aged 36-39 years, but not so in those younger than 36 years of age. This might be due to the fact that the serum androgen levels decline steeply with age, as does the response to FSH stimulation. LH administration enhances follicular androgen production followed by its

aromatization to estrogen. It also controls progesterone production by granulosa cells, which is also FSH dependent. Several studies correlated the occurrence of apoptosis in granulosa cells with the IVF outcome. The incidence of apoptosis was lower in granulosa cells of follicles aspirated from patients who became pregnant after ivf cycle compared with granulosa cells of follicles aspirated from patients who are non-pregnant. Bencomo *et al.* reported that, the percentage of apoptotic cells was significantly less in younger age group (<38 years) compared with older age group (>38 years) and further suggested that apoptosis may be a marker for ovarian age or reserve as granulosa cells of older women are more susceptible to apoptosis. In a study by Ruvolo *et al.* shown that the r-LH administration resulted in a reduction in the apoptosis observed in the cumulus cells of the patients whose clinical pregnancy rate and implantation rate was significantly high compared with the non-r-LH administered group. The beneficial effect of LH was attributed to its direct action on cumulus and granulosa cells, or by the paracrine effect mediated by secreting factors in the theca and oocyte cells viz. by inducing the expression of epidermal growth factor in the theca cell, which has a reported antiapoptotic activity. Recently Gatta *et al.* studied the gene expression profiles of cumulus cells obtained from r-LH treated patients and found that 84 genes were up regulated with the following cellular function: gene expression, cell-to-cell signaling and interaction, cellular growth and proliferation, cell cycle, morphology and death, inflammatory response and molecular transport. Data from the above recent studies indicated the significance of LH at cellular and molecular pathways. Thus, LH supplementation seems appropriate for aged patients and poor responders where it restores the follicular and endometrial milieu and improves the cycle outcome.

Another retrospective observational study evaluating ART patients undergoing stimulation with an antagonist procedure reported clinical pregnancy success of 36% for patients aged 38 years treated with r-hFSH and r-hLH compared with 19.1% ($P = 0.048$) for those stimulated with r-hFSH and human menopausal gonadotrophin (hMG). Conversely there were two studies, Fabregues *et*

al. and Nyboeandersen *et al.* who found no benefit in supplementing rLH in the GnRH agonist long protocol.

Role of luteinizing hormone in polycystic ovary syndrome (PCOS)

The detrimental impact of endocrinological disorder, which is linked to hyper-secretion of LH and ovulatory dysfunction, is attributed to increased LH levels. Studies have found that such women are associated with poor fertilization, oocyte quality and embryo quality, which could be due to underlying mechanisms such as androgen excess induced by LH. However, contrary to previous belief, it was later demonstrated that hyper-insulinemia and not LH hyper-secretion plays a vital role in PCOS pathogenesis. Adding LH in this scenario would lead to OHSS and hence LH should be avoided.

Role of luteinizing hormone

LH supplementation is important in older and poor-responding patients because they usually receive higher FSH doses for COS, show higher progesterone levels at the end of stimulation and subsequently, their endometrium receptivity diminishes. Previous studies have shown the benefical effects of LH supplementation in older patients.

Dosing of luteinizing hormone

In 1998, the European Study Group conducted the first randomized efficacy clinical study to investigate the safety and tolerability of r-hLH supplementation in hypogonadotropic hypogonadal women (WHO group 1 anovulation). The researchers also aimed to assess the minimal effective dose for this patient population. The patients (n = 38) randomly received daily injections of 0 IU, 25 IU, 75 IU, or 225 IU of r-hLH in conjunction with 150 IU r-hFSH/day for up to 20 days. The results were showed that r-hLH helped in:

- Promoting dose-associated increase in the secretion of estradiol and androstenedione by r-hFSH-induced follicles.
- Enhancing ovarian sensitivity to FSH as observed in the number of patients who developed follicles following FSH administration.
- Increasing the successful luteinization of follicles on exposure to hCG.

It was observed that 75 IU r-hLH promoted adequate follicular development and steroidogenesis in 46% of the treatment cycles, with sufficient secretion of estrogen and progesterone in 75-80% of the cycles. Based on the findings, the researchers recommended that 75 IU r-hLH is effective in most of the women by facilitating maximal endometrial growth and optimal follicular development, which is defined as:

- ≥1 follicle of ≥17 mm.
- Estradiol levels of ≥400 pmol/L.
- Mid-luteal phase progesterone level of ≥25 nmol/L.

Furthermore, they suggested that a small percentage of women may require up to 225 IU of r-hLH/day subcutaneously, but emphasized that the high dose of r-hLH was also found to be immunogenic and well tolerated. To achieve an optimal benefit Ramu *et al*. suggested a dose of 75 IU/day of r-hLH for supplementation with r-HFSH

The widely used dosage is a ratio of 2:1 for FSH: LH, i.e., 150 IU: 75 IU starting on day 1 or 6 of stimulation, especially in hypo-hypo patients. A study carried out by Lisi *et al.*, shown that the administration of r-hLH (75 IU/day for 4 days), 1 day before the beginning r-hFSH stimulation, offers some benefits in terms of clinical pregnancies when compared with the patient's undergoing

stimulation with r-hFSH alone. Though starting patients with r-hLH on day 1 maximizes the benefit of increased ovarian androgen production triggered due to the presence of the exogenous LH, it acts synergistically with FSH to promote FSH receptor mRNA expression, follicular development and steroidogenesis.

Numerous studies have demonstrated that r-hLH in combination with FSH is better than hMG with FSH. This might be due to excessive or inconsistent LH activity from the hCG component in hMG may affect ocyte maturation in the latter half of the ovarian stimulation cycle, giving rise to the differences in numbers of oocytes retrieved and success of pregnancy.

Controlled Ovarian Stimulation in Normo-Responders

An optimal response to COS cycles is considered as an oocyte yield between 10 and 15 oocytes. Pre-treatment with estrogen, progesterone or oral contraceptive pills (OCP) prior to COS do not offer any benefits in normo-responders. A recent meta-analysis showed a significantly lower ongoing pregnancy rate with antagonist compared to long agonist protocol. However, this outcome was noted only with the combination of oral hormonal pre-treatment and flexible antagonist protocol, while no such difference was evident between fixed antagonist and agonist protocol. Antagonist protocol is preferred in many IVF clinics worldwide considering convenience and safety aspects.

