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Is Serum Estradiol a good predictor for yield of mature oocytes?

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Abstract

Objective:

This study aimed to examine the association between serum estradiol levels and the number of metaphase II oocytes yielded after in vitro fertilization cycles used in embryo transfer.

Methods:

This observational analytical retrospective study was carried out at the VANI IVF CENTER. It concluded 25 cases and looked into the number of metaphase II oocytes. Statistical analysis was based on the calculation of results done by student t-test in Sigma plot 11.0 software. The significance was observed with $p < 0.05$.

Result: The data of 25 patients were analyzed by using SigmaPlot 11.0. Our study showed a scatter plot between AMH and estradiol E2, AMH and age, AMH and MII, AMH and total retrieved follicles, E2 and age, E2 and MII, E2 and total follicle retrieved and stimulation days, and E2. In which the mean baseline anti-Mullerian Hormone of 6.2±0.63 ng/ml. The average estradiol level on the day of the trigger was 373pg/mL per retrieved follicles and 530pg/mL per metaphase II oocyte.

Conclusion:

Serum estradiol had a positive correlation with mature oocytes. and we may predict serum E2 concentration tentative value per mature is 500±50. we may correlate this outcome with our ART procedures. These results indicate that estradiol levels can be used as an important clinical tool in the prediction of oocyte and mature oocyte yield in ART cycles. The reproductive outcome in ART cycles is largely dependent on the numbers of oocytes and mature oocyte yield. Estradiol levels on the day of hCG appear to correlate with the outcome of ART cycles.

1. Introduction

Infertility is a disease characterized by the failure to establish a clinical pregnancy after 12 months of regular and unprotected sexual intercourse. It is estimated to affect between 8 and 12% of reproductive-aged couples worldwide. Males are found to be solely responsible for 20-30% of infertility cases but contribute to 50% of cases overall. Secondary infertility is the most common form of female infertility around the globe, often due to reproductive tract infections.

The three major factors influencing the spontaneous probability of conception are the time of unwanted non-conception, the age of the female partner, and disease-related infertility. Hormonal causes of female infertility involve ovulatory dysfunctions that may result from dysfunction of the hypothalamic-pituitary-ovarian axis, peripheral endocrine glands, non-endocrine organs, or metabolic disorders. Ovarian stimulation aims at the development of one or more of the ovarian follicles to reach the stage of maturity

culminating in the release of one or more mature oocytes ready for fertilization. Ovarian follicular development is under the control of local factors inside the ovaries, as well as hormones produced from extra ovarian sources, mainly pituitary gonadotropins. Other hormones may play a role in ovarian follicular development; the extent and details of such a role are not fully understood.

1.2. Controlled ovarian stimulation

During in vitro fertilization (IVF) or intracytoplasmic sperm injection (ICSI) therapy, controlled ovarian hyperstimulation (COH) is the standard protocol for generating enough follicles. COH is carried out using a standardized long (agonist) or short (antagonist) regimen, the choice of which is dependent on patient characteristics or reaction during prior cycles. GnRH analogs function via receptor-mediated mechanisms. A single dosage of an agonist causes a rapid decrease in pituitary gonadotroph surface GnRH receptor-binding capability that lasts around 12 hours. The agonist's high-affinity binding to the membrane receptors mediates this down-regulation mechanism. The stabilized receptor-hormone complexes are internalized into the cell and progressively destroyed by proteolysis, and this receptor loss is linked with a significant decrease in pituitary Follicle-stimulating hormone (FSH) and Luteinizing Hormone (LH) content and release. The effects are completely reversible in a short amount of time, and a submaximal treatment can actually be employed to keep LH activity in circulation even in the face of severe negative feedback pressures **[1]**. Although the purpose of developing GnRH antagonists was intended to provide a non-steroid contraceptive medication, it was discovered that GnRH antagonists may be useful in assisted reproduction. The primary goal of utilizing GnRH antagonists in IVF is to prevent an early LH surge. GnRH antagonists work by immediately suppressing pituitary gonadotropin release and quickly restoring normal production of endogenous LH and FSH. GnRH antagonists rapidly and competitively block the GnRH receptor, reducing LH and FSH output within 8 hours. The suppression of LH production is stronger than that of FSH, which is most likely related to the distinct modes of gonadotropin regulation and the extended FSH half-life, or the immunoreactive and bioactive forms of FSH.