Both recombinant follicles stimulating hormone (rFSH) and human menopausal gonadotropins (HMG) or highly purified HMG (HP-HMG) have been used for COS. A greater number of oocytes can be expected with rFSH compared to HMG. Non-inferiority of HP-HMG to rFSH has been established in both antagonist and long agonist protocols in terms of ongoing pregnancy rates. Thus, the choice of gonadotrophins in normo-responders is based on the availability, cost and clinician's

discretion. There exists a positive correlation between FSH dose and oocyte yield. For predicted normal responders, more oocytes are retrieved with daily dose of 200–225 IU FSH compared with 100–150 IU, with no significant difference observed between 225 IU and 300 IU. However, the current evidence suggests a similar pregnancy rate in normo-responders with starting doses of 150 IU or 200IU of FSH.Available evidence does not support incorporation of recombinant LH (rLH) in rFSH protocols for young normo-responders. Role of rLH supplementation in those with profound suppression of endogenous LH remains controversial. Unexpected hypo response in young women (POSEIDON group I) remains a challenge. A retrospective cohort study from India reported that simple increase in dose of FSH or change of protocol may achieve LBR similar to those with good prognosis. A systematic review in which two RCTs specifically addressed the issue of unexpected hypo response in young women reported that addition of rLH may be beneficial. However, the findings should be interpreted with caution considering the limitations of these studies including relatively small numbers.

Minimal/Mild Ovarian Stimulation

Mild stimulation protocols aim to achieve an oocyte yield of <8 per cycle. The data regarding the efficacy of mild/minimal ovarian stimulation in normal responders is limited. A retrospective cohort study from India reports the cost-effectiveness of mild stimulation in a well selected group of normal responders. A recent meta-analysis shows similar live birth rate (LBR) in normo-responders with conventional or mild ovarian stimulation. However, cancellation rate was two-fold in mild stimulation and with reduced oocyte and embryo numbers.This may negatively affect time to pregnancy and cumulative LBR.

Ovulation Triggering

Presence of two or three leading follicles of 18 mm diameter determines the timing of ovulation trigger. The current literature addressing the optimal length of COS is sparse. It is thought that a shorter duration may allow insufficient time for oocyte maturation and endometrial development. While some authors report a decrease in success rate with prolonged duration of stimulation, others found no association between the length of stimulation and treatment outcome. The most commonly used preparation to mimic LH surge, for oocyte maturation is either recombinant or urinary human chorionic gonadotropin (HCG). Both preparations are equally effective for triggering oocyte maturation in COS. A comparison of 5000 IU and 10,000 IU has not shown any difference in OHSS. 4000IU and 6000 IU have shown similar oocyte maturation, with no benefit on OHSS and a possible negative impact on clinical pregnancy rate. The most recent meta-analysis highlights the need for luteal phase optimization when GnRHa is used as a trigger, to maintain an equivalent LBR to that with HCG. Current evidence is very limited regarding the use of dual trigger in norm responders. Conversely, it is noted that a double dose of rHCG does not improve IVF outcomes. We should consider fresh transfers in normo-responders as no difference has been observed in LBR when compared with elective frozen embryo transfer (eFET). Any change in the current practise should be based on the emerging data.

Normal responders: Summary Points

Gonadotropin Starting dose: 225 IU or lower (considering age and BMI).

Pituitary suppression: Long GnRH agonist or Fixed antagonist (based on availability, convenience and clinician's choice)Ovulation trigger: HCG or GnRHa trigger (in antagonist protocol if hyper-response noted).

Controlled Ovarian Stimulation in Poor Responders

Poor response to COS is encountered in approximately 12-20% of women undergoing IVF. The most common aetiology is POR with its varied, often ill-understood underlying mechanisms.[19] It is important to note that more than 50% women with POR in first cycle of IVF will have normal response in subsequent cycles.[65] However, a persistently poor response of three or less oocytes is a predictor of reduced LBR in older women.[65] A comparative study in women undergoing IVF has shown that the ovarian age of Indian women is approximately six years older than their Spanish counterparts.[22] The interventions in management of this challenging group are directed towards improving the recruitment of a homogenous cohort of follicles leading to an increase in oocyte number and live birth.

Pre-Stimulation Strategies

Androgen supplementation is a widely practised approach to improve the outcome in poor responders. Transdermal or oral testosterone and oral dehydroepiandrosterone (DHEA) are the most commonly used molecules; with conflicting evidence regarding any benefit from various RCTs and meta-analyses. Testosterone initiated before or during ovarian stimulation may improve IVF outcomes in poor responders. Duration of its usage may have therapeutic implications. Currently ongoing T-TRANSPORT trial may add to the understanding of androgen supplementation. DHEA is considered as a cost-effective alternative to testosterone and 75 mg daily in micronized form is the most widely used androgen supplement in expected or proven poor responders. A systematic review including 17 RCTs concluded that the benefits of androgen pre-treatment were inconclusive when the studies with high risk of performance bias are removed. The most recent network meta-analysis with included studies using Bologna criteria for defining poor response shows an improved clinical pregnancy rate with DHEA. It is important to note that only two studies in which 82 women received DHEA were

eligible for inclusion. This precluded the authors drawing conclusions on the quality of evidence. A small single centre cohort study from India documents better pregnancy rates subsequent to DHEA supplementation in poor responders with previous IVF failures. Another study measuring serum and follicular fluid concentrations of DHEA in poor and hyper-responders suggests an important role for DHEA in oocyte activation. Rectification of both low and high values may have a positive impact on embryo parameters and LBR. Current evidence is inconclusive on the role of growth hormone supplementation in improving LBR in poor responders. Limited evidence suggests its beneficial role in long agonist protocol. A single study shows possible benefit of Co-enzyme Q10 (CoQ10) in poor responders.

RCTs including studies with uniform definition of poor response and low risk of bias are necessary to define the place of the above supplements in management of poor responders. Cost of these additions and current lack of conclusive evidence to support their use routinely in clinical practice should be considered prior to their incorporation in routine clinical practice.

Steroid Pre-treatment

Progestins, OCPs and oestradiol are routinely used prior to antagonist cycles. A single study comparing antagonist cycles with and without OCP pre-treatment to GnRHa cycles in low responders showed a lower number of oocytes and embryos in untreated antagonist group compared to the other two groups. However, live birth rate was similar in all the three groups.

Stimulation Protocols

Long agonist, short agonist and antagonist protocols are all utilized in IVF for poor responders. Long agonist and antagonist protocols yield similar pregnancy rates. Conventional protocols in poor

responders involve a higher starting dose of FSH compared to normal responders. Addition of rLH from mid cycle onwards to rFSH is a common clinical practice in poor responders to improve LBR despite lack of conclusive evidence in its support. The ESPART trial did not show any advantage to adding rLH to rFSH in poor responders. Use of urinary HCG instead of rLH appears to be a promising approach in improving clinical pregnancy rates. A retrospective study suggests that early initiation of HMG with rFSH is associated with an improved LBR compared to mid-follicular HMG or rFSH alone. However, this observation needs to be validated through appropriately designed RCTs.An alternative approach to conventional stimulation is the use of mild stimulation or modified natural protocols. A low per cycle pregnancy rate, high cancellation, increased time interval to pregnancy and lack of available evidence on cumulative pregnancy rate should all be considered while choosing this option. Protocols incorporating clomiphene and letrozole may be associated with low oocyte yield, high cancellation rate and the lowest pregnancy rate. Dual stimulation offers an attractive opportunity of increasing the number of oocytes within the span of an ovarian cycle in the context of fertility preservation. However, such an approach in the management of poor responders should be used cautiously considering the financial implications and the absence of supporting evidence.

Poor Responders: Summary Points

Gonadotropin Starting dose: Usually 300 IU (age, BMI and previous response may influence the choice of starting dose).Pituitary suppression: Fixed antagonist or long agonist.Ovulation trigger: HCG.Pre-stimulation strategies: Use of testosterone, DHEA, growth hormone and CoQ-10 all lack high quality evidence for their use in routine clinical practice.

Controlled Ovarian Stimulation in Hyper-Responders

Diagnosis of PCOS, a high AMH or AFC values, a previous high response or high number of retrieved oocytes (>15 oocytes) are considered as indicators of a high response.

Choice of COS protocol, dose of stimulant, ovulation trigger will influence the occurrence of OHSS in hyper-responders.

Pre-Stimulation Strategies

Pre-stimulation steroid and metformin administration may have important impact on the course of ovarian stimulation in hyper-responders. Use of metformin before and during ART is a widely used intervention in women with PCOS. The most recent meta-analysis suggests a reduction in OHSS and a non-significant reduction in miscarriages. While no impact on LBR was noted in long agonist protocol, LBR was lower in the antagonist protocol in comparison to a placebo. The limitations were the low quality of evidence and no data on cumulative livebirth.

Pre-treatment with OCP is a common practice in expected or proven hyper-responders to achieve pituitary suppression without increasing the risk of OHSS. Pre-treatment with OCP in antagonist cycles across the entire spectrum of ovarian response is considered to reduce pregnancy rate, LBR and miscarriages. However, a retrospective study in women with PCOS suggests an improved IVF and pregnancy outcomes following pre-treatment with COCP for three months or longer. This assumption needs further exploration before adopting as a standard clinical practice.

Ovarian Stimulation in Hyper- Responders

Ovarian response to urinary HMG and recombinant FSH exhibits certain differences during ovarian stimulation: rFSH results in a larger number of small and intermediate follicles, more mature oocytes, and in women with basal LH <1 IU/L, very low E2 levels with poor folliculogenesis. Results of a single RCT show that HP-HMG results in higher E2 levels but a lesser incidence of OHSS and miscarriage rate in comparison to rFSH and a similar pregnancy rate. Further, a decision-tree model evaluating the financial impact of therapy per live birth after first embryo transfer in the same patient population suggests a reduced cost with HP-HMG in comparison to rFSH. These reported benefits of efficacy and safety need validation through further RCTs.

A reduced starting dose of FSH is both cost-effective and safe in women expected to be hyper-responders. An elective use of antagonist protocol is both effective and safe in hyper-responders. A prospective study from India in a cohort of women with PCOS shows an increased risk of OHSS with long GnRH agonist protocol compared to antagonist protocol. Final trigger for oocyte maturation in hyper-responders is best decided based on the ovarian response. Coasting, reduced dose of HCG, GnRHa trigger and elective embryo cryopreservation have all been used in an attempt to reduce the incidence of OHSS in this subgroup of women. Prediction of OHSS based on the number of follicles and choosing the appropriate strategy for further management may help optimise the outcomes. Though a ‘freeze all’ strategy remains the standard approach, an intensive luteal phase support with the addition of oestradiol or a small bolus of HCG to the standard progesterone therapy is necessary if fresh cycle transfer is considered following GnRHa trigger.

Hyper-Responders: Summary Points

Gonadotropin Starting Dose: 150 IU or lower (based on BMI, AMH/AFC value and previous response).

Pituitary suppression: Antagonist (most widely used - fixed or flexible multiple dose).

Ovulation trigger: GnRHa (HCG if ovarian response is ≤ normal).

Pre-stimulation strategies: ? Metformin for long agonist GnRHa protocol.

The Efficacy of Stimulation Protocols to Improve Oocyte and Embryo QUALITY

The oocyte quality is one of the key parameters determining the embryo quality and is a good predictor of IVF outcome. Bovine and murine studies have shown that ovarian stimulation may negatively impact the fertilization and embryo development, impair implantation and increase chromosomal abnormalities. However, an analysis of trophectoderm biopsies in a large cohort has shown that the intensity of stimulation does not influence the ploidy status. In a large cohort study, a strong association is reported between the number of oocytes and live birth rate; with the best chance of a live birth at 15 oocytes.

While a study from India suggested that antagonist protocols may be associated with better perifollicular vascularity and better-quality embryos, it included small numbers and did not report on LBR. A systematic review of 73 RCTs has not shown any difference in the LBR when antagonist or agonist was used. No difference is noted in embryo morpho kinetics within individuals undergoing IVF when switched between antagonist and agonist protocols.

It is plausible that gonadotropin preparations used in COS may have an impact on oocyte quality. However, similar pregnancy rates have been reported while comparing rFSH and HP-HMG in the MERIT trial. An RCT comparing urinary FSH (hFSH) and rFSH did not reveal any difference in the fertilization rate or implantation rate. A comparison of HMG, hFSH, rFSH, and sequential hFSH/rFSH did not reveal any difference in the oocyte numbers or embryo quality amongst the different groups in a RCT. Even supraphysiological E2 does not appear to have any negative impact on oocyte quality. Addition of rLH to rFSH in older women has not shown to improve clinical outcomes.

Agonists versus Antagonists in *In vitro* Fertilization

GnRH analogues play an important role in COS to prevent premature rise in LH and premature ovulation as evident from the above discussion. A recent systematic review and meta-analysis showed that in normo-responders GnRH agonist protocols result in higher pregnancy. Within this population, antagonist treatment prevents one case of OHSS in 40 patients but results in one less ongoing pregnancy out of every 28 women treated. In women with PCOS and potential high responders, GnRH antagonists do not seem to compromise ongoing pregnancy rates and are associated with less OHSS and therefore should be considered as standard treatment. In addition, they offer the flexibility of using GnRHa for triggering to minimize the risk of OHSS. While antagonist protocols are widely used in the poor responders, long agonist protocol may be equally effective.

Does Luteinizing Hormone Activity Improve the Quality of Oocyte and Embryo?