GnRH agonists stimulate gonadotropin production on a long-term basis, whereas GnRH antagonists operate as chemical hypophysectomy mediators. Overall, both analogs are commonly used in IVF to stimulate folliculogenesis by preventing endogenous LH surge and oocyte retrieval at the appropriate time.

The physician normally chooses whether to utilize a long agonist protocol, an antagonist protocol, or a minimum stimulation protocol for each patient. The choice is based on the advantages and disadvantages of each treatment approach, as well as the patients' responses. Patients undergoing gonadotropin stimulation are classified into three groups according to their responses: (1) high responders, (2) moderate responders, and (3) low responders. The most often used parameters for identifying poor ovarian response are FSH level, oocyte number, cycle cancellation rate, gonadotropin dosage, and E2 level **[2]**.

However, depending on the specialist, the criteria for identifying poor responders may differ. The poor response has been linked to advanced maternal age, which affects oocyte quality and follicle quantity. This has also been found in certain young patients, although the etiology is unclear. Other drawbacks of combining gonadotropin and clomiphene citrate in IVF included an increased risk of multiple pregnancies, which was linked to premature birth, growth retardation, and miscarriage. The relationship between ovarian stimulation and low birth weight is still controversial since it might have a complicated impact on the couple's reproductive history.

1.3. Estradiol and its significance

Estradiol (17-beta estradiol, E2) is the most well-known and potent member of the estrogens. E2 is a steroid hormone that is largely generated by the ovaries during the reproductive lifespan and is important for the development of female secondary sexual characteristics as well as the maintenance of the female reproductive tract **[3]**. including vaginal lining, cervical glands, the lining of the fallopian tube, the endometrium, and the myometrium. E2 is also involved in the initiation and maintenance of postpubescent female secondary sex characteristics such as breast growth, body shape alterations, and bone and fat deposition, as well as plays a crucial function in the maintenance of pregnancy. E2 production happens in the ovaries due to the coordination of two cell types, the follicular granulosa, and theca cells, as well as the control of two gonadotropins, such as luteinizing hormone and follicle-stimulating hormone **[4]**. Androstenedione is released from the thecal cells and enters the mural granulosa cells, where it is converted to E2 by the P450 aromatase enzyme and then released into the follicular fluid and bloodstream.

1.3.1 Role of estradiol in oocyte maturation and Endometrial preparation

Intraovarian factors such as steroids, cytokines, and other growth factors that act at critical phases during follicle formation directly regulate in vivo oocyte growth and maturation **[5]**. Among these factors, E2 also has great importance in oocyte maturation. E2 is involved in both

nuclear and cytoplasmic maturation of oocytes by inducing the [Ca+2]i through CICR activation (Calcium Induced Calcium Release) **[6]**. During InVitro Maturation (IVM), E2 was discovered to act directly on the human oocyte surface to promote fertilization and cleavage. Estradiol mediates its effects principally through the activation of ERα and ERβ receptors which are members of the superfamily of ligand-activated transcription factors. **[7]** suggested that E2 may function synergistically with gonadotropins in promoting cytoplasmic maturation of oocytes via cyclic AMP (cAMP) secretion, which controls oocyte meiosis **[7]**. E2 also has a significant role in implantation through improving endometrial proliferation and uterine perfusion for the priming of embryo implantation. **[1,8]** and independently observed that the implantation rate increases as the peak of E2 elevates.

1.4. Output of stimulation on mature oocytes

The duration of gonadotropin stimulation is related to oocyte maturation, follicular growth, oocyte quality, and endometrial development. Inadequate gonadotropin exposure might result in nuclear or cytoplasmic immaturity of oocytes. Prolonged stimulation duration, on the other hand, may result in post-maturity of oocytes or apoptosis of granulosa cells and oocytes. It may also cause increased progesterone and estradiol levels, as well as impaired endometrial receptivity. Various criteria, including follicular size and estradiol levels, have been used to determine the timing of final oocyte maturation. In poor responders, having a higher baseline E2 level was similarly linked to a shorter stimulation duration. The higher baseline E2 level may represent earlier follicular recruitment caused by endogenous gonadotropin during the late luteal and early follicular phases. This might result in quicker follicular development and a shorter follicular phase during COH. In poor responders, shorter stimulation may reflect improved granulosa cell activity and oocyte quality, which may lead to a better pregnancy result **[9]**.