Considering the vital role LH plays in folliculogenesis, the current trend of conducting COS in an LH-depleted environment (pituitary suppression and COS with recombinant FSH) has been questioned. The role of exogenous LH in COS remains controversial since very low concentration of endogenous

LH are sufficient to sustain adequate follicular growth and development. However, profoundly suppressed LH may compromise the quality of oocytes and thereby ART outcome. A negative effect on the ovarian response and follicular endocrine profile in LH depleted cycles has been reported. A reduction of apoptosis with improved chromatin quality of cumulus cells involved in oocyte maturation in women treated with r-LH has been observed. A review and meta-analysis of studies comparing different gonadotrophins concluded that FSH alone resulted in higher oocyte number, HMG improved the number of mature oocytes and embryos and increased implantation rate, while rLH addition or use of HMG lead to higher pregnancy rate in GnRH agonist cycles. A large retrospective study of more than 4000 patients demonstrated the beneficial effect of LH in low prognosis patients. LH may improve the oocyte quality by leading to activation of ERK1/2 and AKT-pathway and a final proliferative and anti-apoptotic signal.

The ultimate answer to this debate may lie in pharmacogenetics which demonstrates the effect of individual genetic variability. FSH and LH receptor polymorphisms have been implicated in infertility as well as response to COS. An increase in FSH requirement for COS has been demonstrated in women having an LH or AMH polymorphism. An association between LHCGR N312S polymorphism and a higher requirement for rLH in Indian women homozygous and heterozygous for serine was noted in a cross-sectional study. It is to be seen whether customised COS based on the patient's genome would possibly provide the final answer on the need for LH in COS.

Selective and Elective Freeze Policy

Transfer of supernumerary cryopreserved embryos generated as a result of COS in IVF has evolved as an important strategy to enhance cumulative pregnancy rates (CPR) in ART. A shift in cryopreservation technique from slow freezing to vitrification has led to enhanced embryo survival

rates. and better reproductive outcomes in frozen embryo transfer (FET) cycles. Consequently, a global upsurge in FET cycles of approximately 15-40% has been observed.

High steroid levels generated during COS initiate early endometrial maturation, altering the 'window of implantation'(WOI) leading to a negative impact on embryo implantation. The improved pregnancy rate (PR) in FET cycles is presumed to be a result of better embryo – endometrial synchrony. Rise in pre-ovulatory progesterone level in stimulated cycles is also detrimental to implantation. A significantly reduced risk of ectopic pregnancy, preterm birth, low birthweight and small for gestational age babies has been reported in FET pregnancies.

An elective freezing or a 'freeze all strategy' implies cryopreservation of all embryos generated in IVF with subsequent FET in a natural or hormone replacement cycle. Selective freezing refers to freezing of supernumerary embryos following a fresh embryo transfer or freezing of all embryos in specific clinical scenario when an unexpected intra-uterine pathology such as endometrial fluid, polyps or thin endometrium was encountered during COS; rise in pre-ovulatory progesterone level in stimulated cycles or unexpected hyper-response. Elective freezing was initially proposed as an OHSS risk reduction strategy in hyper-responders, in patients undergoing preimplantation genetic testing (PGT) and fertility preservation. Its use is extended to patients with recurrent implantation failure to improve embryo-endometrial synchrony at ET.

One of the earliest systematic reviews and meta-analysis, comparing reproductive outcomes of fresh or elective frozen embryo transfer (eFET) proposed that eFET should be universally advocated because it resulted in an approximate 30% increase in CPR and ongoing pregnancy rate (OPR). Two of the three trials included in this review were on high responder patients whilst one included normal responders. Many other studies followed reporting higher PRs with eFET; most of them included

PCOS patients. However, RCTs done in patient specific groups reveal that eFET does not improve results across the spectrum. An RCT in non-PCOS patients found no advantage of eFET over fresh transfer. SART registry data of 82935 patients revealed that CPR and LBRs were significantly higher only in eFET in high responders (>15 oocytes recovered). In normal (6-14 oocytes) and poor responders (<6 oocytes) on the other hand, CPR and LBRs were significantly higher in fresh ET cycles ($P < 0.001$). Only data of first IVF cycles and ET done within one year were used for analysis. A population based study also reported significantly lower cumulative LBR in normal and sub-optimal responders with eFET. In high responders, cumulative LBR was similar in fresh and eFET. A Cochrane meta-analysis of 2021 concluded that cumulative LBR between eFET and fresh ET are similar with a moderate quality of evidence. However, the meta-analysis was unable to draw any conclusions on the impact of 'freeze all' on the risk of miscarriages, multiple pregnancies and small-for-gestational age.

Elective freezing has other associated disadvantages. There is an inherent risk of complete or partial degeneration of embryos during the freeze thaw process. Added to that there is a delay in cycle completion leading to increased emotional and financial burden. A high rate of treatment discontinuation has also been observed in normal and suboptimal responders (24.4% and 34.1%, respectively) and an increase in pregnancy induced hypertension and large for gestational age babies has been reported. In addition, the luteal support (LPS) in FET cycles may need an individualized approach to achieve the best possible outcomes rather than a standard LPS for all.

Monitoring of Controlled Ovarian Stimulation Cycles

This section provides a brief overview of the monitoring of COS cycles during IVF. Patient comfort, cost implications and the impact on outcome influence the choice of modality. Transvaginal ultrasonography (TVS) forms the mainstay of monitoring ovarian response. Ultrasound assessment of

follicular growth was first introduced in 1978 when a linear relationship between follicle size and circulating E2 levels was reported. There is no evidence that cycle monitoring by TVS alone is any less effective than combined monitoring by transvaginal and oestradiol assay. Till date there is no consensus regarding the optimal number of measurements for each follicle or how best they are performed; but a single measurement is less reliable than two or three measures.

In addition to measuring the number and the rate of growth of follicles and the endometrial thickness, a TVS may be used to evaluate follicular and endometrial blood flow.Baseline ultrasonography (USG) is utilised to confirm that the follicular size is <10 mm, there is absence of ovarian cyst, endometrial thickness <6 mm Rate of growth of endometrium is slow during the first few days, but reaches 1-2 mm/day around 2-3 days before ovulation. Ideal thickness required varies between 8-14 mm. Endometrial thickness of less than 7 mm on the day of HCG is associated with poor implantation.

AIM:

The current work a retrospective study on assessing embryonic outcome based on different variable in patient undergoing IVF treatment was conducted to assess the embryonic outcome in D3 and D5 based on different variables (AGE, BMI, AMH, FSH, LH, E2, P4) range of the women in IVF treatment. The purpose of the study is to increases the success rate of IVF treatment and to know the necessity of different variables during IVF treatment. The study is carried out in sims hospital vadapalani in obstetrics & gynecology department

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OBJECTIVES:

The objective of the study is to

PRIMARY:

Number of oocyte production.

Embryonic outcome

SECONDARY:

To check the success rate of IVF treatment and importance of Age, BMI, Hormone profiles of women undergoing IVF treatment

PROPOSED PLAN:

1. Protocol submission to the institutional ethics committee for ethical approval
2. Past 2 years medical history of ivf undergone patients data should be collected
3. Data collected to be evaluated
4. Statistical analysis

5. Report

MATERIALS AND METHODS

5.1 STUDY SITE:

The current study carried out on women with underwent invite fertilization treatment in obstetrics gynaecology department from sims hospital vadapalani. It is a tertiary hospital in vadapalani and patients come from the in and around surrounding areas.