1.5 Estradiol structure and its biosynthesis

Estradiol is carried from the ovaries to target cells in the blood whereas testosterone. It is primarily bound to sex hormone-binding globulin(SHBG). Estradiol simply diffuses around the target cell plasma membrane and binds to a cytosolic complex then enters the nucleus where it binds to DNA. thus, regulating gene transcription. E2 acts as a growth hormone for female reproductive organs, including the vaginal lining, cervical glands, lining of fallopian tubes, the endometrium, and the myometrium. E2 provides a proper environment for an important role in maintaining pregnancy. Because of these significant intra-ovarian actions, estradiol is regarded as a key indicator of follicle quality.

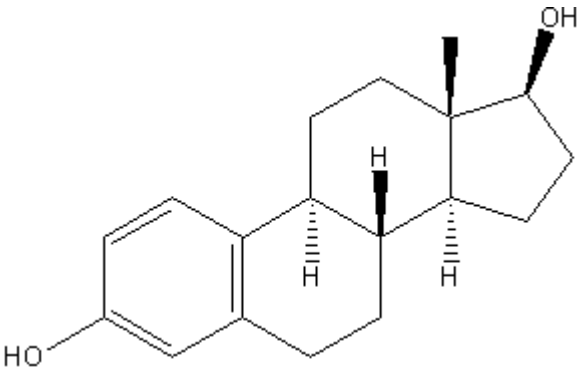


Figure 1: Structure of Estradiol (E2).

Estradiol has two hydroxy groups, one at the C3 position and another one at the 17**β** position, as well as three double bonds in the A ring. Due to its two hydroxyl groups, estradiol is often abbreviated as E2.

During the menstrual cycle, E2 is produced by the growing follicles triggered via a positive feedback system, the hypothalamic-pituitary events that lead to the luteinizing hormone surge, inducing ovulation. In the luteal phase, E2 in conjunction with progesterone prepares the endometrium for implantation. During pregnancy, E2 increases due to placental production.

1.5.1 Biosynthesis of Estradiol (E2)

Synthesis of estradiol starts in theca interna cells in the ovary, by the synthesis of androstenedione from cholesterol. Androstenedione is a substance of moderate androgenic activity. This compound crosses the basal membrane into the surrounding granulosa cells, where it is converted to estradiol, either immediately or through testosterone.

Estradiol is derived from cholesterol metabolic cleavage in which the androstenedione act as the key intermediate molecule. Cholesterol side-chain cleavage occurs in the mitochondria and produces pregnenolone which then moves into the smooth endoplasmic reticulum. Where the pregnenolone converts into progesterone via 3**β**-hydroxysteroid dehydrogenase. Progesterone acts as a substrate for the 17**α**-hydroxylase and converts into 17**α**-hydroxyprogesterone. The specific lyase enzyme that presents in SER, lyse the hydroxyl group of the 17**α**-hydroxyprogesterone and converts it into androstenedione. There are two ways to convert androstenedione into estradiol. In the first pathway, a portion of the androstenedione is converted to testosterone with the help of 17**β**-hydroxysteroid dehydrogenase, which in turn undergoes conversion to estradiol by aromatase. In another pathway, androstenedione is aromatized to estrone with the help of aromatase, which is subsequently converted to estradiol with help of 17β-hydroxysteroid dehydrogenase.

1.6. Correlation between Anti Mullerian Hormone (AMH) and Estradiol

The composition of FF alters during follicular growth and development, particularly in terms of steroid content and certain growth factors. Early investigations based on steroid measures revealed that the microenvironment of the follicle influences oocyte health and future development and maturation prospects. Several previous studies have suggested that the AMH plays an indirect role in the development and maturation of oocytes. AMH expression in granulosa cells increases with the commencement of early follicular growth and drops in granulosa cells of preovulatory follicles. The exact physiological mechanism of AMH in the ovary is unknown, and there is only little experimental evidence to show that AMH expression is down-regulated as the follicle progresses through the developmental phases of the oocyte. An inverse correlation between the E2 and AMH levels was found in the follicular fluid of small antral follicles. Thus, it could be that the reduction in AMH levels is not only a consequence of the depletion of the small antral

follicles. Possibly, the induced in E2 levels during the follicular phase mediates this decline in AMH expression. AMH concentrations are relatively high in FF from small antral follicles, indicating that serum AMH levels reflect the pool of small antral follicles present in a woman's ovaries and supporting the use of AMH as an ovarian reserve biomarker. Furthermore, AMH has a strong negative correlation with estradiol concentrations, implying that FSH may be involved in the downregulation of AMH expression, possibly through estradiol synthesis. Rigg et al., found that AMH was not indicative of embryo morphology or pregnancy outcome in a previous investigation.