5.2 STUDY PERIOD:

This study is done for 6 months in with past 2 years data (Jan 2020-dec 2021) were collected

5.3 STUDY DESIGN:

Retrospective study

5.4 STUDY TITLE:

A Retrospective Study to Find Embryonic Outcome with Different Variables During IVF Treatment.

5.5 DATA SOURCE:

The data required was collected using case collection form

5.6 POPULATION SIZE:

A total of 100 patients were participated in the study, the patients were analysed for the following parameters

1.Age

2.Bmi

3.Antimularian Hormone (AMH)

4.Lh (Luteinizing Hormone)

5.Follicular Stimulating Hormone (FSH)

6.Estrodiol(E2)

7.Progestrone (P4)

8.D3 And D5 Blastocyte Profile

5.7 STUDY CRITERIA:

1.Bmi – Normal / Abnormal

2.Age -25 To 50

3.Indication For Invitro Fertilization Treatment

4. Irregular Periods

5. Hormone Profile

5.8 EXCLUSION CRITERIA:

1. Positive HIV, hepatitis B, C screening test
2. Planned preimplantation genetic testing for embryo
3. Mentally retarder patients
4. Bellow 20 and above 50 aged women

5.9 STUDY METHOD:

A retrospective study was conducted to compare the embryonic outcome (D2 and D3 blastocytes) with different variables. The main aim of this study is the Importance of AGE ,BMI hormone profiles during IVF treatment and to find whether This variables are important for better embryonic outcome and increase in success rate of IVF treatment. For statistical analysis the subjects are split into case group and controlled group.

5.10 ETHICAL APPROVAL:

This study has been approved by the institutional ethics committee of SIMS VADAPLANI HOSPITAL.

5.11 STATISTICAL ANALYSIS:

The aim of the study is ASSESING EMBRYONIC OUTCOME BASED ON DIFFERENT VARIABLE IN PATIENT UNDERGOING IVF.

All data were analyzed using SPSS software. continuous variables were calculated and expressed as mean +_SD. categorical parameters were expressed in percentage. the p value obtained < 0.05 was statistically significant. the results were demonstrated using graphs.

RESULT

Demographic Profile of The Study Subject

Table 1: Demographic S Details of Study Participants (N=100)

PARTICIPANTS CHARACTERISTICS	N	PERCENTAGE (%)
AGE		
21-30	39	39
31-40	56	56
41-50	6	6
BMI (BODY MASS INDEX)		
MOBIDLY OBESE	4	4
NORMAL	24	24
OBESE-I	23	23
OBESE-II	3	3
OVER WEIGHT	46	46

Age patients age was range into three categories like wise 39 patients in 21- 30 years that is 39 % among the total enrolled in the study followed by 56 patients in 31- 40 years of age and 6 patients in 41- 50 years

Figure 1: Pie Chart Showing Categorization of Age Groups of Study Participants (N=100)

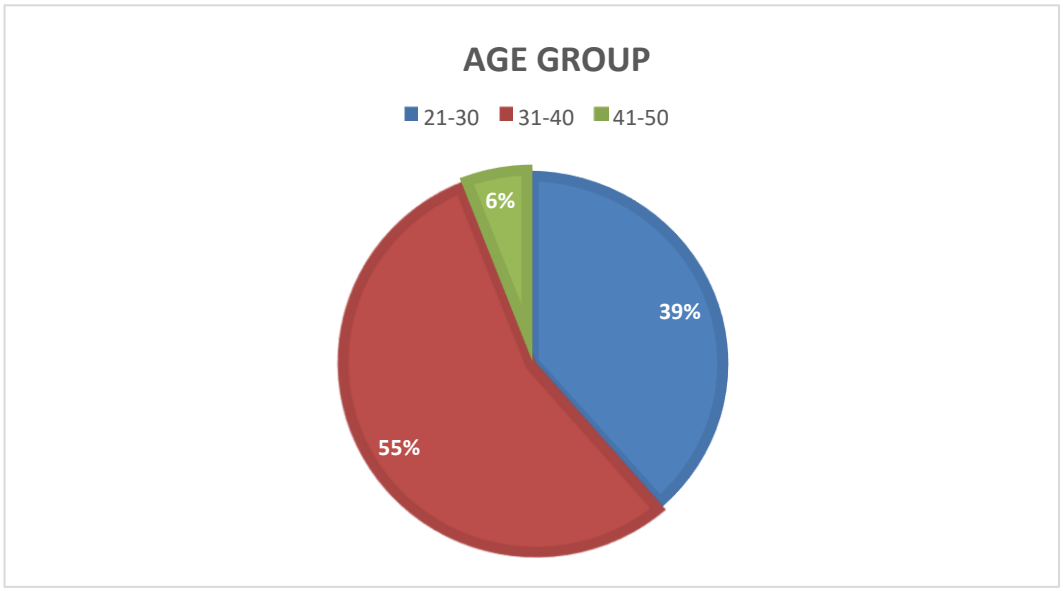
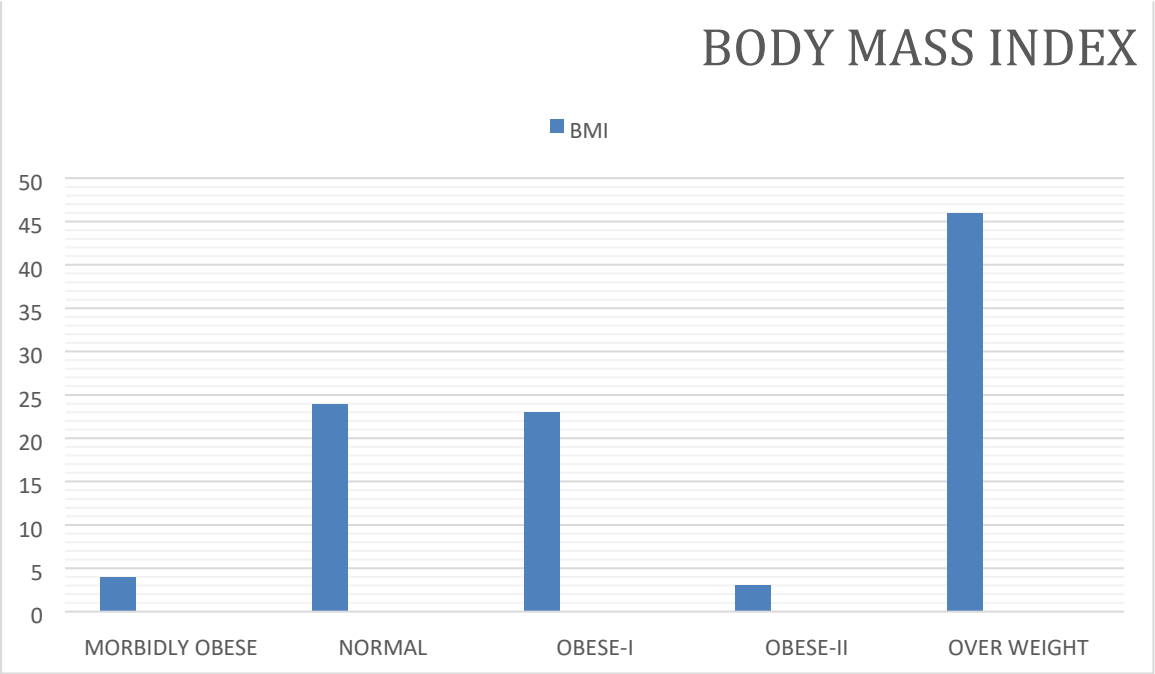


FIGURE 2: BODY MASS INDEX OF STUDY PARTICIPANTS (N=100)



The patients come into 5 categories. majority of patients comes under over weight (N= 46) and normal patients are 24 followed by 23 patients in obese 1 category

TABLE 2: HORMONE PROFILE OF STUDY PARTICIPANTS (N=100)

VARIABLES	N	MEAN±SD
FSH (FOLLICLE STIMULATING HORMONE)	89	7.88±4.62
LH (LUTEINIZING HORMONE)	90	5.72±5.57
AMH (ANTI-MULLERIAN HORMONE)	91	3.80±3.45
E2 (ESTRADIOL)	37	-
P4 (PROGESTERONE)	5	3.80±3.45

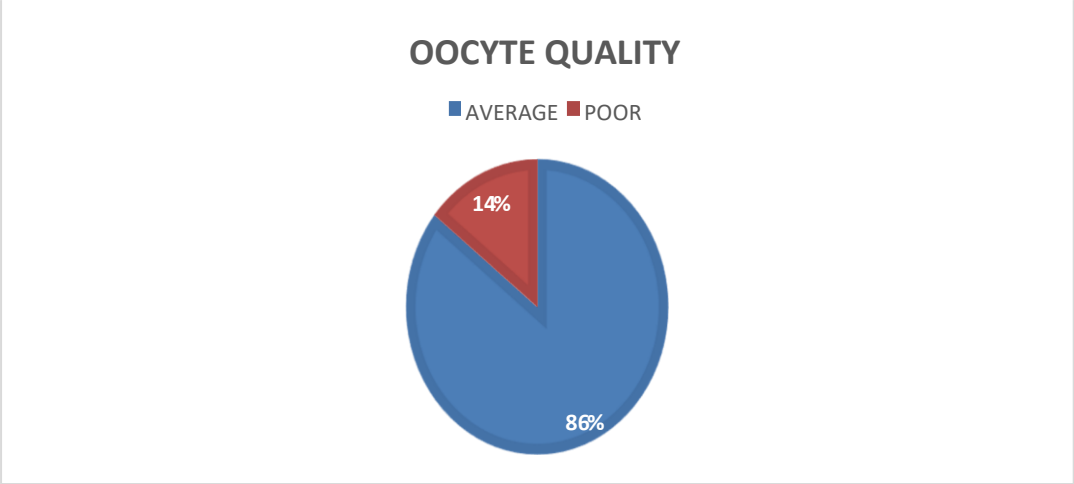
The hormone profile study is done on 100 patients. The hormonal test is done only if there is any necessary therefore out of 100 subjects, we have very limited data for concluding. The FSH hormone test has been done on 89 subjects and their mean difference were found to be 7.88±4.62. LH hormone has been monitored in 90 subjects and their mean difference were found to be 5.72±5.57. Next AMH hormone is measured for 91 subjects and their mean difference are 3.80±3.45. Estradiol hormone measurement is done only for few individuals (37). progesterone values are not mandatory in assessing embryonic outcome therefore only 5 members undergone progesterone testing and 3.80± 3.45 is their mean difference.

TABLE: 3 OOCYTE PROFILE OF STUDY PARTICIPANTS (N=100)

VARIABLES	**N**	**MEAN ±SD**
RETRIEVED	98	9.59±6.21
MATURED	98	7.64±5.05
PERCENTAGE	-	80±15.74

Out of 100 subjects, the retrieved oocytes are seen in 98 patients with mean difference 9.59± 6.21 and 98 subjects had matured oocyte and their mean difference were found to be 7.64±5.05. the total percentage is 80±15.74

FIGURE 3: OOCYTE QUALITY OF STUDY PARTICIPANTS (N=100)



Out of 100 subjects 86 patients shows average oocyte quality and 14 women had poor oocyte quality

TABLE 4: CATEGORIZATION OF AGE GROUPS WITH CLINICAL OUTCOMES

VARIABLES	FREQUENCY	FERT		D3		D5		P Value
		>90%	<90%	>60%	<60%	>70%	<70%	
AGE								
21-30	39	31	7	28	8	7	10	.832
31-40	56	47	9	41	12	8	15	
41-50	5	5	0	4	0	3	0	

Patients age was range into three categories like wise 39 patients in 21- 30 years that is 39 % among the total enrolled in the study followed by 56 patients in 31- 40 years of age and 6 patients in 41- 50 years. the fertilization, D3 and D5 profile is compared with different age group. The p value was 0.832. which is not significant

TABLE 5: CATEGORIZATION OF BMI WITH CLINICAL OUTCOMES

VARIABLES	FREQUENCY	FERTILIZATION		D3		D5		P Value
		>90%	<90%	>60%	<60%	>70%	<70%	
BMI (BODY MASS INDEX)								
MOBIDLY OBESE	4	4	0	2	1	1	1	
NORMAL	24	21	3	16	6	5	4	
OBESE-I	23	19	4	17	4	4	8	.322
OBESE-II	2	2	0	3	0	0	1	
OVER WEIGHT	46	37	9	35	9	8	11	

The patients come into 5 categories. majority of patients comes under over weight (N= 46) and normal patients are 24 followed by 23 patients in obese 1 category. The fertilization, D3 and D5 profile is compared with different BODY MASS INDEX The p value was 0.322. which is not significant

TABLE 6: CATEGORIZATION OF AMH IN REGARD TO CASE AND CONTROL WITH CLINICAL OUTCOMES.