2. Materials And Methods

2.1 Patient Characteristics

This was an observational retrospective study of 25 patients at Vani IVF Centre, Ahmedabad. The data of the patients were used and presented with the due consent of the patients. The study included patients submitted to IVF diagnosed with infertility of any kind (primary or secondary). Additional information such as serum estradiol levels on Day 2, Day 5 or 8 and on trigger day and cycle outcome, number of the retrieved oocyte, number of metaphases II (MII) oocytes, number of metaphases I (MI), number of germinal vesicles oocytes. The clinical characteristics of the study group are shown in Table 1.

2.2 Inclusion Criteria

Patients between the age of 20-28 years were included. Patients were having 1 or 2 biological children included. Patients with not a single time donated their oocytes were included.

2.3 Exclusion Criteria

Patients who have PCOS, comorbidities, and overweight were excluded. Patients who had once undergone controlled ovarian stimulation were excluded.

2.4 Stimulation Protocol

Patients' standard stimulation on day 2 with used recombinant FSH is given subcutaneously used for follicles maturation. hMG (human menopausal gonadotropin) injection is given for the prevention of LH surge and follicle maturation. Cetrorelix is given subcutaneously used for the prevention of premature ovulation in their cycles. Doses were established based on patient baseline characteristics age, weight, FSH, anti-Mullerian hormone, and antral follicle count and were adjusted from the seventh day of stimulation based on ultrasound controls.

2.5 Hormone measurements

Serum estradiol concentrations were determined from venous blood obtained. Serum estradiol measurements were performed in a Sunflower Laboratory. Estradiol levels usually were evaluated on day 2, day 5 or day 8, and day 11(trigger day). E2 concentrations were measured by chemiluminescent microparticle immunoassay. Gonadotropin regimens were individualized on the basis of patient age, ovulation history, and their normal hormonal profile. Gonadotropin therapy began on cycle day 2. Gonadotropin dosages were adjusted based on serial estradiol levels.

2.6 Oocyte Retrieval and Denudation Process

Prior to one day of the oocyte retrieval procedure, a culture dish was prepared. For the culture dish 1ml of single-step media (SAGE 1- Step, CooperSurgical Fertility Solutions, Knardrupvej 2 – 2760 Målov Denmark.) and overlay oil in the center well dish. Prepare Four well dish with single-step media and overlay oil on each well for culturing of denuded oocytes. Both dishes are kept in a benchtop incubator at 37°C. Round bottom tubes were filled with 1 ml of flushing media and kept these tubes on an incubator block at 37°C for collection of the follicular fluid collection process. Transvaginal ultrasound-guided oocyte retrieval was

performed 36 hours after trigger. Before oocyte retrieval flushing media was kept at 37°C for equilibration. Culture dish preparation was done before 30 min of oocyte retrieval. Follicular fluid screening is done under the stereozoom microscope. For the denudation process, hyaluronidase enzyme (SAGE IVF inc., CooperSurgical Fertility Solutions, Knardrupvej 2 – 2760 Måløv Denmark.) was used, keeping the enzyme at room temperature for 15 minutes prior to use, for the denudation dish preparation hyaluronidase 10μl/oocyte was kept in centre of centre well dish and flushing media was kept outer well. Oocytes transfer from the culture dish to the denudation dish using a micropipette. Denudation of oocytes was performed using a Flexi pipette whose denupet diameters were 150μm and 175μm. After denudation oocyte quality is checked under the stereozoom microscope. Oocytes grading was done by referring. Denuded oocytes were transferred to four well dish and kept inside a benchtop incubator at 37°C.

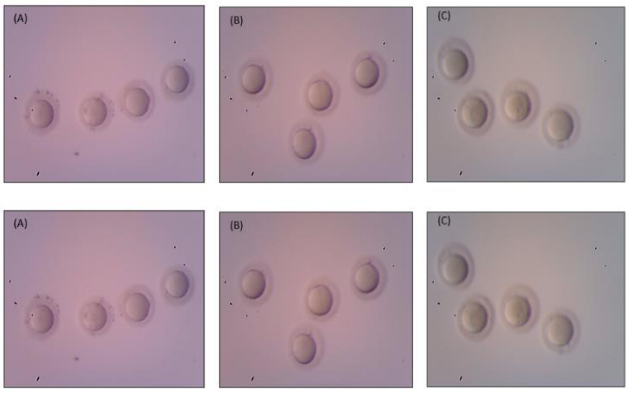
2.7 Statistical Analysis

Patients were selected based on the research method given by the ethical committee. Data were collected from 25 records of female infertility patients between the ages of 22 to 30 who were seen at the Vani IVF centre in Ahmedabad and recorded on data collection cards. We used the student t-test/mean for the patient's age, level of anti-mullerian hormone, and

estradiol on day 2, day 5, and the day of the trigger. We used graphs to demonstrate the relationship between Estradiol and the number of total retrieved follicles, E2 and AMH, and E2 and M2 oocytes. The above plots were used to justify the results. The significance was observed with $p < 0.05$.

3. Results

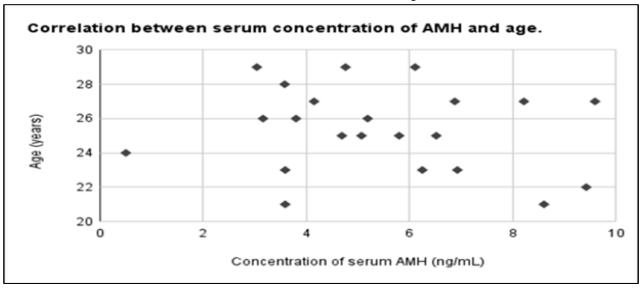
The medical records of 25 patients meeting the following inclusion criteria were analyzed: mean age of 26±4 years women; baseline anti-Mullerian Hormone of 6.2±0.63 ng/ml. The average estradiol level on the day of the trigger was 373 pg/mL per retrieved follicles and 530 pg/mL per metaphase II oocyte. In terms of patient age, the following mean E2 levels were observed on day 2, day 5, and on the trigger day. Our first step was to identify if there was any difference in the morphology of the mature oocyte (M2) based on the age of the female and the stimulation given to them. We did give the same stimulation to these below mentioned three patients, but the age factor was different. Stimulation protocol will be not disclosed due to hospital policies but if the study is moved further to the stage of publication, stimulation protocol will be mentioned in detail for the same. The three patients that we chose were at the age of 22, 28 and 30. The age as we know do play a major role in the quality and quantity of oocytes, but the stimulation plays a major role in the number of oocytes retrieved to the quality of oocytes retrieved.



*[**Figure 2**. Metaphase II oocytes after denudation. (A) Mature Oocyte (M2) from a female aged 22 years. (B) M2 from a female aged 28 (C) M2 from a female aged 30. All three images were captured in Vani IVF Center, Ahmadabad.]*

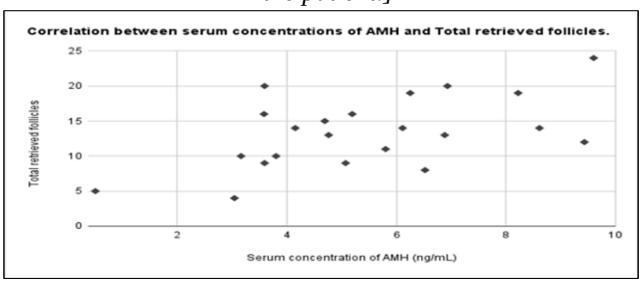
These photos were taken to notify that the research is based on the true facts and that the quality of the mature oocytes was of A grade. Grading of the M2 is difficult, where you must check the Oocyte Cumulus Complex before denudation, zona pellucida thickness, elasticity of the ooplasm and perivitelline space. In this study we have only considered M2 of the quality A grade as shown in the images above. These oocytes are graded in a way which has a higher level of fertilization rates and higher level of pregnancy rates. The average value of the E2 for one single M2 oocyte was calculated keeping only these A grade M2 oocytes in mind.

We have conducted our study in terms of statistical analysis where the main source of the analysis is depicted in terms of scattered plot, as the comparison lies first between AMH and E2 levels in terms of M2 oocytes. Due to the well-established technique of using AMH as the predictor of M2 oocytes or the overall follicular reserve, it becomes important for us to analyse the data of AMH vs Overall Follicles and M2 rate. Also, as we know that AMH values vary with age. So, we have done a complete analysis of AMH vs age, overall follicular retrieval and M2 oocytes. With the help of these data, we analysed further the correlation of E2 with age, overall follicular retrieval and M2 oocytes.