VARIABLES	FREQUENCY (N=93)	FERT		D3		D5		P Value
		>90%	<90%	>60%	<60%	>70%	<70%	
AMH (ANTIMULLERIAN HORMONE)								
CASE	49 (52.68 %)	42	7	39	7	9	9	.349
CONTROL	44 (47.31 %)	36	8	32	11	8	15	

The 93 subjects were analyzed and categorized into control and case. 49 subjects come under case and 44 subjects are considered as control group. This case and control group was compared with fertilization, D3 and D5 and the final p value was said to be 0.349 which is not significant

TABLE 7: CATEGORIZATION OF LH IN REGARD TO CASE AND CONTROL WITH CLINICAL OUTCOMES

VARIABLES	FREQUENCY	FERTILIZATION		D3		D5		P Value
		>90%	<90%	>60%	<60%	>70%	<70%	
LH (LUTEINIZING HORMONE)								
CASE	57	48	9	43	11	9	13	.147
CONTROL	33	27	6	26	6	6	11	

The 90 subjects were analyzed and categorized into control and case. 57 subjects come under case and 33 subjects are considered as control group. This case and control group was compared with fertilization, D3 and D5 and the final p value was said to be .147 which is not significant

TABLE 8: CATEGORIZATION OF FSH IN REGARD TO CASE AND CONTROL WITH CLINICAL OUTCOMES

VARIABLES	FREQUENCY	FERT		D3		D5		P Value
		>90%	<90%	>60%	<60%	>70%	<70%	
FSH (FOLLICE STIMULATING HORMONE)								
CASE	73	60	13	56	14	11	19	
CONTROL	17	15	2	13	3	4	5	.552

The 90 subjects were analyzed and categorized into control and case. 73 subjects come under case and 17 subjects are considered as control group. This case and control group was compared with fertilization, D3 and D5 and the final p value was said to be .552 which is not significant.

TABLE 9: CATEGORIZATION OF ESTRADIOL IN REGARD TO CASE AND CONTROL WITH CLINICAL OUTCOMES

VARIABLES	FREQUENCY	FERT		D3		D5		P Value
		>90%	<90%	>60%	<60%	>70%	<70%	
ESTRADIOL								
CASE	6	4	2	3	3	1	0	
CONTROL	32	24	6	24	5	6	11	.277

The 38 subjects were analyzed and categorized into control and case. 6 subjects come under case and 32 subjects are considered as control group. This case and control group was compared with fertilization, D3 and D5 and the final p value was said to be .277 which is not significant

TABLE 10: CATEGORIZATION OF PROGESTERONE IN REGARD TO CASE AND CONTROL WITH CLINICAL OUTCOMES

VARIABLES	FREQUENCY	FERT		D3		D5		P Value
		>90%	<90%	>60%	<60%	>70%	<70%	
PROGESTERONE								
CASE	4	3	1	4	0	2	1	.319
CONTROL	2	2	0	1	1	0	1	

The six subjects were analyzed and categorized into control and case. 4 subjects come under case and 2 subjects are considered as control group. This case and control group was compared with fertilization, D3 and D5 and the final p value was said to be .319 which is not significant

6.DISCUSSION

In the three decades following the birth of Louise Brown, innovations in ART have overcome numerous seemingly insurmountable barriers to allow couples the chance to have families. Significant developments in the first decade led to greater efficiency and expanded. Accessibility of in vitro fertilization to the general public. Refinements in laboratory technology and clinical practice have allowed IVF to evolve into a medical procedure that is efficient, safe, readily accessible, and relatively affordable. During the initial years of experimentation, at best approximately 50% of embryos survived the freeze/thaw process and resulted in a pregnancy rate of 13.4% per embryo transfer procedure, as only 4.6% of the individual thawed embryos implanted (Friedler et al 1988) (37). IVF protocols are constantly under review in an attempt to improve follicular recruitment, whilst primarily to increase live birth rates. To check the efficacy in vitro fertilization, The following BMI, Age and hormones profiles [FSH, LH, P4, E2, AMH] are measured.

In IVF treatment the oocytes are retrieved from the ovary artificially and semen's are collected from male. The retrieved oocytes and sperms are artificially fussed in a sterile manner. At day 1 the 1st cleavage is formed and in day 3, two to four cell stage is formed. Early stage of blastocyst is seen in day 5 and last stage blastocyst (hatching) is formed in day 6 and 7. finally on 8 and 9th day the implantation of blastocyst has to be done or the fertilized oocyte are frizzed (frozen) for future use (38).

In this study the clinical data such as d3 and d5 fertilization is monitored and stated as embryonic outcome. This outcome is compared with different parameters such as AGE, BMI, hormone profile (AMH, LH. FSH, E2, P4). The goal of the study is to find whether the above parameters are important for conducting IVF treatment or not and its effects are monitored. Total of 100 subjects were recruited in this study and their data are collected in retrospective process. There are seven variables need to be compared individually with the d3and d5 clinical outcome. Age is one of the major

factors that effects the fertilization percentage therefore the subjects are split into two groups as case and control. As most of the patient falls under the age of 31 to 40 and an average oocyte quality observed in 86%pateint. the age group is categorized with clinical outcome in which the p value is 0.832. By this we came to know that age is not an important category for IVF treatment and shows no significant.

Again the BMI is categorized with clinical outcome .out of 100 subjects 46 patients comes under overweight and the p value is said to be 0.322. which is a negative outcome and shows no significant. By comparing the hormone profile with clinical outcome, the mean difference for the hormones is FSH (7.88±4.62), LH (5.72±5.57), AMH (3.80±3.45), P4 (3.80±3.45). The oocyte profile of the study participants was calculated and their mean standard difference of oocyte retried is 9.59± 6.21 and matured oocyte is 7.64±5.05

The p value of anti-mullerian hormone (0.349) has been calculated by grouping the subject into case and control. It shows alternative hypothesis. We hereby came to know that AMH Hormone is not mandatory for oocyte production and pregnancy outcome.

Although increasing luteinizing hormone and progesterone and estradiol was associated with increased blastulation and increase pregnancy outcome. But in other case there was no effect on oocyte production and embryonic outcome with increase in p4, E2 and LH hormone. The subjects who have decreased P4, E2, LH hormones also response well to ovarian stimulations and have same chances of conceiving (39). Calculated p value for Progesterone, Estradiol and Luteinizing Hormone said to have no significant. Similarly Follicular Stimulating Hormone should be decrease during ovulation. The increased FSH indicates menstruation and infertility. But in this study, we got negative impact and has no significant. the FSH hormone value are not mandatory for IVF success rate the elevated FSH shows zero impact on embryonic outcome

7. RESULT

The total number of study population was 100. The majority of the population was found to be 31 to 40 age. The predominant BMI among study population of overweight (46%). The mean FSH was 7.88.62 . The mean of LH was 5.725.57. The mean of AMH was 3.803.45. The mean of p4 was 4.105.66. The association between Age and BMI with hormone profile was found to be not significant . The clinical outcomes of fertilization rate D3 and D5 key performance indicator was found to be not significant and hormone profile variable

Using different variables the embryonic outcome on D3 and D5 was found to be no significant.