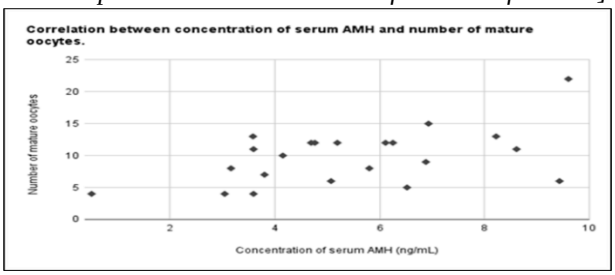


[***Graph 1***: *Correlation of serum AMH concentration with patient's age. Here the X-axis represents the concentration*

of serum AMH in ng/mL and the Y-axis represents the age of the patient.]

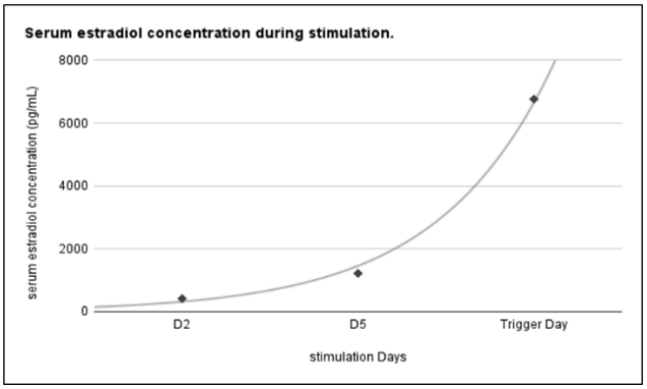


[***Graph 2***: *Correlation between serum AMH concentration and total number of retrieved follicles. Here the X-axis represents the concentration of serum AMH in ng/mL and the Y-axis represents the total number of retrieved follicles.]*



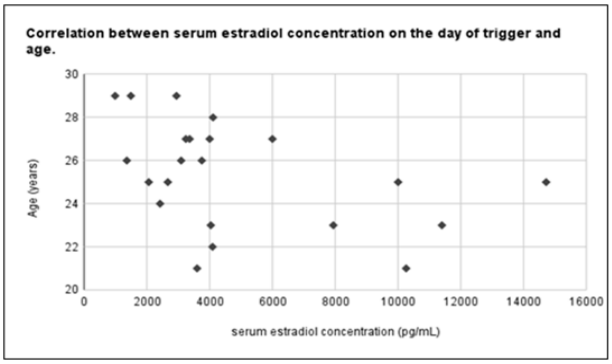
[***Graph 3***: *Correlation of serum AMH concentration with the number of mature oocytes (Metaphase II oocytes). Here the X-axis represents the concentration of serum AMH in ng/mL and the Y-axis represents the number of mature oocytes from total retrieved follicles.]*

After analyzing the data of 25 patients with their AMH vs age, increase in the E2 levels at different stimulation stages. The result came out to be as expected, where the levels of E2 were increasing with the increasing stimulation days and also in turn with the increase in the maturity of the oocytes. The below graph mentions the increase in the E2 levels at day 2 of cycle (without any stimulation given), day 5 (after 3-4 days of stimulation with only FSH) and on the day of trigger (after FSH and LH combination stimulations).

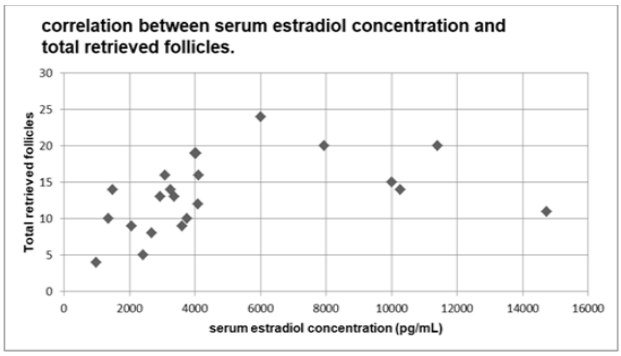


[***Graph 4:*** *Serum estradiol concentration during controlled ovarian stimulation was significantly increased. The Days of stimulation indicated on the X-axis and Y-axis represent the mean value of serum estradiol concentration in pg/mL.]*

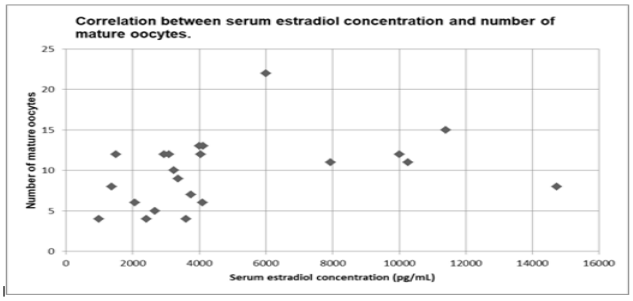
Now the correlation between E2 vs age, overall retrieval and mature oocytes was taken into consideration. E2 levels were also age dependent and our results show a very close correlation.