8.CONCLUSION

As oocyte production and embryonic outcome is the main objective of this study. The patients were assessed with physical and lab investigations and grouped based on case control study. Among 100 subjects nearly 86 percent came out with average oocyte quality and increased oocyte production. The fertilization percentage got increased in day2 and day5 profile and this elevation in outcome does not depend on variables such as AGE, BMI, AMH, LH, FSH, P4 and E2. This study shows no significant.

9. LIMITATIONS

Several limitations were present in the present study. one is the nature of the respective analysis. possible confounding factors may not have been taken into consideration in the present study including fertilization percentage, embryonic outcome, oocyte productions. Therefore we believe that the embryonic outcome in clinical level do not depend on the variables considered in this study. the limitations is that indications drawn in the present study were based on limited cases and data from a single centre. the sample size included in the present study may not be able to discriminate the differences in measurements. Therefore, the conclusion s from this study are not definitive but indicative, and these findings need to be confirmed by a large cohort study.

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GLOSSARY

Terminologies:

1. CONCEPTION:

During IVF, an egg is removed from the woman's ovaries and fertilised with sperm in a laboratory. The fertilised egg, called an embryo, is then returned to the woman's womb to grow and develop. It can be carried out using your eggs and your partner's sperm, or eggs and sperm from During IVF, an egg is removed from the woman's ovaries and fertilised with sperm in a laboratory. The fertilised egg, called an embryo, is then returned to the woman's womb to grow and develop. It can be carried out using your eggs and your partner's sperm, or eggs and sperm from donors.

2. FERTILIZATION:

During in vitro fertilization, eggs are removed from mature follicles within an ovary (A). An egg is fertilized by injecting a single sperm into the egg or mixing the egg with sperm in a petri dish (B). The fertilized egg (embryo) is transferred into the uterus (C).

3. OOCYTE PRODUCTION:

An oocyte is produced in a female fetus in the ovary during female gametogenesis. The female germ cells produce a primordial germ cell (PGC), which then undergoes mitosis, forming oogonia. During oogenesis, the oogonia become primary oocytes.

4. OVULATION:

Ovulation is the process in which a mature egg is released from the ovary. After it's released, the egg moves down the fallopian tube and stays there for 12 to 24 hours, where it can be fertilized.

5. GRANULOSA:

Ovulation is the process in which a mature egg is released from the ovary. After it's released, the egg moves down the fallopian tube and stays there for 12 to 24 hours, where it can be fertilized.

6. ANTRAL FOLLICLE COUNT:

The antral follicle count is a fairly simple test to perform with high quality ultrasound equipment. It allows us to evaluate a woman's ovarian reserve – her supply of eggs for the future. Tests of ovarian reserve do not measure the quality of the eggs, they only measure the quantity.

7. MENOPAUSE:

Menopause is the time that marks the end of your menstrual cycles. It's diagnosed after you've gone 12 months without a menstrual period. Menopause can happen in your 40s or 50s, but the average age is 51 in the United States. Menopause is a natural biological process.

8. ESTROGEN:

Estrogens are a group of hormones that play an important role in the normal sexual and reproductive development in women. They are also sex hormones. The woman's ovaries make

most estrogen hormones, although the adrenal glands and fat cells also make small amounts of the hormones.

9. MYOMETRIAL QUIESCENCE:

Myometrial quiescence is a physiological stage of the myometrium during pregnancy. It is a period of active relaxation of the myometrial smooth muscle cells; myometrial quiescence is responsible for maintaining pregnancy.

10. IMMUNE MODULATION:

Using drug therapy to change how the immune system responds to the presence of cancer cells.

11. MISCARRAIGE:

Miscarriage is the spontaneous loss of a pregnancy before the 20th week. About 10 to 20 percent of known pregnancies end in miscarriage. But the actual number is likely higher because many miscarriages occur very early in pregnancy — before you might even know about a pregnancy.

12. OBSTRETIC GYNAECOLOGY:

Obstetrics and gynecology, medical/surgical specialty concerned with the care of women from pregnancy until after delivery and with the diagnosis and treatment of disorders of the female reproductive tract.

13. BLASTOCYST:

Three days after fertilization, a normally developing embryo will contain about six to 10 cells. By the fifth or sixth day, the fertilized egg is known as a blastocyst — a rapidly dividing ball of cells. The inner group of cells will become the embryo. The outer group will become the cells that nourish and protect it.

14. PREGNANCY RATE:

Multiply the numbers for births, abortions and fetal deaths by their respective proportion of the year a woman is pregnant for each pregnancy outcome by month, and then sum them.

15. P VALUE:

P-value is the level of marginal significance within a statistical hypothesis test, representing the probability of the occurrence of a given event. $P > 0.05$ is the probability that the null hypothesis is true. 1 minus the P value is the probability that the alternative hypothesis is true. A statistically significant test result ($P \leq 0.05$) means that the test hypothesis is false or should be rejected. A P value greater than 0.05 means that no effect was observed.

CASE RECORD FORM

I.P No:

DEPARTMENT:

HEIGHT/WEIGHT:

BMI:

DIAGNOSIS ON ADMISSION:

PERSONAL HISTORY & HABITS:

- Smoking:- □ yes □ no
- Alcohol:- □ yes □ no

Sleep cycle:- □ regular □ irregular

FAMILY HISTORY: HTN/ DM/ THYROID SEXUAL HISTORY:

Frequency / Dyspareunia

BRIEF HISTORY: (comorbid disease)

- Advanced age:
- Diabetes:- □ yes □ no
- Hypertension:- □ yes □ no

- Thyroid:- □ yes □ no
- Ovarian cysts:- □ yes □ no
- Tuberculosis:- □ yes □ no

ARE YOU ALLERGIC TO ANY MEDICATIONS: □ yes □ no

- **OG HISTORY:**
- Menarche:
- Last Menstrual Period (LMP):
- Menstrual cycle – Regular / Irregular Scanty / moderate/ heavy
  Painful/ painless
- Age at marriage:
- Abortions:
- Gravida:
- Have you ever used birth controlled pills:- □ yes □ no if (yes) mention with which drug

PREVIOUS SURGERIES: □ yes □ no

PREVIOUS INFERTILITY TREATMENT:

OI / IUI / IVF / ICSI

PHYSICAL EXAMINATION:

VITAL SIGNS:

- Temperature:
- Blood pressure (mm/hg):
- Pulse (beats/min):
- Respiratory rate (breath/min):

LAB INVESTIGATION:

Hormone profile:

- FSH-
- LH-
- E_2-
- P_4-
- AMH-
- **GAMETES:**

Female:

- No. of Oocytes retrived.
- No. of matured Oocytes.

EMBRYO OUTCOME IN PERCENTAGE

- Fertilization:
- D_3 Cleavage conversion rate:
- D^5 Blastocyst:

NAME AND SIGNATURE OF THE PERSON FILLING THIS FORM WITH DATE