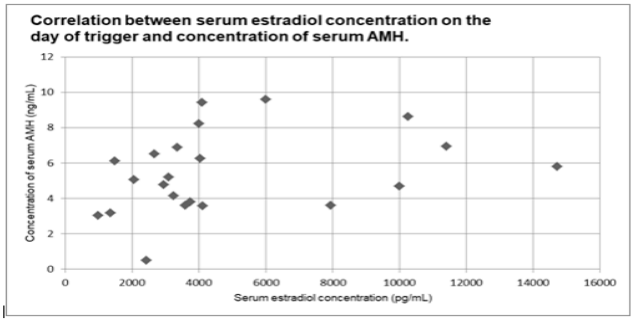
[***Graph 5****: Correlation between Patient's age and their concentration of serum estradiol on the day of trigger. The age of 23 to 28 years has high serum estradiol concentration on the day of trigger. In the graph, the X-axis represents the serum level of estradiol in pg/mL and the Y-axis represents the patient's age in years.*



[***Graph 6:*** *Correlation between concentration of serum estradiol on the day of trigger and number of total follicles retrieved. In this graph, the X-axis represents serum estradiol concentration in pg/mL and the Y-axis represents the total number of retrieved follicles.]*



[***Graph 7***: *Correlation of the serum estradiol concentration and the number of mature oocytes. between the range of 2000 to 4000 pg/ml of serum estradiol concentration tend to mature more oocytes. Here the X-axis represents the concentration of estradiol concentration in pg/mL and the Y-axis represents the number of mature oocytes.]*



[***Graph 8***: *Correlation of serum estradiol concentration on the day of trigger with the concentration of serum AMH. Here the X-axis represents the concentration of estradiol concentration in pg/mL and the Y-axis represents the concentration of serum AMH in ng/mL.]*

4. Discussion

Controlled ovarian stimulation (COS) is a critical step in obtaining mature follicles using current assisted reproduction technologies. This is closely linked to supraphysiological estradiol levels. Measuring serum E2 levels is part of basically every assisted reproduction center's fundamental protocol; in addition to estimating the number of expected oocytes, it is also beneficial in preventing inherent consequences from stimulation cycles. In the present study, we observed the correlation of the concentration of serum estradiol with the number of mature oocytes yielded from the total number of follicles retrieved. This study could help to predict the mature oocyte during controlled ovarian stimulation procedure in assisted reproductive technology (ART). We also examine the ratio of E2/AMH on the maturation of oocytes from total retrieved follicles.

During controlled ovarian stimulation hMG injection is given for the growth and maturation of follicles. Commercially available hMG which contains a mixture of FSH and LH. Gonadotropins have the potential to bind to the follicle-stimulating hormone receptor and promote the growth of the follicles in the process of folliculogenesis. Activated Follicle Stimulating Hormone (FSH) receptors produced estradiol from the granulosa cells. Estradiol has a significant role in the development of follicles during folliculogenesis. hMG also binds to the Luteinizing Hormone (LH) receptor, constitutively present in theca cells, and stimulates the expression of factors as well as enzymes involved in progesterone and androgen production. The combined action of the above hormones contributes to the final maturation of pre-ovulatory follicles up to ovulation **[1]**. However, in our study, we calculated the mean value of the serum E2 concentration on the various days during stimulation such as on Day 2, Day 5, and on the day of trigger. Serum E2 level significantly increases on the day of trigger in comparison to initial days of controlled ovarian stimulation [Graph 4]. The sudden rise of serum estradiol occurs due to subcutaneous administration of decapeptyl. Decapeptyl contains the active ingredient triptorelin, an analog of GnRH, which binds the GnRH receptors and triggers the production of LH as well as FSH. An increased level of FSH promotes the growth of follicles which produce more estradiol during the follicular maturation Establishing a more specific serum E2 value as a prognostic marker at the time of controlled ovarian stimulation provides for a more accurate picture of the expected outcomes. Higher levels of E2 were found in follicles from which oocytes with higher fertilization rates were obtained. These observations however have not been confirmed by other studies. The embryo was not related to levels of follicular E2. Regarding pregnancy rates, elevated E2 levels in Follicular Fluid were associated with an increased chance of pregnancy. Furthermore, we studied the serum E2 concentration on the trigger day with the patient's age and it suggested that the age of the patient between 24-28 years might have a concentration of serum E2 on the trigger day range between 2000-4000 pg/ml [Graph 5]. As the age of the patient rises above 28 years, the level of the estradiol is reduced. Also, we examined the concentration of serum E2 on the day of the trigger which was between 2000-4000 pg/ml and had received more total follicles after the oocyte retrieval procedure [Graph 6]. This might occur due to FSH which releases from the anterior pituitary **[10]** and stimulates the granulosa cells to convert androgen sex hormone, released by theca cells to estrogen sex hormones. As follicles grow, granulosa cells continue to produce more E2. After E2 concentration is raised it will allow the preovulatory follicle to ovulate which leads to LH surge in the spontaneous cycle as performed by COS in patients for the yield of more mature oocytes. If there were a higher number of follicles and the serum E2 concentration on the day of the trigger ranged between 2000-4000pg/ml, we may expect that a greater number of mature oocytes would be obtained from those follicles. E2 plays an important role in the maturation of oocytes by inducing levels of [Ca+2]i through Calcium-induced Calcium Release. We get the most mature oocytes when the serum E2 concentration is between 2000 to 4000 pg/ml [Graph 7].

The AMH level of the patient indicates ovarian reserve and helps the gynecologist to customize the COS protocol. As per our results, the age range between 24 - 28 years shows a considerable level of AMH, ranging between 4 - 8 ng/mL, which might predict the estimated number of follicles [Graph 1]. As age increases the mean number of follicles, and mature oocytes decrease, indicating that age is an important ovarian reserve marker **[11]**. Thus, AMH has a substantial positive relationship with the number of follicles and mature oocytes. Moreover the scattered plot of serum level of E2 and serum

level of AMH indicate that the patients whose serum AMH concentration was between 4 - 8 ng/mL have tended to show 2000 - 4000 pg/mL of serum estradiol concentration during the day of trigger [Graph 8]. Furthermore, the number of total retrieved follicles increase with serum concentration of AMH increases and show positive correlation with serum AMH level [Graph 2]. Previous studies mention that AMH is associated and an important marker of ovarian reserve **[11,12]**. The patients with above 8 ng/mLof the serum AMH level show high number of mature oocyte (Metaphase II) from the total retrieved follicles. However, as per the plot, the optimal number and good quality of oocytes represented by serum AMH ranged between 4 - 8 ng/mL [Graph 3].

In the last where there is AMH concentration vs. E2 concentration (on the day of trigger), we analyzed that the AMH Values and E2 concentration gives us the estimate about the mature oocytes. Using this graph, we can easily predict that at the AMH 4 we can estimate about 4000 pg/ml E2 on the day of trigger and that would give us about 8 mature oocytes.

Thus, the age of 24-28 years which indicates highly fertile age, because during 24-28 years of age, ovaries have a considerable number of premature follicles which could be measured by the serum level of AMH. In the case of infertility, the number of follicles decreases at this age,due to that the number of granulosa cells also decreases and results in decrease in concentration of serum AMH. Thus, serum AMH levels have positive correlation with age and the total number of follicles present during COS. In addition, serum E2 also increased with increase the number of retrieved follicles during the day of trigger. However, E2 shows positive correlation with age that has been proved by our study. The mature oocytes only can release the appropriate amount of E2 represented in our study that the serum E2 positively correlates with mature oocytes from total retrieved follicles. Hence, we can predict the outcome of mature oocytes by serum E2 concentration.

5. Conclusion

In conclusion, the present study showed a positive correlation between estradiol levels and the number of total retrieved follicles, and the number of mature oocytes. From the study we may predict that serum estradiol concentration is 500±50 pg/ml per mature oocyte. In addition, measuring concentration of serum estradiol levels on the day of trigger may be predicting the total number of mature oocytes before the oocyte retrieval procedure. By using the AMH and E2 level estimates we can increase the clinical pregnancy rates and also can give better results to the patients **[13-17]**.

This study is one of its kind, whereas of now no one has predicted the E2 levels of M2 oocytes. This is a short study with a small sample size of 25 patients, we will be taking this study further for the perfect E2 level estimates. The E2 level helps us to manage the stimulation protocols, decrease the OHSS (Ovarian hyperstimulation syndrome) rates and also will help us to get the maximum yields of M2 oocytes.

6. References

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